

Analysis of Conventional and Biodegradable Microplastics in Solid Sample Matrices: A Contribution to Method Standardization

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Abstract

Microplastics are the new emerging pollutants ubiquitously detectable in aquatic and terrestrial ecosystems. Fate and behavior as well as ecotoxicity are of increasing environmental concern particularly in sediments and soils as natural sinks. For a global environmental risk assessment reliable and easy to apply analytical methods are mandatory to obtain comparable data. This is based on the isolation of microplastics out of the solid sample matrices prior to instrumental detection. Thus, this study provides a validated approach for density separation, which emerged from a comparative study using different salt solutions to isolate conventional and for the first time biodegradable microplastics from different solid sample matrices, i.e., sand, artificial soil, and compost. Four solutions (water, sodium chloride, sodium hexametaphosphate and sodium bromide) of different densities were applied followed by oxidizing digestion. Finally, the impact of the procedures on size and surface properties of microplastics was tested. Dependent on the sample matrix, highest recovery rates of 87.3-100.3 % for conventional polymers and 38.2–78.2 % for biodegradable polymers were determined with sodium bromide. It could be shown that the type of solid sample matrix influences the recovery rates and has to be considered when choosing a sample preparation technique.

Introduction

Since the start of the large-scale production of plastic with about 2 Mt in the 1950s, production and use have rapidly grown due to benefits like low weight, hygienic use and easy plasticity (Geyer et al. 2017). In 2019, production of plastics reached a maximum with 368 Mt worldwide (PlasticsEurope 2020). Generally, plastics are released deliberately and accidentally from different origins, through different pathways into the environment, which results in a ubiquitous plastic pollution (Schell et al. 2020). It is estimated that out of the generated 6300 Mt plastic waste up to 2015, nearly 80 % were accumulated in the environment (Geyer et al. 2017). Studies confirmed the persistence of plastics in nature. The main degradation process is assumed to be fragmentation into smaller plastic particles so called “microplastics” (MPs) which are polymers with a size between 1–1000 µm (Hartmann et al. 2019). However, some definitions contain sizes below 5 mm or 10 mm (Braun et al. 2018).

To overcome littering problems, biodegradable plastics have been developed with a current share of 0.6 % of the global plastic market (PlasticsEurope 2020; Bioplastics 2019). With 43.8 %, starch blends are produced the most, followed by polylactid (PLA) with 24.0 % and polybutylensuccinate (PBS) with 11.4 % of biopolymer production (Haider et al. 2019). Biodegradable alternatives for conventional plastics in mulching for agricultural industry have been developed like MaterBi (MB) by Novamont GmbH (Morra et al. 2016). Theoretically, biodegradable plastics will be converted to water and carbon dioxide by biological forces like microorganism metabolism and can become part of the carbon cycle. However, the degradation potential of plastics strongly depends on the chemical structure (e.g. molecular mass or crystallinity) as well as environmental conditions (e.g. temperature or UV radiation). Number and composition of microorganisms and enzymes present in the corresponding compartment also influence the degradability of plastic (Thakur et al. 2018; Haider et al. 2019). In case of biodegradable polymers,

studies suggest that additives will increase the persistency and might make them potentially undegradable under natural conditions (Lambert and Wagner 2017). Recent studies point out an even higher risk to the environment of some biodegradable plastics than conventional plastics due to faster fragmentation rates into MPs and incomplete degradation into water and carbon dioxide (Qin et al. 2021; Liao and Chen 2021). Still, formation and effects of biodegradable plastics and MPs to environment and human health are widely unknown.

Sampling strategies, sample preparation methods and measurement techniques for MP analysis differ strongly within the scientific community (Thomas et al. 2020). Currently used techniques to measure MPs lack harmonization and show certain limits, especially for biodegradable MPs, ranging from limited size detection to limitation of detected polymer types (Büks and Kaupenjohann 2020; O’Kelly et al. 2021). Consequently, previous studies calculated strongly varying concentrations e.g. of 0.34–690,000 particles/kg soil and < 0.54-67,500 mg plastic/kg in different soil matrices (Zhang et al. 2018; Zhou et al. 2019; Piehl et al. 2018; Fuller and Gautam 2016). Likewise, the effects of MPs on the environment can only be assessed with difficulty. In first studies it was shown that MPs in soil or sediments have an effect on survival, growth and reproduction of organisms (de Souza Machado et al. 2018; Boots et al. 2019; Barceló and Picó 2019; Wang et al. 2021; Zhang et al. 2021). It is assumed that MPs are accumulating and transported along trophic levels of the food chain (Huerta Lwanga et al. 2017). Besides, MPs distribute vertically and horizontally within soil. The presence of MPs has potential geochemical and biophysical altering effects on soil structure, might influence soil aggregation, water holding capacity and soil nutrient cycle (Rillig et al. 2017; Guo et al. 2020). A recent study has shown that even biodegradable MPs have a negative effect in soil structure e.g. by altering soil ecological function and biogeochemical cycling (Zhou et al. 2021).

A reliable method for MP analysis in solid sample matrices was not established until today, but is urgently needed to guarantee comparability between scientific investigations. As there is only a low percentage of MPs in solid samples, MPs needs to be isolated from the soil matrix. Since MPs are also distributed heterogeneously, mixed samples with the highest possible sample mass will lead to more meaningful results regarding content of MPs in solid samples. This in turn leads to increased effort in sample preparation as organic and inorganic matrices have to be removed for analytics. Especially high organic contents within the sample matrix lead to interfering signals and have to be removed (Thomas et al. 2020). However, many of the previous studies have only processed small sample amounts from 3–50 g (Ding et al. 2020; Liu et al. 2019; van den Berg et al. 2020; Scheurer and Bigalke 2018; Corradini et al. 2019). Several techniques can be applied for sample preparation like sieving, visual sorting, extraction, electrostatic separation etc. (Quinn et al. 2017; Thomas et al. 2020). Most common techniques are density separation followed by oxidative digestion. Density separation is based on the fact that solid sample matrices have significantly higher specific densities, e.g. bottom sediments (2.65 g/cm^3) than most of plastics ($0.05\text{--}1.70 \text{ g/cm}^3$) (Chubarenko et al. 2016). However, organic matter is not separated from plastics with density separation as it shows similar densities of about $1.0\text{--}1.4 \text{ g/cm}^3$ or rather 1.6 g/cm^3 (Bläsing and Amelung 2018; Cerli et al. 2012). Thus, oxidative digestion can be applied. Effects of

sample matrices on efficiency of the separation method need to be assessed. Moreover, there is no valid method available for measuring concentrations of biodegradable MPs in solid matrices.

In this study we aim to generate a validated and easy to apply sample preparation method to detect conventional and biodegradable MPs in solid sample matrices. Therefore, a minimum standard has to be found, which enables a worldwide application to collect comparable data on MPs pollution worldwide. We compared the applicability of different solutions with increasing density for density separation as well as oxidative digestion under various conditions. The solutions used for density separation were water (H₂O), sodium chloride (NaCl), sodium bromide (NaBr) and sodium hexametaphosphate (SHMP). These are considered to be harmless to humans and the environment according to the Globally Harmonized System of Classification, Labelling and Packaging of Chemicals (GHS). This is considered to be particularly important because the users have different chemical backgrounds. NaCl and NaBr solutions provided high recovery rates of MPs in other studies and are relatively cheap (Scheurer and Bigalke 2018; Liu et al. 2018; Thomas et al. 2020) while SHMP is applied as a dispersing agent for soils and sediments (Scheurer and Bigalke 2018; Vermaire et al. 2017). Recovery experiments were conducted by spiking solid sample matrices with different organic contents with conventional and biodegradable MPs in two separate sample set ups. In addition, the effect of sample preparation on MPs was tested. The effect should be as low as possible, especially when performing further identification tests of MPs.

Methods

The experimental design to separate MPs from solid sample matrices includes preparation of three different sample matrices and addition of artificial MPs. Separation steps of density separation and digestion of organic matter were performed. Four different solutions were applied according to their density and toxicity. Recovery rates as well as changes in size and surface structure of MPs were analyzed with stereo microscope and ATR-FTIR (Fig. 2). A chemical identification of potential MPs is associated with the cost of an analyzer, but assumed to be mandatory, because of the high error rates of 20–70 % through visual sorting (Bläsing and Amelung 2018). An ATR-FTIR analyzer is relatively cheap e.g. compared to an automated μ FPA-FTIR microscope. Therefore, 500 μ m was chosen as the lowest size limit as it represents the optical resolution maximum of this technique.

To meet the standards of analytical quality assurance, every combination of plastic mixture, extraction solution and substrate was tested in triplicates with a blank for every series of tests to investigate contamination. Beyond, sources of MPs contamination were excluded (see 2.6).

2.1 Preparation Of Mps

Different types of conventional and biodegradable plastic polymers were produced from mainly post-consumer products (Table 1). Conventional polymers were chosen according to their relevance and the probability of appearance as MPs in the environment. With PE/PP (—) as light

density polymers and PVC/PET (—) as high-density polymers, it can be estimated if the method is able to recover a variety of plastics with different densities. Densities were measured due displacement of volume with a pycnometer (50 ml) and ethanol (99 %; TH. Geyer). Furthermore, three biodegradable polymers consisting of a range of newly produced polymers (PLA, PBS, MB) were applied in density separation validation tests from solid samples for the first time.

Reference MPs were prepared in four steps: first, products were cut. Second, pieces were frozen in liquid nitrogen and ground (Table 1). Third, size classes of 500–800 μm and 800–1000 μm were differentiated by sieving. Fourth, particles were visualized under a stereo microscope (Zeiss Stemi SV8 with Olympus SC30, $f = 100, 80\times$, Oberkochen, Germany). Particles with a maximum length of 1.69 cm \pm 0.40 cm and a minimum width of 1.08 cm \pm 0.15 cm in the upper size class and a maximum length of 1.24 cm \pm 0.33 cm and a minimum width of 0.77 cm \pm 0.14 cm were gained with this method as the image analysis showed. All MPs were applied as fragments except MB, which was used as a film, because it is disposed as biowaste bags.

Two polymer composite samples were mixed, one with conventional and the other with biodegradable polymers. Concentrations of 400 particles/kg soil and 0.01 % w/w, respectively, were concluded to reflect realistic concentrations of MPs in solid sample matrices and were also applied in other recovery tests (Liu et al. 2019). These assumptions resulted in a MPs count of 25 particles per polymer, consisting of 10 particles in the size range of 800–1000 μm and 15 particles between 500–800 μm . As it is likely that more small particles occur in soil (Braun et al. 2018), more particles of the smaller size class were applied.

Besides the densities of used MPs, the color, chemical spectra as well as the sizes of particles before and after sample preparation were determined (see 2.7).

Table 1
Parameters of preparation of reference MPs

polymer	abbreviation	device	speed [rpm]	origin	color	density
polyethylene	PE	centrifugal mill (Retsch ZM 200)	8000	pellet	bright yellow	0.862
polypropylene	PP	knife mill (Retsch GM 200)	6000	pipe	blue	1.083
		centrifugal mill (Retsch ZM 200)	6000			
polyethyleneterephthalate	PET	scissor		bottle	green	1.216
		centrifugal mill (Retsch ZM 200)	6000			
polyvinyl chloride	PVC	cutting		pipe	red	1.369
		centrifugal mill (Retsch ZM 200)	10000			
polylactid	PLA	scissor		coffee-to-go lid	white	1.300
		centrifugal mill (Retsch ZM 200)	10000			
polybutylensuccinate	PBS	centrifugal mill (Retsch ZM 200)	6000	pellet (Bionolle)	white	1.234
MaterBi	MB	scissor		biowaste bag	white-	
		centrifugal mill (Retsch ZM 200)	10000	(Edeka)	green	1.241

2.2 Preparation Of Solid Sample Matrices

Three solid sample substrates (sand, OECD artificial soil and compost) were examined, representing different environmental compartments (beach/sediment, terrestrial soil and natural sample with high content of organic matter).

An amount of 250 g was applied per sample. This is together with Möller et al. 2021 the largest sample mass that was investigated compared to previously published studies that measure MPs concentrations in soil with density separation.

a) Sand substrates

To determine the influence of different particle sizes in the matrix, sands in different size classes ranging between 60–300 µm, 400–800 µm and 700–1200 µm (Scherf Quarzsand) as well as silicon dioxide in a size range of 53–74 µm (Eijelkamp synthetic sand) were purchased. These were combined to sand sample substrates according to sand fractions in OECD artificial soil (Table 2). In relation to OECD 222, the size fraction of 0.05–0.2 mm has to make up at least 50 % which was evenly divided between the two obtained fractions in this section. The composition of the other 50 % followed the formula of 100 µm 5 % of total substrate composition. Therefore, sand was dried at 200°C over night and sieved with a vibratory sieve shaker (Retsch AS 200) for 10 min, amplitude 1.2.

Afterwards, the sand sample substrates were placed in a muffle furnace at 550°C for at least 6 h to remove potential MP contamination. Finally, the composite plastic samples were added to the sand sample substrates. The mixture was stirred manually for 30 s.

Table 2
Composition of OECD artificial soil and sand substrate

proportion [%]	mass for sand substrate [g]	mass for OECD artificial soil [g]	size fraction [µm]	
25	62.5	43.75	53–74	sand
25	62.5	43.75	80–200	sand
5	12.5	8.75	200–300	sand
10	25	17.5	300–500	sand
10	25	17.5	500–710	sand
5	12.5	8.75	710–800	sand
10	25	17.5	800–1000	sand
10	25	17.5	1000–1200	sand
		35		kaolinite
		15		halloysite nanoclay/kaolin clay
		25		sphagnum peat
total mass [g]	250	250		

b) OECD artificial soil

The OECD artificial soil is based on elaborations of an artificial soil in OECD 222: Earthworm Reproduction Test (*Eisenia fetida/Eisenia andre*). This test was established for the ecotoxicological test of chemicals but shows the advantage of creating a reproducible soil matrix with proportions of soil that can interfere measurements of MPs like clay particles and soil organic matter (SOM) (Thomas et al. 2020).

The OECD guideline presets a content of 20 % kaolin which was split between kaolinite (CAS No. 1318-74-7) and halloysite nanoclay/kaolin clay (CAS No. 1332-58-7) (Sigma Aldrich). Kaolinite ($\text{Al}_2\text{O}_7\text{Si}_2 \cdot 2\text{H}_2\text{O}$) and halloysite nanoclay/kaolin clay ($\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4 \cdot 2 \text{H}_2\text{O}$) are both 1:1-layered aluminosilicate minerals which belong to the kaolin group. The proportion of kaolinite (70 %) was selected to be higher than of halloysite nanoclay/kaolin clay (30 %) as it is the most widespread clay mineral in soils (Dixon 1989).

As there was no further definition of finely ground, the size of sphagnum peat (Floragard Floratorf) was oriented to the descriptions of OECD 232: Collembolan Reproduction Test in Soil which specifies finely ground to a particle size of 2 – 1 mm. Therefore, sphagnum peat was sieved to 1.00-3.15 mm due to available mesh size.

All proportions were dried to constant weight.

A mixture of sand size classes with same proportions as the sand substrate was prepared (Table 2) and placed in a muffle furnace at 550°C for at least 6 h. Afterwards, 35.00 g kaolinite and 15.00 g halloysite nanoclay/kaolin clay were added as well as 25.00 g of sphagnum peat. Additionally, 1.25 g of CaCO₃ were added which corresponds to 0.5 % of soil mass for obtaining pH 6.0 – 0.5. The pH was determined according to OECD 222 by mixing 20 g of artificial soil with 100 mL KCl (1M) for 5 min thoroughly. The mixture was left to settle for 2–4 h and then measured with a pH-meter (pH 526, WTW), calibrated at pH 4.0 and 7.0.

The formation of soil aggregates was accelerated by wetting and drying soil. The applied amount of water was oriented to 60 % of water holding capacity as demanded in OECD 222 which was assessed with HYPROP 2 (Meter Group). Two samples of OECD artificial soil (250 g) were put in a sample ring and placed into water for complete saturation for three days. Afterwards, measurements were conducted over 13 days. The water holding capacity at pF 1.8 was calculated to 32.7 %. Therefore, 45 mL of distilled water and the composite plastic sample were stirred manually together with the substrate for two minutes. Afterwards, 5–10 mL of distilled water were used to rinse the spoon. The sample was let stand for four days to create agglomerates with components and dried at 60°C for 24 h.

c) Compost

Compost was derived from a biowaste plant in Lower Saxony, Germany (ALBA, Watenbüttel). The compost consists of biowaste from private households as well as garden waste (66 %) and was fermented at > 55°C for 14 days according to the composting plant operator. Characteristics show a content of organic matter triple the amount of OECD artificial soil. Furthermore, pollution with plastics is documented in the share of foreign matter with 0.08 % dry weight. The moisture content of 38.5 % was obtained by drying compost to a constant weight.

Compost was dried at 60°C and sieved. The composite plastic sample and 100 mL of distilled water were added to 250 g of compost dry weight (size class < 1 mm) and stirred manually for 1 min to create agglomerates containing MPs whereas the amount of added water corresponds to the moisture content before drying. Afterwards, samples were dried again (60°C, 24 h). As compost was not newly mixed from components in contrast to OECD artificial soil, no standing time was applied.

2.3 Preparation Of Density Solutions

Density separation was performed with distilled water () and brine solutions of different densities. Saturated solutions were made by dissolving the respective salt into a volume of 1 l distilled water. In case of NaCl (> 99%, z. A., Ph. Eur.; CHEMSOLUTE®) and NaBr (99%, p.a., ACS; Roth) the amount of salt per water volume (358 g NaCl /L H₂O and 905 g NaBr/L H₂O) was depending on the specific solubility of each salt. The solutions were stirred in a beaker (2 l) on a magnetic stirring plate overnight resulting in solution densities of () for NaCl and () for NaBr. As there was no information available about solubility of SHMP (65–70% P₂O₅ basis; SIGMA-ALDRICH), the substance was added to distilled water sequentially and stirred until no further salt dissolved and an excess formed at the beaker bottom ().

To lower the amount of disposed chemicals as well as costs, separation solutions of SHMP and NaBr were recycled and reused throughout the test series of density separation. Solutions were decanted onto a pleated filter (Macherey-Nagel MN 615 1/4, diameter 385 mm). The salt was retrieved in a rotary evaporator (Rotavapor RE 111; Büchi Labortechnik AG). Nevertheless, solutions that were used for density separation with compost were not recycled as the dark color indicated pollution with humic substances from compost.

2.4 Density Separation, Digestion And Recovery

The recovery rates of MPs in solid sample matrices by density separation with distilled water and different salt solutions were determined. Therefore, spiking experiments were performed, where 250 g of solid sample matrix was spiked with the composite plastic sample. First, 500 mL of the respective separation solution was added into a 2 L glass beaker and stirred with a rectangular stirring bar at 200 rpm. Afterwards, each spiked solid sample was transferred together with another 500 mL separation solution into the glass beaker, resulting in a total volume of 1 L separation solution per sample. Glass beakers were also used in other studies (Chen et al. 2020; Huang et al. 2020; Liu et al. 2018; Zhou et al. 2020) for density separation of MPs in soil as they are cheap, directly to purchase and easy to handle without the need to design complicated individual apparatus assemblies. Thus, they meet the requirement to be available worldwide.

Then, samples were stirred on a magnetic stirring plate at 400 rpm for 30 min as MPs can be strongly sorbed to the matrix (Liu et al. 2018). Even though polymers were artificially added to solid samples in these test series and it is hypothesized that strong sorption effects might not take place, it is likely that this time span is necessary when analyzing real soil samples. Afterwards, the stirring bar was removed and rinsed with the respective density solution as well as the beaker walls. Beakers were let stand to allow density separation for about 24 h, chosen according to (Hurley et al. 2018; Liu et al. 2018). The settling time allows the MP particles to float up to the solution surface and heavier sample matrix particles, like inorganic contents, to sink to the beaker bottom.

The upfloating MPs were taken with a spoon from the top layer of the solution surface. For sand substrates, MPs were then separated with tweezers, washed in distilled water and stored in petri dishes. For OECD artificial soil and compost samples, organic matter was additionally surfacing to MPs. Remaining residues were collected by suction of a peristaltic pump in a second removal step (Masterflex Console Drive easyload 7518-00; Cole-Parmer GmbH; Wertheim; Germany). A glass tube connected to a hose was moved around the solution surface to gain floating MPs. The supernatant was directly filtered on a filter (Whatman 589/1, diameter 125 mm) using a Buchner funnel and a vacuum pump. When filtration was completed, the filter was thoroughly washed with distilled water to remove salty residues and dried at room temperature. Finally, MPs were sorted from the filter visually. Added MPs were identified according to known color and shape. Films were considered according to lighter color and flexibility when touched with tweezers.

H₂O₂ (min. 30% w/w; CHEMSOLUTE®) was used as a reagent for oxidizing digestion of organic matter. It was successfully applied in many studies recovering MPs from solid sample matrices (e.g. Hurley et al. 2018; Han et al. 2019; Liu et al. 2018; Liu et al. 2019) and showed removal rates of 96–108 % of SOM (Hurley et al. 2018).

Handling and amount of the digestion solution had to be adopted depending on the substrate. Compost samples were placed in a beaker (2 L) and H₂O₂ (30 %) was added depending on the amount of floating organic matter (Ratio 1:4) and stirred at 50–60 rpm. The samples were heated for 24 h at 60°C and let stand for 3 days at room temperature. OECD artificial soil samples were also heated at 60°C for 24 h, but 100 mL H₂O₂ were not sufficient to cover the sample, so the digestion solution volume was increased to 200 mL H₂O₂ (30 %) (Ratio 1:8). Residues were filtered using a vacuum pump onto filters (Whatman 589/1, diameter 125 mm).

Afterwards, MPs were sorted out with tweezers visually with the naked eye and under a stereo microscope. Then, a second density separation was applied. Residues were mixed with 500 mL of the respective density solution, stirred manually and let stand for 1–2 minutes. MPs were then sorted from the surface and washed in distilled water, dried at room temperature and stored in petri dishes for analysis.

Preliminary experiments showed that density separation eliminated most of the sample matrix compared to digestion. There was one exception in compost samples with NaBr as density solution. Thus, density separation was applied before digestion in all experiments except compost with NaBr.

2.5 Tests On Influence Of Sample Preparation

To test the influence of sample preparation on MPs, several potential impact factors were examined individually and also in combination. The individually tested impact factors were dry storage at 60°C, applied separation solutions for density separation, H₂O₂ (30 %) at room temperature and H₂O₂ (30 %) at 60°C. Three particles per polymer type in a size > 1 mm were put into a petri dish and tested for eight days

for each impact factor except with H₂O₂ at 60°C which was tested for 3 d. The duration was based on the time periods of the separation process and corresponds to at least twice the time for each condition in the separation process described above. The combination was a sequence of dry storage at 60°C, the separation solution NaBr that extracts the most MP contents and H₂O₂ at room temperature and at 60°C with same durations as for individual factors, respectively. Afterwards, particles were washed in distilled water, dried and analyzed.

2.6 Contamination Mitigation

To prevent contamination of the samples with MPs during the ongoing experiments, cotton lab coats and butyronitrile gloves were worn in the laboratory and lab surfaces cleaned with distilled water (Torres and De-la-Torre 2021). Equipment was cleaned in a dishwasher and dried in a drying oven. Furthermore, only filtered distilled water (20 μm) was applied in test series and for cleaning. All tests of density separation and removal of organic matter were performed under a flow bench and a fume hood. The time a sample was exposed to air was limited as much as possible to prevent atmospheric contamination. Additionally, beakers were covered with aluminum foil during stirring and settling. Furthermore, the air in the lab was filtered (Philips air purifier 2000 AC 2887; Koninklijke Philips N.V.; Amsterdam; Netherlands) as well as the supplied air from outside. Isolated MPs were stored in glass petri dishes until analysis. Besides, a blank sample was performed for each test series.

2.7 Microscopy And Ftir Polymer Identification

MP composite samples and MPs for influence tests were manually sorted onto glass petri dishes and photographed under a stereo microscope (Zeiss Stemi SV8 with Olympus SC30, f = 100, 80x, Oberkochen, Germany) with analysisPRO before and after application of the separation protocol. Special attention was paid to lay all particles the same way to ensure comparability for tests of influence of sample preparation. For other samples of recovery tests, this could not be ensured due to extent of particles.

Then, the photographs were evaluated by automatic computer image analysis to determine the damaging effects on the size of the MPs due to the impact factors. Image analysis was done using the open source computer vision library OpenCV (opencv.org): Contours were localized with the openCV function findContours, and the particle area was calculated from the contour. Maximum particle length was identified by automatic stepwise contour rotation and the openCV function boundingRect. All pictures had identical scale (84 pixels/mm), and contours with an area smaller than 500 pixels (0,07 mm²) were regarded as noise and excluded from the processing. The particles were fragmented and not round which is more likely to reflect their occurrence in the environment. Some particles were also frayed. The fabricated MPs are multidimensional so a two-dimensional picture can only represent a certain aspect of the particles. Thus, two parameters of area and maximum length were considered to reduce effects of observational error.

Besides, MPs were characterized chemically with ATR-FTIR (Bruker Tensor 27 with MVP-Pro, resolution 4 cm^{-1} , 16 Scans, area $4000 - 380\text{ cm}^{-1}$). Several recovered MPs from the MP composite samples were measured with ATR-FTIR to ensure sufficiency of visual classification. In addition, each polymer for the influence tests was determined by measuring three particles on two sides. The following six spectra were accumulated to reference spectra.

2.8 Statistical Analysis

For recovery tests, means, standard deviation and recovery rates of MPs were calculated for each sample and compared for every combination of substrate and solution. Besides, numbers of recovered MPs for the first and second removal from the solution surface were compared. For tests of influence, an analysis of changes in surface structure was performed with ATR-FTIR. Likewise to reference spectra, six spectra per polymer and impact factor were accumulated and afterwards compared with reference spectra. The similarity of spectra was tested with Pearson's correlation coefficient likewise to Vermeiren et al. 2020.

Results And Discussion

Recovery rates of MPs

Increasing densities of solutions were associated with increasing recovery rates of conventional and biodegradable MPs (Fig. 3). Similar results for conventional polymers were shown in other studies (Han et al. 2019; Vermeiren et al. 2020; Radford et al. 2021). Recovery rates were higher for conventional polymers than for biopolymers in every solution and substrate. As there were no other studies testing recovery rates of biodegradable polymers in soil, no comparisons can be conducted. Recovery rates were across all density solutions the highest in following order: sand substrate > artificial soil > compost. This corresponds to the higher organic content.

In this scientific work, conventional polymers were recovered highest with NaBr (94.8 % – 10.7 %). Similar results with recovery rates > 90 % were achieved in other studies, showing comparability of this investigations made. MPs > 500 μm were recovered with 90.7 % in ZnCl_2 (————) in an organic rich sediment (Vermeiren et al. 2020). Another study with NaBr as density solution also recovered PE, PP, PET and PVC with > 90 % (Liu et al. 2019). In a solution of NaI with ———, (Zhou et al. 2020) recovered PVC with 75.8 % and PE with 112.4 % from soil (particle size 0.5-2 mm). In these test series, PVC showed even higher recovery rates (91.1 % – 12.8 %) with NaBr. Therefore, the usage of NaBr is recommended in further experiments due to highest recovery rates of MPs in every substrate and simple handling. Additionally, for separation of low-density polymers PE and PP, application of NaCl as density solution is sufficient as recovery rates of 93.6 % – 8.5 % illustrate. This justifies the application of NaCl as the most used salt for density separation of MPs from sediments (Cutroneo et al. 2021).

Looking at recovery rates of different polymers, more polymer types were retrieved with increasing density of the applied solution (Fig. 4).

For sand substrates, PE was the only polymer that was recovered with > 90 % in H₂O. In another study with pure sand, PE and PP were applied in a similar concentration and retrieved with > 87 % (Zhang and Liu, 2018). Since the density of PP with _____ was lower than in these investigations (_____), density separation with water was able to achieve these results. Only PP and PE particles are recovered in NaCl with > 90 %. PET and PVC show no sufficient retrieval with 18.0 % 13.1 %, likewise to other works (Liu et al. 2019; Han et al. 2019).

Recovery rates in SHMP and NaBr for conventional polymers are both high with higher recoveries of PVC in NaBr (100 % 0 %) compared to SHMP (88.0 % 8.0 %) due to higher density of the solution. Biodegradable MPs were only retrieved in SHMP (89.8 % 18.6 %) and NaBr (78.2 % 28.6 %). In NaBr solution, salt crystals on beaker walls or solution surface hindered identification. This was not observed in SHMP accounting for higher recovery rates.

Lowest recovery rates in SHMP of 70.7 % 23.4 % and NaBr of 45.3 % 27.2 % are measured for MB which also showed highest standard deviations. This can be attributed to the fact that MB films could not be sufficiently visually identified due to the white-translucent color in liquids. Again, formation of salt crystals of NaBr interfered identification.

Matching results compared to sand substrate as for H₂O can be observed in artificial soil with a similar recovery rate of 106.7 % for PE. Comparable recovery rates in soil of > 90 % of PE beads (850–1000 µm) with H₂O were also measured by (Hurley et al. 2018). However, recovery rates of PE were lower in other solutions (85–101 %). PE particles were hardest to identify of conventional polymers due to their color which matched the brown-yellow color of residues of organic matter the most. In addition, gas formation in second density separation hindered visual identification of particles on the solution surface in salt solutions. Except for PE for this reason, conventional polymers all showed highest recovery rates in NaBr with > 92 %.

Biodegradable MPs were also highest recovered on NaBr with 17–85 %. MB showed lowest recovery which can be again attributed to decreased visibility. In the residues after digestion, only MB films with green color could be identified. An effect of color on recovery rate was also described by (Han et al. 2019) which showed lowest recovery for PE due to same white color as mineral particles left in the sample. Liu et al. 2019 also recorded lower recovery rates of films compared to fibers and particles. Similar reasons applied to PLA and PBS.

There are greater differences for recovery rates with SHMP and NaBr in OECD artificial soil compared to sand substrate. PET and PVC showed notable higher recovery rates in sand substrate (NaBr: 97.3 % 5.5 %; SHMP: 43.3 % 30.5 %). The same was observed for PLA and PBS (NaBr: 76.0 % 12.1 %; SHMP: 32.7 % 27.5 %). These results cannot be explained by density of solution or the amount of

floating organic matter as there were no differences between solutions which could have influenced recovery rates of MPs. It can only be speculated that presence of organic matter in general or kaolin had an influence on behavior of floating MPs. Higher standard deviations of MP concentrations in every OECD artificial soil sample for SHMP and NaBr compared to sand can be referred to depending visual identification. This highlights the need to consider characteristics of soil organic matter when choosing a separation method (Thomas et al. 2020). Additionally, Radford et al. 2021 identified a decreased efficiency of removal of organic matter by H_2O_2 in the presence of SHMP and classified the solution as unsuitable. Similar effects could also be observed in these test series.

Recovery rates in compost samples show a matching trend to OECD artificial soil with increasing recovery rates in increasing density but they were lower in every solution in compost compared to OECD artificial soil. The decreased recovery rates can be attributed to the content of organic matter which was also shown in other studies (Radford et al. 2021). Compost had a content of organic matter triple the amount of artificial soil. In addition, with the increasing density of solutions, more organic matter of compost was surfacing. Thus, more organic matter had to be filtered and digested after density separation. In addition, there were residues of compost left after digestion. Removal of organic matter showed varying efficiency, depending on applied density solution but also depending on sample in general. This is attributed to varying temperatures of heating plates with $5^\circ C$ and incomplete degradation of filters during digestion which hindered identification of polymers. For example, likewise to artificial soil, MB particles could only be identified when having a green color, resulting in recovery rates < 30 %.

As mentioned before, the sequence of density separation and removal of organic matter was changed for compost samples with NaBr solutions as preliminary tests revealed surfacing of 80–90 % of the sample. Based on research findings by (Hurley et al. 2018), it was assumed that there is no considerable effect of the sequence on efficiency of separation of MPs. However, samples were then easier to handle. Residues of organic matter were also surfacing in density separation as not the whole sample was digested.

No MPs greater than 500 μm were detected in blanks of artificial soil and sand and no intentionally added MPs in blanks of compost.

A disadvantage of the method is that it currently relies on visual sorting from solution surface as well as from filters after removal of organic matter and is therefore only applicable for identification of MPs > 500 μm . Separation of residues of organic matter from the sample has to be increased to make an automated analysis of smaller MPs possible.

Depending on sample and density solution, different amounts of organic matter were floating. In artificial soil, the whole amount of organic matter was surfacing in all test series while in compost it depended on density of solution. This depicts a different behavior of organic matter based on the type and composition. Thus, the application of different methods to remove organic matter can be helpful. To increase recovery rates and decrease visual dependency, conditions of digestion have to be improved to

digest organic matter completely like increase heating to 24 h or using more reagent. Otherwise, a second digestion after density separation can be implemented.

Considering the recovery rates of the second removal from solution surface, 1–3 % of all recovered particles were recovered in second removal step with the same density solution, depending on the substrate (Table 3). Thus, this step is not concluded to be necessary for further studies. This data was not collected in other studies although several extraction steps were performed which can be time-consuming (Chen et al. 2020; Huang et al. 2020).

Table 3
MPs after second removal of solution surface

substrate	recovered particles	share of recovered particles [%]	share of affected samples [%]
artificial soil	9	1.0	6.0
compost	25	3.0	15.5

Impact Of Sample Preparation On Mp Size

Likewise to Liu et al. 2019, the impact of H₂O was set as a reference point indicating no impact of sample preparation. Changes in size of polymers varied 2 % for both parameters (area and maximum length) with H₂O which suggests this as an observational error. All impact factors except H₂O₂ at 60°C showed only changes of size in this margin of uncertainty.

With H₂O₂ at 60°C, MB particles decreased by 12.2–27.1 % (Fig. 5). This is also reflected in the decrease of size of MB particles with the combination of impact factors (temperature of 60°C; NaBr; H₂O₂ at room temperature and at 60°C) (Fig. 6). At room temperature, no effects on MPs by H₂O₂ were shown considering the preconditions (Fig. 7). The same conditions of H₂O₂ at 60°C for 3 d were applied by (Liu et al. 2019). No considerable changes of conventional polymers were shown (these were defined > 10 %). The same applies for considered conventional polymers in tests of (Hurley et al. 2018) with a time period of 24 h.

For PET particles in tests of combination of impact factors, a substantial increase in area of + 26 % was measured although no change in maximum length was detected. In addition, this effect was not recorded for other impact factors. No specific cause could be identified.

Spectral Changes Of Polymer Structures

The comparison of spectra of impact factors with reference spectra reveals changes for PE, PP and MB in H₂O₂ at 60°C as well as combination of factors based on Pearson's correlation coefficients > 0.98 (Fig. 8).

There are two additional peaks at a wavelength between 1213 and 1153 cm^{-1} for MPs with H_2O_2 at 60°C as impact factor. These could be attributed to C-O-bonding (Hesse et al. 2012) and showed an oxidation of molecules at the surface of particles. It is likely that more radicals of H_2O_2 are produced at a higher temperature which increases reactivity. It was shown that in concentrated solutions of H_2O_2 and at an elevated temperature, oxidation of PE can occur (Saunders 1988). Abiotic oxidation is also the first step for degradation in the environment (Gewert et al. 2015). Another peak at 503 cm^{-1} could not be allocated due to missing information about this wavelength (Hesse et al. 2012). Nevertheless, there is no effect visible in the spectrum of the test series with combination of impact factors. Despite same settings, it could be observed during experiments that temperatures of heating plates varied 5°C which could have influenced changes in this case.

For MB, a peak at 3317 cm^{-1} is missing in the polymer spectra for H_2O_2 at 60°C and combination of factors which can be attributed to changes of hydrogen bonds (Hesse et al. 2012). Furthermore, there are differences in the intensity of transmittance for MB due to the method of fixation in the ATR-FTIR.

The same approach was conducted with H_2O_2 at room temperature by (Vermeiren et al. 2020) with similar results of Pearson's correlation coefficient > 0.900 for considered conventional polymers. Across all measured conventional polymers, correlation coefficients with 0.931 – 0.025 were detected which is lower than in these experiments with 0.996 – 0.003, so effects on polymer bonds were to a lesser extent. IR-spectra of conventional polymers with H_2O_2 at 60°C also showed “no major deviations from control” (Hurley et al. 2018). Effects of H_2O_2 on MPs were also not detected by (Radford et al. 2021).

The results of spectral changes fit results of size change for MB with H_2O_2 at 60°C as impact factor. However, oxidation of PE was not visible in measuring of size in tests of influence. All in all, influence tests revealed that application of H_2O_2 at 60°C showed decrease of size of MB and changes in hydrogen bonds as well as oxidizing effects at the surface of PE.

Thus, it is concluded that lower recovery rates of biodegradable polymers mainly depend on appearance of polymers with reduced visibility during analysis of samples and not on chemical properties of the polymer.

For further studies, experiments of removal of organic matter with Fenton's reagent are recommended. With the same efficiency, temperatures could be decreased which can decrease effects on particles (Hurley et al. 2018).

Changes In Size Of Mps From Recovery Tests

Measurements of recovered MPs have not been described in other studies before. Compared to previously described tests of influence, there is additional mechanical stress on MPs.

A change in size < 3.8 % was regarded as observational error as this was the highest alleged growth of particles. Only means of polymers with recovery rates 100 % were compared. Considering area and maximum length, only particles of PE in OECD artificial soil show decreases in size with 5.1–20.4 % (Table 4). Combining with tests of influence, the decrease in size can be attributed to mechanical stress during density separation by stirring of brittle PE particles. This is in coherence with tests of recovery where it was shown that several PE particles were destroyed. Thus, materials like separatory funnels and spiral conveyors which are not compressing particles between stirrer and vessel should be reconsidered (Enders et al. 2020; Möller et al. 2021).

Samples with < 100 % recovery rate per polymer were not included as changes in size are then mainly influenced by the size of recovered MPs. For this reason, no measurements of MB could be conducted and results of influence tests not be proven. However, the evaluation method cannot exclude the possibility that there is also an effect on the other polymers.

Table 4

Decrease in size of recovered particles with recovery rates > 100 % per sample. Decreases in size > 3.8 % are bold. In rows without standard deviation, only one sample showed recovery rates > 100 %.

polymer	substrate	solution	maxlen [%]		area [%]	
PE	sand	H2O	3.4	3.0	5.6	4.5
PE	artificial soil	H2O	17.3	3.8	20.4	12.8
PE	sand	NaCl	0.7	2.5	-0.9	4.9
PE	sand	SHMP	6.7	6.9	3.1	5.7
PE	artificial soil	SHMP	7.4	0.3	13.7	4.7
PE	sand	NaBr	3.5	2.9	4.2	1.1
PE	artificial soil	NaBr	5.1		13.4	
PP	sand	NaCl	-2.5	2.9	-1.7	2.8
PP	artificial soil	NaCl	-0.4	0.4	0.7	1.3
PP	sand	SHMP	-0.3	1.5	1.3	0.5
PP	artificial soil	SHMP	3.0	3.9	1.9	7.2
PP	compost	SHMP	0.3	2.6	3.0	0.2
PP	sand	NaBr	-1.1	0.7	-3.8	0.7
PP	artificial soil	NaBr	0.3	5.0	1.5	6.3
PP	compost	NaBr	3.4	0.7	10.3	2.7
PET	sand	SHMP	1.1	0.2	1.3	0.2
PET	sand	NaBr	0.9	2.0	-1.8	2.2

polymer	substrate	solution	maxlen [%]		area [%]	
PET	artificial soil	NaBr	1.8	1.3	4.0	3.7
PET	compost	NaBr	2.4		8.1	
PVC	sand	NaBr	-0.9	1.0	-2.2	2.6
PVC	artificial soil	NaBr	3.2		2.3	
PLA	sand	NaBr	-1.7		-2.6	
PBS	sand	SHMP	0.7	1.2	-0.4	1.3
PBS	sand	NaBr	-0.4		0.5	

Conclusion

There is a need of standardized procedures for MPs analysis to collect data for a true picture of the scale of MP contamination of the global environment. Thus, methods should be applicable around the globe. The introduced protocol provides a simple and useful tool for separation of conventional and biodegradable MPs from solid sample matrices with different composition representing different environmental compartments (beach/sediment, terrestrial soil and natural sample with high content of organic matter), which are supposed to be sinks for MPs. It was shown that recovery rates of MPs increase with increasing density of separation solution and decrease with increasing content of organic matter within the sample matrix. To isolate a wide range of environmentally relevant polymers, solutions with a density $> 1.5 \text{ g/cm}^3$ are recommended.

This study showed recovery rates of 94.7 % ± 10 % with NaBr as density solution for conventional polymers with a density of $0.86\text{--}1.37 \text{ g/cm}^3$ with an inexpensive, easy set up for density separation with a salt solution that does not harm the environment and has minimal effect on particle size and composition. Concentrations of 0.01 % w/w MPs in solid matrices were recovered which shows applicability for realistic concentrations in solid matrices.

Indeed, it could be shown that the method needs to be optimized for isolating biodegradable polymers. However, this is the first study to isolate biodegradable MPs from solid sample matrices. Although the share of bioplastics from globally produced plastics is currently $< 1 \%$, it is expected to grow. Thus, it is important to further investigate formation and quantification of MPs from bioplastics in solid sample matrices.

With a size limit of $500 \mu\text{m}$ and the set up with beakers, the method applies as a common minimum consensus. To get a full picture of MP contamination with respect to particles $< 500 \mu\text{m}$, the procedure has to be specialized on the sample matrix. As the set up possibly leads a higher effort for sample

isolation and causes misidentifications, more specialized apparatus, such as separating funnels, are recommended. In addition, more elaborate and expensive analytical methods have to be applied.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

DT: conceptualization of the research activities and the manuscript, supervising experiments, writing and editing of manuscript. BS: performing experiments, data analysis, writing and editing of manuscript. MK: data analysis, reviewing manuscript, RK: reviewing manuscript, JB: funding acquisition, reviewing manuscript.

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Figures

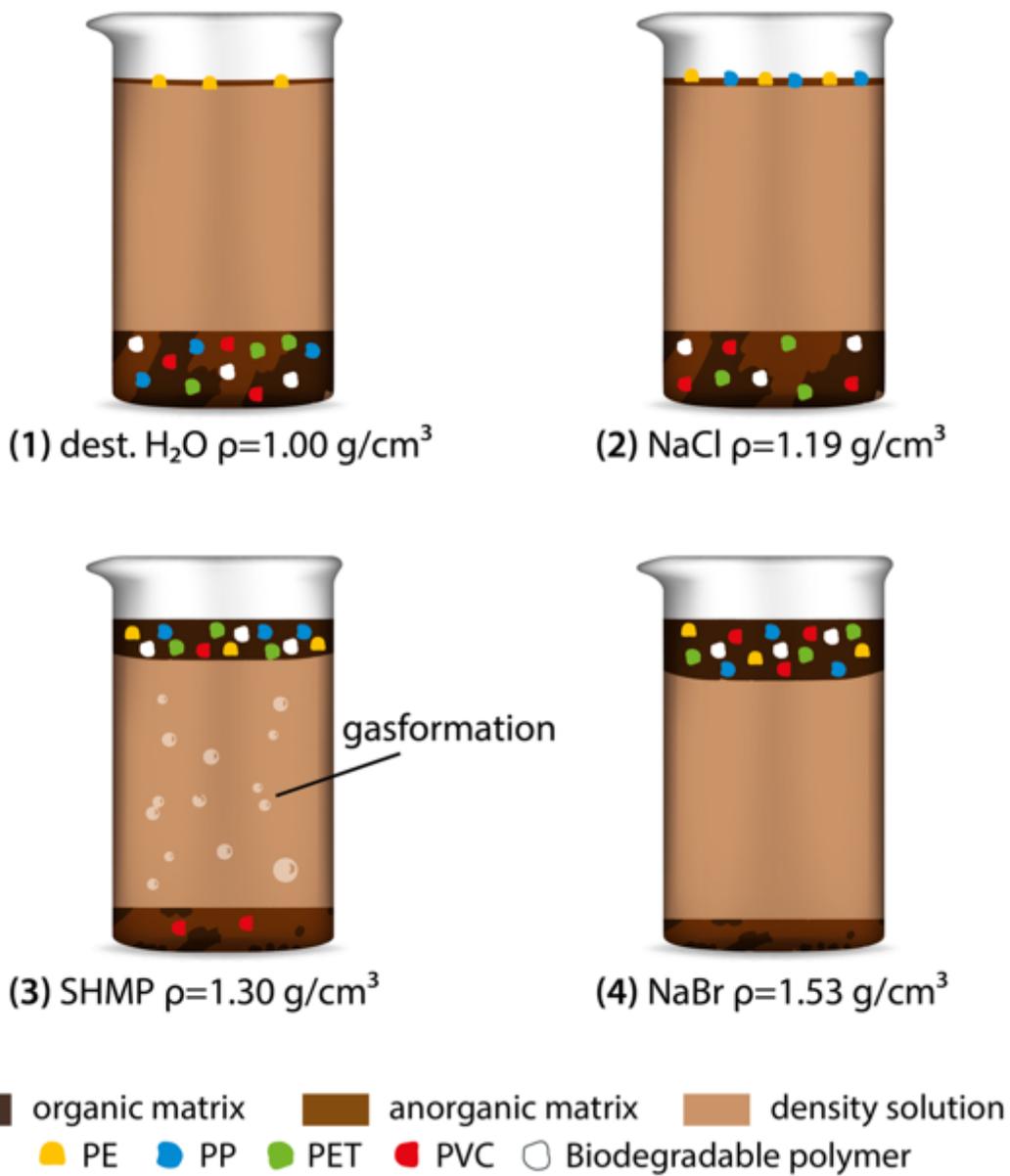


Figure 1

Graphical abstract

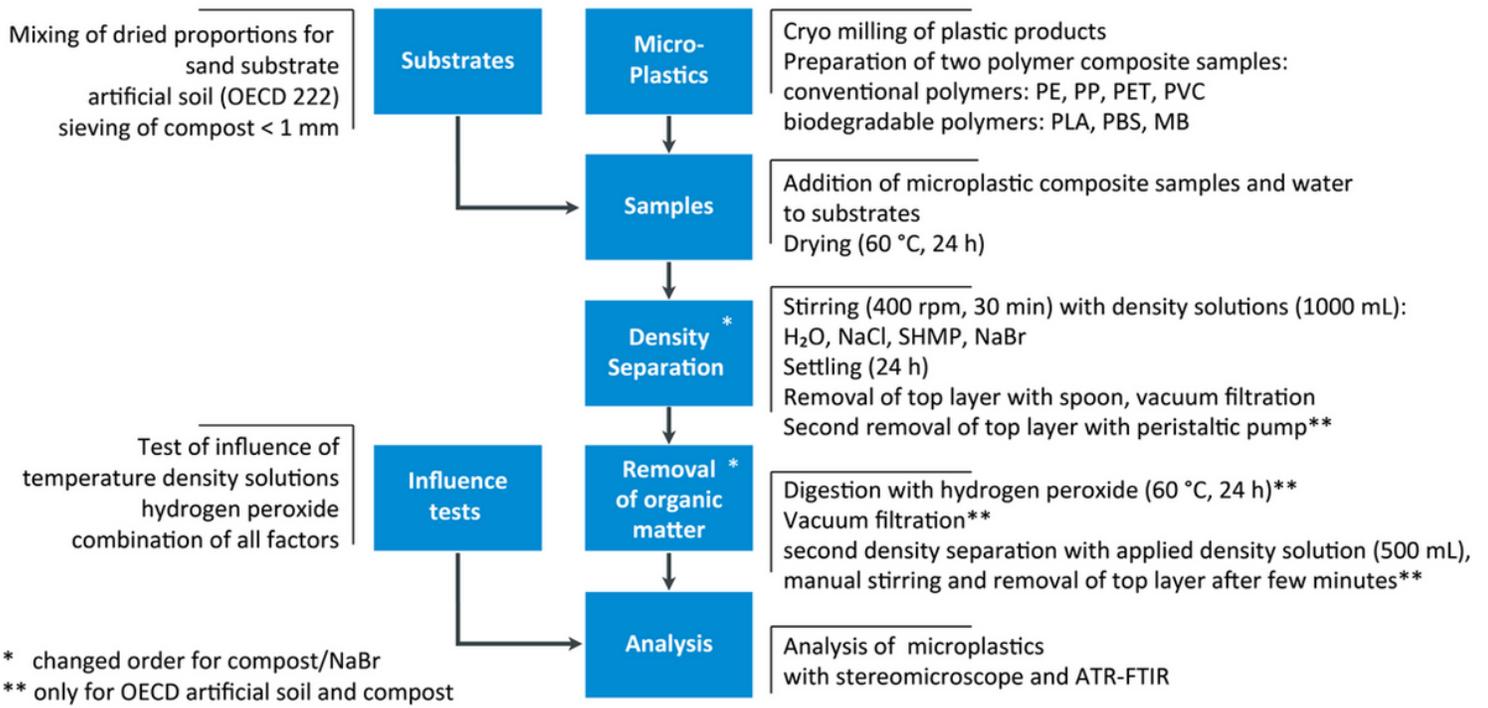


Figure 2

Flow chart of separation method

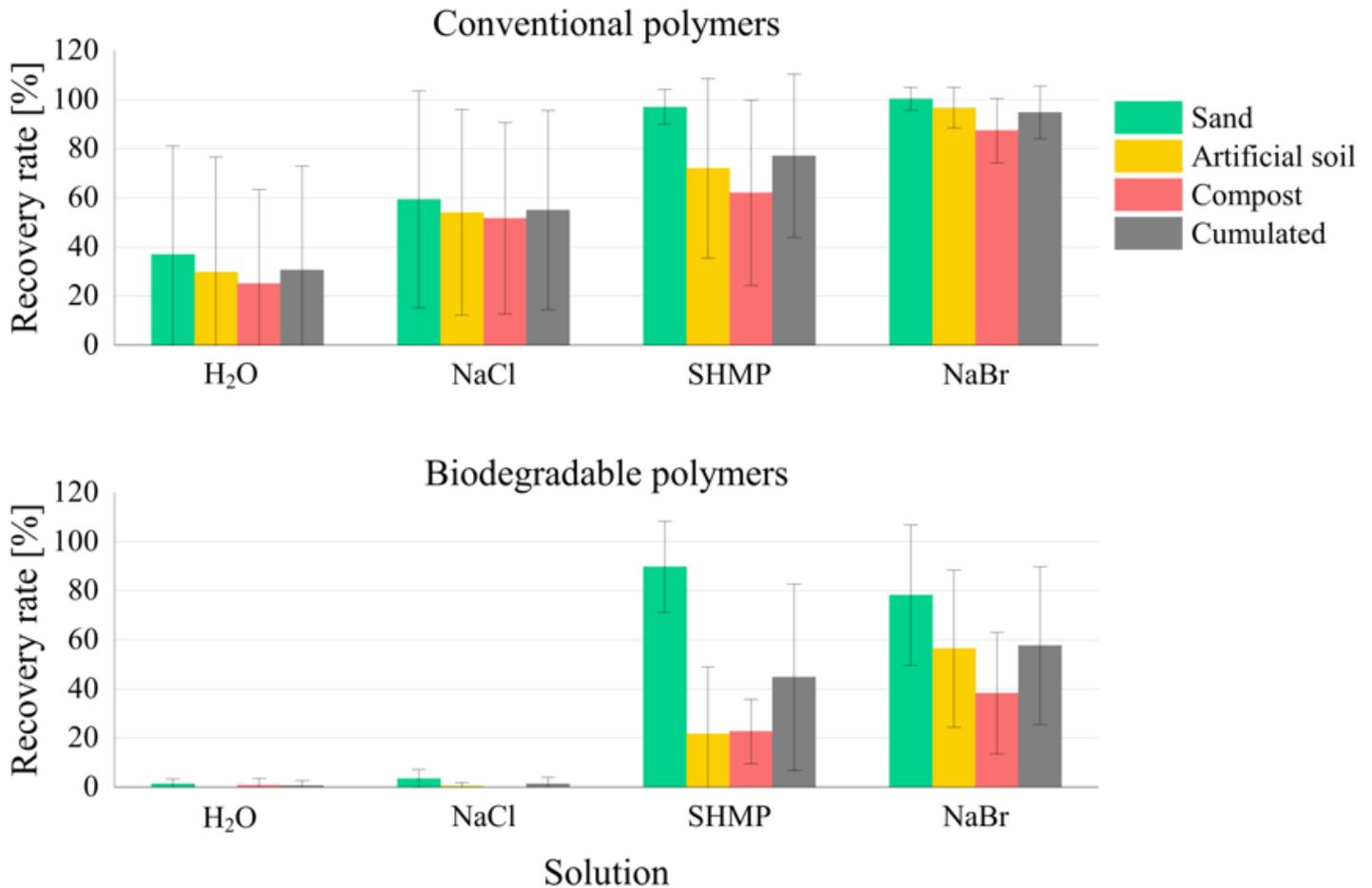


Figure 3

Recovery rates of conventional and biodegradable MPs depending on solution and substrate

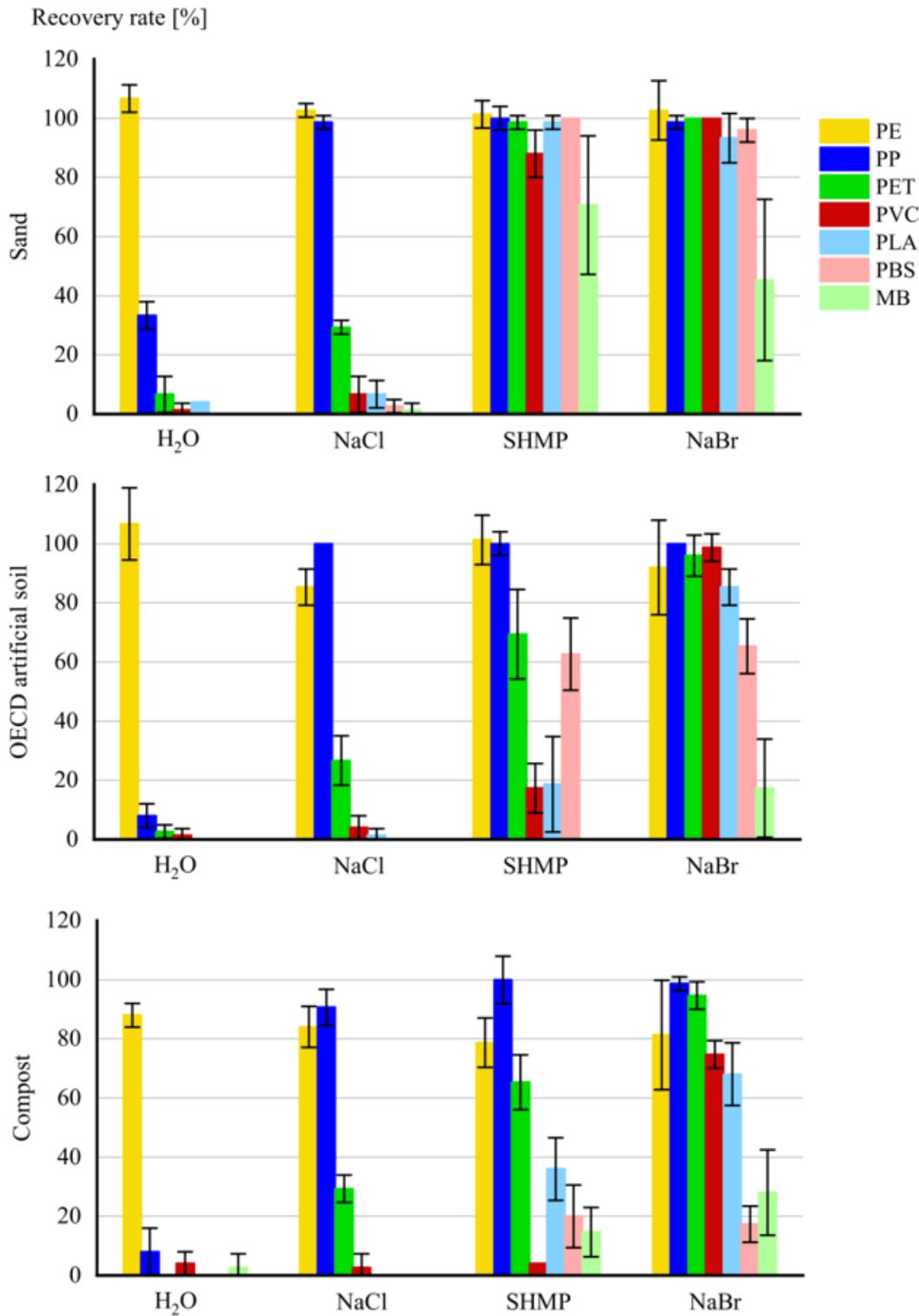


Figure 4

Recovery rates of MPs sorted to substrate and solution

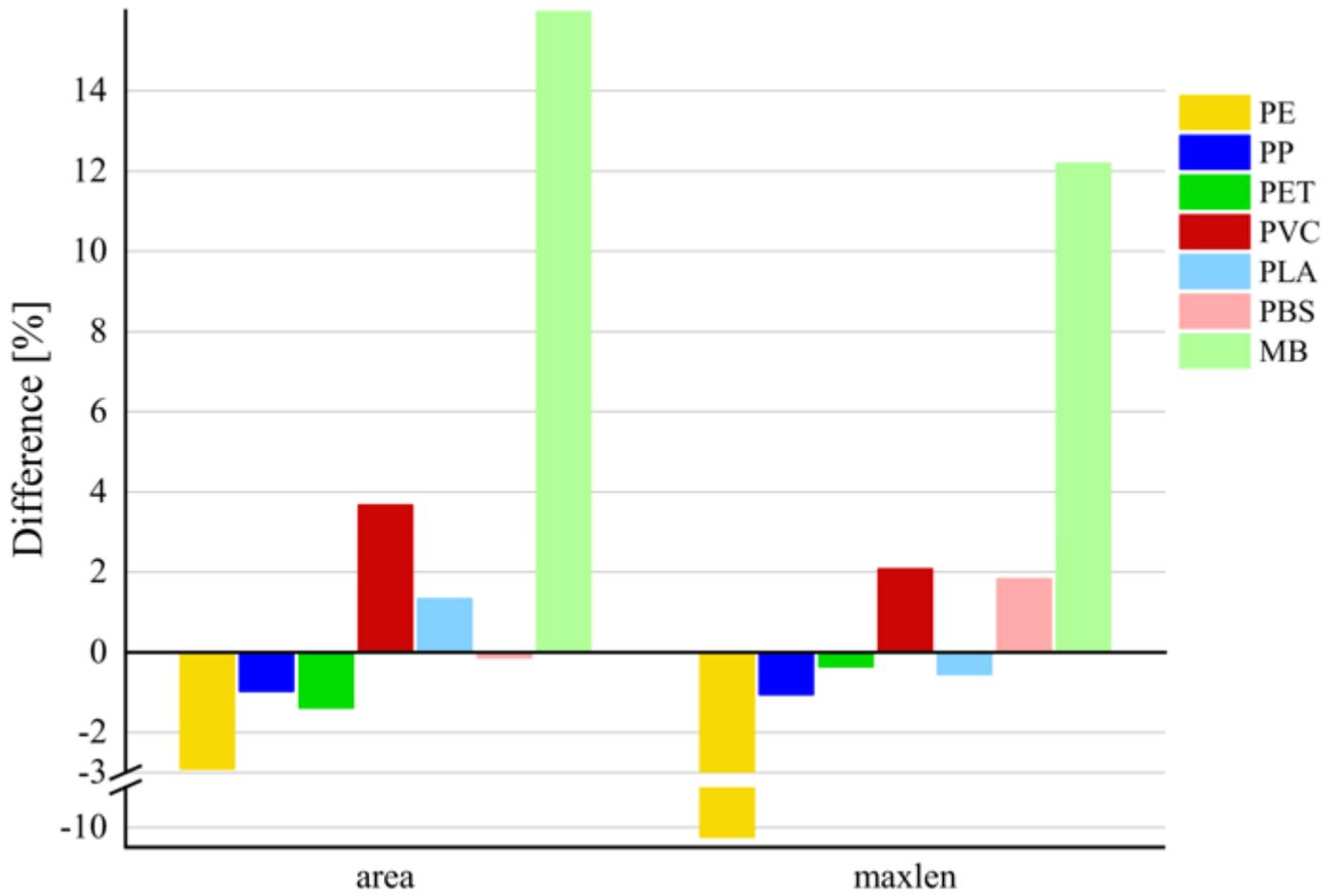


Figure 5

Decrease in size due to influence of H₂O₂ at 60 °C

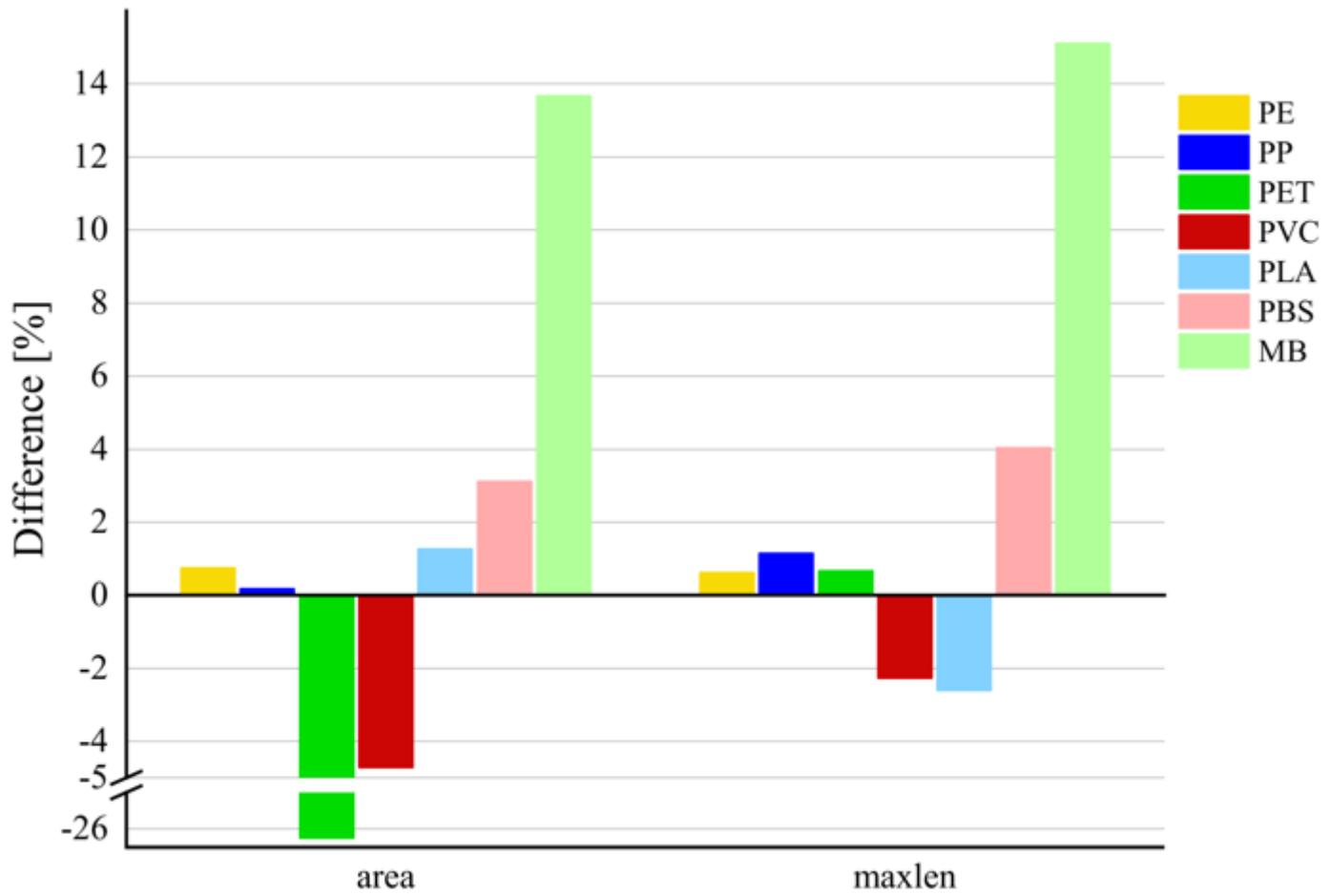


Figure 6

Decrease in size due to influence of combination of factors

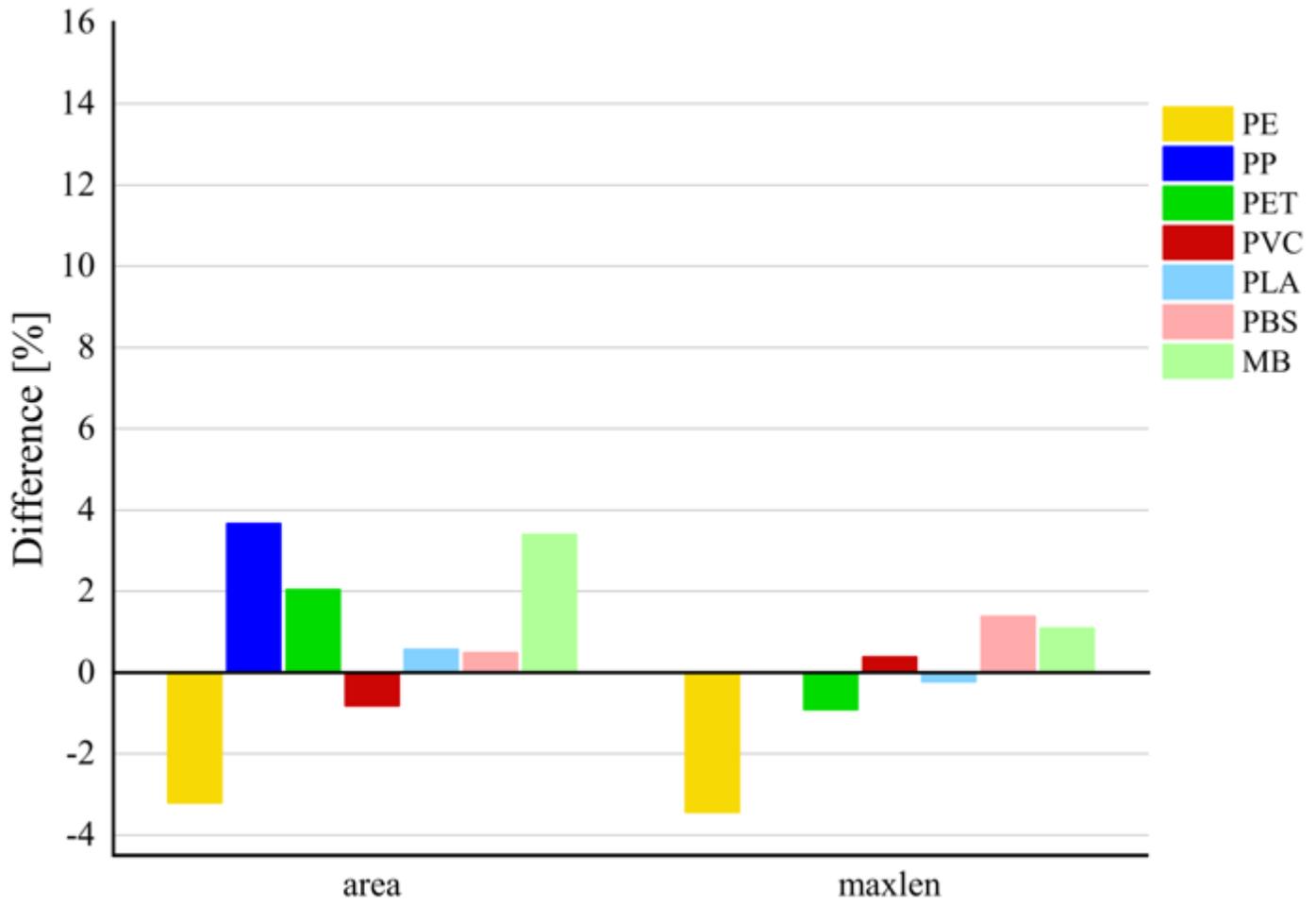


Figure 7

Decrease in size due to influence of H₂O₂ at RT

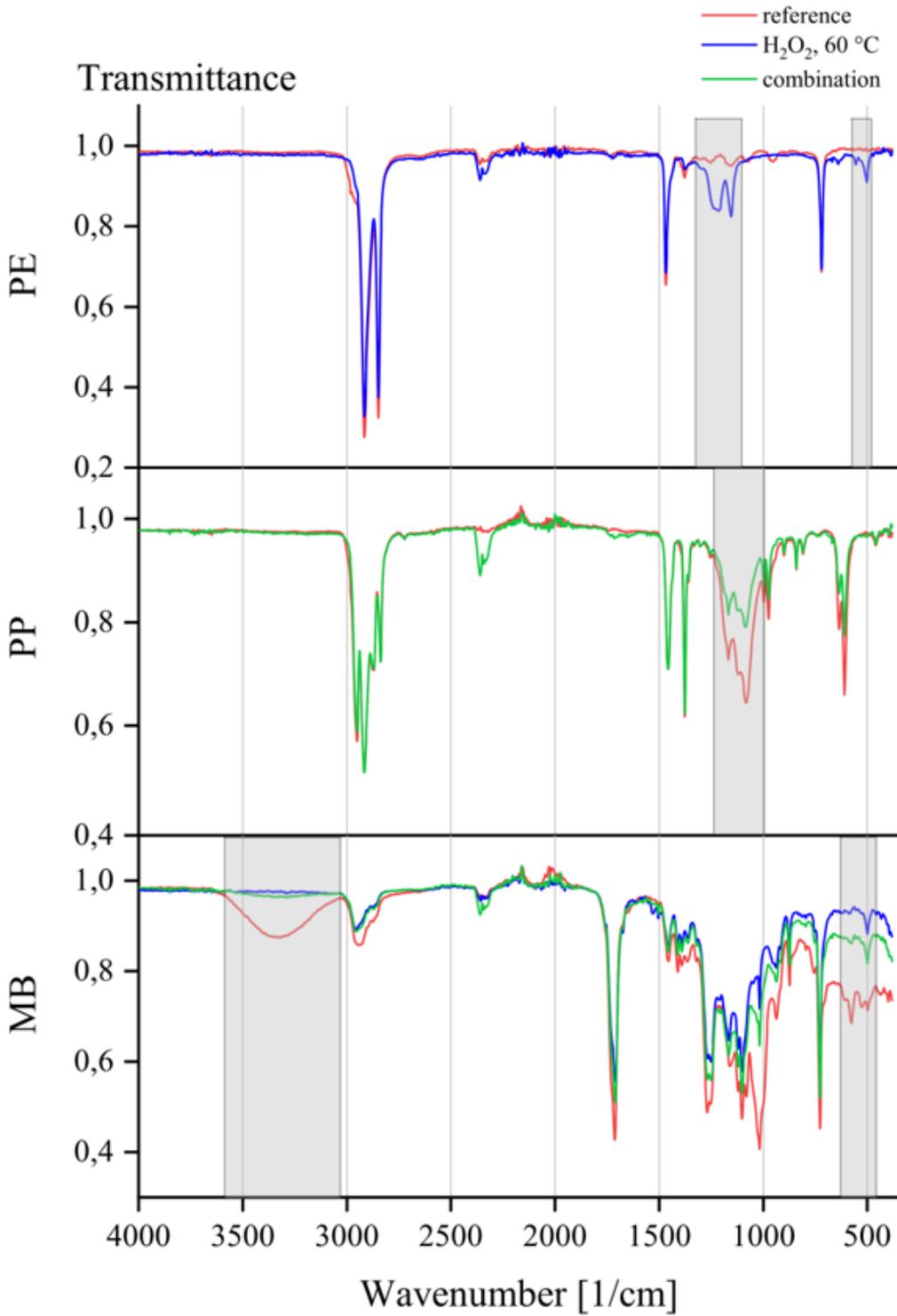


Figure 8

Comparison of IR-spectra of PE, PP and MB with Pearson's correlation coefficients > 0.98 compared to reference. Areas with visible differences are marked.