

Microplastics in freshwater systems
– origin, fate and impact –
“a methodological research work”

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List of abbreviations

ATR	Attenuated total reflection
AWI	Alfred-Wegener-Institut für Polar-und Meeresforschung
BBP	Benzylbutylphthalate
BfG	Federal Institut of Hydrology
CAS-NO	Chemical Abstracts Service, Registration number
CFC	Continious flow centrifuge
Cs	Cesium
DBP	Dibutylphthalate
DEHA	Diethylhexyladipate
DEHP	Diethylhexylphthalate
DEP	Diethylphthalate
DMP	Dimethylphthalate
DNOP	Di-n-octylphthalate
dw	dry weight
E2	17 β -estradiol
EEQ	Estradiol equivalent concentration
FT IR	Fourier-transform infrared spectroscopy
H ₂ O ₂	Hydrogen peroxide
HPLC	High-performance liquid chromatography
KHCO ₂	Potassium formate
KOH	Potassium hydroxide
KWS	Korona-Walzen-Scheider
l/min	liter per minute
LC 50	lethal concentration that kills 50% of organisms during the observation period
MP	Microplastics
MPSS	Munich Plastic Sediment Separator
NADPH	Reduced form of Nicotinamide adenine dinucleotide phosphate
NOAA	National Oceanic and Atmospheric Administration
OECD	Organisation for Economic Co-operation and Developmen
PE-HD	Polyethylene of high density
PE-LD	Polyethylene of low density
PET	Polyethylene terephthalate
PLA	Polylactic acid
PMMA	Polymethyl methacrylate
PTFE	Polytetrafluorethylene
PVC	Polyvinylchloride
PyGCMS	Pyrolysis gaschromatography coupled with mass spectrometry
Q-ToF	Quadrupol-Time-of-flight mass spectrometer
rpm	rounds per minute
TDS-GC-MS	Thermal desorption gas chromatography mass spectrometry
TDT	Total daily intake
TED	Thermo extraktion desorption
TGA	Thermogravimetric analysis

TOC	total organic carbon
UV	ultraviolet
WSV	Wasser- und Schifffahrtsverwaltung des Bundes
WWTP	Waste water treatment plant
YES	Yeast Estrogen Screen

Explanation

The entire work was carried out by myself as part of the research and development project "Microplastics and its degradation products in federal waterways and coastal waters -Origin, fate and impact", at the Federal Institute of Hydrology, Am Mainzer Tor 1, 56068 Koblenz. Parts of the present work are already published in a scientific journal (Felsing et al., 2017) or are in publication progress. In these publications, I am the first and responsible author to have done most of the data creation, most discussions, and writing of the draft publication. Some parts of experiments were conducted by bachelor and trainee students ((Joscha Stiedl (sediment contact test with PS, PMMA and PLA) and Esther Breuninger (sediment contact test with PET and PVC)) and supported by me. Most of the results have already been presented at international and national conferences in lectures and posters by myself and/or my direct project partners.

Publications

Felsing, S., Kochleus, C., Buchinger, S., Brennholt, N., Stock, F., Reifferscheid G., 2017. A new approach in separating microplastics from environmental samples based on their electrostatic behaviour. Volume/Issue 234C. Environmental Pollution 234C (2018) pp. 20-28.

Conference paper

Breuninger, E.; Bänsch-Baltruschat, B.; Brennholt, N.; Hatzky, S.; Kochleus, C.; Reifferscheid, G.; Koschorreck, J., 2017. Addendum Plastics in European Freshwater Environments ISSUE PAPER (Final Version)

Poster

Nicole Brennholt, Christian Kochleus, Stefanie Felsing, Friederike Stock, Axel Schmidt und Georg Reifferscheid. Zeitliche Entwicklung der Mikroplastikbelastung in ungestörten Sedimenten der Trave. Setac GLB Neustadt a.d. Weinstraße, 2017.

Joscha Stiedl, Christian Kochleus, Friederike Stock, Stefanie Felsing, Georg Dierkes, Nicole Brennholt, Thomas Ternes und Georg Reifferscheid. Etablierung und Optimierung einer Nachweismethode von Mikroplastik aus Biotaprobieren. Setac GLB Neustadt a.d. Weinstraße, 2017.

Stefanie Hatzky, Christian Kochleus, Esther Breuninger, Julia Bachtin, Georg Reifferscheid, 2016. Potential effects of PVC microplastics particles on Lumbriculus variegatus. 7th Late Summer Workshop 2016. 25. – 28. September in Haltern am See, 2016.

Johanna Girardi, Nicole Brennholt, Christian Kochleus, Stefanie Hatzky, Georg Reifferscheid, 2016. Comparison of different procedures for the preparation microplastics particles in freshwater samples. 7th Late Summer Workshop 2016. 25. – 28. September 2016 in Haltern am See

Oral presentations

Christian Kochleus, Joscha Stiedl, Esther Breuninger, Stefanie Felsing, Friederike Stock, Georg Dierkes, Georg Reifferscheid, Jochen Koop, Thomas Ternes, Nicole Brennholt. Beeinflusst Mikroplastik den Süßwasseroligochaeten *Lumbriculus variegatus* Setac GLB Neustadt a.d. Weinstraße, 2017.

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Stefanie Hatzky, Christian Kochleus, Julia Bachtin, Esther Breuninger, Nicole Brennholt, Georg Reifferscheid, 2016. Microplastics in inland waterways and coastal waters – origin, fate, impact. Micro 2016 Lanzarote.

Stefanie Hatzky, Christian Kochleus, Julia Bachtin, Esther Breuninger, Nicole Brennholt, Georg Reifferscheid, 2016. Microplastics in inland waterways and coastal waters – origin, fate, impact. Setac Nantes 2016.

Nicole Brennholt, Beate Bänsch-Baltruschat, Esther Breuninger, Stefanie Hatzky, Georg Reifferscheid, Jan Koschorreck, 2016. Overview on plastics in European freshwater environments -Results of a survey. Setac GLB Tübingen 2016.

Summary

Since the 1970s, it is a well-known fact that the volume of plastic waste in the form of microplastics too (MP < 5 mm) is increasing in our environment. But only in recent years has this topic become the focus of marine and inland aquatic research. In order to determine whether pollution by MP is present, initially only the surface of the water was sampled with nets of mesh sizes $\geq 300 \mu\text{m}$. While it is very easy to take sediment samples, processing them is much more difficult. Here, too, the research in the application is limited in terms of the smallest MPs because it is too difficult to isolate the small plastics from the rest of the material. However, these particles (< 300 μm) have become a global problem in the aquatic environment. Sampling and processing causes many problems, especially in water with a high biomass content. To date, there is no standardized methodology to sample the entire water column and to determine the size classes or distribution of the particles. The potential effects of the particles on the organisms of the different trophic levels are also unknown. Since the actual amount of MP in the water compartments or in the sediment is unclear, laboratory tests are often carried out with unrealistically high/low particle numbers or take place under unnatural conditions. In this work, two methods have been successfully developed: a) to enable the sampling of the entire water column without limitation in terms of particle size or density and b) to separate MP (< 5 mm) from environmental samples without much effort using a modified Korona–Walzen–Scheider (KWS) device originally used in recycling management. Furthermore, a sediment contact test (OECD (2007) Test No. 225) was adapted to determine the effects of MP on the organism *L. variegatus*. In a preliminary study, it was first determined whether the plastic types (PVC, PET, PMMA, PLA, and PS) used in the experiment have poisonous additives (phthalate ester) and whether they dissolve in the water phase. With the high-performance liquid chromatography (HPLC) method, different phthalates could be detected in the water contaminated with PVC products. Furthermore PVC had a negative effect on mortality beginning with a concentration of 0.1 mg/g dw (sand) with an LC 50 of 1.7 mg/g dw. Other types of plastic such as PET, PS, PMMA, and the bioplastic PLA show no effects on organisms even at concentrations of more than 15 mg/g dw (about 23,000,000 particles per 16 g of sand). None of the used concentrations have been recorded anywhere in the environment. It was also examined whether the different plastic concentrations have a negative influence on the fitness of the organisms. For this purpose, the glycogen content of the surviving worms was determined. The results show that only the increasing concentration of PVC causes a negative effect.

Zusammenfassung

Seit den 70er Jahren ist bekannt, dass sich Plastikmüll in Form von Mikroplastik (MP <5 mm) vermehrt in unserer Umwelt befindet. Doch erst in den letzten Jahren ist dieses Thema zum Schwerpunkt in der Meeres- und Binnengewässerforschung geworden. Um einen Einblick zu erhalten, ob eine Verschmutzung durch MP vorhanden ist, wurde zunächst nur die Gewässeroberfläche mit Netzen abgefischt, deren Maschenweiten meist nicht weniger als 300 µm betragen, da diese andernfalls zu schnell mit Schwebstoffen verschlossen werden. Sowohl Probenahme als auch Aufbereitung bereiten daher vor allem in Gewässern mit hohem Biomasseanteil viele Probleme. Im Gegensatz dazu sind Sedimentproben relativ einfach zu nehmen, deren Aufarbeitung stellt dabei den weitaus schwierigeren Teil dar. Des Weiteren ist die Forschung aktuell noch sehr eingeschränkt, was die Extraktion des MP aus der Proben-Matrix, insbesondere jener MP-Partikel <300 µm, anbelangt. Nachweislich sind diese kleinen Partikel zu einem weltweiten Problem in der aquatischen Umwelt geworden. Bis heute gibt es keine standardisierte Methodik zur Beprobung der gesamten Wassersäule und um die Größenklassen sowie die Verteilung der Partikel zu ermitteln. Es ist ebenfalls nicht hinreichend erforscht, welche möglichen Effekte die Partikel auf die Organismen der verschiedenen Nahrungsstufen haben. Da die tatsächliche Menge von MP in den Wasserkompartimenten und ebenfalls im Sediment nicht ausreichend quantifiziert ist, werden die Laborversuche oftmals mit unrealistisch hohen/niedrigen Partikelzahlen durchgeführt, sodass zumeist keine repräsentativen Ergebnisse erwartet werden konnten. In dieser Arbeit wurden aufgrund dieser methodischen Schwierigkeiten erfolgreich zwei Verfahren entwickelt um a) die gesamte Wassersäule beproben zu können, ohne Einschränkung in Hinblick auf die Partikelgröße oder -dichte und b) ein Gerät aus dem Recyclingmanagement modifiziert, um es für die vereinfachte Trennung von MP (<5 mm) aus Umweltproben einsetzen zu können. Des Weiteren wurde der Sediment-Kontakttest (OECD (2007) Test No. 225) zur Ermittlung der Auswirkungen von MP auf den Organismus *L. variegatus* adaptiert. In einer Vorstudie wurde dazu zunächst ermittelt, ob die verwendeten Plastikarten (PVC, PET, PMMA, PLA und PS) im Versuch schädliche Inhaltstoffe (z. B. Phthalate) aufweisen und ob diese sich in der Wasserphase lösen. Mittels Hochleistungsflüssigkeitschromatographie (HPLC) konnte bestätigt werden, dass sich vor allem aus PVC- Produkten einige, zum Teil toxische, Additive im Wasser lösen und dort nachweisbar sind. Zudem stellte sich heraus, dass PVC, mit hohem Anteil an Additiven, ab einer MP- Konzentration von 0,1 mg/g Trockengewicht (TG) die Mortalität erhöht bei einer letalen Konzentration (LC 50) von 1,7 mg/g TG. Plastikarten wie PET, PS, PMMA sowie das Bioplastik PLA, mit einem sehr geringen Anteil an Additiven, zeigen auch bei Konzentrationen von über 15 mg/g TG (ca. 23.000.000 Partikel in 16 g sand) keine nachweisbaren

Effekte auf den Süßwasseroligochaeten *Lumbriculus variegatus*. Keine der eingesetzten Konzentrationen konnte bisher in der Umwelt ermittelt werden. Ebenfalls wurde untersucht, ob die verschiedenen Plastikkonzentrationen einen negativen Einfluss auf die Fitness der Organismen ausüben. Hierzu wurde die Fitness, anhand des Speicherstoffs Glykogen, der überlebenden Würmer ermittelt. Die Ergebnisse zeigen, dass sich nur bei hohen Konzentrationen von PVC der Glykogengehalt reduziert.

1 Introduction

Plastics play an important role in human society because of their numerous social benefits (Andrady and Neal, 2009). Plastic, a man-made material, is inexpensive, strong, durable, lightweight, and easy to manufacture (Thompson et al., 2009). As a result, plastics production has increased since the 1950s, rising in particular from 225 million t in 2004 to 322 million t in 2015—an increase of 43% over the last decade (PlasticsEurope, 2016). However, this estimate does not take into account the synthetic fibres widely used in the textile and fish industries (Dris et al., 2016) and hence underestimates between 15% and 20% for each year are likely (Industrievereinigung Chemiefaser, 2013). Low estimates suggest that floating marine plastics weigh between 70,000 and 270,000 t (Cozar et al., 2014; Eriksen et al., 2014; Van Sebille et al., 2015). Small plastic particles, called microplastics (MPs), account for a total of 51 trillion particles of plastic waste (Van Sebille et al., 2015). MP is defined as a plastic particle smaller than 5 mm (Arthur et al., 2009). Increasing attention has been paid to MPs over the past decade following the publication of Thompson et al. (2004). Plastic microparticles originate from two distinct pathways, primary and secondary sources. Primary sources of MP include a) plastics directly made in micrometric size, including plastic pellets (Barnes et al., 2009; Cole et al., 2011), b) MP of peeling cosmetics (Chang et al., 2015; Fendall and Sewell, 2009; Napper et al., 2015; Zitko and Hanlon, 1991), and c) clothing fibres in wastewater treatment plants (Browne et al., 2011; Carr et al., 2016). Secondary MP results from the degradation of larger pieces by mechanical attrition, biodegradation, photochemical oxidation in the environment and all kinds of abrasion like fibers and tire wear (Andrady et al., 2011; Barnes et al., 2009; Bouwmeester et al., 2015; Breuninger et al., 2016; Browne et al., 2011; Cole et al., 2011; Kalliopi and Karapanagioti, 2017; Lambert and Wagner, 2016; Pathak, 2017; Song, 2017). MP can also break down into even smaller parts called nanoplastics (Gigault et al., 2016; Koelmans et al., 2015; Lambert and Wagner, 2016). So, after the actual use, the waste ends up as a primary or secondary MP (Crawford and Quinn, 2017; Dris et al., 2015; Gasperi et al., 2015; Gesamp, 2015; Vince and Hardesty, 2017) in the environment through the most diverse entry paths and reaches the seas e.g. via the rivers (Figure 1)(BKV, 2017; Koelmans et al., 2014b). Estimated 70–95% of the marine MP comes from land-based inputs (Andrady et al., 2011; Duis and Coors, 2016; Mehlhart and Blepp, 2012). MP contamination can be detected anywhere in the world; in open waters, sediments, and consequently also in organisms (Ivar do Sul and Costa, 2014).

The knowledge about its actual amount, distribution or potential risk is far from being a basis for specific management options. The methods currently used to sample MP in the sea and inland waters are very similar. Mainly, they involve plankton nets with mesh sizes of 300–500 µm for the water surface. This requires much more research, especially for smaller particles (< 300 µm) (Peng

et al., 2017; Wang et al., 2017). The use of nets is a problem, especially in areas of high biomass content, which causes the mesh to clog quickly, making it impossible to use smaller mesh sizes to capture even the smaller particles. It is also not possible to sample the entire water column with current methods. As already mentioned, the surface of aquatic systems is mainly sampled the upper 50 cm of the water body; therefore, it has to be assumed that only those types of plastic with density $< 1 \text{ g/cm}^3$ are covered. Studies on the distribution of MPs are so far based only on estimates. Sediments or beach sand samples are simple to collect but only give an indication of the amount of MP per kg.

The motivation for this work comes from this background, that a standardization and harmonization of the sampling methods are needed to make them more effective, as this is the basis for successful and comparable studies in the field of MP research (Rochmann et al., 2017). Therefore, the focus is laid on method development for microplastics sampling and preparation from the aquatic environment. For example, a new methodological approach is needed to be able to sample primary plastic as well from cosmetic products ($< 300 \mu\text{m}$). Suspended matter can be separated from the water phase under specified conditions by different methods. The choice of the method of sample collection depends on the question and the investigation target. The suspended matter content of a stream is determined primarily by the flow rate and thus by the discharge of water. Suspensions are not evenly (homogeneously) distributed in the water cross-section, neither horizontal nor vertical. For large cross-sections, a composite of a mixed sample can be obtained by pooling several individual samples. The choice of the 'right' sampling strategy i.e. the representativeness for a larger water range, is crucial. The important part is the development of a novel sampling methodology. In order to sample the entire water column, the nets have to be replaced by an adapted procedure. This should also cover the entire size range and preferably should not be limited by plastic densities (Table 2). In this work, the flow-through centrifuge technique, already a standard method in suspend particulate matter sampling, is established for MP sampling (Breitung et al., 2015). The main aim of this strategy is the investigation of all sizes and types of MP in the total water column. Regarding the sediments, for the first time, sediment cores were taken by divers for MP extraction. These represent the MP occurrence in a temporal course and a documentation of the quantitative and qualitative evolution of MP particle entry into aquatic ecosystems and their sediment deposition is possible.

As mentioned above, the high biomass fraction then causes difficulties in the separation of MP from the sample. Further processing, e.g. the chemical digestion or density separation is very complicated, time- and cost-intensive (Hanvey et al., 2017) because the proportion of organic and inorganic material in these samples (water and sediment samples) varies highly. Especially in

sediments, the biomass fraction is very high (Underwood et al., 2017; Wagner et al., 2014). In order to isolate the MP from the field samples, chemical processes are currently being used in which the biological material is destroyed by bases or acids and then density separated by means of different heavy-duty solutions (Hidalgo-Ruz et al., 2012; Imhof et al., 2012). However even with these solutions, it is not certain that all plastic particles are detected; thus, densities are achieved up to only about 1.7 g/cm^3 and there are far denser plastics such as PTFE (A. Rodríguez-Seijo and R. Pereira, 2017). In addition, the chemical treatment of the samples may cause the MP to further decay or even dissolve (Rocha-Santos and Duarte, 2015; Nuelle et al., 2014). For these reasons another key consideration in simple MP separation is that the sample mass should be reduced and the biological materials removed without altering the properties of the particles. Reducing the sample mass would significantly reduce the amount of chemicals needed for further processing. Moreover, less harmful and less expensive solvents are needed (Felsing et al., 2017). Because of the difficulties in sample preparation, alternative approaches were sought. A promising process was found in the recycling industry. In this work, a modified device (KWS) was introduced that uses the electrostatic properties of MPs to separate them from different sample materials (Felsing et al., 2017; Köhnlechner and Sander, 2009). It usually has dimensions of several square meters and a capacity in the tonne range. To determine whether this method is also applicable to environmental samples, a KWS measuring device has been provided. The aim is that the physical separation method can successfully replace the chemical density separation method without having an influence on the structure, age, or growth of MP or be limited by the sample composition or particle densities. The KWS reduces a sample successfully by about 90 % during the separation process.

According to many authors, small MP particles are of particular importance due to increasing bioaccumulation potential with decreasing size. Apart from this, bioaccumulation could possibly lead to a trophic transfer into food webs, which has been proved under laboratory conditions for marine organisms (Duis and Coors, 2016). In addition to physical influences, plastic additives, intermediates formed by degradation processes, or chemicals adsorbed to plastic materials after oral ingestion may have harmful effects (chemical influences) on organisms (Andrady 2011). One additional part of the work is about capturing the real potential leaching of environmental plastic waste (soft baits were used which enter daily into waters). It is important to determine what the material consists of, what is leached, and how much of it leached. An important prerequisite is to make the laboratory conditions as natural as possible. Furthermore, it should be determined whether the leached substances pose a risk, by analyzing the estrogenic activity in the water. In this work, the focus lies on the detection of different phthalate ester that may be leached of plastics. It then has to be determined whether these substances can have a negative impact on organisms.

As already mentioned many examples of plastic additives occurring in the environment (Hermabessiere et al., 2017). The basis of a supplementary effect study is the ingestion of MP by *Lumbriculus variegatus* and the exploration which effects occur through what (chemical or physical endangering). The main part of this research is the further development of a classic sediment contact test OECD (2007) Test no. 225.). The aim of this experimental setup is the in vivo characterization of the effects of polyvinyl chloride (PVC), polystyrene (PS), polyethylene terephthalate (PET) polymethyl methacrylate and (PMMA) and a bioplastic polylactic acid (PLA) particles on *Lumbriculus variegatus*—especially with regard to different MP concentrations, between direct and indirect routes of exposure, and between all tested plastic materials. In addition, the identification and quantification of leached chemicals are evaluated by means of Yeast Estrogen Screen (YES) or chemical analysis. YES offers reliable information about the endocrine potential of substances in water-based matrices (TZW, 2015). It is established as a biological test procedure.

Table 1: Microplastics extracted from 15 m³ surface water of the river Rhine and identified with PyGCMS.

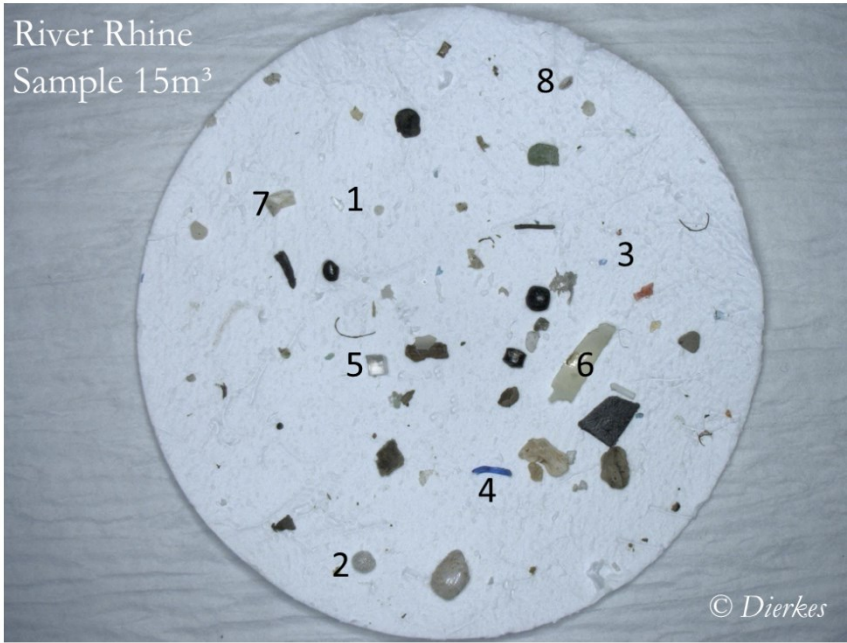
No.	Material	
1:	Ethylene propylene copolymer	
2:	Polystyrene	
3:	Polystyrene	
4:	Polyethylene	
5:	Polypropylene	
6:	Polyethylene	
7:	no MP	
8:	Polyethylene	

Table 2: Summary of the densities of the most commonly used polyester.

Kind of plastic	Abbreviation	Density g/cm ³
Polyvinyl chloride	PVC	1.38
Polyethylene terephthalate	PET	1.37
Polylactic acid	PLA	1.21-1.43
Polymethyl methacrylate	PMMA	1.18
Polystyrene	PS	1.05
Polyethylene	PE	0.95
High-density PE	HDPE	0.94
Low-density PE	LDPE	0.9-0.93

1.1 Current state of research

1.1.1 Sources of micro-, meso-, macroplastics in the aquatic environment

Plastic sources are commonly discussed in the context of pollution due to marine plastics. Land-based sources (including beach litter) account for about 80% of plastic waste (global input) (Andrady et al., 2011). It can be assumed that the entry of plastics into the oceans through rivers contributes significantly to the pollution of the oceans. In this section, an overview is given of the most important sources of plastic release into the environment, insofar as they are relevant to freshwater environments, including their catchment areas (Breuninger et al., 2017). The information is based on various reports and reviews. In the meantime, the reported values should be considered as an indicator of the potential size of each source of pollution. Estimates of personal care products (PCP) estimates have also been made, which are believed to have the smallest share of MPs, while the emissions of tire wear are the largest. The relevance of this tire dust as an emission source for MPs has also been reported by other authors. According to Sundt et al. (2014) and Lassen et al. (2015), car tire wear is the most important land-based source of MP in Denmark and Norway (500-2,250 t per year). Table 3 shows six different sources of MP emissions in Europe and some estimated amounts. Another, mostly forgotten source is the use of artificial soft baits by the increasing fishing activity in freshwater systems, which also contain partially toxic additives. According to estimates, there are tons of lost fishing lures in German waters. It is not clear what exactly happens to them (Raison et al., 2014). Currently, there is no estimate of the amounts of meso- and macroplastics released into the environment. The results of a study by Faure et al. (2015) show that relevant amounts of meso-plastics (5–25 mm) are present in lakes and rivers. In seven Swiss lakes, an average of 44 g/m² (relative to the water surface) of mesoplastic was measured. In contrast, the average MP concentration was 26 mg/m² (in terms of water surface area). In addition, the mean concentrations of four Swiss rivers were 0.43 mg/m³ for mesoplastics and 1.4 mg/m³ for MPs (Breuninger et al., 2017).

Table 3: European emissions of microplastics into the marine environment from six different sources (Sherrington, 2016 = data source).

Emission source	Year	Lowes estimated value t/a	Highest estimated value t/a
Tire wear	2012	25,122	58,424
Pellets	n. n.	24,054	48,450
Textiles	2010	7,510	52,396
Wall paint	2002	12,300	28,600
Road marking	2006	7,770	18,069
Cosmetics	2012	2,461	8,627

1.1.2 Pathways of microplastics to the environment

Figure 1 shows the different ways how plastics enter the environment. There are several reports of exposure of freshwater environments to wastewater from public and/or industrial wastewater treatment plants (AWI et al., 2014; Bannick et al., 2015; Leslie et al., 2012; Schwaiger et al., 2016). Pollution of surface waters by plastics can also be caused by atmospheric inputs. For example, plastic waste or MPs can be transported by wind from areas of infrastructure, agriculture, and industry, from uncovered landfills, or during waste collection. Modelling methods are developed to estimate the entry of plastics into the oceans. ‘From Land to Sea—A model for the detection of land-based plastic waste, 2016’ was designed by the consultancy Consultic Marketing & Industrieberatung GmbH (2016) on behalf of the chemical and plastics industry in Germany and Austria. The modelling approach systematically seeks to identify land-based plastic inputs into the marine environment from improperly disposed litter. The approach intends to characterize and quantify major pathways of micro-, meso-, and macroplastics into marine environments and to distinguish the inputs of rivers from the total input from coastal regions. In addition, the model includes European data on coastal zoning, population density, and socio-economic situations (Cieplik et al., 2016). The knowledge gained so far is validated in the next step, whereby the model parameters are checked and if necessary modified; the model itself is extended as soon as other data become available.

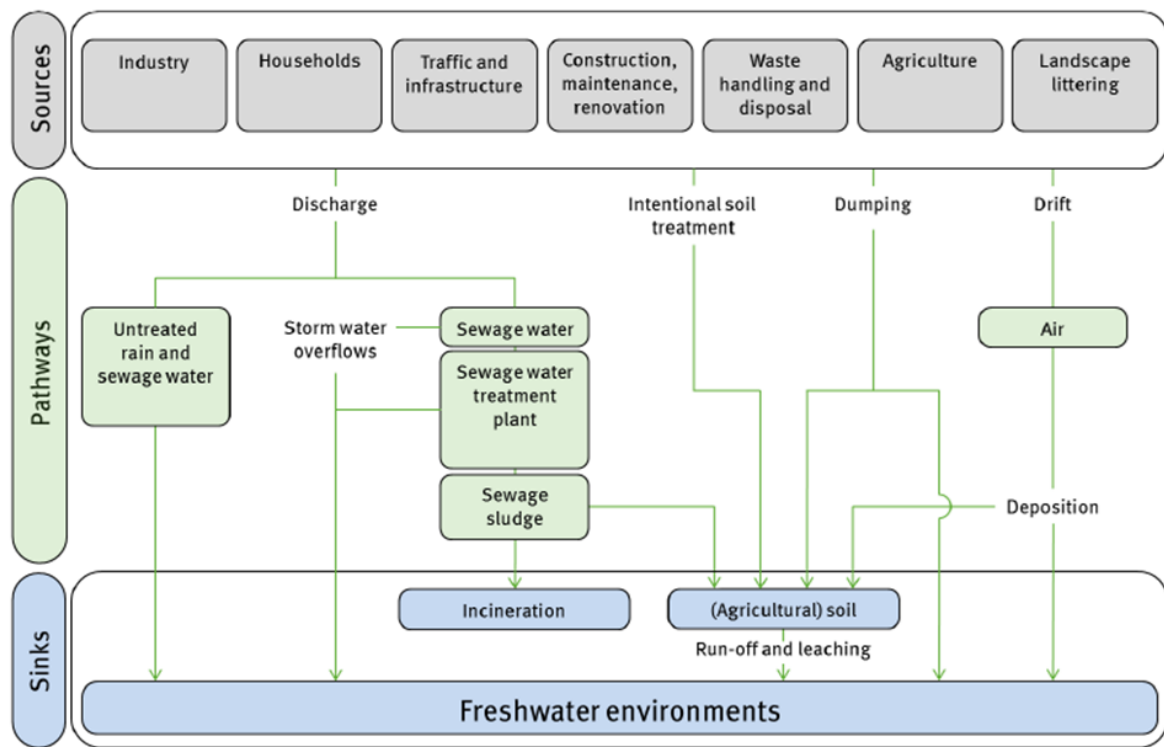


Figure 1: Possible pathways of microplastics into freshwater environments and catchment areas (Bänsch-Baltruschat et al., 2017)

1.1.3 Methodology of sampling microplastics

Plankton networks of various mesh sizes (Hohlenbluhm et al., 2015a) and the so-called Manta Trawl (Fischer et al., 2016) have been used in most of the previous studies on MP capture in water bodies. As already mentioned above, in waters with high biomass content, this method has the disadvantage that the nets clog quickly. For this reason, most studies are limited to a particle size range of 300-5000 μm . Bänsch-Baltruschat et al. (2016) summarize the common methods. However, since small particles in particular can play a major role for the organisms (Duis and Coors, 2016), this gap (Figure 2), which is currently not comprehensively recorded, must be closed. Some researchers have already created protocols for the sampling of surface waters, to sample MPs in aquatic systems, but as mentioned, only for particles larger than 300 μm (Reifferscheid et al., 2017); (Table 4). Also for sediment or beach sampling different methods were available (Hengstmann et al., 2018).

Table 4: Brief summary of the methods used in microplastics research until 2015 (Breuninger et al, 2017).

	AWI et al. (2014)	Hohenblum et al. (2015a)	Löder et al. (2015)	Mani et al. (2015)
Sampling location	Sewage water treatment plant outlet	Danube River	North Sea	Rhine River
Sampling device	Cartridge filter 10 µm	nets 500, 250, and 41 µm	net 500 µm	net 300 µm
Density separation	ZnCl ₂	NaCl	sieving 500 µm	NaCl
Organic digestion	Sodium dodecyl sulphate, enzymatic degradation, H ₂ O ₂	None	enzymatic degradation	enzymatic degradation
Water removal	sieving, filtration on aluminium oxide filter	thermal drying	filtration on aluminium oxide filter	sieving 300 µm
Detection/identification	Identification: > 500 µm: ATR-FT-IR < 500 µm: FPA-µFT-IR	detection: visual identification: ATR-IR of sub-samples (particles > 2 mm)	identification: µFT-IR	detection: visual (particles > 300 µm) identification: random controls by FT-IR)

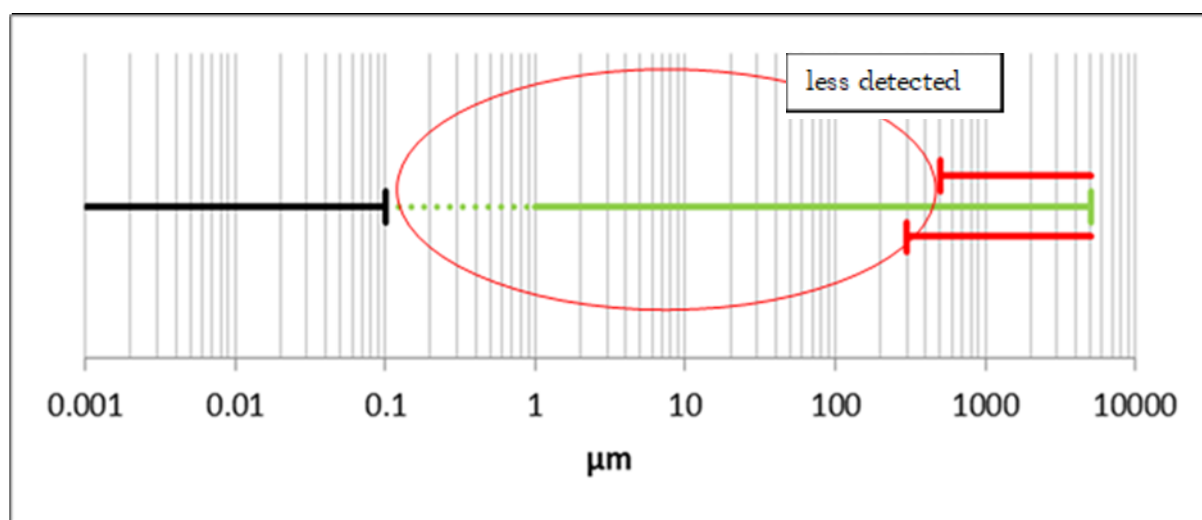


Figure 2: Overview of microplastics size classes shows the gap that cannot be captured by current research.

1.1.4 Sample preparation and microplastics isolation procedures

Freshwater samples and sediments consist of a high mass of organic components (Underwood et al., 2017; Wagner et al., 2014). To extract the MP from the samples, it is necessary to reduce this matrix by chemical digestion and afterward density separation, which are both costly and harmful (Hanvey et al., 2017). The process of chemical digestion is carried out with acids or bases, which dissolve the excess biomass into its components. The resulting residual material, which is resistant to these chemicals (most plastics), is additionally subjected to a density separation. Currently, the separation of plastic particles is also based on some chemical processes that use solutions with different densities (Table 3) (Hidalgo-Ruz et al., 2012; Imhof et al., 2012). Therefore, the samples with different materials are placed in a medium-density fluid. Less dense materials like MPs separate from the denser material by floating on the surface. Changing the density of the liquid, usually a salt solution, enables the separation of particles with different densities (Table 5). This technique has been used in 65 % of the studies in which MPs were separated from sediments (Hidalgo-Ruz et al., 2012). NaCl solution (density = 1.2g/cm^3) is most commonly used for this purpose (Reifferscheid et al., 2016). However, this method may not be applicable to MPs with higher densities than the commonly used solutions, like alkyd resin, polyester, and polytetrafluoroethylene. To address this problem, higher density salt solutions were used for the extraction of MP sediments, such as sodium polytungstate (1.4g/cm^3) (Corcoran et al., 2009; Zhao and Li, 2015), zinc chloride ($1.5\text{--}1.7\text{g/cm}^3$), (Imhof et al., 2012; Liebezeit and Dubaish, 2012), calcium chloride ($1.30\text{--}1.35\text{g/cm}^3$) (Stolte et al., 2015), and sodium iodide (1.8g/cm^3) (Claessens et al., 2013; Nuelle et al., 2014). Recently, NOAA has recommended the use of 5.4Mol lithium metatungstate (1.62g/cm^3) for density separation. The results obtained with these solutions are relatively different, and the plastic particles used in recovery experiments are also relatively large ($200\text{--}1000\mu\text{m}$) (Quinn et al., 2017). In addition, chemical treatment may result in partial or complete degradation of some MPs, thereby preventing their further analysis (Rocha-Santos and Duarte, 2015; Nuelle et al., 2014).

Table 5: Overview of the densities of currently used solutions in microplastic research.

Solution	Density (g/cm ³)
Water (H ₂ O)	1.0032
Sodium chloride (NaCl)	1.1708
Calcium chloride (CaCl ₂)	1.3
Sodium bromide (NaBr)	1.37
Sodium polytungstate (SPT)	1.4
Sodium iodide (NaI)	1.566
Zinc chloride (ZnCl ₂)	1.5–1.7
Lithium metatungstate	1.62
Zinc bromide (ZnBr ₂ 25%)	1.71
Calcium formate (Ca(HCOO) ₂)	1.91

1.1.5 Identification of microplastics

There is currently no single method for the identification of MPs. However, it is widely accepted that visual identification is inappropriate because up to 70% of all visually identified particles are false-positive (Hidalgo-Ruz et al., 2012). Recently, five methods are being increasingly applied: micro-Fourier transform infrared microscopy or infrared spectroscopy (μ -FT-IR) (Lusher et al., 2013; Renner et al., 2017), Raman microspectroscopy (Van Cauwenberghe et al., 2013; Imhof et al., 2016), Pyrolysis gas chromatography mass spectrometry (PyGCMS) (Bart 2006) and a combination of thermogravimetric analysis (TGA) with thermal desorption gas chromatography mass spectrometry referred to as TED-GC-MS (Dümichen et al., 2015) and the microPhazir (Haseler unpublished). Since each of these methods has advantages and disadvantages (Table 6), combinations are recommended (Bänsch-Baltruschat et al., 2017).

Table 6: Overview of the currently used methods for MP identification with their advantages and disadvantages (Bänsch-Baltruschat et al., 2017) = supplemented).

Analytical methods	Advantages	Disadvantages
μ FT-IR spectroscopy	Easy handling even for inexperienced personnel; fast measurement possible by using a focal plane array detector Qualitative & quantitative analysis of plastic particles of various chem. composition and shape (spherical, irregular, foils, fibres)	Difficulty in detecting black particles; theoretical limit of detection of $\sim 3\ \mu\text{m}$, practically not achievable ($\sim 20\ \mu\text{m}$); quantification of MP mass not possible
Raman microspectroscopy	Spatial resolution down to $1\ \mu\text{m}$; qualitative and quantitative analysis of plastic particles of various chem. composition & shape (spherical, irregular, foils, fibres); information on size distribution of particles (see Imhof et al., 2016)	Interference of fluorescence from (micro)biological and (in)organic contaminations; measurements are time-consuming and require trained personnel; quantification of MP mass not possible
Pyrolysis GC-MS	Identification of all kinds of synthetic materials in field samples and their additives; quantification of MP mass possible for cleaned up samples	Quantification of large samples including their matrix not possible; sample volume is limited by crucible size
TED-GC-MS	Current state of knowledge: relatively high sample masses compared to Pyrolysis GC-MS (about 200 times higher); identifying and quantifying characteristic decomposition products of spiked PE in complex environmental samples possible (see Dümichen et al. 2015)	Current state of knowledge: Until now, only PE has been tested; pre-concentration will be necessary to measure real environmental samples (see Dümichen et al. 2015)
MicroPhazir	Easy handling even for inexperienced personnel; direct identification in the field with fast measurement. Non-destructive analysis with near-infrared spectroscopy	Analyser for the plastic/polymer identification $< 1\ \text{mm}$. quantification of MP mass not possible. Difficulty in detecting black particles

1.1.6 Toxicological effects of microplastics on organisms

The accumulation of plastic in water environments is one of the great challenges of the early 21st century. Due to the high persistence of plastic materials in the environment (Akhbarizadeh et al., 2017; Moore et al., 2017), the assessment of possible adverse effects on aquatic biota is of particular interest. So far, risk awareness and research have mainly focused on plastics in marine environments. While numerous studies and reports on MPs have been published in the marine environment, little is known about the occurrence and ecological risks of plastic particles in rivers and lakes. In 2012, the Convention on Biological Diversity (CBD) reported the impacts of marine waste on 663 species, of which more than 80% were associated with plastic waste (CBD, 2012). The uptake of relatively small plastic particles (depending on the size of the organism) has been reported for a variety of marine organisms and a small number of freshwater species. Due to their

small size, MPs can be picked up by marine organisms such as zooplankton, bivalves, and worms (De Witte et al., 2014; Devriese et al., 2015; Graham and Thompson, 2009; Rochman et al., 2015; Van Cauwenberghe and Janssen, 2014; Van Cauwenberghe et al., 2015) and higher trophic organisms such as fish (Boerger et al., 2010; Carpenter et al., 1972; Dantas et al., 2012; Foekema et al., 2013; Lusher et al., 2013; Neves et al., 2015; Possatto et al., 2011; Rochman et al., 2015). The uptake of MPs by freshwater species under laboratory conditions has been demonstrated by Imhof and Laforsch (2016) for annelid worms (*Lumbriculus variegatus*), crustaceans (*Daphnia magna*, *Gammarus pulex*), ostracods (*Notodromas monacha*), and gastropods (*Potamopyrgus antipodarum*). Studies on potential adverse effects have been performed on different marine species under field and laboratory conditions, but efficacy studies on freshwater organisms are still rare (Wiesheu et al., 2017). These effects on organisms are related to the particles themselves (physical agents), as well as the adsorbed additives or impurities (chemical agents) on the particle surface. Potential physical effects include entanglement and ingestion (external and internal effects). Depending on the size-ratio of plastic particles to organism and the shape of the particles, ingestion may cause obstruction or damage to the respiratory tract of filter feeders or the digestive tract in birds and mammals. The latter can lead to altered feeding behavior such as false satiety, which leads to starvation and finally death of the organism (Gregory et al., 2009).

For plastic production mainly additives such as plasticizers, flame retardants, stabilizers, antioxidants, and pigments are used. Phthalates, bisphenol A (BPA) and nonylphenols are the most common additives derived from the environment (Berge et al., 2012; David et al., 2009; de Boer et al., 1998; de los Ríos et al., 2012; Mackintosh et al., 2004; Net et al., 2015; Xie et al., 2005, 2007); they pose a threat to the environment and organisms (Lithner et al., 2011; Meeker et al. 2009; Oehlmann et al., 2009). Phthalic acid esters (PAE) or phthalates are a family of plastic additives mainly used in PVC production as plasticizers (Arbeitsgemeinschaft und Umwelt, 2006); Many studies on the phthalate contamination of the marine environment show concentrations ranging from a few pg l^{-1} to about $10 \mu\text{g l}^{-1}$, with DEHP being the most concentrated phthalate found in marine waters (Table 7). Klammer et al. (2005) also report the presence of phthalates (DMP, DEP, DBP, BBP, DEHP and DOP) in North Sea sediments, with DEHP being the most concentrated phthalate at $170\text{--}3,390 \mu\text{g kg}^{-1}$. Phthalates in marine sediments from the Gulf of Mexico were detected on average at 7.6 and $6.6 \mu\text{g kg}^{-1}$ dw for di-n-butyl (Hermabessiere, 2017). Phthalate (DnBP) and DEHP (Giam et al., 1978) were found in the Bay of Singapore and reached $890\text{--}2,790 \mu\text{g kg}^{-1}\text{dw}$ for DEHP (Chee et al., 1996). Whether the plastic additives found in marine sediments are from diffuse sources (wastewater, atmospheric deposition, sewage sludge, etc.) or leachate from plastic waste is unclear, although there is increasing evidence (Al-Odaini et al., 2015)

suggesting that MP and plastic waste are generally likely to be sources of plastic additives in the marine environment. In 2015, 8.4 million t of plasticizer were used worldwide, of which di-2-ethylhexyl phthalate (DEHP) was the most commonly used plasticizer, accounting for 37.1 % of the global plasticizer market (ECPI, 2016). Europe accounted for 1.3 million t of the global plasticizer market in 2015 (ECPI, 2016), but DEHP was not the most widely used plasticizer in Europe, as indicated by its 20 % decline in consumption between 1999 and 2004. DEHP has progressively been replaced by diisononyl phthalate (DiNP), diisodecyl phthalate (DiDP), and di(2-propylheptyl) phthalate (DPHP), accounting for 57 % of plasticizer consumption in Europe in 2015 (Working Community and Environment, 2006; ECPI, 2016). Possible effects on organisms have been reported, mainly in the context of marine studies. Plastic additives, intermediates formed by degradation processes, or chemicals adsorbed to plastic materials after oral ingestion may have harmful effects (chemical influences) on organisms (Andrady, 2011). For example, a study on the additives phthalate-based plasticizers shows effects on the development and reproduction of a large number of wild animal species (Schulte-Oehlmann et al., 2009). So far, few effects have been observed on freshwater species under laboratory conditions. Exposure to environmentally relevant concentrations leads to decreased growth rates, hatching inhibition, altered feeding behavior, and changes in innate behavior, such as reactions to olfactory threats. In a study on the uptake and effects of MPs in zebrafish (*Danio rerio*), inflammation and lipid accumulation in fish liver, oxidative stress, and impaired lipid and energy metabolism were observed (Lu et al., 2016). MPs can potentially transfer chemicals that are adsorbed on its surface (Mato et al., 2001; Teuten et al., 2007, 2009) or evolve plastic additives. However, little attention has been paid to the transfer of plastic additives to marine organisms compared to hydrophobic organic compounds (HOCs), although many additives have been classified as hazardous (Lithner et al., 2011). Plastic toxicity has increased in recent years. Recent studies using leaching experiments demonstrate that various plastics are toxic to a variety of organisms (Table 8). Li et al. (2016) used the seven categories of recyclable plastics (HDPE, LDPE, PP, PVC, polycarbonate (PC, PET, and PS) to study the effects of their leachate on the survival and colonization of *Amphitritalanus amphitrite* larvae. Of all types of plastic, PVC was the most toxic for the larvae.

In addition, a possible tissue transition from MP uptake was observed in laboratory experiments on marine organisms. These studies suggest that tissue transfer depends on the particle size of the MPs taken (specific for species or taxonomic groups) (Duis and Coors, 2016). It is expected that only small MP particles can enter the body tissue from the digestive tract. Studies on the freshwater species *Daphnia magna* reveal that polystyrene (PS) spheres (0.02 μm and 1 μm particles) appear to pass through the intestinal epithelium (Rosenkranz, Chaudhry et al., 2009). Tissue transfer from

MP particles is of particular interest, as it may possibly cause even more severe effects within the organisms such as inflammation, rather than the mere uptake and excretion of particles (Wagner et al., 2014).

1.1.7 Description of the analyzed phthalate ester

Dimethyl phthalate DMP is e.g. used in pesticides, fragrances and cosmetics and pharmaceutical products. It is also used for the production of explosives and as a plasticizer in various products. It has a negative impact on mammal sperm quality when taken orally. In addition, it can lead to an influence of fish phagocytizing cells and thus disturb the immune defense of the animals (Watanuki et al., 2003). Diethyl phthalate DEP has the same fields of application as the DMP. The uptake leads to endocrine disorders and changes in the metabolism in fish (Barse et al., 2007). Di-butyl phthalate DBP may cause considerable health issues by inhalation or ingestion. In addition, it has an irritating effect on the skin and mucous membranes. The DBP is toxic to aquatic organisms. There are demonstrable developmental, reproductive and embryotoxic effects (Adams et al., 1995). The use of DBP is prohibited in toys for children under three (www.Umweltbundesamt.de). Due to the high fertility hazard and the possibility of harming the unborn child the benzyl butyl phthalate BBP was banned in 2005 in some consumer goods and baby articles. In the latest draft of the RL 2002/95 / EG RoHS the BBP is included in the substance ban (www.Umweltbundesamt.de). Di (2-ethylhexyl) phthalate DEHP can lead to the formation of hermaphrodites in juvenile fish. This means that hormones for the formation of gender are influenced by the absorption of DEHP in the organism. (Norrgrén et al., 1999, Norman et al., 2007) The use of DEHP is prohibited in toys for children under the age of three (www.Umweltbundesamt.de). Di iso nonylphthalate DINP is a colorless liquid with a faint odor. It is preferably used as a plasticizer in PVC production and has a high bioaccumulation potential (www.Umweltprobenbank.de). In children's toys, the DINP is prohibited, however, there are no safety instructions (www.Umweltbundesamt.de). For Bis(2-ethylhexyl) adipate DEHA, effects on offspring in reproductive toxicology studies have been identified as critical endpoints. Due to the ubiquitous occurrence of these plasticizers, therefore, a damaging effect, in particular with regard to subsequent generations (Article 14 (4) Regulation (EC) 178/2002), cannot be ruled out; the affected consumer group are pregnant women and infants. Due to its solubility in oil from the packaging, it is found in many foods (eg. cheese). In food, a maximum level of 18 mg/kg is allowed (BfR, 2006).

Table 7: Compilation of research results on the occurrence of additives (phthalate ester) in the environment (Hermabessiere et al., 2017).

Location	DMP	DEP	DnBP	DiBP	BBP	DEHP	DnOP	Reference
Tees Bay, UK	$<1 \times 10^{-3}$	0.025–0.5	0.47–0.55	0.66–1.1		0.98–2.2		Law et al. (1991)
North Sea, Germany	0.2×10^{-3}	0.67×10^{-2}	1.7×10^{-3}		0.05×10^{-3}	2.2×10^{-3}		Xie et al. (2005)
Surface waters, the Netherlands	0.004–0.49	0.07–2.3	<0.066 –3.1		0.001–1.8	0.9–5	0.002–0.078	Vethaak et al. (2005)
Bay of Biscay, Spain	$(7.5 \pm 0.4) \times 10^{-3}$	$(33 \pm 3) \times 10^{-3}$	$(83 \pm 7) \times 10^{-3}$		$(8 \pm 1) \times 10^{-3}$	$(64 \pm 4) \times 10^{-3}$	$(3.6 \pm 0.4) \times 10^{-3}$	Prieto et al. (2007)
Coastal seawater, Mediterranean Sea, Spain	0.003–0.14	0.024–0.48			0.001–0.10	0.03–0.62		Sánchez-Avila et al. (2012)
Port sea, Mediterranean Sea, Spain	0.004–0.012	0.024–0.87			0.003–0.80	0.06–5.97		Sánchez-Avila et al. (2012)
River – sea interface, Mediterranean Sea, Spain	0.005	0.07–0.16			0.003–0.07	0.02–0.21		Sánchez-Avila et al. (2012)
Ligurian Sea, Mediterranean Sea, Italy ^a						18.38 ± 44.39		Fossi et al. (2012)
Sardinian Sea, Mediterranean Sea, Italy ^a						23.42 ± 32.46		Fossi et al. (2012)
Barkley Sound, Canada			0.18–3.0			0.01–0.95		Keil et al. (2011)
Puget Sound, USA						0.06–0.64		Keil et al. (2011)
Klang River estuary, Australia						3.10–64.3		Tan (1995)
Caspian Sea, Iran	0.49	0.52						Hadjmohammadi et al. (2011)
Arctic	40×10^{-6}	138×10^{-6}	51×10^{-6}	22×10^{-6}	8×10^{-6}	448×10^{-6}		Xie et al. (2007)

Table 8: Short compilation of past attempts to study additives and their influence on different organisms (Hermabessiere et al., 2017).

Species	Plastic type	Exposure time	Exposure level	Endpoints	Reference
<i>Daphnia magna</i>	PC, PVC, PU, PE, LDPE, PMMA, PET, HDPE, PTFE, ABS, PP, MDPE	24 and 48 h	70–100 g L ⁻¹	Mortality	Lithner et al. (2009)
<i>Daphnia magna</i>	PP, HDPE, PVC, ABS, Epoxy resin	24 and 48 h	Up to 250 g L ⁻¹	Mortality	Lithner et al. (2012)
<i>Nitroca sinipes</i>	PP, PVC, PS, PET, PUR, LDPE, HDPE, ABS, PLA, Unknown	96 h	100 g L ⁻¹	Mortality	Bejgarn et al. (2015)
<i>Amphibalanus amphitrite</i>	PET, HDPE, PVC, LDPE, PP, PS, PC	24, 48, 72 and 96 h	0.1–0.5 m ² L ⁻¹	Settlement	Li et al. (2016)
<i>Perna perna</i>	Virgin (PP) and beached pellets	48 h	25% of pellets (v/v)	Embryo development	Gandara e Silva et al. (2016)
<i>Pseudochromis fridmani</i>	PE (two different origins)	48 h	—	Mortality	Hamlin et al. (2015)

2 Material and Methods

2.1 Sampling

2.1.1 Sampling of water bodies

A flow centrifuge of the Federal Institute of Hydrology (BfG) was used for the sampling on the Lahn river. It is a flow centrifuge from the Padberg company (type CEPA Z61), combined with an external pump (Neptun NCGP-E110 INOX) (Figure 3). It was first ensured that the method samples all plastics (high and low density) by means of recovery (Figure 4). The power was supplied via an external generator or a fixed three-phase connection (Figure 5) for example when sampling at measuring station locations or a ship. It works at a flow rate of 12 l/min and 17.000 rpm. A suspended solid content of 30–100 g of wet substance was obtained per run. The flow centrifuge is firmly installed on a trailer and has a total weight of about 900 kg. Sampling on Federal Waterways, such as the Lahn river, takes place ship-based. For this purpose, the trailer is transported by crane to a ship of the Wasser- und Schifffahrtsverwaltung des Bundes (WSV) and firmly secured. Sampling involves collecting water at three positions in the longitudinal and vertical profile at three different depths. In the flow centrifuge, particles are separated from the aqueous phase due to the centrifugal force on the inner wall of the separator. The separator (clarifier) is lined with a Teflon film (PTFE). All parts of the pump and centrifuge that come into contact with the water/suspended solids mixture are made of stainless steel and/or provided with Teflon (PTFE) in order to avoid contamination by other plastics. The water is pumped continuously through the separator. The acceleration in the separator is a multiple of the gravitational acceleration. Through the use of high-performance flow centrifuges, rebound rates of up to 98 % were reached (Breitung et al., 1997; Bierl et al., 2015). In order to sample at greater depths, an external pump is connected in front of the centrifuge, which supports the water supply of the centrifuge. The exact flow rate of one m³ was detected by the centrifuge. The water is passed through a steel funnel which collects all particles <5 mm. For physical reasons, the centrifuge only collects particles with a density >1 g/m³. The residual water that leaves the centrifuge must therefore be collected separately and contains particles that are lighter than water, with a density <1 g/m³. At the four positions, sediment samples were also taken with the Van Veen Grab. One can thus obtain an entire cross-section of a sampling location. Sampling was performed at the Lahn river upstream and downstream of the effluent of a wastewater treatment plant (WWTP)(Figure 6) to determine a possible point source of MP. Additional two horizontal samples were taken to cover the entire river Lahn in a transverse profile. It was sampled in two depths (directly below water surface and -3 m). The suspended matter was collected in the centrifuge and transferred directly to glass dishes. The net (5 µm) which collected the outcoming water of the CFC was rinsed and the

particulate matter also transferred to a glass jar. Between each sampling, all materials were rinsed with ultrapure water.

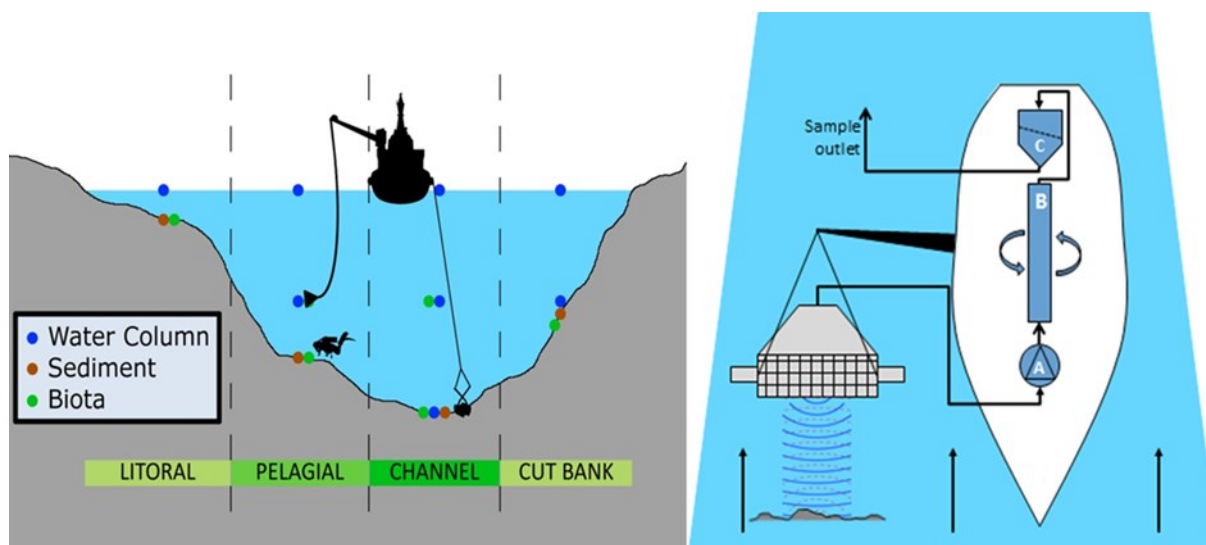


Figure 3: Adapted continuous flow centrifuge (CFC) method to obtain suspended matter samples for the sampling of microplastics from the aquatic environment. On the left is the overview of how to sample; on the right is the scheme of the modified strategy with a steel funnel A) external pump, B) the CFC, C) and a 5µm sieve after the outlet for catching the rest water.

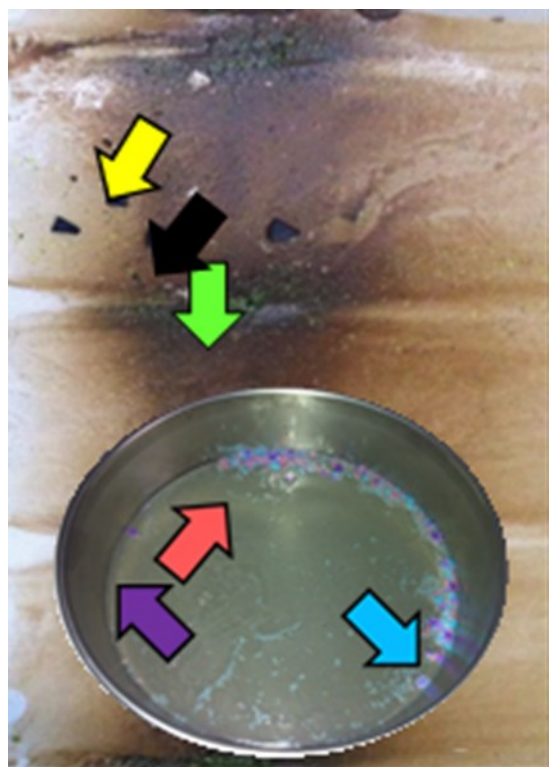


Figure 4: The picture shows the tested kinds of plastic on the teflon foil of the continuous flow centrifuge and on a sieve which collects the low-density particles of the out coming water. The colored arrows are described in table 2.

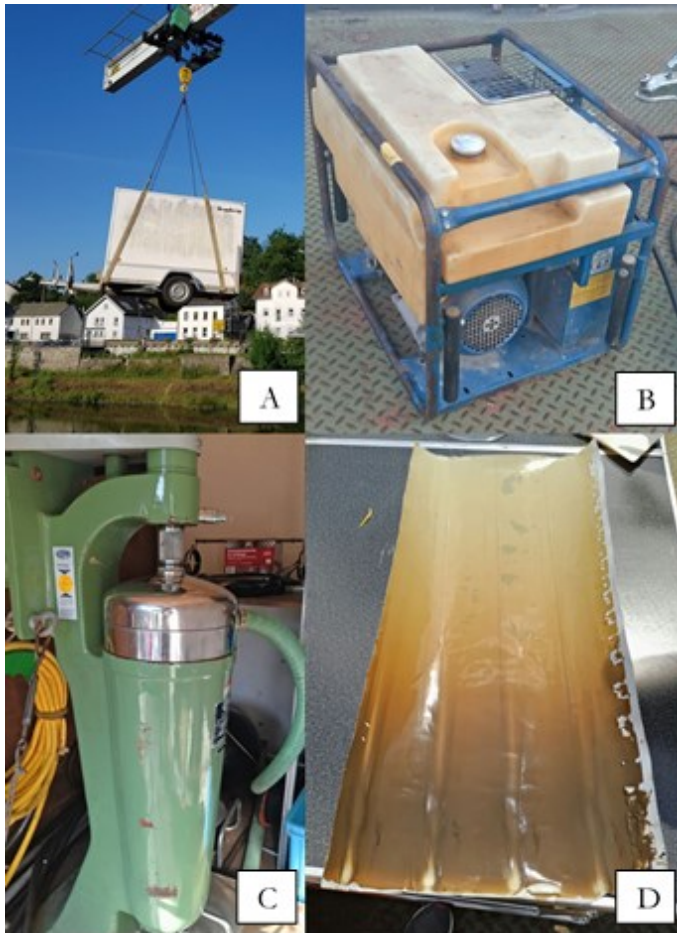


Figure 5: Overview Of the sampling process. The four pictures show A) a crane lifting the centrifuge onto the ship of the WSV. B) a diesel operating generator that starts up the pump. C) CFC where river water is passed through and D) the suspended matter collected on the teflon foil by the centrifuge.

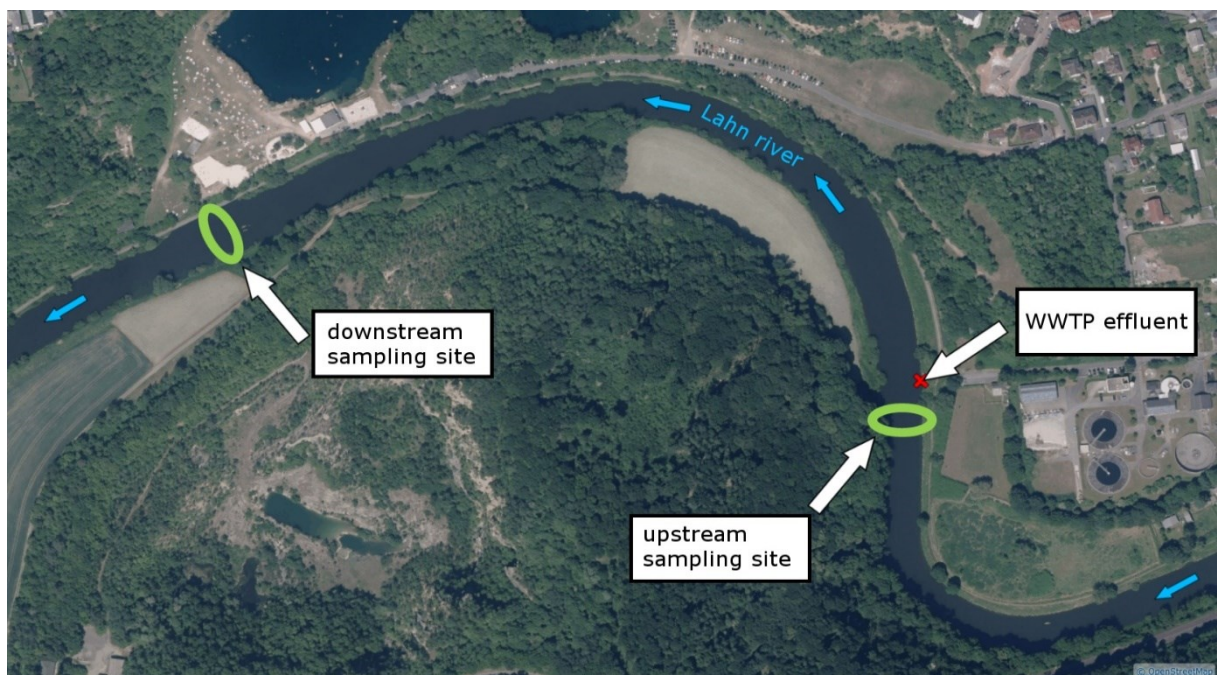


Figure 6: The picture shows the sampling sites (upstream and downstream) on the river Lahn and the effluent of a waste water treatment plant (Source: Google Maps).

Sediment core sampling

The sediment cores were taken by divers during a test run of the centrifuge by divers. The undisturbed sediment cores come from the Trave river (about 4m depth) (Figure 7). The used cores (hard PVC) are 20 cm in diameter and about 22 cm long. The lids are removed and the lancing tube is slowly pushed into the sediment. Then the upper lid is replaced and the sediment lingers in the core. The core is then closed with the lower lid and frozen until further processing. Subsequently, these are divided into 1.5–2 cm thick layers. The age of each layer was determined with gamma spectroscopy (cesium 137 dating) and the amount of MP content was determined. The sediment was dried and the MP was isolated using the KWS (Felsing et al., 2017). The digestion of organic material was done using 10 Mol KOH and 30% H₂O₂. For the density separation, 1.6 g/ml KHCO₂ was used (house method). Pressure filtration on glass fibre filter was at 5 bar overpressure. Only visual identification with the microscope was done at first.

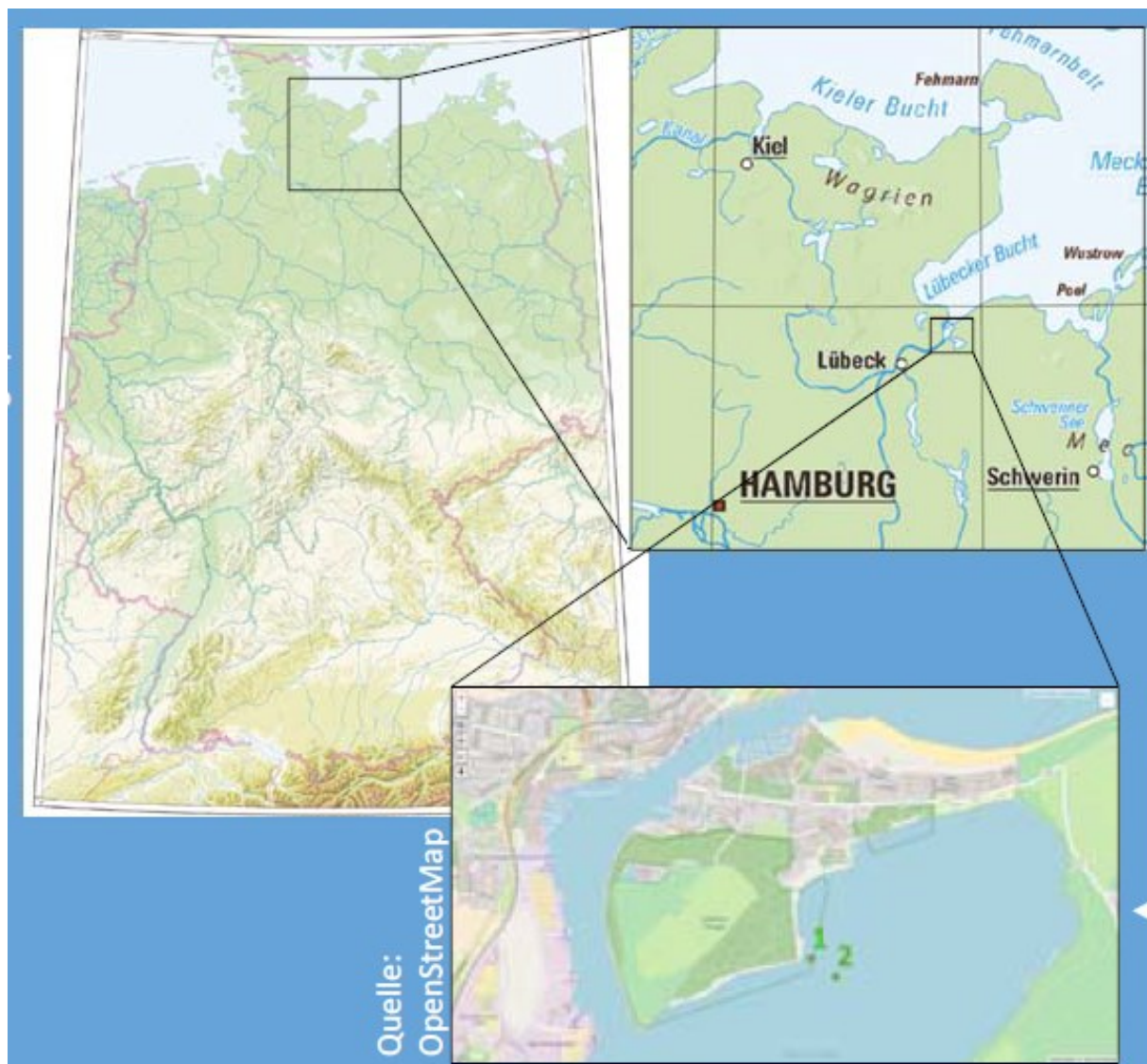


Figure 7: The maps show the sampling positions on the Trave river, Brennholt et al, 2017.

2.1.2 Identification

The qualitative identification of the small particles, which can no longer be determined visually, is done presently in the BfG by using a μ FTIR and microphasir.

2.1.3 Sample preparation

To separate MP from different sample material the Korona–Walzen–Scheider (KWS), a metal/plastic separator, was used. The device used here has the dimensions $60 \times 60 \times 60$ cm and a weight of 57 kg. The funnel has the dimensions $20 \times 15 \times 20$ cm, so that samples up to this volume can be introduced directly. The collecting trays can be emptied continuously so that theoretically very much larger quantities can be separated. Operation takes place via a touch panel from sigmatek, which stores all settings (voltage, speed, vibration) and allows for export via USB (hamos, 2016). The mixture to be separated is fed via a vibratory conveyor to a rotating grounded metal drum and transported into the area of a corona electrode. Here, the particles are electrostatically charged due to their substance-specific properties (Chosakai, 1976, 1984, Domenico and Schwartz, 1990). Because the drum is grounded, the particles discharge as soon as they leave the electric field, which discharges more conductive materials faster than less conductive ones. They are discarded by the rotational movement according to their discharge rate in different sample collectors. The less conductive the particles are, the more slowly they are removed; later, they are divided into the corresponding fraction: the sample fraction (non-conductor fraction of MP particles and small amounts of organic and inorganic residues) and the discarded residual fraction (conductor fraction) (Domenico and Schwartz, 1990), which is free of plastic (Köhnlechner and Sander, 2009). After several test runs, the optimal settings for the KWS for the separation of environmental samples were found. The vibration of the conveyor belt was set to 900 rpm and the drum speed to 66.5 rpm (Figure 8). A voltage of 20 kV was applied to the electrode (Flachberger and Köhnlechner, 2009).

In order to validate the effectiveness of the KWS for MP separation from field samples and to ensure the reliability of the procedure, a comprehensive recovery attempt was made. For this purpose, four different materials were compared—purified quartz sand, sand from the beach, river sediment, and freshwater particulate matter (Figure 9, A–D); 150 g of each material were spiked with 10 MP particles of different types (see MP production) and four different size classes (63–200 μ m, 200–630 μ m, 0.63–2 mm, and 5 mm). The added plastic particles were then separated from the material by the KWS in terms of their conductivity (Flachberger and Koehnlechner, 2009; Koehnlechner and Sander, 2009; Schubert et al., 2001). For the best possible result of the sample reduction, each fraction was run three times. For the determination of the recovery, three replicates each were run and presented as an average. Depending on the composition of the sample, the

separation time varied (Figure 10). The weights of the fractions were collected after each step and the particles counted (Felsing et al., 2017).

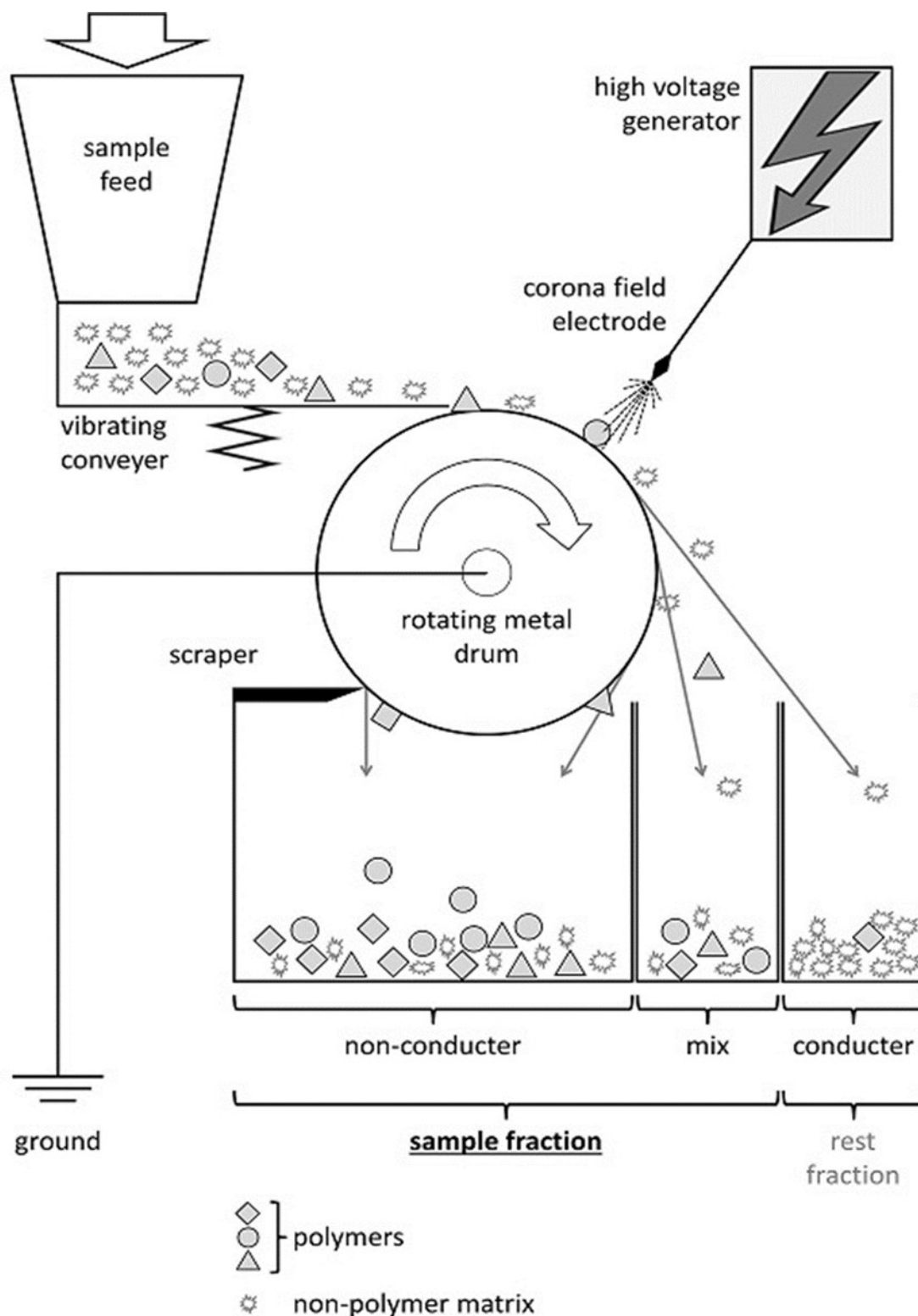


Figure 8: Scheme of the Korona–Walzen–Scheider (KWS) electrostatic metal separator (hamos GmbH). The KWS 255 sorts material into non-conductor, mix, and conductor fractions. Since the mix fraction may still contain microplastics particles, it is combined with the non-conductor fraction to form the sample fraction, Felsing et al., 2017.

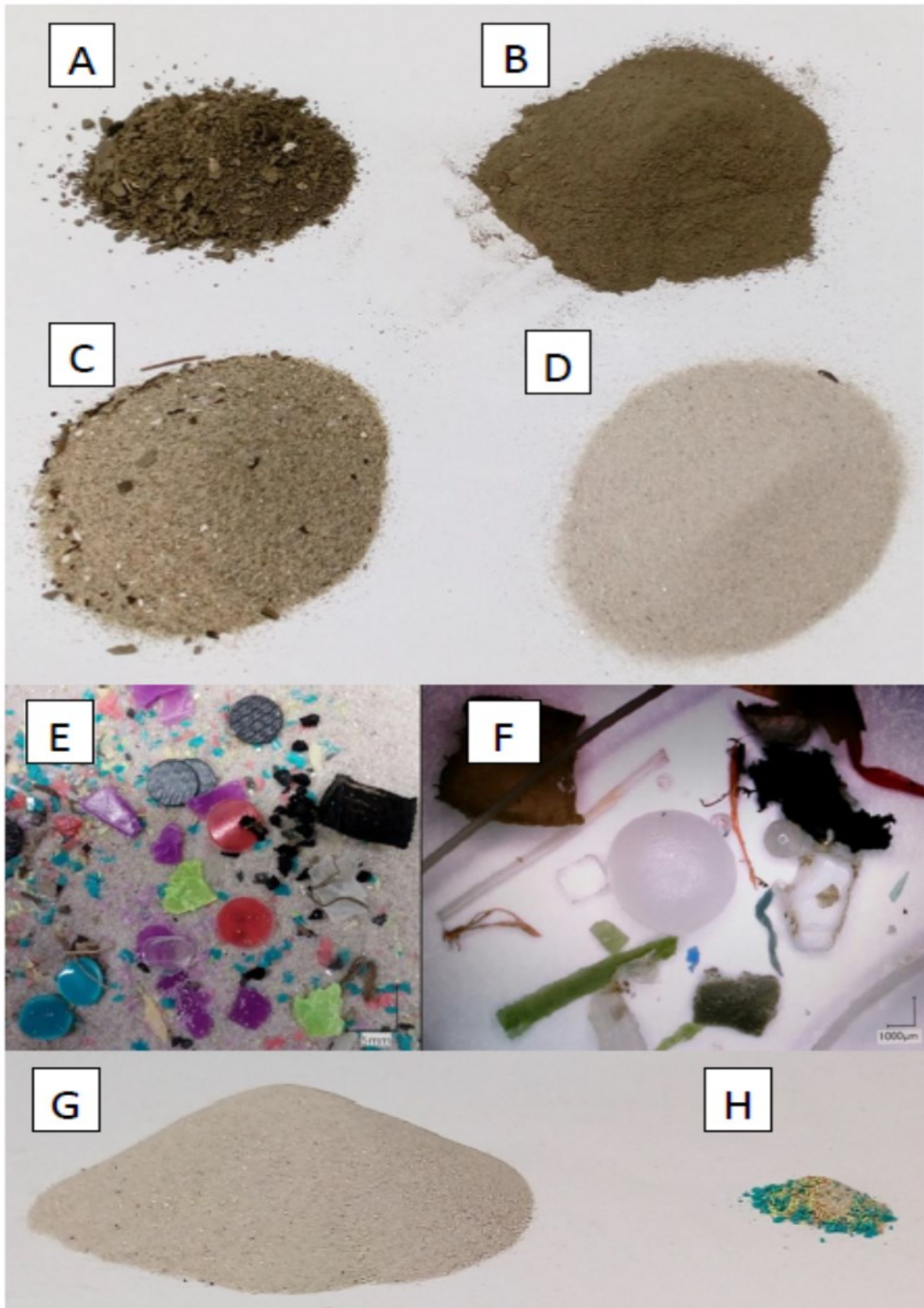


Figure 9: The four different materials used for recovery by the KWS; (A) freshwater-suspended particulate matter (SPM), (B) 259 freshwater sediment, (C) beach sand, and (D) quartz sand. Microplastics (MP) particles of different shapes were tested. (E) 260 Self-made MP particles and (F) isolated aged MP particles from the Rhine river. (G) Rest fraction of 147.6g quartz sand. (H) 261 Sample fraction containing 2g of MPs and less sand; both images were made after Separation Step 1 (Felsing et al., 2017).

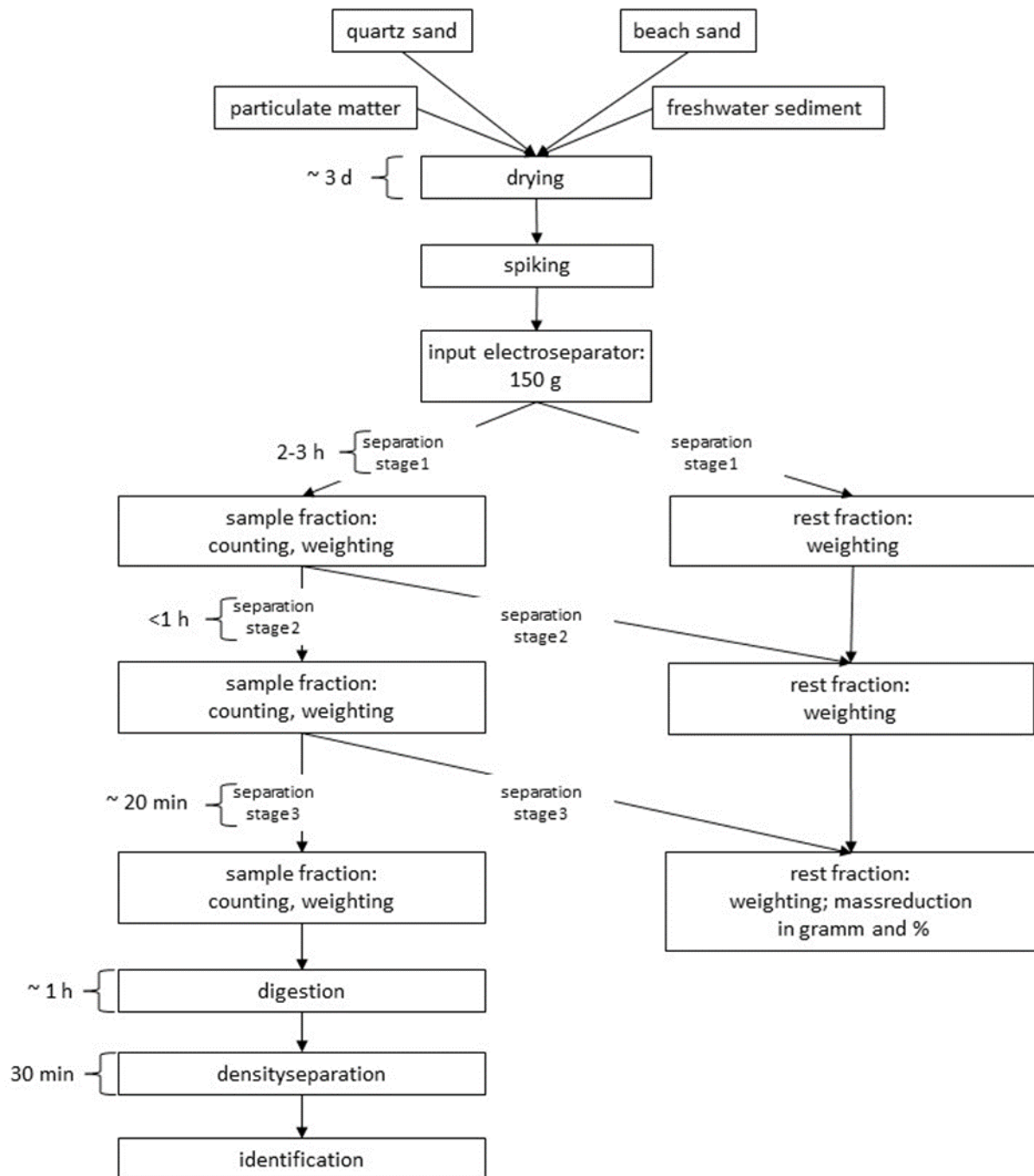


Figure 10: Scheme of the separation steps using the KWS, Felsing et al., 2017.

2.2 Laboratory design: Leaching additives from different polymers

In the experiment investigating the leaching of phthalate ester from plastic material, 11 different soft baits (rubber fish) from different manufacturers were used. First, they were analyzed for their composition by PyGCMS. This information serves as the basis for the phthalate analyses. For leaching attempt, 1 g of the material was weighed from each bait. If the rubber fish were made of

different materials, they were weighed in equal parts to 1 g. The sample material was then incubated in a glass with 75 ml of ultrapure water to exclude impurities from the outside. From each sample, three replicates were made and three blanks were used for comparison. The vessels were then shaken in a day-night (daylight imitation: Inkubationsschüttler Multitron pro, Infors HT, Basel) rhythm 16/8 h at 15 °C (average temperature in the Rhine river). After four and eight weeks, 1 ml of each sample was taken for high-performance liquid chromatography mass spectrometry-mass spectrometry (HPLC MS MS) analysis for determination of additives and 6 ml for the YES test.

2.2.1 Identification of MP using PyGCMS

The pyrolysis-GC-MS(PyGCMS) is an analytical method by means of which almost the entire sample material (solid or liquid) from small sample amounts (30 µg to 1 mg) can be characterized without prior sample preparation. It gives information about the type of plastics, the composition of materials, additives contained or foreign substances (contaminations). A wider temperature range offers the possibility to use this technique for sensitive materials and extremely temperature-stable materials. Particles extracted from the field samples were identified by the following method. A total of 10–30 µg of sample material were flash-pyrolysed at 600 °C using the pyrolysis injector EGA/PY-3030D from Frontier Lab (Koriyama, Japan). The pyrolysis products were separated by gas chromatography using a 7890B gas chromatograph (Agilent Technologies, Santa Clara, USA) and a 30 m Ultra ALLOY-5 capillary column with an inner diameter of 250 µm and a thickness of 0.25 µm (Frontier Lab, Koriyama, Japan). GC oven temperature of 40 °C was maintained for two minutes, increased to 320 °C by 20 °C per minute and maintained for 13 minutes. Detection was performed by high resolution mass spectrometry using a 7200 Q-ToF (Agilent Technologies, Santa Clara, USA) operating in electron ionization (70 eV) and full scan (m/z 50-500) mode. Identification of the plastics was done by database-matching with the F-Search database v3.4.2 delivered by Frontier Lab (Koriyama, Japan). For the detection of leached phthalate ester HPLC was used.

The identification and quantification of additives was done with a HPLC MS/MS (according DIN-Norm 38407-36, DIN 2014). It is an analytical method for the separation and determination of molecules by a combination of liquid chromatography (HPLC) and mass spectrometry (MS). The chromatography is used for the separation of molecules in a mixture and the subsequent mass spectrometry for the identification and/or quantification of the substances. For the sampling directly from the water sample a house own method was used. Parameter: The volume of the direct injection amounted to 10 µl with a column temperature of 30 °C. ESI+ (electrospray ionization with positively charged ions) was adjusted in a Multiple Reaction Monitoring (MRM) mode and a

linear calibration of 0.5–500 ng/μl was used with D4-Diethylphthalat (D4-DEP) as internal standard. A Trapcolumn Poroshell 120 EC-C18 from Agilent Technologies was slotted ahead of the injector for blank elimination. For the analysis, a Zorbax Eclipse Plus C18 (Agilent Technologies) column was used.

2.2.2 Measurement of estrogen active substances using the YES-test

The estrogenicity was tested weekly by the Yeast Estrogen Screen (YES) performed according to the provisional ISO standard ISO/TC 147/SC 5 N 804 - ISO/WD YES Test-1 (working draft, unpublished as of 2017) with *Saccharomyces cerevisiae*. In this in vitro test system, the gene of the human estrogen receptor (hER) was integrated into the main chromosome of the yeast *S. cerevisiae* by molecular genetic methods. In addition, an expression plasmid was inserted, with an estrogen responses element (ERE) and the reporter gene lacZ. LacZ encodes the enzyme β-galactosidase. If an estrogen-active substance activates the hER (an additive from MP), a complex is formed that binds to the ERE in the plasmid. This leads to the transcription of the lacZ gene and thus to the synthesis of β-galactosidase (Spangenberg, 2014). By adding the chromosome substance chlorophenol red-D-galactopyranoside (CPRG), the activity of the enzyme can be determined, since β-galactosidase cleaves the CPRG while releasing chlorophenol red. The colour changes from yellow to red can then be determined photometrically and helps to determine the estrogenic activity (Routledge and Sumpter, 1996).

2.2.3 Laboratory test design of ecotoxicological investigations

2.2.4 Primary studies on additives

In the first step, the types of plastic used were analyzed for their composition by means of pyrolysis gas chromatography mass spectrometry (PyGCMS) (see 2.3.1) and characterized accordingly. It was then determined whether additives are leached of MP particles and to what extent. For this purpose, six different plastics (5 mm) were incubated with water in 20 ml glasses. These were shaken in a day-night rhythm 16/8 h at 20 °C. After four and eight weeks, a sample was taken for the HPLC measurement and the water was analyzed for seven different phthalates (DNOP, DEHP, DEHA, BBP, DBP, DEP, DMP). The identification and quantification of the phthalates was carried out using HPLC-MS-MS (see 2.1.1).

2.2.5 Description of the used polymers

Polylactic acid (PLA, CAS No.: 26100-51-6) belongs to the class of thermoplastics. The monomer can be produced by microorganisms from glucose by fermentation or directly by polycondensation.

It is polymerized from renewable raw materials and thus belongs to the biogenic plastics. The polymer is produced by the esterification of lactic acid monomers. PLA has a density of 1.2–1.4 g/cm³ and therefore sinks in the water body and is available for benthic organisms. It is 100 % biodegradable and in combination with the biogenic origin of the monomers it is a true bioplastic. The used PLA comes from disposable cups from PAPSTAR (PAPSTAR GmbH, Kall, GER). Analysis by PyGCMS shows pure material of only PLA. No impurities or additives were detected in the material.

Polymethyl methacrylate (PMMA, CAS No.: 9011-14-7) is a high-molecular-weight petrochemical polymer. It is polymerized from monomers of methacrylic acid methyl ester. With a density of 1.2 g/cm³, PMMA is also available for benthic organisms. The molecular formula of the monomer is C₅H₈O₂ (GESTIS Substance Database, 2017a). PMMA is a polymer commonly used for acrylic glass. The acrylic should not contain plasticizers or other byproducts. The results of PyGCMS analysis bear out this statement.

Polystyrene (PS, CAS No.: 9003-53-6) is also a high-molecular-weight petrochemical polymer with a density of 1.05 g/cm³. Polystyrene is difficult to ignite, insoluble in water, and resistant to acids, bases, alcohols, and mineral oils. It is unstable in most solvents (GESTIS Substance Database, 2017b). The used polystyrene comes from disposable plates, which are comminuted in the cryomill. Polystyrene is used for a large number of disposable products such as cups and cutlery, and also in the packaging industry. The analysis of polystyrene by PyGCMS shows only the pyrolysis product of the trimer of styrene and thus that it is pure polystyrene. The plastic contains no plasticizers or other added by-products and is therefore suitable for the experiment. Polyvinyl chloride (PVC, CAS No. 9002-86-2) is a thermoplastic polymer.

The PVC plastics are divided into hard and soft PVC. In this work, a soft PVC (pond liner) has been used. Soft PVC contains up to 40 % plasticizers (phthalate ester), for example DEHP and DINP, which lead to elastic behavior of the material. It is practically insoluble in water but soluble in organic solvents (acetone as well as ester and stain remover). It is resistant to concentrated and diluted bases, oils, and aliphatic hydrocarbons. However, it decomposes upon exposure to oxidizing mineral acids. Polyethylene terephthalate (PET, Cas No. 25038-59-9) is a thermoplastic of the polyester family. It has a high breaking strength and dimensional stability at a temperature above 80 °C. PET is unstable against the attack of strong inorganic acids, in particular sulfuric acid or nitric and hydrochloric acid.

2.2.6 Proof of ingestion of MP by *L. variegatus*

To determine whether a sediment contact test with spiked sediment makes sense for *L. variegatus*, an ingestion test was first carried out with two different types of plastic (PVC, PET). Ten

individuals were transferred to a vessel with water and ‘fed’ with the particles (63–200 µm). The animals were then observed under a fluorescence microscope to watch the ingestion simultaneously.

2.2.7 Sediment contact test with *L. variegatus*

The test species used in this study is the freshwater oligochaete *L. variegatus* (Müller; Egeler et al., 2005). This species is tolerant of a variety of sediment types and is commonly used for sediment toxicity and bioaccumulation tests (U.S.P.A., 2000, OECD, 2004). In contrast to epibenthic organisms, endobenthic aquatic oligochaetes such as *L. variegatus* dig into the sediment and collect sediment particles beneath the sedimentary surface. This ensures the exposure of the test organisms to the test substance via all possible routes of exposure (e.g. contact with and ingestion of contaminated sediment particles, but also via pore water and overlying water). The sediment contact test was carried out according to OECD (2007) and adapted as a randomized, controlled, and blinded trial. Each group of 15 worms was randomly allocated to the different MP concentrations, thus preventing known and unknown prognosis factors by the worms being distributed unequally across the test tubes. Otherwise, a selection bias could occur, which would lower the internal validity (Gauch and Bast, 2014). The random assignment was done by means of the Randomization in Treatment Arms (RITA) software. This software ensures the transparency and traceability of the randomization process and the reproducibility of the results (Pahlke et al., 2004). The underlying method of randomization is the so-called Biased Coin Design, in which the current frequencies of the respective experimental approach are taken into account. The controlled design was ensured through the aforementioned three blanks. All glasses were numbered so that the included MP type and concentration would not be visible to the examiner (blinded trial).

The basis is the sediment toxicity test, which is usually carried out with pollutants in natural sediments. This means that it is determined how the pollutants found in the sand affect the organism. In the test here, it is also about finding out if the particle (MP) itself causes effects (by direct uptake over the sand) or indirectly (via possible leaching of pollutants into the water column). The latter requires some modifications of the directives are necessary. For this purpose, the worms were brought to a developmental stage (Phillips et al., 1993). The sediment was mixed with six different concentrations of PVC, PET, PS, PMMA and PLA (63–200 µm) to determine the direct exposure (Test Approach 1). Indirect exposure was assessed by fixed MP in the water column (Test Approach 2) (Figure 11). The sand (unspiked and spiked) was placed in 200ml jars and filled with water (manufactured according to OECD standards). Each jar was provided with 15 worms and ventilated throughout the experiment. The temperature was kept steady at 20 °C. A day-night

rhythm of 16:8 h was set and the exposure duration was 28 days. From each concentration, three replicates were made and a total of three blanks were scheduled for control. Water parameter controls and feeding were done by regulation (OECD, 2007, Test No. 225). The survival rates of individual, biomass, and fitness parameters as well as in vitro estrogenicity of leached chemicals (DEHP) were investigated. These analyses were carried out according to in-house regulations.

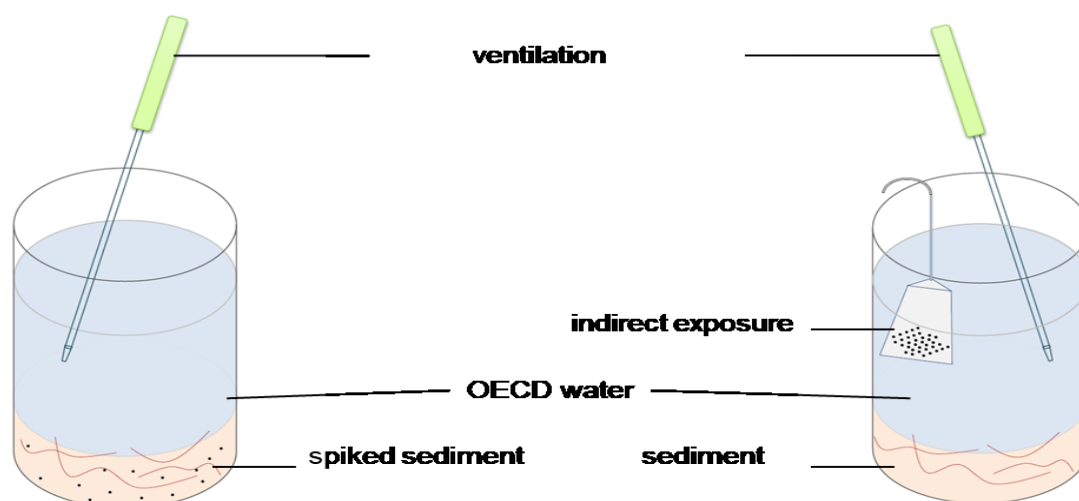


Figure 11: Experimental setup of sediment contact tests. On the left the direct exposure is shown (Test Approach 1) and on the right the indirect exposure (Test Approach 2).

2.2.8 Detection of the glycogen content of *L. variegatus*

As indicators of physiological fitness during the contact test, the form of reserve substances (glycogen) were measured in the surviving worms. For the preparation of a glycogen hydrolysate, 30 μl of the crude homogenate of *L. variegatus* were removed and transferred to a 1.5 ml reaction vessel. For the hydrolysis of the glycogen, 15 μl of KHCO_3 , 250 μl of acetate buffer, and 5 μl of amyloglucosidase were added. For optimal incubation, the sample was placed in a 40 °C water bath for two hours. To terminate the reaction and denature the remaining enzyme, the sample was added to low boiling water for about five minutes. To remove cell debris, centrifugation was then carried out at 14,000 rpm for 10 minutes. The supernatant was removed, transferred to a 0.5 ml reaction vessel, and stored on ice. For the measurement, the glucose and the Tris-Acetate-EDTA (TAE) measurement buffer were then prepared according to house-owned Standard Operation Procedure (SOP). In the photometer (Analytik Jena), the determination of the free glucose via the NADPH concentration then took place at 339 nm. Since the concentration of NADPH is stoichiometrically equal to the glucose concentration, Lambert–Beer's law allows us to determine the exact glucose concentration of each sample. The method is described in detail by Keppler and Decker (1984).

2.2.9 Contamination during all processes

In order to prevent contamination, the work was carried out continuously according to the guidelines of the NOAA (Masura et al., 2015; Woodall et al., 2015). All used objects and devices were carefully cleaned between all working steps.

3 Results

3.1 Sampling

3.1.1 Sampling success in water column samples

The data represent the number of counted particles and are therefore shown without error bars. The results represent the number of particles per square meter. Different amounts of four kinds ((primary; Figure 12: A, B) and secondary; Figure 12: C, D)) of MP (fragments, fibers, foils and spheres) were sampled in the river Lahn. In three meter deep downstream of the WWTP effluent, the highest amounts were found in the nets. Most of the detected particles were fragments (303 particles/m³), fibers (142 particles/m³) on the right shore, 70 fragments and 56 fibers on the left shore (Figure 13). These values are up to 37 times higher than the amounts of particles upstream of the WWTP effluent (Figure 14) in the same depth. Also upstream of the WWTP in the surface water are less particles detected than downstream. A total of 97 fragments, 60 fibers, 3 foils and 2 spheres were determined (left and right shore)(Figure 15). Overall, more particles are detected in the net.

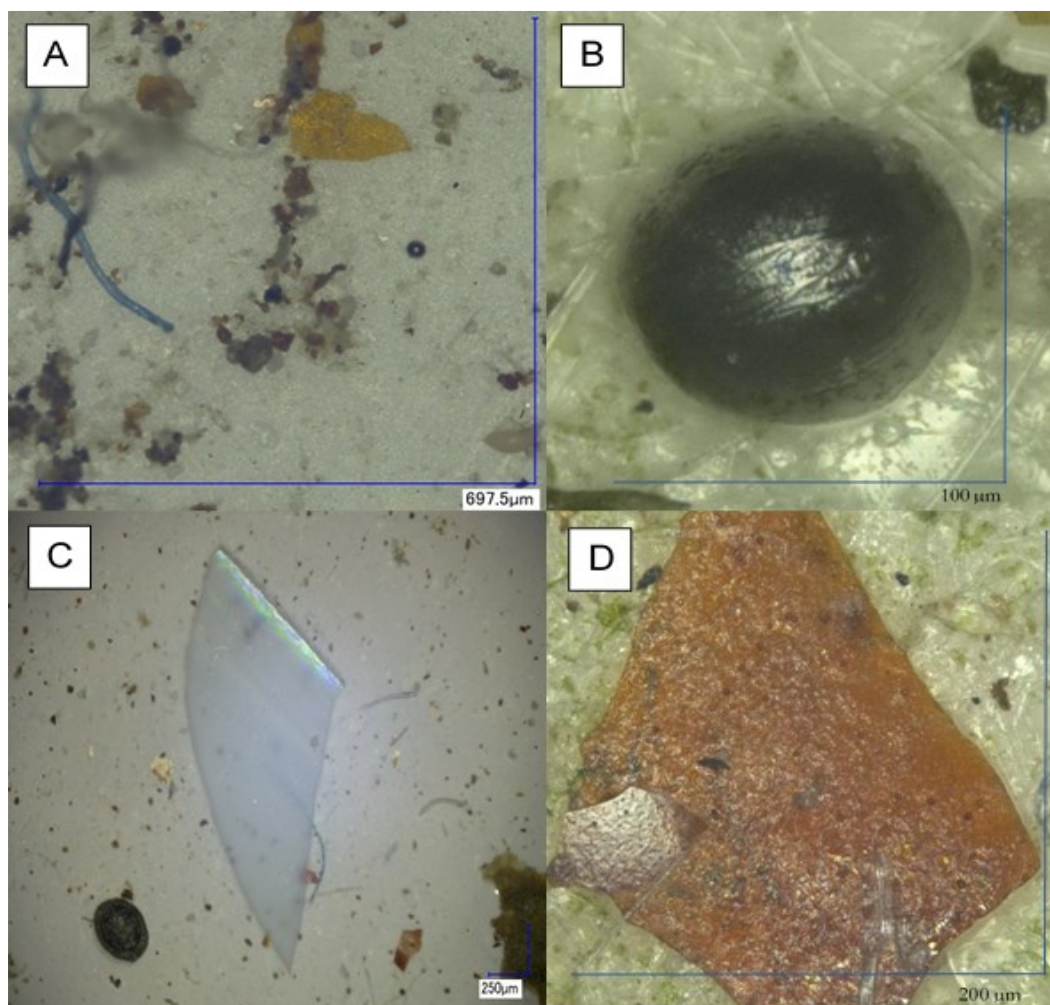


Figure 12: The pictures show some plastic particles of different kinds and size isolated from the river Lahn using the modified CFC method. A) Overview of fiber, fragment and sphere, B) primary plastic, sphere, C) fragment, D) foil.

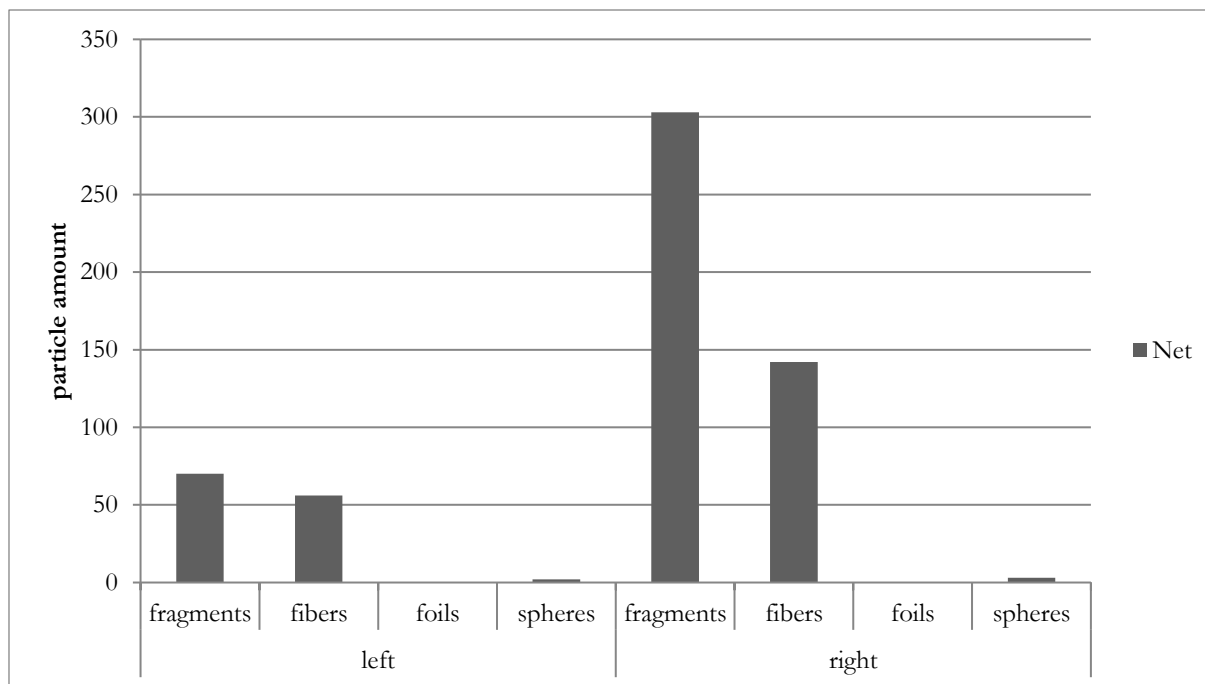


Figure 13: The figure shows the amount of the MP-particles/ m^3 (fragments, fibers, foils and spheres) sampled in 3 meters deep water of the river Lahn using the modified centrifuge system. Illustrated are the results from the centrifuge and the net at the downstream sampling point left, right) after the WWTP effluent (see Figure 6).

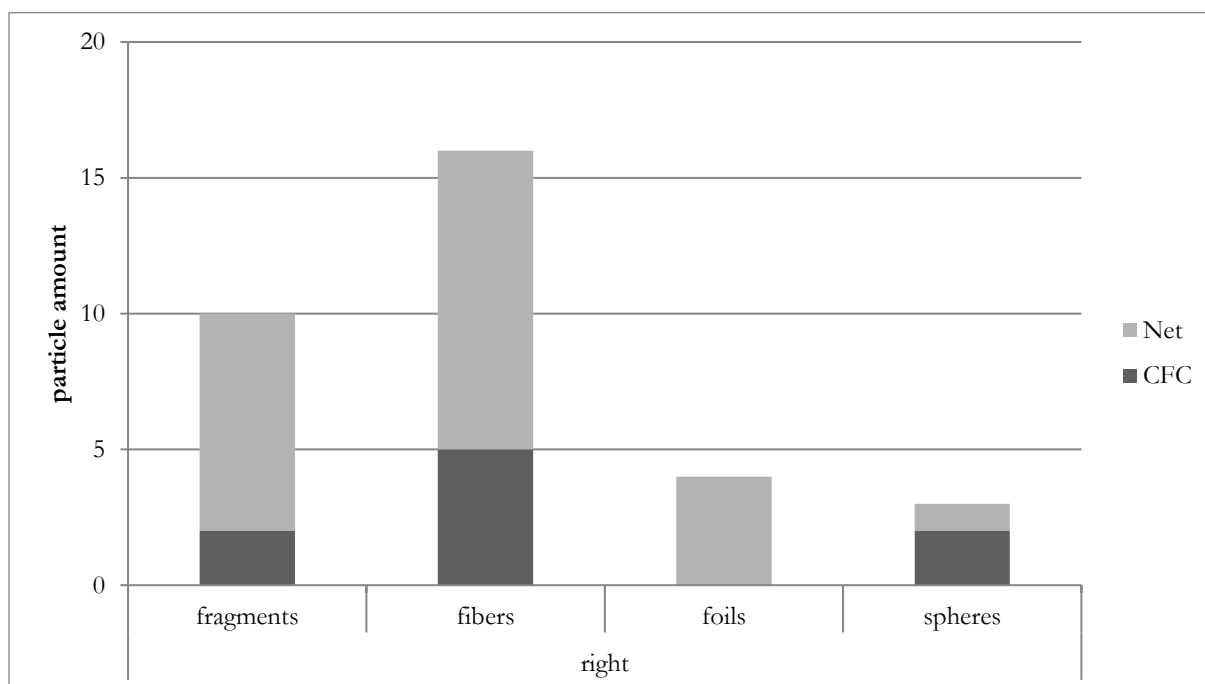


Figure 14: The figure shows the amount of the MP-particles/ m^3 (fragments, fibers, foils and spheres) sampled in 3 meters deep water of the river Lahn using the modified centrifuge system. Illustrated are the results from the centrifuge and the net at the upstream sampling point (left, right) before the WWTP effluent (see Figure 6).

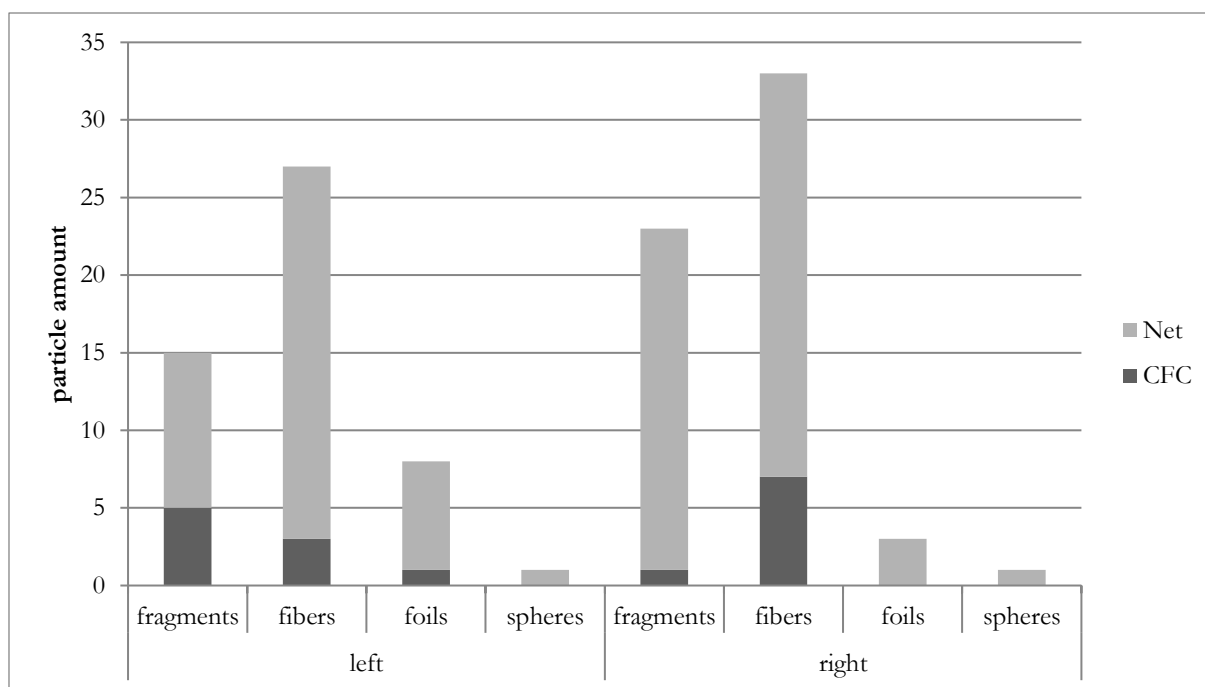


Figure 15: The figure shows the amount of the MP-particles/ m^3 (fragments, fibers, foils and spheres) sampled in the surface water of the river Lahn using the modified centrifuge system. Illustrated are the results from the centrifuge and the net at the upstream sampling point (left, right) before the WWTP effluent (see Figure 6).

3.1.2 Distribution of MP in sediment samples

The results show a significant increase in particle abundance to date (Figure 16) and the plastic particles differ in kind, size and shape (Figure 17, Table 9). This means that with the increasing consumption of plastics over the last years, the occurrence of these, in form of microplastics, is also increasing in the environment. This fact should be a reason to better control the way from plastics to its recycling. With a half-life of 30.17 years, the peak of the ^{137}Cs concentration in the sediment could also shows us two events—in 1959—1963 (atomic bomb tests, ~10-20 cm) and 1986 (Chernobyl, 4-6 cm).

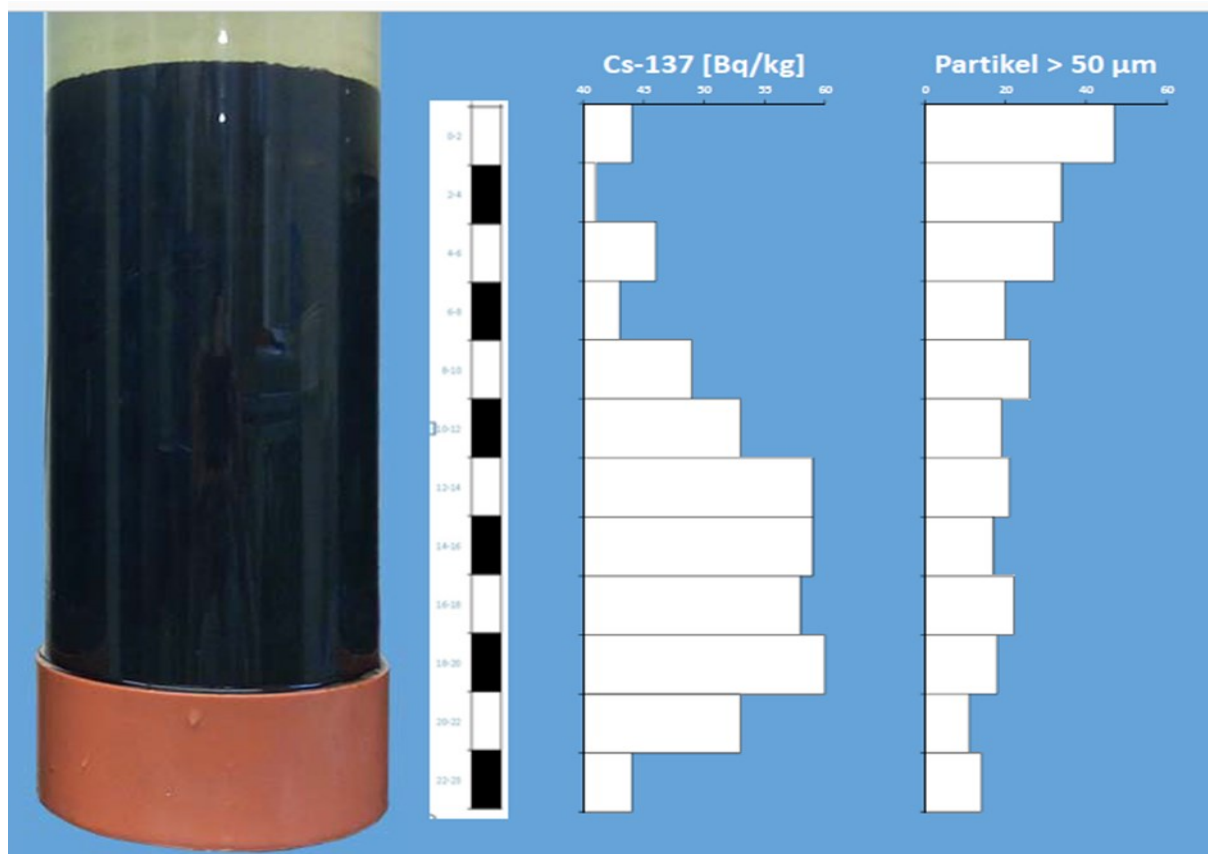


Figure 16: The picture shows a sediment core taken by divers in the Trave river. Also shown are the results of the Cs-137 dating (in the middle) and the MP contribution (on the right) (Kochleus et al., 2017).

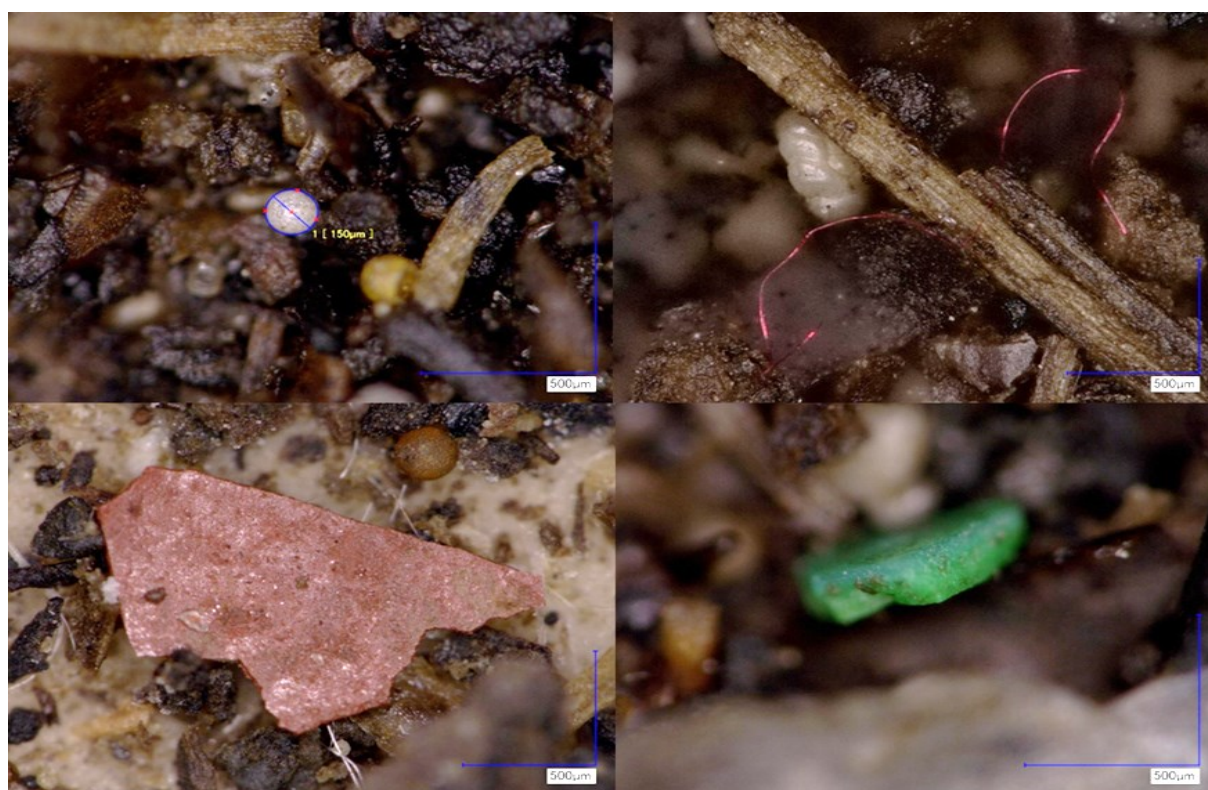


Figure 17: The pictures show MP extracted from the sediment cores from the river Rhine including information about depths. A) sphere (14–16 cm), B) fiber (20–22 cm) C) fragment (20–22 cm) D) fragment (22–23 cm) (Kochleus et al., 2017).

Table 9: Type of particles found ($> 50 \mu\text{m}$) in the sediment core sorted by commonness.

Depth/cm	1 common	2 common	3 common
0–2	fragments	sphere	fibers
2–4	fragments	fibers	
4–6	fragments	fibers	
6–8	fibers		
8–10	fragments		
10–12	fragments	fibers	sphere
12–14	fragments	fibers	
14–16	fibers	sphere	
16–18	fragments	fibers	sphere
18–20	fibers	fragments	
20–22	fibers	fragments	
22–23	fibers		

3.1.3 Sample preparation

The results show that electroseparation is a reliable alternative method to reduce even relatively large sample volumes. For all materials, a mass reduction of almost 99 % was achieved (Figure 18). For example, a 150 g sample of quartz sand after the third step could be reduced by $98.4 \pm 0.1 \%$ to $2.34 \pm 0.17 \text{ g}$. The freshwater sediment was reduced from an initial mass of 150 g to a final mass of $2.33 \pm 0.13 \text{ g}$, which corresponds to a reduction of $98.4 \pm 0.1 \%$. For beach sand, the 150 g sample was reduced to $2.00 \pm 0.04 \text{ g}$ —a reduction of $98.7 \pm 0.2 \%$. Similarly, the 150 g particle sample was reduced to $2.51 \pm 0.23 \text{ g}$ ($98.3 \pm 0.1 \%$). The results also show successful accumulation of MPs across the separation runs, with the best results achieved with beach sand, although there is no significant difference between the four materials. During the process, the small loss of sample material in the device (Figure 19) did not affect the recovery of the added plastics. In addition, depending on the grain size of the material, the method yields different results with respect to the percentage mass reduction in the individual steps. After the first separation step with the KWS the mass reduction of the residual material is $>90 \%$. The yield achieved with the KWS is also very promising, since the recovery of the largest size category ($2,000\text{--}5,000 \mu\text{m}$) was 100 % and that of the size class $630\text{--}2000 \mu\text{m}$ was almost 100 % for all tested materials. One particle of PP and one of PS were lost in freshwater particle or freshwater sediment samples. In the size fraction $200\text{--}630 \mu\text{m}$, a PP particle was lost in the freshwater SPM sample, whereas for the smallest size fraction ($63\text{--}200 \mu\text{m}$), the recovery was 100 % across all materials. MP particles from the Rhine river were also recovered at 100 %. Only for the $630\text{--}200 \mu\text{m}$ fraction, there was a loss of one out of 10 PP and PS particles, and a particle of PP was lost in the $200\text{--}630 \mu\text{m}$ fraction (Figure 20). Figure 21 gives an idea of the enrichment of particles during the separation. The experiment with the isolated plastic shows that the shape of the MP does not affect the separation process, the age of the

particles, or the possible growth of biota on their surface. Neither the particle size distribution nor the TOC of the materials affects the recovery. For beach sand and quartz sand, a TOC of <0.1 % was determined, while for freshwater particles and freshwater sediment, TOC values were 4.64 % and 5.25 %, respectively (Table 10).

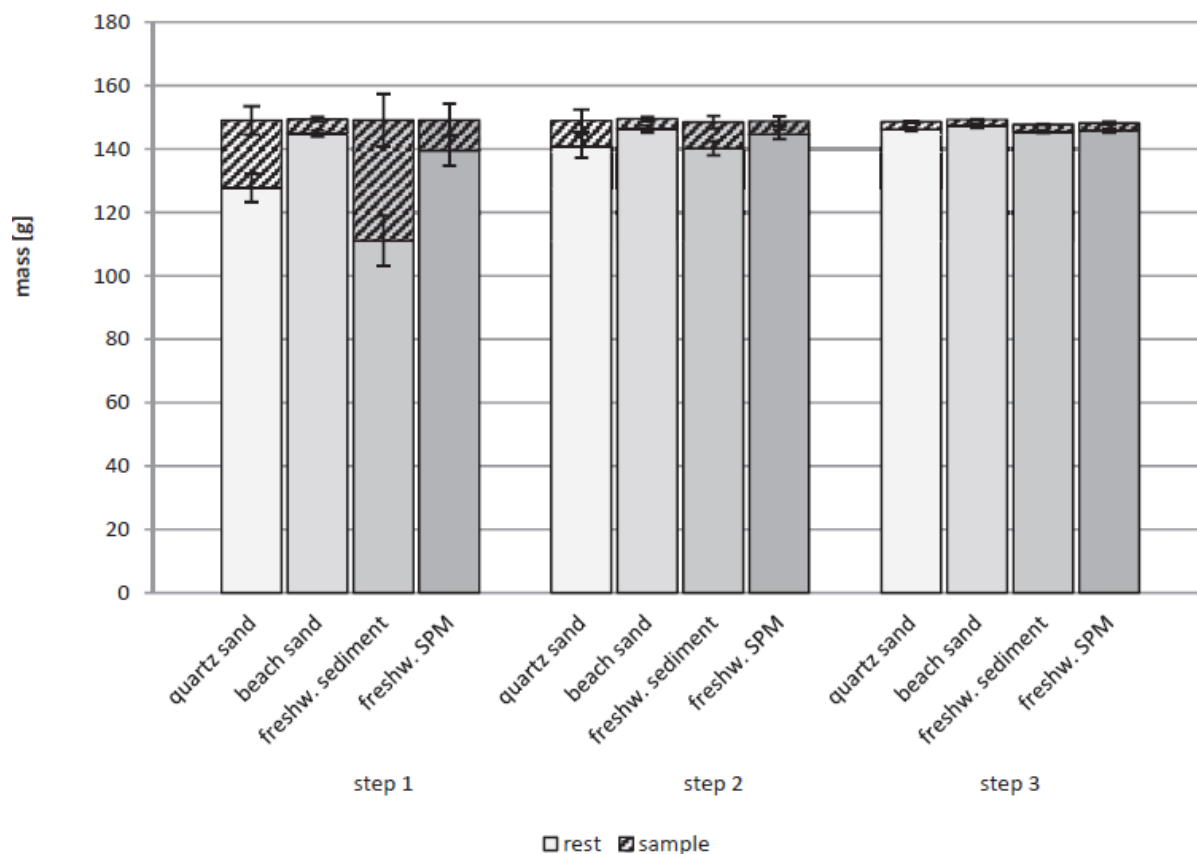


Figure 18: Mass reduction of quartz sand, beach sand, freshwater (freshw.) sediment, and freshwater-suspended particulate matter (SPM). The mean mass \pm S.D. of the remaining sample (hatched) and the rest (non-hatched) fractions after one, two, and three steps of electroseparation for all four types of matter is shown. After the third separation, 2–2.5g remained from the original 150g sample, independent of the type of matter, Felsing et al., 2017.

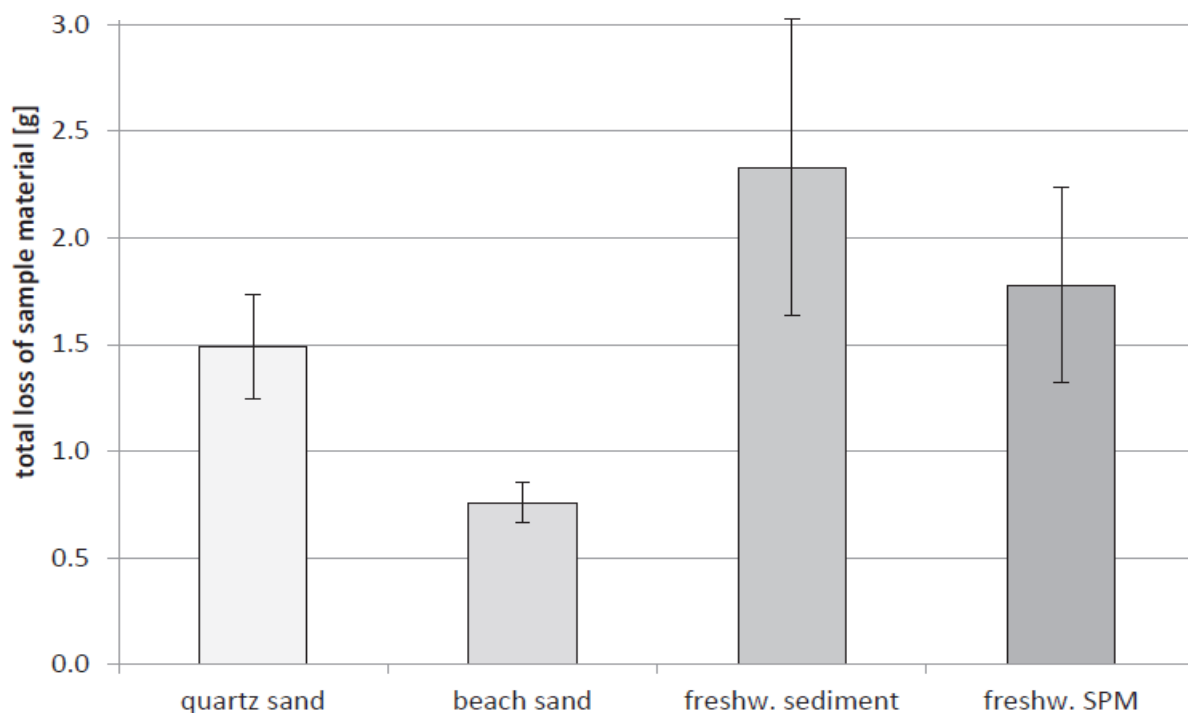


Figure 19: Mean loss of original 150g sample material during the separation process using the KWS for all four tested sample types. The mean \pm S.D. after three steps of electroseparation is displayed, Felsing et al., 2017.

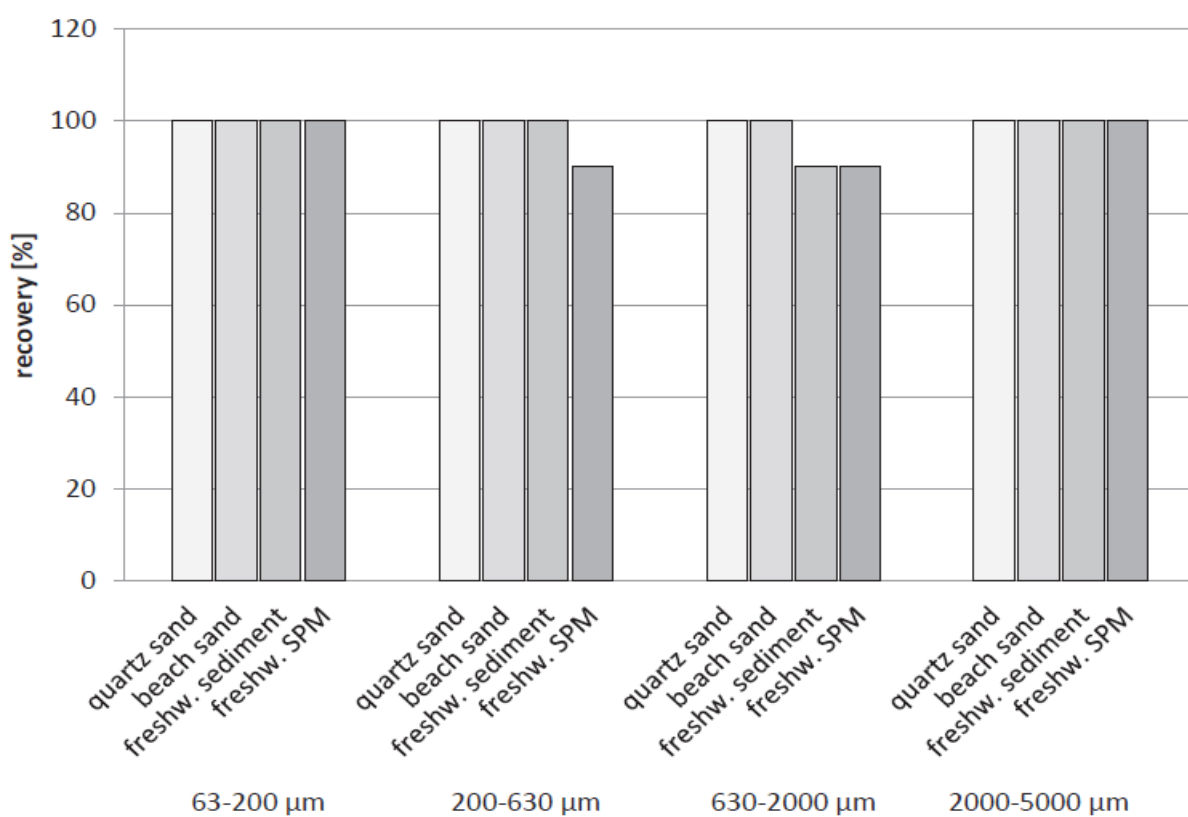


Figure 20: Recovery of MP particles in four size ranges from four different sample materials after the third separation step using the KWS. The mean value, expressed as a percentage, is shown. Standard deviations cannot be shown because in all three cases of less than 100% recovery, one single particle was lost in each replicate, Felsing et al., 2017.

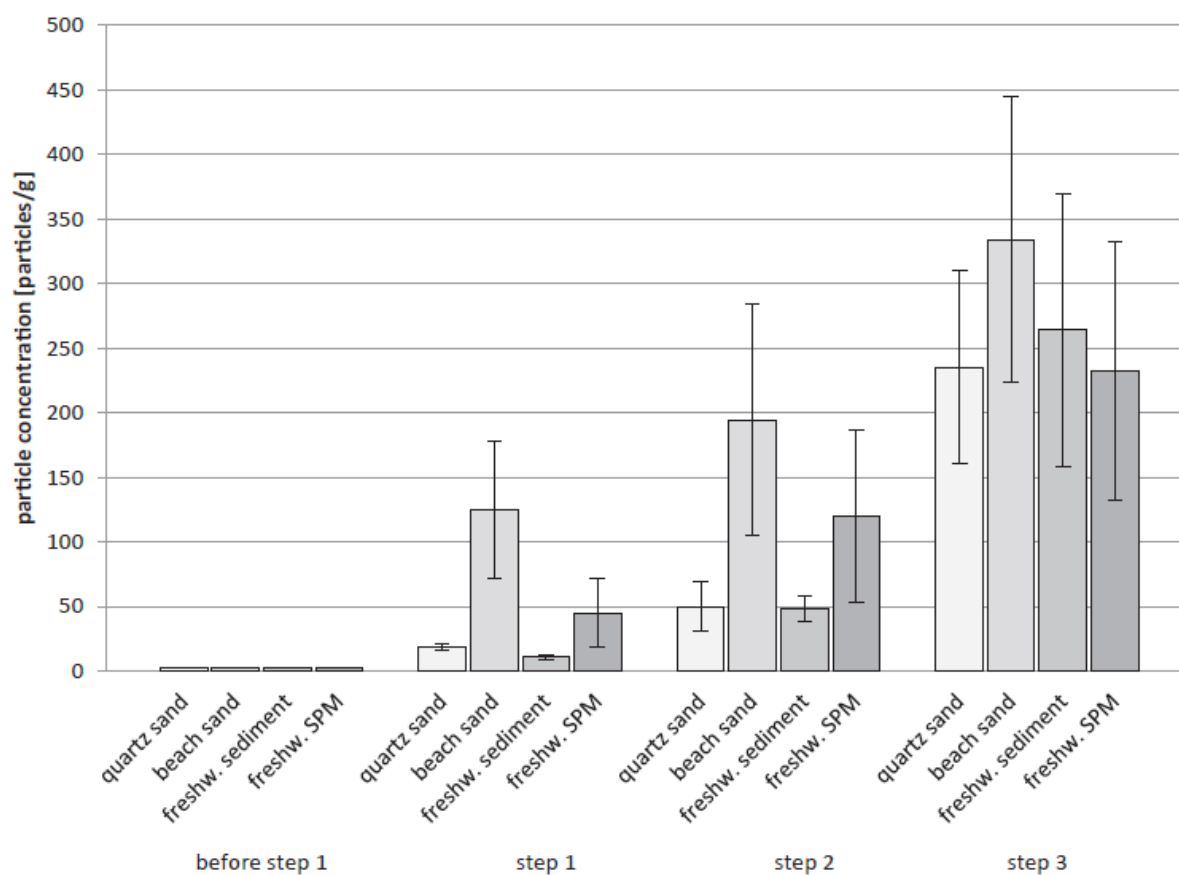


Figure 21: Enrichment of the spiked particles in the four materials (particles of MP/material in g) over the three separation steps using the KWS, Felsing et al., 2017.

Table 10: Grain-size distribution of the four sample materials used in the KWS recovery experiment, Felsing et al., 2017.

Sample material	630-2000 μ m	200-630 μ m	63-200 μ m	20-63 μ m	<20 μ m	Σ	Loss
	[%]	[%]	[%]	[%]	[%]	[%]	[%]
Quartz sand	0	16.6	82.9	0.3	0.3	100	0.0
Freshw. Sediment	0	2.9	25.7	17.9	47.1	93.6	6.4
Beach sand	0.1	41.4	57.6	0.1	0.3	99.5	0.6
S. particulate matter	0	1.7	37.0	15.4	39.4	93.6	6.4

3.2 Modifications of the electroseparator KWS already implemented

The KWS was initially an exposition device. This was served analogously at the beginning. This means that all parameters have been set using knobs in eight possible parameters. The modified device is now operated via a touch panel. The KWS was not designed for fine sediments; it was internally designed to be open, resulting in the loss of sample material when separating fine materials such as sediment or suspended matter. To optimize the KWS on field samples, the steel funnel was converted with a jacket so that it can be removed by a handle for cleaning. On the funnel, there is now a lid that prevents entries from the outside. The shuttering prevents the loss of dust on the way to the roller and into the corona field. Even before and after the roller, the use of plates also prevents article loss. The forces in the corona field can lead to distraction of particles. This is prevented by installing the KWS in the interior and making it more closed by means of further conversion measures. For ease of use, the three compartments have been replaced by two (Figure 22, Figure 23, Figure 24).

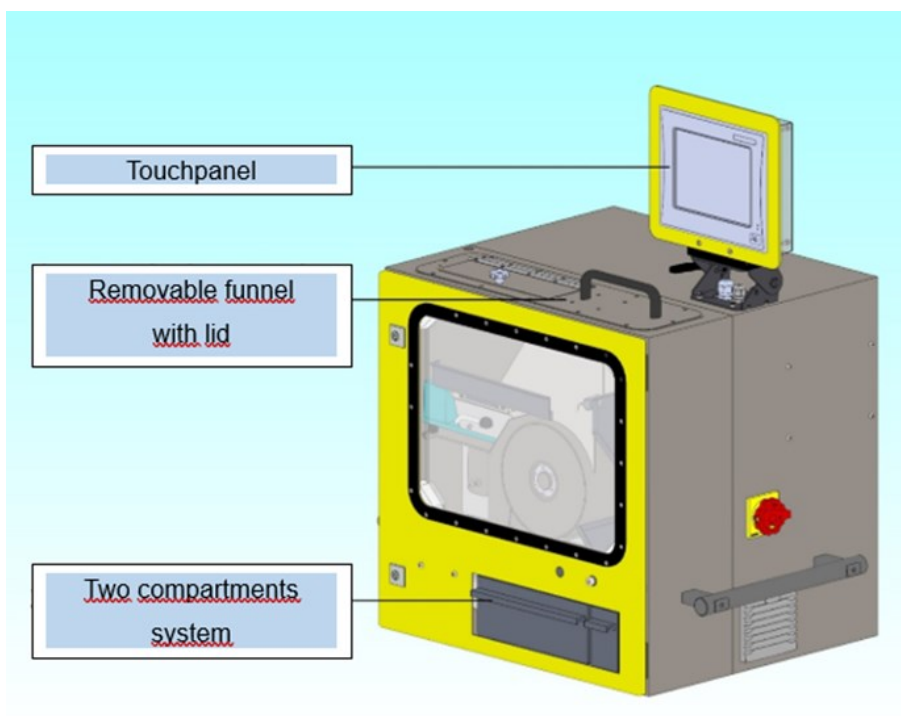


Figure 22: Overview of the modifications of the KWS. Compared to the old device, the new KWS is operated via a touch panel. In order to prevent the loss of sample material, there is now a shot removable for cleaning and two instead of three drip trays.

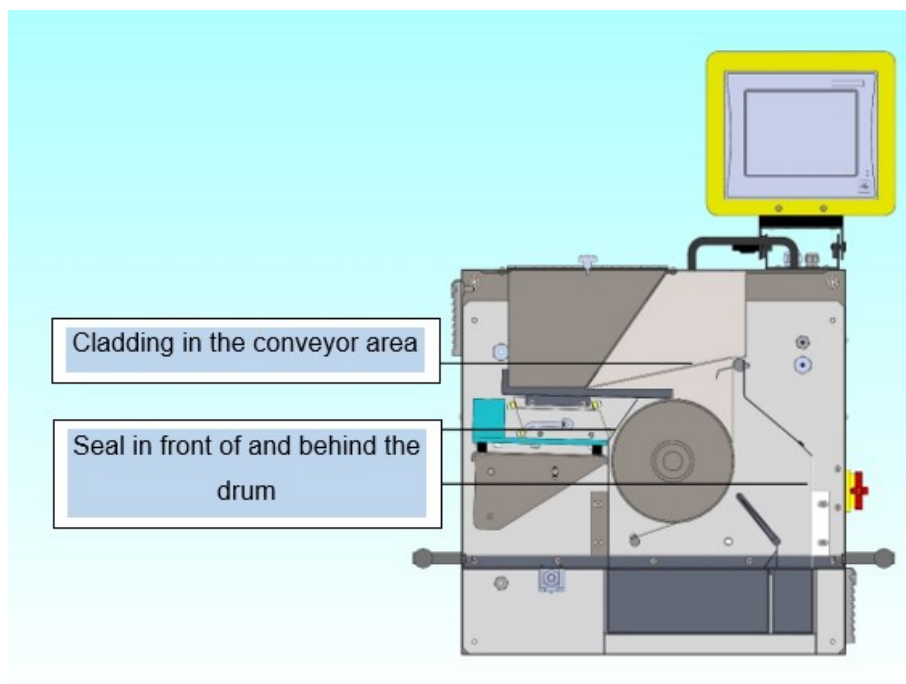


Figure 23: Overview of the modifications of the KWS. In the new KWS, additional walls have been installed to prevent the loss of sample material.

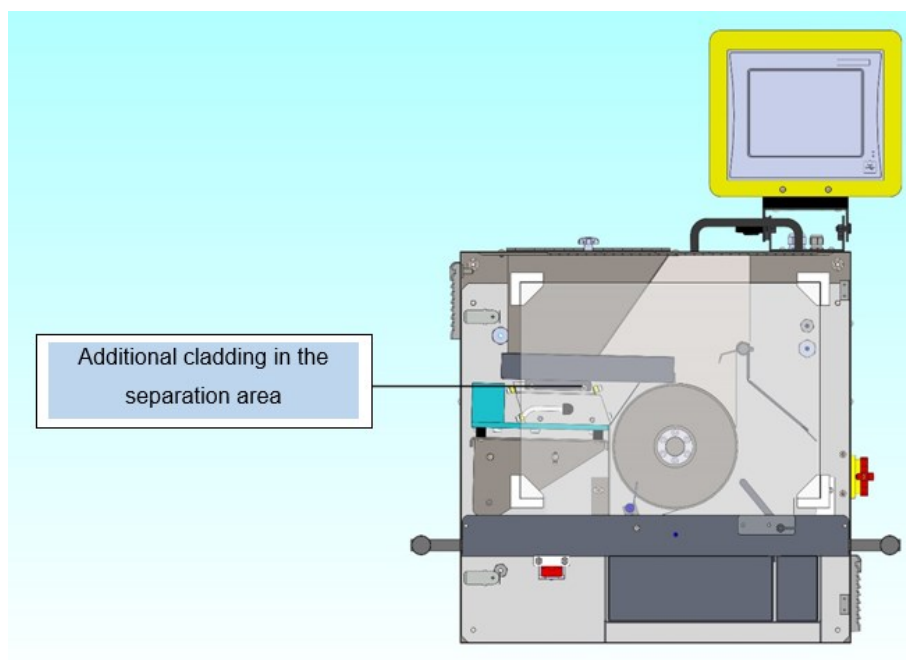


Figure 24: Overview of the modifications of the KWS. An additional door inside prevents the loss of sample material.

3.3 Leaching from bait material

The basic material of the baits is PVC added with different additives or antioxidants (Table 11). The results show that not all baits were leaching phthalates. Only for samples 1, 2, 5, 8, 10 and 11 are the values within the measurable range. Fish bait 1 leached an amount of $4.78 \pm 1.3 \mu\text{g}/\text{ml}$ after one week (W1) up to an amount of $40.51 \pm 11.9 \mu\text{g}/\text{ml}$ after eight weeks (W8) of DINP in the water (Figure 25). In the water body which was contaminated by fish bait 2, different ranges of DMP (W1 = $36.2 \pm 12.3 \text{ ng}/\text{ml}$ and W8 = $299.8 \pm 22.29 \text{ ng}/\text{ml}$) were detected. DEHA was determined for W1 = $13.8 \pm 5.3 \text{ ng}/\text{ml}$ up to W8 = $207.3 \pm 57.3 \text{ ng}/\text{ml}$. DEHP was found in lower ranges with W1 = $16.9 \pm 4.8 \text{ ng}/\text{ml}$ and W8 = $74.7 \pm 34.4 \text{ ng}/\text{ml}$ (Figure 26). In the sample of fish bait 8 a high amount of DEHP was determined (W8 = $824.5 \pm 297.6 \text{ ng}/\text{ml}$) (Figure 27). The highest levels of DEHA were detected in the fish bait 11 sample (W1 = $0.21 \pm 0.1 \text{ ng}/\text{ml}$ and W8 = $10.91 \pm 2.2 \text{ ng}/\text{ml}$). The results also show that there is an increase of phthalate ester over time (Figure 28). In addition, an increase in estrogenic activity over time was detected in the water phases with fish bait 3 (W8 = $15 \pm 3.8 \text{ ng}/\text{l}$) and 10 (W8 = $153 \pm 18.4 \text{ ng}/\text{l}$) (Figure 29, Figure 30). The results of the other samples were below the detection limit or the same as the detected blank.

Table 11: Results of the PyGCMS show the compositions of the different baits.

Sample/ Fish	Main component	additions
1	DINP	Di ethyl phthalate
2	DEHT	Bis(2-ethylhexyl) phthalate
3	DEHT	Bis(2-ethylhexyl) phthalate
4	DINCH	Phthalic acid, 2-methylbutyl octyl ester
5	DINP	long-chain alcohols
6	DINCH	Adipic acid, dioct-4-yl ester
7	Tributyl acetylcitrate	Adipic acid, 3-heptyl nonyl ester
8	DEHP	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
9	DINCH	Phthalic acid, isopropyl octyl ester (Spuren)
10	DIOP	long-chain alcohols
11	DINP	long-chain alcohols

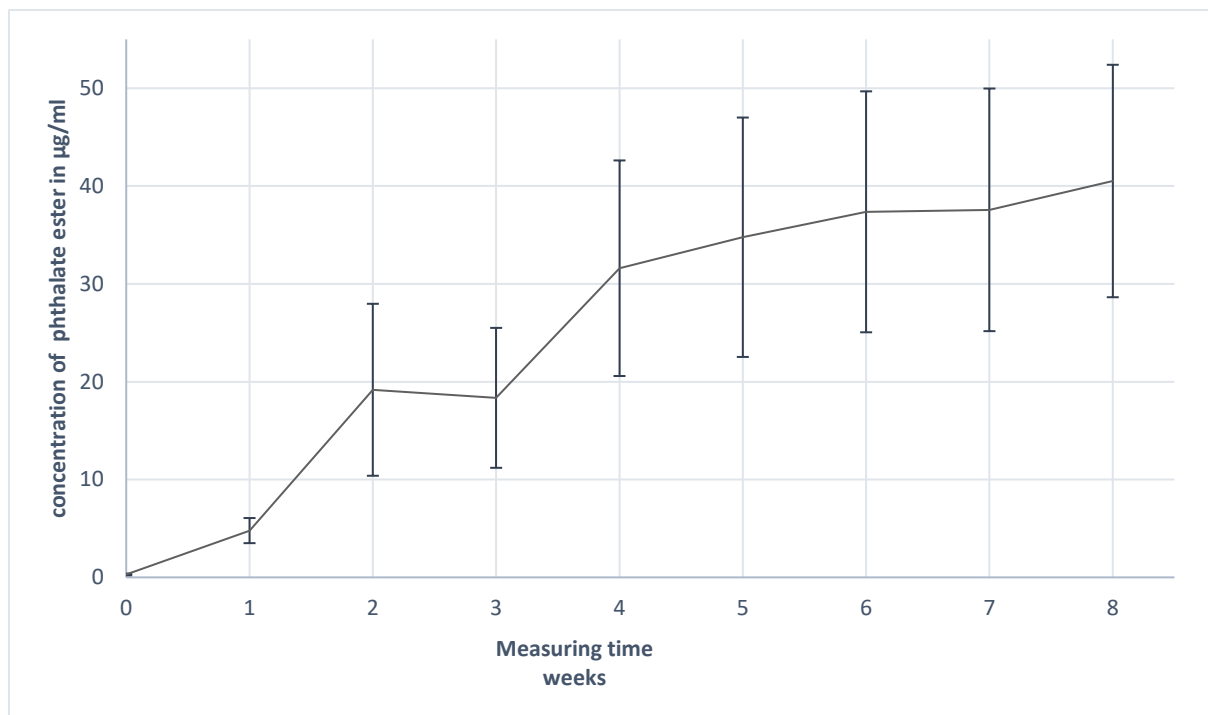


Figure 25: The figure shows the concentration of Diisononylphthalate (µg/ml) leaching into the water phase of fish bait 1. Standard deviations are shown based on $n=3$.

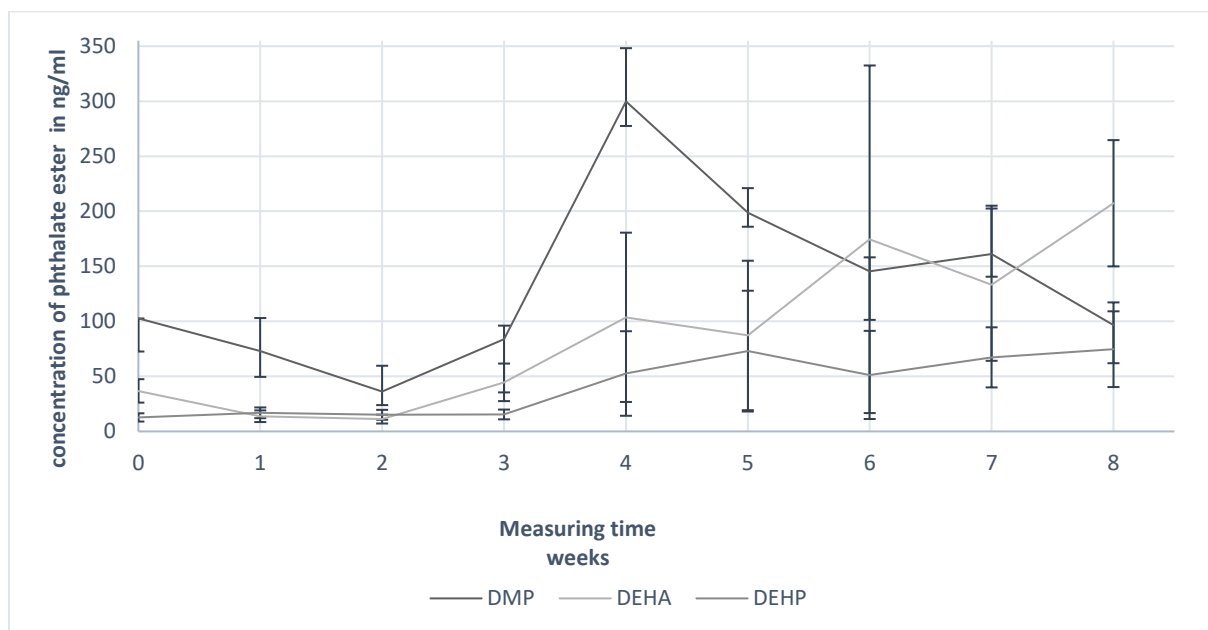


Figure 26: The figure shows the concentrations of DMP, DEHA and DEHP (ng/ml) leached out in the water phase of fish bait 2. Standard deviations are shown based on $n=3$.

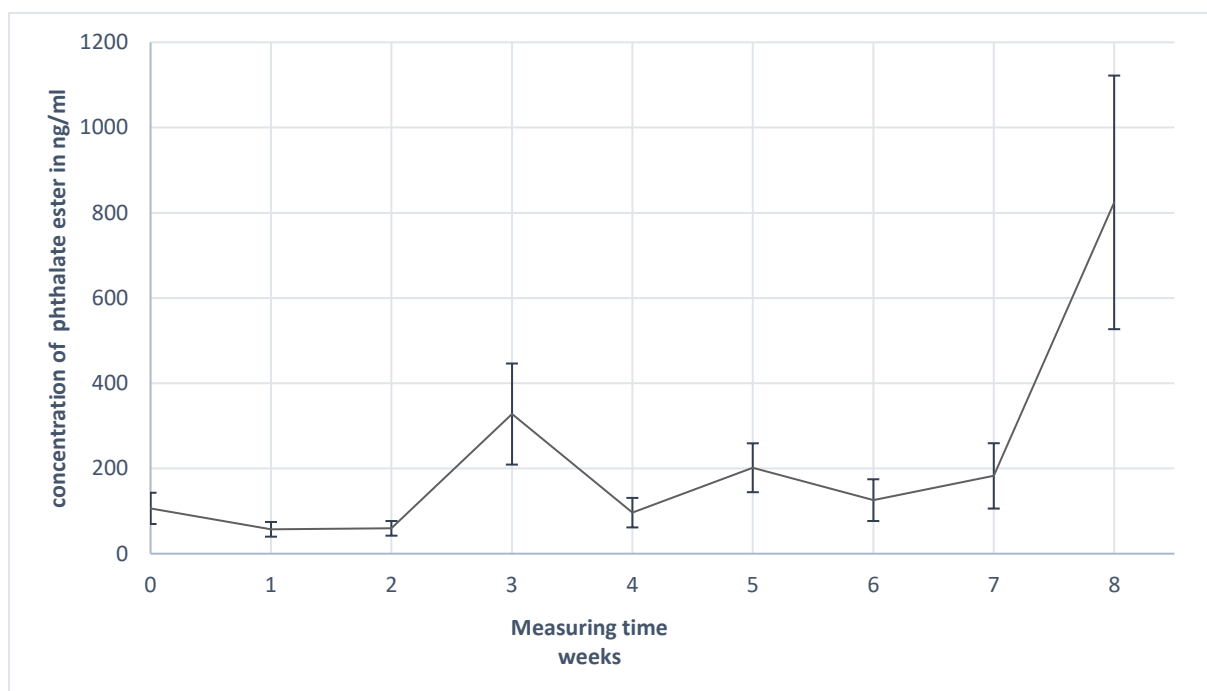


Figure 27: The figure shows the concentrations of DEHP (ng/ml) leached out in the water phase of fish bait 8. Standard deviations are shown based on $n=3$.

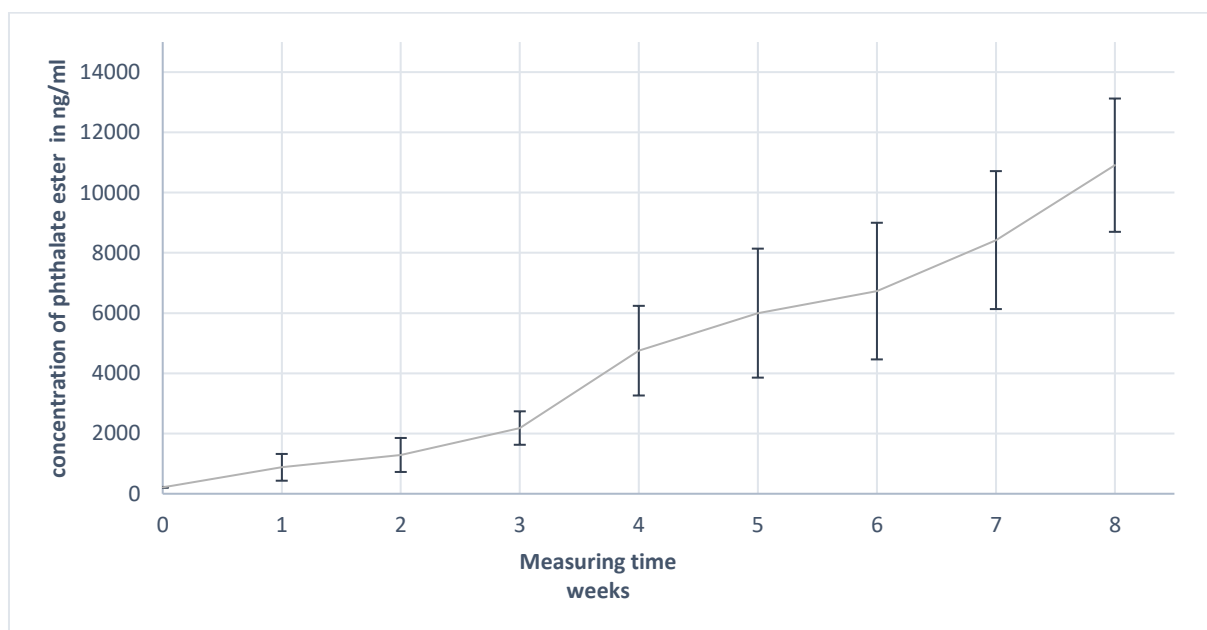


Figure 28: The figure shows the concentrations of DEHA (ng/ml) leached out in the water phase of fish bait 11. Standard deviations are shown based on $n=3$.

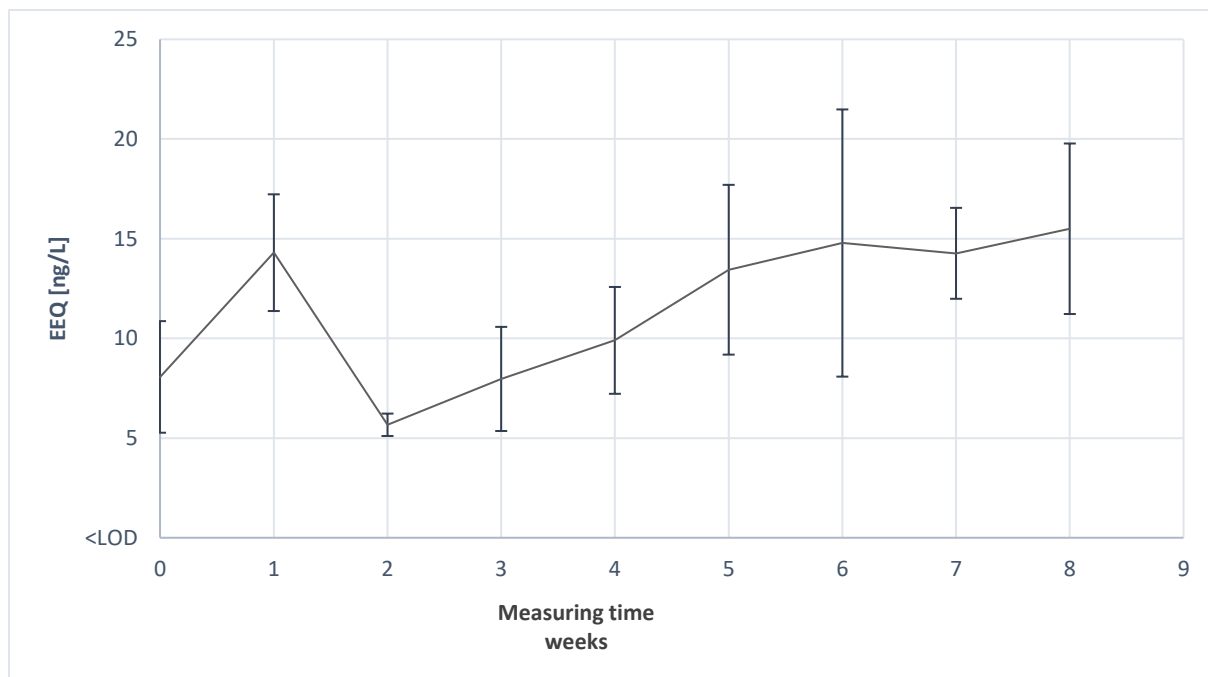


Figure 29: The figure shows the concentration (ng/l) of an estrogen active substance in the water phase of fish bait 3. Standard deviations are shown based on n=3.

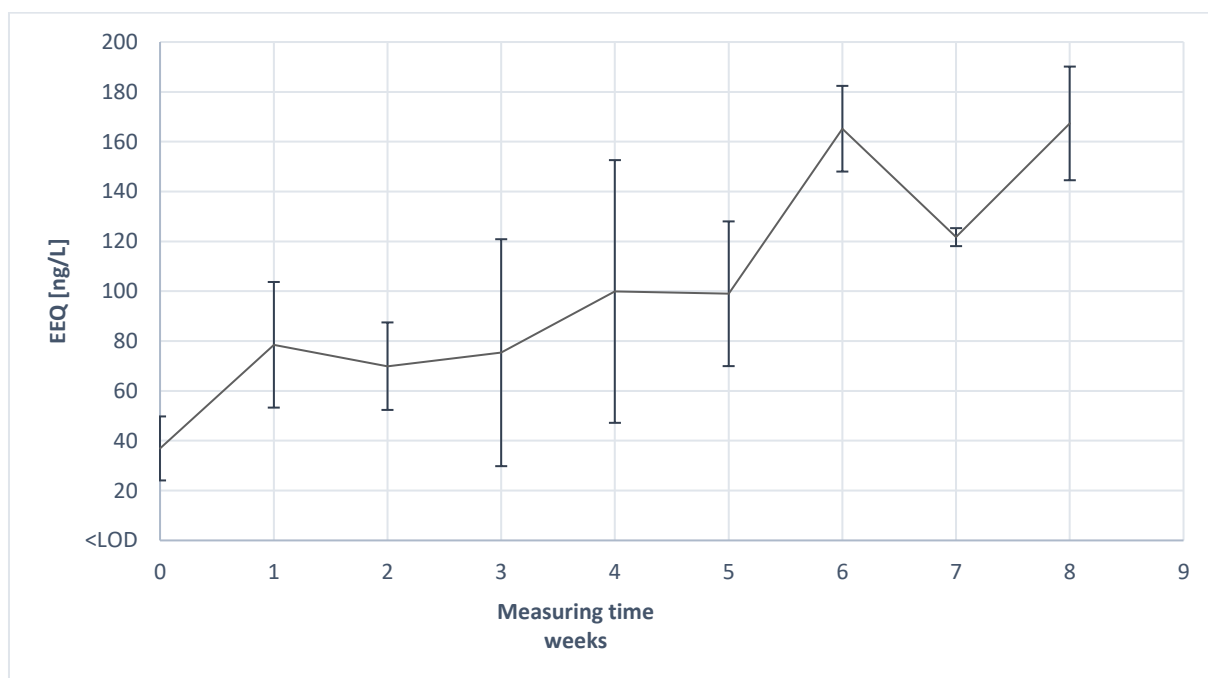


Figure 30: The figure shows the concentration (ng/l) of an estrogen active substance in the water phase of fish bait 10. Standard deviations are shown based on n=3.

3.4 Effects of plastic on model organism *L. variegatus*

3.4.1 Detected additives

In the experiment, all seven phthalates (DNOP, DEHP, DEHA, BBP, DBP, DEP, DMP) analyzed were detected in organisms. The results show that different degrees of washout of additives occur of the synthetic materials in the water phase, with PVC containing the highest impurities (Figure 31). In most cases, there is an increase in concentrations over time in some of the samples like the DMP found in PET, PP and PS. Also the factor light seems to strengthen the leaching because the amount increase from dark to light.

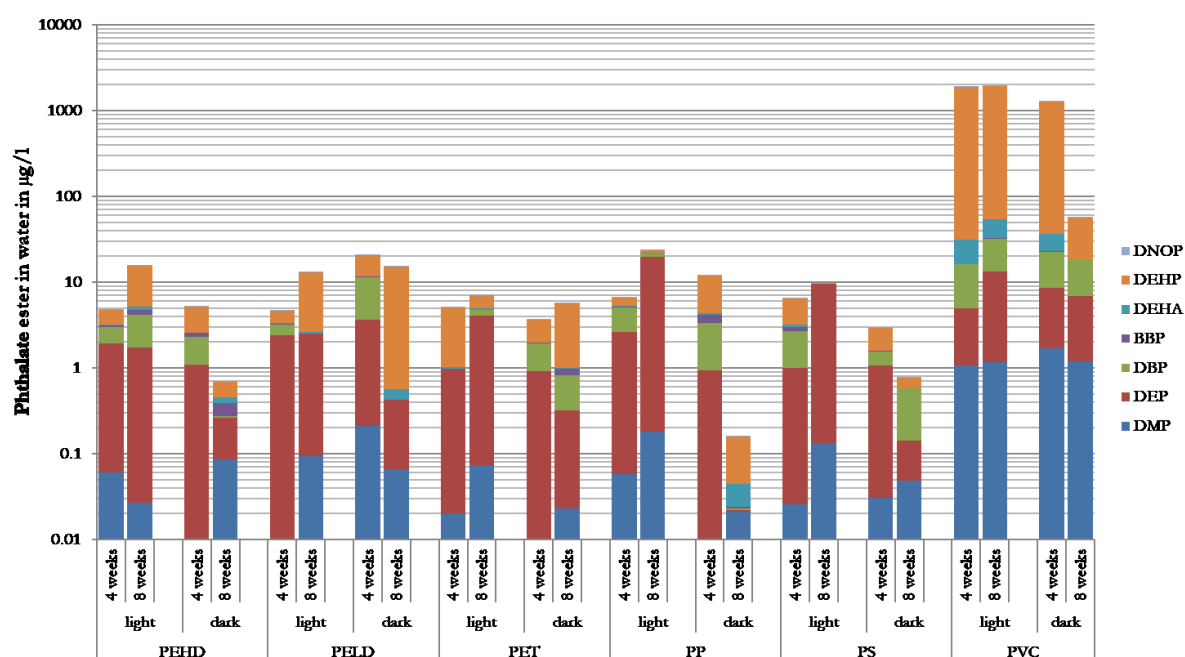


Figure 31: Leaching test of additives from six different types of plastic in water. The MP particles (5 mm \varnothing) were incubated for eight weeks at 20 °C with a 16:8 h day-night rhythm. The figure shows the measured values of eight common phthalate ester after four and eight weeks.

3.4.2 MP characterization using PyGCMS

The pyrolysis and adjustment of polymer database provides clear results on the starting material of the self-produced MP used from conventional household products (Figure 32, 1-5).

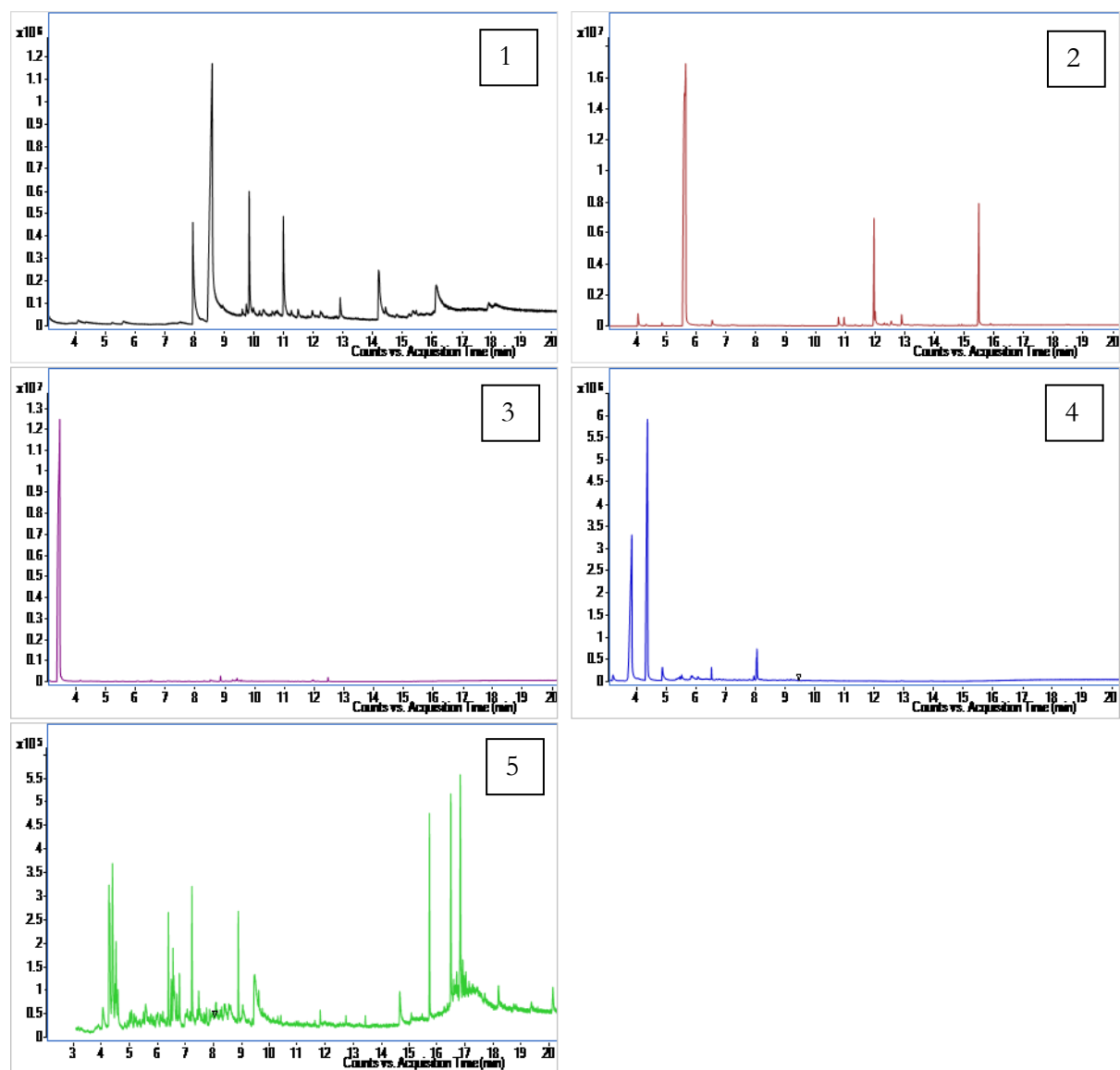


Figure 32: Results of the PyGCMS are shown as chromatograms of 1) PET, 2) PS, 3) PMMA, 4) PLA and 6) PVC, used for the sediment contact test.

3.4.3 Evidence of the uptake/ingestion of MP by *L. variegatus*.

The results show that the organisms eat and excrete the two types of plastic. The residence time of the particles has not been determined.

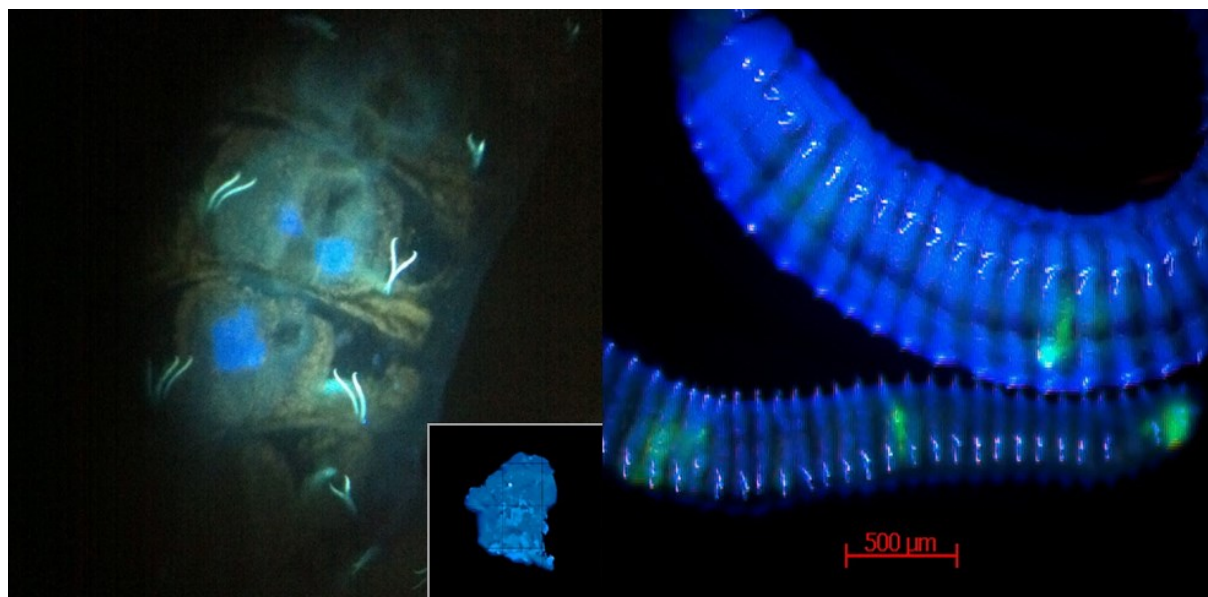


Figure 33: The picture shows the ingestion of PVC (on the left) and PET (on the right) by *L. variegatus*. The images were taken with a fluorescence microscope.

3.4.4 Results of the sediment contact test

The results show that there are differences between the plastic species and their influence on the organism. An LC₅₀ of 1.7 mg/g dw of the endpoint of surviving individuals was determined in PVC-spiked sediment whereas PET, PS, PMMA and PLA-spiked sediments showed no significant effect (Figure 34). Table 12 gives an overview of how many particles are in the weighed amount of PVC and PET (p/g dw). These numbers shown in Table 12 are almost identical to the other three types of plastic (PS, PMMA and PLA). The increasing concentration had negative effects on the number of individuals only in PVC. The higher the concentration, the greater the effect on the organism. For PET, even the highest concentration C₆ had no effect on mortality. The polymers PMMA, PS as well the bioplastic PLA have also no effect on the mortality or fitness of *L. variegatus* (Figure 35).

Table 12: The table shows the particle concentrations of PVC and PET in mass and amount used in the sediment contact test.

Label	Mass (PVC/PET) mg/g dw	PET particles p/g dw	PVC particles p/g dw
C ₀	0	0	0
C ₁	0.02	23.000	26.000
C ₂	0.08	115.000	130.000
C ₃	0.83	1.150.000	1.300.000
C ₄	1.67	2.300.000	2.600.000
C ₅	8.33	11.500.000	13.000.000
C ₆	16.67	23.000.000	26.000.000

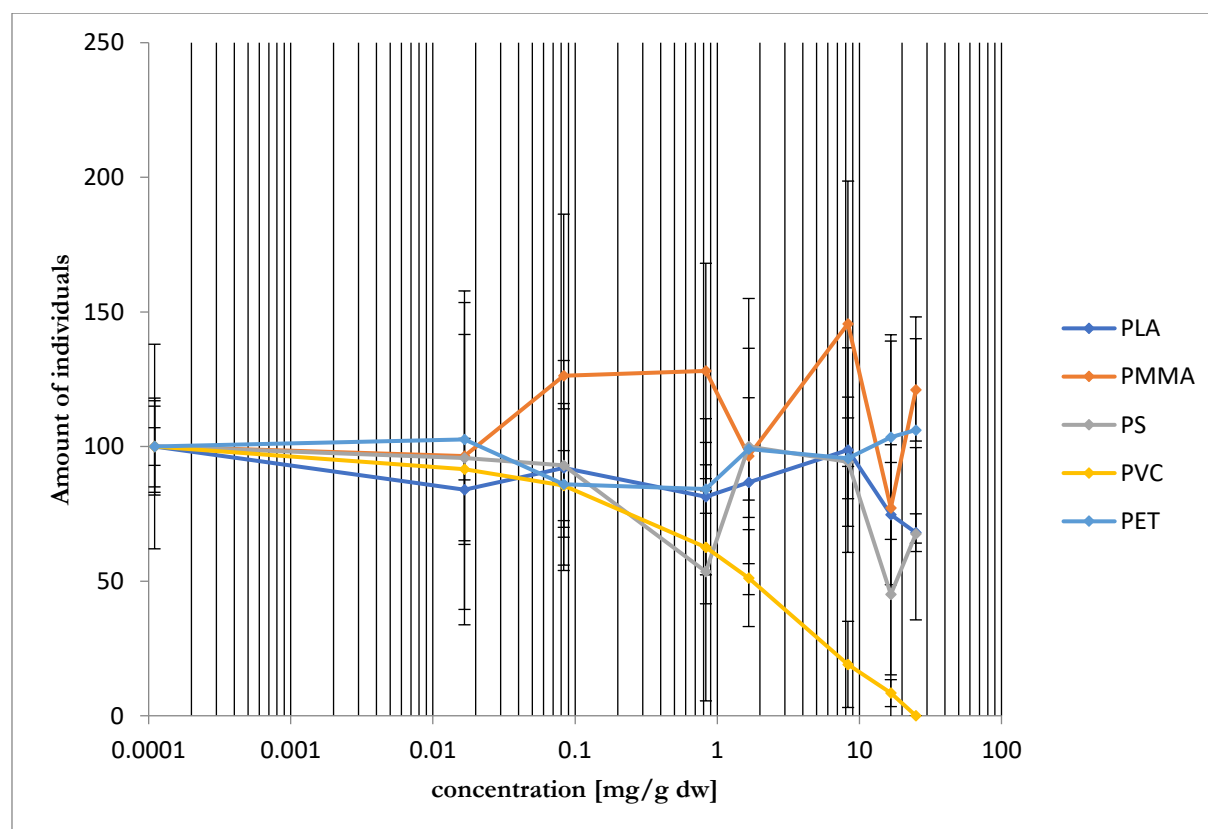


Figure 34: The figure shows the mortality (by number of individuals based on 100%) of *L. variegatus* after the sediment contact test. The mortality decreases with increased concentration of PVC. It shows a LC50 of 1.7 mg/g dw. There are no effects with increasing particle concentration of PET, PMMA, PS and PLA.

3.4.5 Glycogen content of *L. variegatus*

The basic glycogen content refers to the blanks. The results show that the four plastic types (PET, PS, PMMA, and PLA) have no effect on the glycogen content. Also, the increasing concentration of the particles does not affect the fitness of the animals. However, in the case of the PVC, it could be shown that the increasing concentration in the sediment has a negative influence on the organism and its fitness (Figure 35).

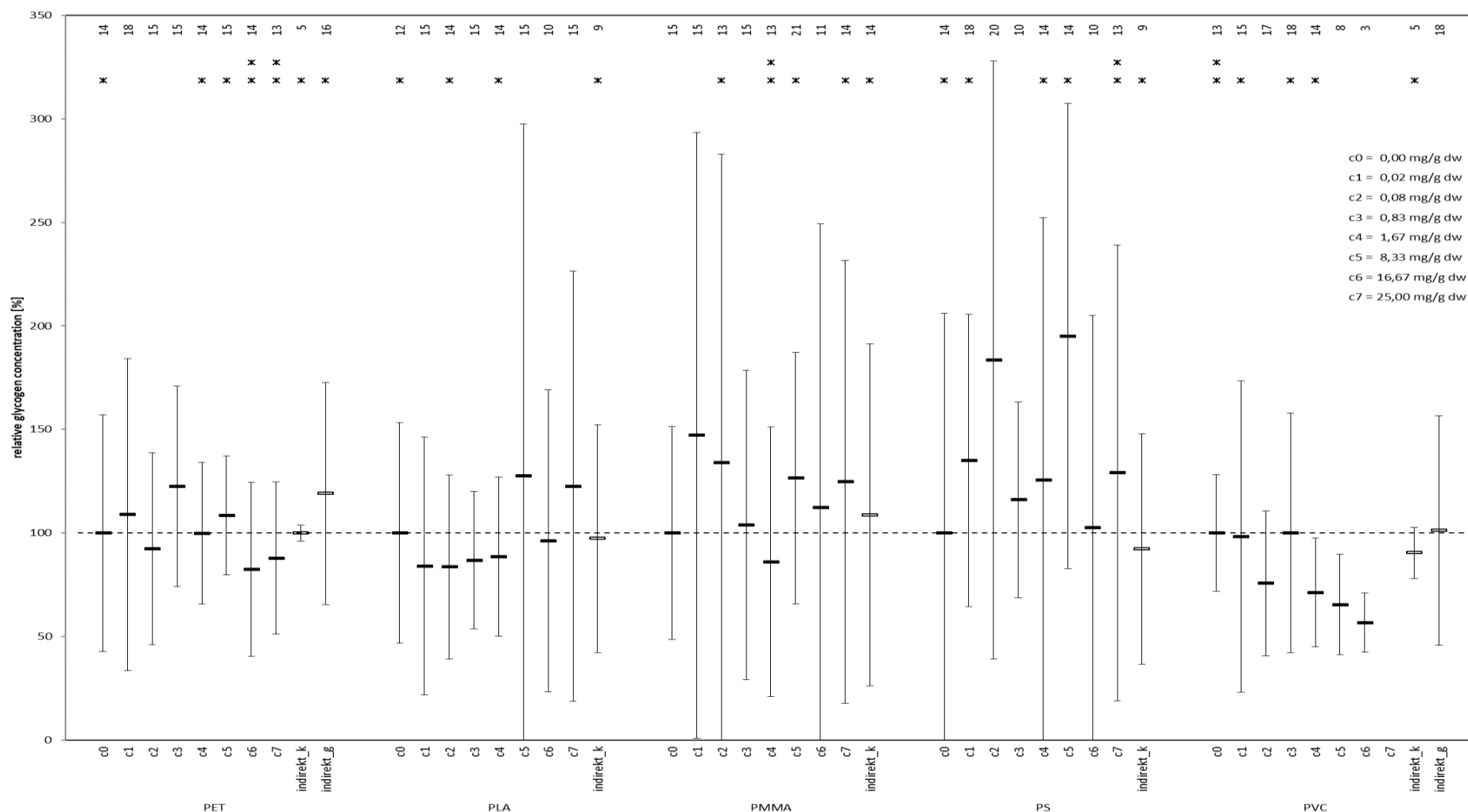


Figure 35: Glycogen content in *L. variegatus* after 28 days. Only with PVC is there a—though not significantly—recognized trend. PET/PVC and PLA/PMMA/PS were examined simultaneously and by one person each. 'Indirect' indicates that the respective maximum concentration was investigated in indirect exposure, the index k stands for particle size $<200\ \mu\text{m}$ as in direct exposure, index g stands for particles of size $200\text{--}630\ \mu\text{m}$. Mean values without outliers ($p < 5\%$; Q test; marked with *) are shown, including standard errors as well as n —the number of measurements considered.

4 Discussion

MP is found almost everywhere in the environment due to the increasing use of plastics and inadequate recycling management. The potential hazards, as a result, cannot be fully estimated yet. However, recent studies give an indication of the hazardousness of the particles to organisms. Due to the difficulties in quantifying and qualifying the MP in the environment, there are not enough data available to estimate the risk potential. The aim of this work is to develop methodological solutions for MP research, especially in sampling and sample preparation. Additionally there are investigations how organisms could be affected by plastic in the environment included.

4.1 Sampling methodology and success

Different methods were developed and tested, the use of which can immensely facilitate future investigations in MP research.

CFC-Method

Sampling with a continuous flow centrifuge (CFC) enables us to record the quantitative and qualitative distribution of the MPs in the water column. The entire spectrum of the particles can be recorded and thus can provide the prerequisites for realistically conducted effect studies. The method with the centrifuge can well replace techniques involving nets. There are no longer any problems with large quantities of organic material in the water. An advantage of sampling with a centrifuge is that centrifuges are already being used in almost all federal states for the purpose of suspended particulate matter monitoring (Claus, 2009; Schubert et al., 2012). In the course of these surveys it would thus be possible to integrate the MP sampling without much additional effort while an entirely new purchase would be very expensive.

For sampling suitable locations have to be chosen. The trailer must be balanced and stand secure so that the centrifuge does not waver. Hoses used may contaminate the sample. However, since all materials are determined beforehand, they are not recorded in the analysis/identification (blank value control). Negatively you get only small amounts of sample material, but without loss of information. For future studies, anyway, a comprehensive recovery of the modified CFC method and a blank value analysis must be carried out to determine the standard error of the device.

The results of the centrifuge method show that it is successful in sampling the entire water column. All particles (high and low density) <5 mm can be sampled by the modified centrifuge strategy. The data are random samples to show that the method can capture the MP as planned. In-depth analyzes using PyGCMS have subsequently shown that a certain percentage has been identified falsely positive (Figure 12-D). This requires further controls. The samples taken at the water surface

show that the proportion of particles found in the net was higher than in the CFC. At a depth of three meters, however, more particles were detectable in the centrifuge. This fits in with the descriptions (2.1.1) that the centrifuge captures the particles $>1 \text{ g/cm}^3$ and the net captures the particles $<1 \text{ g/cm}^3$. Based on the results that were found in the net, it is clear that a higher load is detectable downstream behind the effluent of the WWTP which is thus determined as a point source for fragments and fibers. For these reasons treatment plants must be subjected to intensive controls and remediated so that no MP enters the waters from these sources. Whether the found of primary plastic comes from cosmetic products is difficult to comprehend. What is known, however, is that it has been manufactured by industry as such and is now increasingly found in the aquatic environment. A special improvement of the sewage treatment plants can safely prevent the entry into the waters. Furthermore MP is knowingly lost during transport, for example. Stronger guidelines regarding production and export should therefore be introduced. All results are used to describe a distribution of the MP (size, type, shape) in the water column including the sediment samples.

KWS-Method

The presence of MPs in different aquatic systems has been frequently confirmed (Schneiderman and Hillmyer, 2017; Verster et al., 2017). However, a reliable assessment of the environmental risks of MPs requires quantitative and qualitative analyses of their occurrence including a detailed study of their size distribution (Duis and Coors, 2016). Many strategies have been developed for the sampling and quantification of MPs in waters, freshwater/marin sediments and beach sand (Löder and Gerdts, 2015; Besley, 2017), but the different organic and inorganic compositions of environmental samples have complicated sample preparation, making it mostly inefficient. Many alternative methods have been proposed, but none has been widely accepted or standardized (Hanvey et al., 2017; Hidalgo-Ruz et al., 2012; Rochman et al., 2017; Shim et al., 2017).

The new method introduced in this work uses the electrostatic properties of MPs to separate them from different sample materials (Felsing et al., 2017; Köhnlechner and Sander, 2009). Coarse-grained samples such as beach sand or freshwater-suspended solids can be analyzed directly, e.g. by PyGCMS (Nuelle et al., 2014; Tagg et al., 2015), Fourier-transform infrared or Raman microscopy (Kappler et al., 2016). Further studies on the recovery of added MPs are required for a final assessment of the electroseparation method including a comparison of the widely used Munich plastic separator (Imhof et al., 2012) and our KSW-based electroseparation method in order to assess whether the recovery results are the same for each kind of sample material. These future experiments should use the most common types of MPs in different concentrations and sediment types as well as field samples from different sources. The loss of $<1 \%$ of the fine-grained

material during the separation process may be considered negligible as it does not result in a loss of added plastic particles in the size range of 63–5,000 μm . Due to the simple technique and the consistent physical properties of polymers it can be assumed that all smaller particles $<63\mu\text{m}$ are successfully separated. This should still be reviewed. Since the KWS can only separate dry material, the samples must be completely dried before separation. Although this is the most time-consuming step and can take several days, the process is otherwise time-saving and the samples are easy to handle. If it comes to clumping, the sample can be carefully processed with a mortar. Other advantages of the process are that only a 230 V power supply is required. Labor costs are minimal and neither special protective clothing nor monitoring is required. In addition due to the physical separation principle of KWS and the greatly reduced sample mass after electroseparation significantly lower levels of more or less harmful heavy solutions such as zinc chloride or sodium polytungstate are used and then discarded (GESTIS, 2016a; GESTIS, 2016b).

The results of this study show that electroseparation is a valid and reliable method to isolate a very wide range of commonly used MPs of different density, size, shape and age from environmental samples that differ in their content of organic matter and grain sizes. The varying compositions of field samples do not negatively affect either the separation process or the recovery rates. Even samples with high biomass compounds are efficiently reduced and the MPs successfully enriched in the final fraction. Unlike other methods, e. g. those based on chemical density separation, the recovery rate of the KWS is very high ($> 99\%$), even for very small ($63\mu\text{m}$) MP particles. This efficient recovery reflects the high quality of this new method, which has been used for the first time in a scientific study. By reducing the sample mass by up to 99 %, KWS simplifies the handling of large field samples without special safety measures or hazardous fluids with material damaging effects during the separation process. In addition, the application is simple, requires no special skills and is of a short duration (a few hours), which contributes to its efficiency. To further research the harmful effects of plastic waste on aquatic and terrestrial environments, electric separation using KWS can help to assess the worldwide abundance of plastic waste in different ecosystems with less effort. In conclusion the electrical separation method can replace the chemical digestion. The device (KWS) is currently proven for particles up to a minimum of $63\mu\text{m}$. The composition of the samples used and the proportion of biological material has no influence on the success of the separation process. All types of plastic, including aged plastic can be separated with the KWS. The using of the new sampling technique leads to the production of new or extensive results. The assumption that tiny primary plastic makes up only a small amount of the MP entry could be undoubtedly refuted. For future studies, however, a comprehensive blank value analysis must be carried out to determine the standard error of the device.

The physiological functioning of animals is altered by consumption of MPs, either by the leaching of accumulated organic pollutants from the particles into the gastric mucosa, as shown for fish, by the physical blockage of the digestive system, or simply by taking up space in the stomach that could otherwise be used by nourishment (Bakir et al., 2014; Wright et al., 2013). As MPs can facilitate the bioaccumulation and/or biomagnification of harmful contaminants (Koelmans et al., 2013) it is relevant whether plastic waste and its small fragments are a source or sink of hazardous chemicals for the environment and for organisms (Crawford and Quinn, 2017; Hong et al., 2017; Lambert, 2017). Since the electrostatic separation by the KWS does not require liquids that could dissolve substances adsorbed on the surfaces of the particles, the separated MPs can subsequently be analyzed for these contaminants. In further investigations a division of the MP fractions into the various types of plastics with an electrostatic plastic-plastic separator would improve the precision and efficiency of the analyses of the various plastics due to their individual conductivity (Schubert et al., 2001).

Core sampling

Investigating sediment cores is a good way to view MP evolution (quantity, kind, and size) over time. The frequency of the particles increases over time and thus reflects the increase in the use of plastics since the 1950s. To obtain even more accurate and more comprehensive data longer cores made of stainless steel are currently used for further studies at the BfG (Brennholt et al., 2017). The prerequisite for sampling with cores is that the sediment is suitable for this purpose. Thus locations must be determined without coarse grained subsoil for example. Depending on the depth of the water it is no longer possible to sample from the ship and divers must be used. Whereas divers are in turn dependent on the flow velocity and flow characteristics.

4.2 Microplastics and possible effects on organisms

The presence of accumulated plastic additives in the environment or organisms has often been used as a proxy of plastic pollution in the marine environment resulting from the release of additives from plastic waste. While direct toxicity may occur due to the physical effects of plastic ingestion (Wright et al., 2013), indirect toxicity may be observed with respect to the release of hazardous chemicals from plastics. Since most plastic additives are not chemically bound to the plastic, they can end up in the environment and become available to organisms (Table 6) (Hermabessiere et al. 2017). The experiments in the laboratory show that some of the detected additives are toxic plasticizers that are no longer authorized in Germany or subject to strict regulations. DINP and DIDP are suspected to accumulate to a high degree in organisms and to be long-lived in soil and sediments. Due to the high input quantities for soft PVC and the structural similarity to DEHP it can be expected to spread strongly in the environment. For the DEHP a

tolerable daily intake (TDI) of 50 µg/kg bw per day was established. In both cases the DEHP may be responsible for the results of the YES. Samples 2, 5 and 9 were tested positive for estrogenic substances with the YES-test. There is a ban on baby products and children's toys because of the reproductive toxicity properties for DEHP. For precautionary reasons the Umweltbundesamt (UBA) advocates avoiding the environmental impact of DIDP and DINP. Although there are no hazard labels for DINP, it has a TDI of 150 µg/kg bw per day. The phthalates found were awarded a high environmental risk. It can therefore be assumed that the high content in fish bait also increases the proportion of toxic phthalates in the water. It is not shown in this experiment how long it takes for a rubber fish to be completely decomposed. It is also not clear how much additives are introduced the water phase through a whole bait, whether they accumulate there and have an influence on the reproduction of organisms. This has to be investigated separately. Due to the material properties the baits sink to the riverbed after a possible loss by the angler. Over a longer period of time and until the final decay they could release toxic/estrogenic ingredients there continuously, possibly exerting a negative influence on benthic organisms.

PVC can contain 10–60% phthalates. Because phthalates are not chemically bound to the polymer matrix, they can easily enter the environment during manufacture, use and disposal (Net et al., 2015). The release of the phthalates from soft PVC cannot be prevented. The UBA therefore advocates where (technically) possible and reasonable for a gradual replacement of soft PVC with alternative plastics (such as polyethylene or polypropylene) (UBA, 2007). PAEs have been found in a variety of environments, as reported by Net et al., 2015. This is alarming because some phthalates have been defined as endocrine disruptors in low concentrations (Oehlmann et al., 2009; Hermabessiere et al., 2017). Phthalates (DMP, DEP, DiBP, DnBP, BBP, DEHP, DnOP, and DnNP) have been found in a variety of organisms, ranging from 18 different species of primary producers (plankton and macroalgae) to the dogfish shark (*Squalus acanthias*), but no biomagnification has occurred in the phthalates studied by the food web (Mackintosh et al., 2004). Recently Cheng et al. (2013) also reported phthalates (DMP, DEP, DiBP, DnBP, 2-methoxyethyl phthalate [DMEP], DHP, BBP, DEHP, DnOP, DNP) in concentrations ranging from 0.2 to 1.223 µg g⁻¹ ww. Therefore, the transportation and fate of plastic additives extracted from plastic waste should be treated with care in future field, laboratory and modeling work. In this study only phthalates were sought in the water samples. However the results of the pyrolysis have shown further additions in the baits. One could expand the analysis even more. And even if some of the toxic phthalates are banned in the EU, products with questionable ingredients always seem to be imported to Germany/Europe. For the effect research it can be assumed that in a closed

experimental setup the values would be even higher. Since phthalates are very volatile substances the concentration drops at each working step.

The particle concentrations used for the experiment are far from the realistic numbers found in the environment. If one were to repeat the experiment with realistically selected particle numbers, none of the plastic kinds would probably have a negative effect on the mortality of *L. variegatus*. Whether the leaching really has no influence is not known exactly. Since the experiment is a very open system, it can be assumed that the substances evaporate too quickly to be effective at all. In the experiment further estrogenic effects were detected. It is unclear whether this influences *L. variegatus* because it is not known if this worm has an estrogenic receptor. Hence it would also be useful to choose an organism that reacts to estrogen-active substances. Physiological indicators of fitness present a measure of an organism's response to a changing environment. An analysis of how these organisms allocate and store their energy resources provides an understanding of how they cope with such environmental changes. The amount of stored energy is the most important physiological indicator of fitness. Glycogen and triglycerides are the main substrates in insects and crustaceans to store surplus energy and release it in times of need (Hervant et al., 1999; Koop et al., 2008). This approach seems to be the most efficient route and has successfully been applied to invertebrate samples before (Winkelmann and Koop, 2007; Koop et al., 2008). It is proposed that this allows an almost real-time observation of negative developments because physiological indicators react very quickly (within 1 to 3 weeks) to environmental changes (Hervant et al., 1999). The toxicological studies have shown that especially additive-rich plastic particles can pose a potential hazard and the results of the glycogen content analyses reflect the mortality rate of the sediment toxicity test. This will be the case especially in waters with high plastic load. Otherwise it must be further investigated from which concentrations the certain pollutants and particles are really dangerous. If one compares the results of 3.4.1 with the mortality of the worms in the experiment, there seems to be a positive correlation between the occurrence of the additives and the mortality rate. However the effect of the additives on the organisms seems to act only through direct uptake (ingestion) and not indirectly over the water column. But based on the presented results, it has not become clear whether the additive or the estrogenic effect has an influence on the organism. That is why further studies are needed here. The Polymer and Food Protection Consortium and several studies (Wagner et al., 2006; Tchounwou et al., 2014) warn against the heavy metals that will also be leached of the plastic over time. Especially PVC can have high concentrations of toxic cadmium, titanium and zinc (Heinrichs and Hermann, 1990). Heavy metals have been shown to negatively impact animal and plant life as well as humans (Singh et al., 2010). A next step and continuation of this work is the analysis of the used plastics on the occurrence of

heavy metals. These results can then be used to construct a suitable effect study or compared to existing literature on environmental impact. With regard to the quantities of PVC and other plastics it can be assumed that the values of heavy metals in the aquatic environment will increase if they are continuously fed into the environment. This urgently requires further studies. In general the modification of the sediment contact test was successful and can now be used for further MP studies.

In this study an improvement for sampling is shown and indications of possible contaminants from plastic as well as effects on biota are demonstrated. To capture the real danger of increasing pollution by MP more studies are needed especially to be able to estimate the entries of MP in the open sea. The presented approaches can be useful for this purpose.

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Declaration/Selbstständigkeitserklärung

I hereby declare that I have completed the work submitted here independently and have composed it without outside assistance. Furthermore, I have not used anything other than the resources and sources stated and where I have taken sections from these works in terms of content or text, I have identified this appropriately.

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