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Abstract

Microplastic particles have emerged as persistent pollutants in the environment, and biodegradable plastics represent a potential alternative to non-biodegradable plastics. However, standardized protocols for extracting microplastic particles from complex environmental matrices such as soil remain underdeveloped. This study aimed to develop and optimize an extraction method for microplastic particles in soil, focusing on polybutylene adipate-co-terephthalate, a biodegradable polymer. Four different extraction sequences were tested, and the experiments were conducted using three types of soil with differing compositions (LUFA 2.1, LUFA 2.2, and LUFA 5M) with four sample sizes (1g, 2g, 5g, and 10g). Fluorescent polybutylene adipate-co-terephthalate particles were added to each soil sample, and the recovery rate was quantified using particle counting under a Stemi SV 11 stereo microscope. The results showed that for each soil type, the sequence of organic matter removal followed by density separation in a funnel consistently provided the highest recovery rates, exceeding 80% in 5g samples. Centrifugation produced slightly higher recovery rates in less complex soil (LUFA 2.1), but exhibited greater variability in soil with higher clay and organic matter content (LUFA 2.2 and LUFA 5M). The optimized protocol, which involved organic matter removal followed by density separation in a funnel, was then applied to soil from an agricultural test site of the University of Natural Resources and Life Sciences in Vienna. This soil was spiked with five different types of polymers: polybutylene adipate-co-terephthalate, polylactic acid, polypropylene, low-density polyethylene, and polystyrene. The fluorescence dye used for polymer identification was 4-dimethylamino-4'-nitrostilbene, a fluorescent dye that produces distinct emission colors depending on the polymer type. The overall recovery rate across the five polymers was 80 ± 4%, with polylactic acid yielding the highest recovery at 93 ± 12%. These findings confirm that the composition of soil strongly influences the recovery efficiency of microplastic particles. The optimized protocol using a 5g sample size offers a reproducible and cost-effective method for extracting biodegradable plastics from soil and can be adapted for various polymer types and soil compositions.

Kurzfassung

Mikroplastikpartikel haben sich zu persistenten Schadstoffen in der Umwelt entwickelt, biologisch abbaubare Kunststoffe stellen eine potenzielle Alternative zu nicht biologisch abbaubaren Kunststoffen dar. Allerdings sind standardisierte Protokolle zur Extraktion von Mikroplastikpartikeln aus komplexen Umweltmatrizes wie Boden noch unterentwickelt. Ziel dieser Studie war es, eine Extraktionsmethode für Mikroplastikpartikel im Boden zu entwickeln und zu optimieren, wobei der Schwerpunkt auf Polybutylenadipat-co-terephthalat, einem biologisch abbaubaren Polymer, lag. Es wurden vier verschiedene Extraktionssequenzen getestet, und die Experimente wurden mit drei Arten von Böden unterschiedlicher Zusammensetzung (LUFA 2.1, LUFA 2.2 und 5M) mit vier Probengrößen (1g, 2g, 5g und 10g) durchgeführt. Jeder Bodenprobe wurden fluoreszierende Polybutylenadipat-co-terephthalat Partikel zugesetzt, die Rückgewinnungsrate wurde durch Partikelzählung unter einem Stemi SV 11 Stereomikroskop quantifiziert. Die Ergebnisse zeigten, dass für jeden Bodentyp die Entfernung organischer Stoffe gefolgt von einer Dichteseparation in einem Trichter durchweg die höchsten Rückgewinnungsraten ergab, die bei 5-g-Proben über 80 % lagen. Die Zentrifugation ergab etwas höhere Rückgewinnungsraten bei weniger komplexen Böden (LUFA 2.1), zeigte jedoch eine größere Variabilität bei Böden mit höherem Ton- und Organikgehalt (LUFA 2.2 und 5M). Das optimierte Protokoll, das die Entfernung organischer Stoffe gefolgt von einer Dichteseparation in einem Trichter umfasste, wurde dann auf eine landwirtschaftliche Bodenprobe angewendet, die an einem Teststandort der Universität entnommen worden war. Dieser Boden wurde mit fünf verschiedenen Polymertypen versetzt: Polybutylenadipat-co-terephthalat, Polymilchsäure, Polypropylen, Polyethylen niedriger Dichte und Polystyrol. Der zur Polymeridentifizierung verwendete Fluoreszenzfarbstoff war 4-Dimethylamino-4'-nitrostilben, ein Fluoreszenzfarbstoff, der je nach Polymertyp unterschiedliche Emissionsfarben erzeugt. Die Gesamtausbeute für die fünf Polymere betrug 80 ± 4%, wobei Polymilchsäure mit 93 ± 12% die höchste Ausbeute erzielte. Diese Ergebnisse bestätigen, dass die Zusammensetzung des Bodens einen starken Einfluss auf die Ausbeute von Mikroplastikpartikeln hat. Das optimierte Protokoll mit einer Probengröße von 5g bietet eine reproduzierbare und kostengünstige Methode zur Extraktion biologisch abbaubarer Kunststoffe aus dem Boden und kann an verschiedene Polymertypen und Bodenbeschaffenheiten angepasst werden.

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1 Introduction

Introduced in the 19th century, synthetic plastics have experienced steady growth in production and application due to their superior properties, such as durability, flexibility, and low production cost [1]. Global production reached approximately 2 million tons in 1950, and it is estimated to have grown to 400 million tons in 2022 [2]. Since the early 1950s, more than 9.1 billion tons of plastic have been produced, with only 9% recycled, 12% incinerated, and the remaining are still in use or have accumulated in landfills, dumpsites, or the natural environment [3]. The continued growth in plastic production, combined with its environmental persistence and the global lack of efficient recycling and waste management systems, highlights the unsustainability of the current plastic life cycle [2], [3], [4]. Plastic pollution is therefore recognized as a critical threat to the environment worldwide. In 2025, “Beat Plastic Pollution” was chosen as the main theme for World Environment Day.

Plastic can be classified into four categories based on their origin and degradability: (1) fossil-based, non-biodegradable; (2) fossil-based, biodegradable; (3) bio-based, non-biodegradable, and (4) bio-based, biodegradable [5]. Fossil-based, non-biodegradable plastics, commonly called conventional plastics, are currently the most produced plastics due to their low production cost, durability, and wide applications. However, their resistance to biodegradability makes them persistent environmental pollutants. Importantly, the assumption that bio-based polymers can also be biodegradable is misleading, highlighting the need for more precise categorization and communication towards the public.

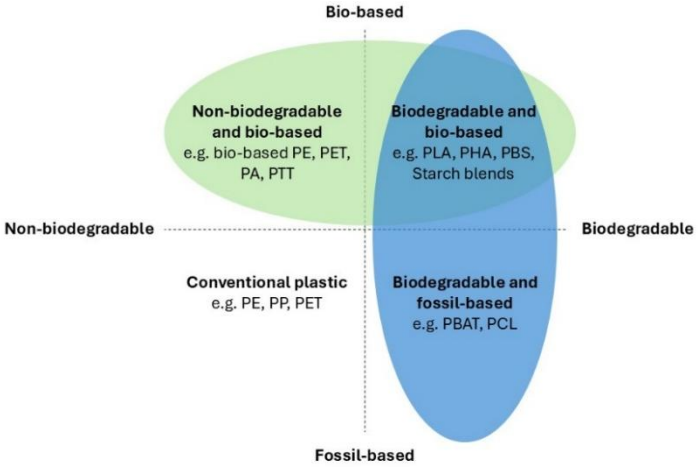

Figure 1.1: Classification of plastics based on their origin and biodegradability.

Disregarding their type, plastic waste will eventually fragment into microplastics (MPs) due to weathering and abrasion. While biodegradable plastic can eventually decompose under suitable conditions, non-biodegradable plastic will persist in the environment without complete degradation. These MPs could enter terrestrial environments via multiple pathways and can affect the soil ecosystems, flora, fauna, and potentially humans [6]. Most of this research is preliminary; the current understanding of MPs concentration and degradation ability in soil systems remains limited. Studies indicated that once taken up by the plants, MPs can accumulate in the roots and most likely block cell connections or cell wall pores for the transport of nutrients [7]. MPs can act as intermediaries that transmit bacteria, viruses, proteins, and xenobiotics that can be infused into plants, leading to a high risk of bioaccumulation and the potential to enter the food chain [6].

The lack of complete comprehension of MPs' threats highlights not only the need for a technical solution, but also includes system frameworks such as the circular economy. The concept of the circular economy is gaining popularity in response to the pressing problem of plastic pollution. This conceptual framework aims to minimize resource consumption by promoting reuse, recycling, and extending the life cycle of products for as long as possible [8]. For plastic material, it means increasing durability for long-term use. However, while durability benefits applications such as infrastructure and construction materials, single-use plastic can also become a significant problem, especially in recycling processes, where the purity of plastic becomes a critical issue. It is challenging to obtain high quality plastic from recycling because it requires the separation of different types of plastics and then the removal of contaminants, such as additives and pigments. That is why improving plastic quality, controlling the amount of plastic waste generated, and improving recycling technology must also be focused on. Biodegradable plastics offer a potential solution, as they have similar properties to fossil-based, non-biodegradable plastics and reduce plastic residues in nature by degrading instead of being persistent.

Another aspect of the circular economy is the aim to up-cycling organic waste into compost to use it in agriculture [9]. However, a concern is that the generated compost may contain MPs, due to incomplete removal during treatment. Given that, when compost is applied to the farmland, MPs can be uptaken by crops and, through the food chain, eventually enter the human body. Therefore, although the circular economy opens many opportunities for technological innovation to reduce plastic pollution, it may increase the input of MPs into terrestrial systems [10], [11]. Alongside compost, another alternative is wastewater treatment plant (WWTP) sludge, but its use is in the process of becoming restricted or banned worldwide due to environmental and health concerns. In the US, Maine became

the first state to ban the use of sewage sludge in farming [12], [13]. In Germany, from 2029, sludge from WWTP serving more than 100,000 population equivalents for agriculture will be banned [13]. WWTP sludge is not only subject to bans on its direct application to agricultural land but also restricted in composting processes. As restrictions on sludge applications expand, organic waste compost is expected to receive greater attention.

MPs have been detected in a wide range of environmental matrices, and various methods have been developed for extraction and analysis. While simpler matrices like ocean sediment do not require a complex procedure, high organic matter (OM) matrices such as compost or soil require a more elaborate extraction procedure to ensure high recovery rates and analytical clarity. Despite the wide recognition of MPs in soil, the information about their concentration, distribution, and degradability is still limited. For this reason, a homogenized methodology for the extraction from soil is needed. Allowing us to better study and understand the impacts of MPs on terrestrial systems. This thesis aims to fill the current gap, building and further optimizing the process of extracting MPs from soil.

2 State of knowledge

2.1 Plastic and Microplastic

Plastics are synthetic polymers commonly produced from processed natural materials [14]. The primary sources for fossil-based, non-biodegradable plastics are petroleum, flammable gases, and coal, making their production unsustainable in the long run.

2.1.1 Microplastics

One of the most concerning problems related to plastic contamination is the eventual presence of the so-called MPs in the environment. They are defined as plastic particles in the size range from 1 µm to 5000 µm, which can be directly released into the environment from products (primary) or generated by the fragmentation of larger pieces (secondary) [1]. To address the issue of MPs' contamination in natural environments, there is a need for innovative solutions, from mitigation to removal approaches.

MPs are released into the environment through various pathways, with plastic products, including bags, bottles, and electronics, being one of the main sources. These plastic products are exposed to various weathering processes and mechanical forces, causing their fragmentation and generating MPs. In addition, the friction between tire and road surface also releases tire wear, a sort of MPs, accumulating in road dust and urban runoff. For instance, according to a study by Järlskog et al. (2021) in Gothenburg, Sweden, MP concentrations are 1500 MPs/L in stormwater, 51,000 MPs/L in wastewater from road

cleaning, and 2.6 × 10^6 MPs/kg in sweepsand. During and after the COVID-19 pandemic, the medical industry showed increased plastic waste since the demand for medical products such as masks, gloves, and disinfectant solutions increased sharply. A new mask alone has been reported to release 223 ± 98.79 MPs/piece, these values are concerning, given the amount of consumption during that period. An indirect source includes household activities such as laundry, cooking, and the use of personal care products. MPs from these activities have been detected at WWTP inlet. Moreover, waste management facilities could also introduce MPs into the environment through incomplete waste treatment processes. As a result, MPs could be released back into the environment. Agricultural activities like using plastic films as mulch, fertilizers containing plastic are another source of MPs that enter the environment. These plastic materials can be introduced deeper through plowing, plant root extension, animal activities like bioturbation by earthworms, or infiltration by irrigation [6], [15], [16].

Although the effects of MPs on humans are not yet fully understood, one thing that has been studied is that MPs are present in soil environments [9], [15], and MPs have also been found in trees and living organisms [17], [18].

2.1.2 PBAT as an example for biodegradable plastics

A potential approach to reducing the release of MPs is the development of biodegradable plastics. While these materials can still fragment into smaller pieces similar in appearance to those from non-biodegradable plastics, their environmental fate differs under suitable conditions. It has been shown that they can break down completely into carbon dioxide, water, and biomass, rather than persisting for decades or centuries [19].

Biodegradable plastics are often designed to achieve this breakdown in controlled conditions, such as industrial composting facilities, where temperature and humidity are controlled and support the degradation. They can be derived from renewable resources (bio-based plastics) like corn, sugarcane, or produced from petroleum-based sources with chemical structures tailored for biodegradation. Unlike traditional plastics, their complete degradation minimizes long-term accumulation and offers a more sustainable alternative in addressing plastic pollution.

Polybutylene adipate-co-terephthalate (PBAT) is extracted from common petrochemicals and obtained by condensation of butanediol (BDO), adipic acid (AA), and terephthalic acid (PTA). The synthesis of PBAT begins with an esterification reaction between BDO and acids (PTA and AA) to create short polymer chains of oligomers. Then, in the polycondensation step, the reaction is usually carried out at a temperature above 190 °C under vacuum conditions, during which PBAT is formed. These conditions are necessary to facilitate condensation reactions and the removal of lighter molecules (water) in the form of products [20]. In Ratshoshi et al. (2024) research, two scenarios of using sugarcane biorefinery are: (1) producing PBAT by creating BDO, AA, and PTA from molasses, and (2) producing only BDO. The study demonstrated that bio-based PBAT can be produced from molasses in an integrated sugar plant at a cost comparable to fossil-based PBAT. However, in terms of investment risk, BDO is a more practical option in the short term [21].

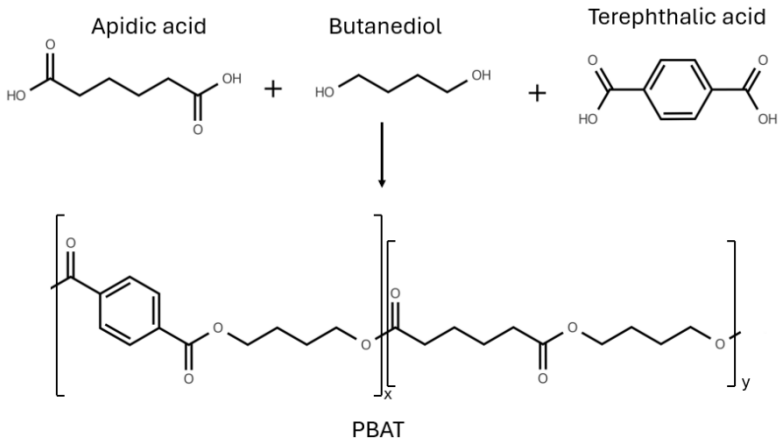

Figure 2.1: Synthesis and chemical structure of PBAT (created with Moldraw)

PBAT has similar physical properties to commonly used polymers, and in addition, is biodegradable due to its ester link. The ester groups in its structure make it easier to biodegrade through hydrolysis, usually by extracellular enzymes. Thanks to the aromatic ring in its structure, PBAT also has notable mechanical properties. Its tensile strength is 21 MPa, the elongation at break is 670%, the flexural strength is 7.5 MPa, and the flexural modulus is 126 MPa [20]. While its tensile strength is a bit lower than polypropylene (PP) (26 – 41.4 MPa), its elongation at break is significantly high, almost reaching the maximum of PP (15 – 700%) [22]. These properties help prove that PBAT is a flexible, durable, and strong polymer, easy to handle by conventional manufacturing methods such as extrusion, blowing film, and injection molding to create environmentally friendly products [20].

PBAT can be degraded under industrial composting conditions, and this is mainly caused by microbial degradation and hydrolysis [23]. Aliphatic segment promotes the biodegradability of microbial activities, and the ester part helps PBAT biodegrade through

hydrolysis [23]. Yue Wang et al. (2025) identified three phases in the degradation pathway of PBAT MPs in soil [24]. The initial phase is microbial colonization and structural fragmentation, shortly after burial. Next is the critical release phase, when fragmentation via enzymatic hydrolysis is accelerated. During this stage, PBAT MPs are the most abundant. Enzymatic hydrolysis has become a dominant mechanism in this step. Finally, it is the critical degradation phase. At this phase, the abundance of MPs decreases as the polymer is broken down. Q. Deshoulles et al. (2022) demonstrate that hydrolysis of PBAT occurs on its ester groups. Since PBAT has three distinct ester groups on its structure, hydrolysis can potentially occur at three different positions [25]. After experiments, it is clarified that hydrolysis initially occurs on the ester group located between the terephthalate and adipate groups. Once this is broken down, it is followed by the ester situated within the adipate group [25]. Microbial activity leads to the mineralization of PBAT into CO_2 and water. People usually blend PBAT and polylactic acid (PLA) since PLA degradation products could promote the hydrolysis of PBAT [26].

Introducing products made from PBAT is a statement of effort to reduce contamination caused by non-biodegradable plastic waste. Currently, PBAT is used to produce products such as packaging, and these products are intended to be biodegradable. In China, the production of PBAT in 2020 was 150,000 tons and reached 1.143 million tons in 2022 [27], [28]. Another application for PBAT is mulching film. While polyethylene (PE) film must be collected and disposed of after use, biodegradable plastic like PBAT can be plowed directly into soil. Another potential market for PBAT is single-use, biodegradable cutlery, which can be disposed of along with organic waste through an industrial composting process. It is thriving in places such as Europe, where regulations restricting the use of single-use plastic have been issued [27].

2.2 Soil

Industrial composting is an established process that transforms organic waste into stable products to be used in agricultural soil. This study focuses on soil, especially agricultural soil, because it is directly affected by compost from organic waste and sludge. These fertilizers are then introduced into the soil to provide nutrients for the crops, and from there, crops are consumed by humans and animals. It is estimated in Gui et al. (2021) that the amount of MPs introduced to agricultural land using domestic waste compost products can reach $1.43 \times 10^7 – 1.93 \times 10^8$ MPs/ha/year, with the dominant particle size ranging from 0.05 to 0.5 mm [29].

The experiment by Castan et al. (2021) found that MPs did not significantly increase the mobility of organic contaminants. The reasons given for this are, first and foremost, that the desorption of most organic pollutants is too fast for MPs to transport contaminants in slow flow and fast flow conditions. The second reason is that MPs themselves, although it is said that MPs are ubiquitous, have very limited ability to move in the soil, especially when MP particles are larger in size than the soil pore diameters. Therefore, soil is also considered an MP filter because of its ability to hold and retain particles. Additionally, the sorption coefficient of bulk soil and soil OM is higher than that of most plastics. This makes it harder for MP to compete and take up organic contaminants in soil, which means they cannot act as a vector for contaminants [32].

2.2.1 Types of soil used in the study

This study selected three soil types to evaluate MPs' recovery rate: LUFA 2.1, LUFA 2.2, and 5M soil. They have different compositions, and these variations allow assessment of the protocol's performance under diverse conditions. Table 2.1 shows the basic properties of the three soil types [33], [34]. Each component interacts with MPs differently, creating a complex environment that affects extraction efficiency.

Table 2.1: Basic properties of LUFA 2.1, LUFA 2.2, and LUFA 5M soil

	Soil type	**pH**	**Sand (%)**	**Silt (%)**	**Clay (%)**	**OM (%)**
LUFA 2.1	Loamy sand	4.7 ± 0.1	86.1 ± 0.7	10.2 ± 1.3	3.7 ± 0.8	0.83 ± 0.29
LUFA 2.2	Sandy loam	5.6 ± 0.4	78.3 ± 1.0	13.7 ± 1.0	8.0 ± 1.5	1.81 ± 0.44
LUFA 5M	Sandy loam	7.3 ± 0.1	53.5 ± 3.2	35.3 ± 2.3	11.1 ± 0.7	1.09 ± 0.25

2.2.2 Plastic and microplastics in soil

The two techniques commonly used to detect MP particles can be grouped into number-based and mass-based. The number-based method is often used due to the simpler analyzer steps and calculations. The concentration expressed as the number of particles can recognize the size distribution of MPs and provide more information when studying the movement of MPs. However, the number-based method may become inaccurate over time as one MP can fragment into multiple MPs, even though the total mass is unchanged, and this can lead to an overestimation of particle abundance. In addition, while many dissolved chemical pollutants are expressed by volume or mass, the concentration expressed in number-based does not indicate an association between MPs and pollutants. Therefore, converting the concentration from number to mass will help clarify the flow/behavior of MPs in different environments, whether it has been degraded or added, instead of only reflecting the change in number due to fragmentation [35], [36], [37].

The mass-based concentration tends to be more influenced by larger MP particles due to their greater contribution to the total mass, whereas for smaller particles, unless present in high quantities, contribute less to the total mass. In contrast, number-based approaches are more sensitive to smaller particles, as each particle is counted equally regardless of its size. It is difficult to convert from number-based to mass-based concentration since it requires information about the number of MP particles, their shape, size, and density, and it is impractical when dealing with a large amount of particles. Reporting concentrations by both number-based and mass-based will increase comparability between toxicity and occurrence studies. The use of inappropriate concentration figures can hide or intensify certain types of toxicity, causing it to be underestimated or overestimated. Therefore, it is recommended to collect as much relevant data as possible (mass, number, volume, size, shapes, etc.), and studies on MPs should report both types of number-based and mass-based concentrations for easier comparison and reference [35], [36], [37].

Many studies have found MPs in agricultural soil [38], [39], [40]. The sources of introducing MPs into the soil are very variable. Still, the primary source of MPs in the soil, especially agricultural soil, is from the use of plastic mulch [40] and fertilizers [41]. Table 2.2 summarizes the reported concentration of MPs in soil and compost in several studies. As most of the studies did not provide both number and mass reports, an approximation was made to compare better. Table S2 in the supplementary section provides the calculation from number-based to mass-based concentration. Additionally, both the number of MPs and mass of MPs across all size ranges tend to be higher in compost compared to soil. This observation supports the idea that compost may act as a source of MPs in agricultural soil. Over time, MPs introduced into soil through compost can be transported vertically or horizontally by agricultural activities and natural processes, potentially causing widespread distribution within the soil profile.

Table 2.2: Information on sample collection of the published studies

	Size (µm)	**MPs conc. (MPs/kg)**	**MPs conc. (mg/kg) estimated**	**Ref.**
Soil	10 – 2000	30500 ± 13400 – 74900 ± 59100	19.16 – 47.06	[39]
	>20	136.7 ± 41.7 – 256.7 ± 62.2	43.1 ± 13.2 – 498.8 ± 120.9	[38]
	>30	888 ± 500 – 2242 ± 984	583.43 ± 328.50 – 1473.03 ± 646.50	[40]
	>500	0 – 11	0 – 178.90	[10]
Compost	>20	191 – 6400	410.98 – 60243.20	[18]
	>30	1253 ± 561 - 2800 ± 616	823.24 ± 368.58 – 1839.65 ± 404.72	[40]
	>500	14 – 895	227.70 – 14556.25	[10]

Not only do MPs pose risks of containing harmful substances that plants might take up, but MPs also have an impact on soil health. Large quantities of MPs can block soil pores, reducing the infiltration capability for rain and irrigation water and negatively impacting the soil's water-holding capacity. MPs act as vectors for various pollutants due to their high specific surface area and hydrophobicity, adsorbing toxic chemicals, leading to complex combined toxicological effects, and facilitating the mobility and bioavailability of contaminants in soil [42]. In addition, MPs can reduce bacterial populations and alter their community structure, and changes in soil porosity and moisture can lead to microhabitat loss for indigenous microorganisms [43]. On the other hand, MPs can also serve as an ecological habitat for soil microorganisms, forming what is sometimes called the "plastisphere", and some bacteria are even capable of degrading MPs, using the plastic as a carbon source [42]. It is important to note that the overall impact of MPs on soil is subject to great variability and requires more in-depth research to fully understand whether these impacts are positive or negative.

2.3 Microplastic extraction methods

Given what MPs can do to soils, crops, and even humans, understanding MPs' presence, degradation, and transport in soil is essential. And to do that, we need to extract MPs from the soil for subsequent analysis. Currently, there is no standardized method for quantifying plastic in soil [44].

Developing an effective extraction protocol for biodegradable plastics is essential to ensure accurate quantification and analysis across a wide range of polymers in complex environmental matrices. Among biodegradable polymers, PBAT is increasingly used in environmentally friendly products. If a method can extract PBAT, it is possibly applicable to other polymers, since PBAT has several challenging characteristics. PBAT contains ester bonds that are easily hydrolyzed and oxidized, making it prone to degradation when exposed to •OH generated during the organic matter removal (OMR) step of the extraction process. Additionally, PBAT has a relatively high density, approximately 1.25 g/cm³ [45], which means that if a separation procedure can recover PBAT, it likely has the potential to recover polymers with similar or lower density like PP (d = 0.895 – 0.920 g/cm^3), Low-density polyethylene (LDPE) (d = 0.915 – 0.935 g/cm^3), polystyrene (PS) (d = 1.040 – 1.050 g/cm^3), PLA (d = 1.24 g/cm^3).

Many studies have come up with different methods for extracting MPs from various types of matrices (Table S1 summarizes them). However, to be able to widely apply the formula to complex environments such as soils, especially soils with high concentrations of OM,

such as agricultural soil, it is necessary to have a method that both produces a reasonable recovery rate, can be applied at large scales.

2.3.1 Density separation

Density separation (DS) is one of the most commonly used methods for extracting MPs from environmental matrices. The principle of DS is based on differences in density between MPs and other soil components. A key requirement for effective DS solutions is the high solubility of the salt in water, and these aqueous salt solutions are used to create liquids with densities higher than water. Many solutions are used for DS, such as of NaI, which has a density of up to 1.8 g/cm^3, but the cost of NaI is very high and inefficient to apply to large-scale and long-term research. Another solution that has also received a lot of attention and study due to its high density is zinc chloride ($ZnCl_2$), with a solution density of d = 1.6 g/cm^3. Zhang et al. (2024) have studied the extract of PP, PE, PS, and PET and found that the recovery rate of these four polymers is >90% [18]. However, $ZnCl_2$ is hazardous and corrosive [46]. NaCl solution with a density of 1.2 g/m3 has also been studied for DS; still, it is insufficient for the recovery of PBAT, which has a higher density [44], [47]. $CaCl_2$ solution with a density of 1.4 g/cm^3 is a solution that satisfies all requirements, such as having a high enough density to separate common polymers, being safe to operate without affecting MPs, and being cost-effective [39], [47]. For these reasons, $CaCl_2$ is the separation solution used in this study. Polyvinyl chloride (PVC) and other high density polymers were excluded as they are incompatible with $CaCl_2$, and PVC is not commonly analyzed in MPs studies. This is because PVC is mainly produced for long term applications such as pipes and construction materials, which are designed for durability [48]. Therefore, the presence of PVC in soil is expected to be minimal.

Regarding the exact technique used for DS, there are two standard methods: funnel-based and centrifugation. The funnel-based DS (DF) method is one of the most used techniques for extracting MPs. It is simple to conduct and inexpensive. However, it requires a long waiting time, typically overnight or at least 24 hours, for high-density substances to fully settle. The process depends only on gravity to separate materials. The floating MPs can then be collected and analyzed [47], [49]. Another technique that has been gaining attention in recent research is centrifugation (DC). This technique makes it easy to separate MPs within 5 to 10 minutes. Thanks to centrifugal force, DS becomes more efficient, especially for small particles that are not MPs and may take too long to settle or may not settle at all using funnel-based methods. However, while centrifugation is faster, it may offer less precise separation compared to the slower, funnel-based technique. Another common concern is that most centrifuge tubes are plastic-based. Thus, the generated friction

between the particles and the tube walls can lead to particle release and contamination of the sample by coming from the tube itself [19], [39].

2.3.2 Fenton reaction

Fenton and Fenton-like reactions have emerged as potential candidates for removing OM from complex matrices such as compost, soil, sludge, and wastewater [50]. The Fenton reaction, which uses ferrous ions (Fe^{2+}) and hydrogen peroxide (H_2O_2) under acidic conditions, generates reactive oxygen species (ROS). The Fenton reaction is cyclic, and the reactions that take place during it are shown below [51]:

$$Fe^{2+} + 2H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH^- \quad (1)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2\bullet + H^+ \quad (2)$$

There are two main types of Fenton reaction: classical Fenton and modified Fenton. Classical Fenton involves the activation of hydrogen peroxide (H_2O_2) by ferrous (Fe^{2+}) ions to produce ROS [51]. Modified Fenton reactions use external energy sources to accelerate catalytic reactions. In this category, we find Photo-Fenton, Electro-Fenton, and Sono-Fenton, among others. These types of Fenton reactions are developed to improve oxidation efficiency. In the Photo-Fenton process, a solution of Fe^{2+} and H_2O_2 is exposed to ultraviolet (UV) radiation, which increases the formation of HO• through photo-reduction of Fe^{3+} to Fe^{2+} and H_2O_2 photolysis [51]. The Electro-Fenton method improves the Fenton process's efficiency by continually producing H_2O_2 in situ at the cathode by electrochemical reduction of O_2. The Sono Fenton method uses ultrasonic dissociation of water and molecular oxygen to enhance the generation of HO• and generate H_2O_2 in situ [51]. The classical Fenton was selected in this study due to its proven efficiency, being less aggressive, does not affect PBAT particles, and relatively simpler setup.

2.3.3 Sequences

In the process of extracting MPs from soil, the order of the processing steps can influence the efficiency of the extraction and analysis. The two most common orders are Organic matter removal before density separation (OMR–DS) and density separation before organic matter removal (DS–OMR).

Since the density of OM in soil typically ranges from 1.10 to 1.50 g/cm³ [52], similar to certain types of plastic, the removal of organic compounds is essential for the DS step to be successful. In the case of a complex matrix like soil, especially agricultural soil, this is even more important because the OMR step not only removes organic compounds but can also weaken the interactions between organic compounds and MPs, thereby making the DS step easier when MPs are not trapped between soil particles. Chandrakanthan et al.

(2024) and Ruffell et al. (2024) employed a 0.05 M Fe(II) solution in conjunction with 30% H_2O_2 to extract MPs from their samples [49], [53]. Following the extraction, they separated the samples based on density using NaI, to be able to extract PVC [54]. Previous studies by Chandrakanthan et al. and Ruffell et al. have reported recovery rates exceeding 90% across all matrices [49], [53]. Furthermore, Wohlleben et al. (2023) utilized the Fenton reaction for OMR, while applying $CaCl_2$ for DS, achieving a recovery rate of 78.2 ± 3.5% [47].

DS is typically conducted before OMR when the sample matrix has low organic content and is predominantly composed of sand. Sand particles exhibit minimal interaction with MPs, facilitating efficient separation during density-based extraction. In contrast, matrices rich in clay or silt present stronger physicochemical interactions with MPs, which can hinder their separation unless OM and fine particles are removed beforehand. Prosenc et al. (2021) applied the DS–OMR sequence to soils with a separation solution of $ZnCl_2$, which yielded a recovery efficiency of more than 93.3 ± 5.2% for both soil and compost [41]. Meanwhile, Kononov et al. (2022) also used the same sequence with the separation solution of Canola oil and unsaturated NaCl and have an efficiency of 95.2 – 98.3% for LDPE, 95.2 – 98.7% for PP, and 76.0 – 80.5% for PVC [55].

The following abbreviations are used to describe the methods used in this research. OMR-DF refers to organic matter removal followed by density separation using funnels, OMR-DC refers to organic matter removal followed by density separation using centrifugation, DF-OMR refers to density separation using funnels followed by organic matter removal, and DC-OMR refers to density separation using centrifugation followed by organic matter removal.

3 Aims and Hypotheses

Hypothesis one

OMR before DS will increase the MP recovery rate in high organic carbon soil. This is expected because pre-treatment with OM removal minimizes the interference from organic material, soil particles with MPs, thereby reducing microplastic losses during separation.

Hypothesis two

Centrifugation will have a higher recovery rate of MPs than funnel-based separation due to its more efficient density separation.

Hypothesis three

The extraction protocol will perform differently across soil types. Sandy soils are expected to yield higher recovery rates and reproducibility due to lower organic content and weaker microplastic binding, while higher clay-content soils will show lower recovery rates due to strong binding interactions.

Hypothesis four

Sample size significantly affects microplastic quantification's representativeness and statistical reliability in soil samples. Larger sample sizes are anticipated to provide more representative data due to the heterogeneous distribution of MPs in soil.

To test all four hypotheses, a recovery experiment was performed using four extraction methods applied to three soil types (LUFA 2.1, LUFA 2.2, and 5M) with four different sample sizes (1g, 2g, 5g, and 10g), each conducted in triplicate. A known amount of fluorescent PBAT was spiked into the samples and subjected to the different extraction methods. MP particle loss at each step of the extraction process was monitored to quantify the total loss and to determine at which specific steps the losses primarily occurred.

4 Materials and Methods

4.1 Materials

Iron(II) sulfate heptahydrate (99%) (CAS No.:7782-63-0), hydrogen peroxide (H_2O_2) solution (30 wt.%), and 4-dimethylamino-4'-nitrostilbene (DANS) (CAS No.:2844-15-7) were supplied by Sigma-Aldrich. Calcium chloride dihydrate ($CaCl_2$) (CAS No.:10035-04-8) and glass microfiber filters with particle retention of 1.0 µm (47 mm diameter) were supplied by Thermo Fisher Scientific. Hydrochloric acid (HCl) 10% was diluted from hydrochloric acid 30% (CAS No.: 7647-01-0) from Supelco. All solutions were prepared using Milli-Q water. Metal salt solutions of 0.05M were prepared freshly on the day of the corresponding experiment. LUFA 2.1, LUFA 2.2, and 5M soil were bought from LUFA Speyer standard soil with no or minimal MPs. An ultrasonication from Bandelin, a Stemi SV 11 Spectro microscope from Zeiss, and a Sorvall Lynx 6000 centrifuge from Thermal Scientific were used.

4.2 Organic matter removal

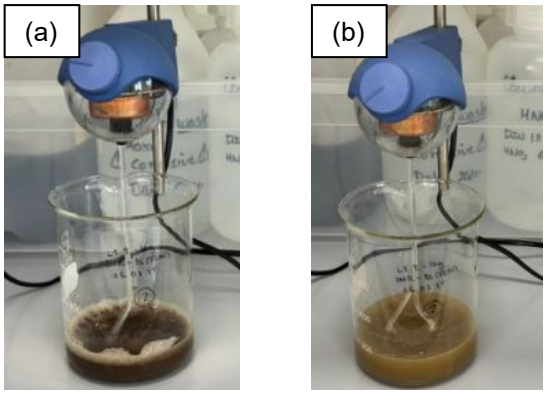

Figure 4.1: Organic matter removal of a 10g sample, LUFA 2.2 with method OMR followed by DS using funnels (a: initial, b: after 5 doses)

Soil samples were sieved through a 1 mm sieve. Fluorescence-stained PBAT with a size range of 125 – 500 µm was spiked into soil. A stock mixture was prepared by adding 30mg of PBAT MPs into 50g of clean soil and putting it in a glass bottle, followed by hand-shaking to make the mixture as homogeneous as possible. From the homogenized mixture, sub-samples from 0.05 to 0.5g were randomly taken, counted under a Stemi SV 11 Spectro microscope, and then transferred into individual tubes for storage. These pre-counted, spiked soil samples were then used in extraction experiments within one week. Each sample size will have a different amount of MPs (1g: 10 – 15 MPs, 2g: 20 – 30 MPs, 5g: 50 – 65 MPs, and 10g: 100 – 120 MPs).

A stock solution of Iron(II) sulphate heptahydrate 0.05 M was prepared fresh daily. Five doses of 5mL Fe(II) solution as catalyst and 15mL H_2O_2 as oxidant were added in 30 minute intervals [56]. pH is adjusted to 3 – 4 with HCl 10%. All samples were stirred under overhead stirrers since the Teflon-coated magnetic stirring bars can generate MPs (PTFE in particular) when used with abrasive particles like sand, soil or when in contact with the beaker directly [57]. Glass-coated stir bars are also an alternative, but they are fragile and prone to fragmentation during mixing.

4.3 Density separation

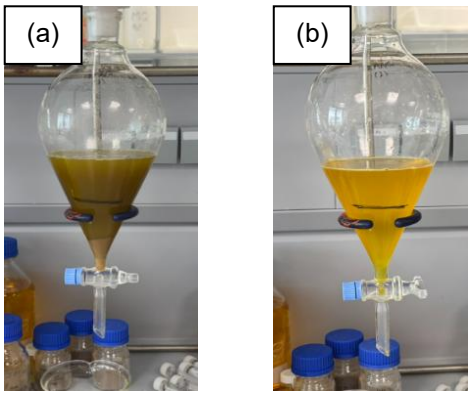

Figure 4.2: Density separation funnels, method OMR – DF, sample 5g, LUFA 2.1 (a: initial, b: after 48 hours)

Figure 4.3: Rinse Fe(II) solution with 25 µm mesh, method OMR – DC (sample 10g – 5M soil)

The key difference between the funnel-based and centrifugation methods lies in the use of the 25 µm mesh for rinsing. In the OMR–DC sequence, the mesh is used to rinse the Fe(II) solution from the OMR step. In contrast, in the DC–OMR sequence, it is used to rinse out excess $CaCl_2$ solution prior to OMR. Detailed steps for the four methods are provided in the procedure section.

4.4 Microplastic observation and identification.

4-dimethylamino-4′-nitrostilbene (DANS), a fluorescent dye, is an effective tool for identifying and characterizing MPs. The dye absorbs into polymers, and its solvatochromic property causes its fluorescence emission spectrum to be positively shifted (redshift) in relation to the polarity of the polymer. This property causes DANS-stained MPs to fluoresce with varying colors upon UV illumination. Staining is simple, requiring only one hour of incubation at 60°C [58]. In this study, a stock solution of DANS was prepared at a concentration of 1 mM in ethanol and diluted to 25 µM for staining. The staining was performed by applying the diluted dye solution dropwise directly onto the glass microfiber filters with the pore sizes of 1.0 µm, followed by incubation in an oven at 60 °C for 2 hours.

After incubation, the filter was observed under a Stemi SV 11 Spectro microscope with UV light to visualize the stained MPs based on their distinct fluorescence emissions.

4.5 Experiment setup and procedure.

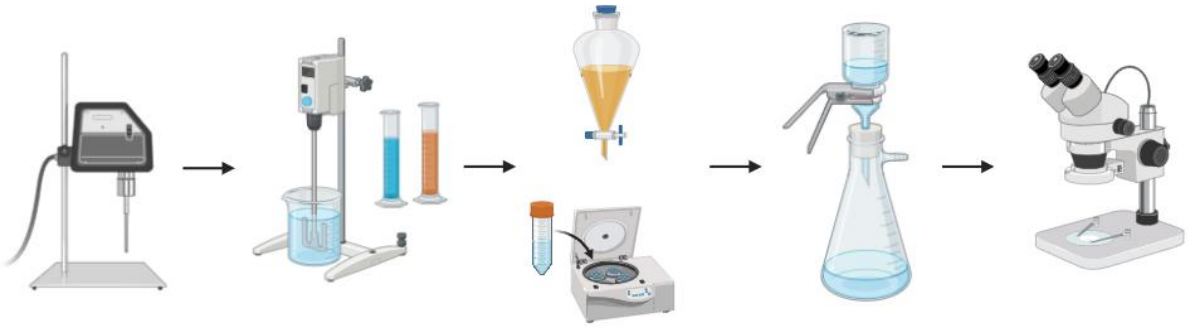

Figure 4.4: Overview of experiment setup and procedure (created with biorender)

4.5.1 OMR before DS (Density separation using funnel-based)

The step by step procedure is as follows: (1) Soil samples were put in beakers, filled with 40 mL Milli-Q, then sonicated using an ultrasonicator at 40W for 5 minutes. (2) Samples were then transferred to 600 mL beakers. (3) OMR was conducted through Fenton reaction. (4) $CaCl_2$ was added directly to the beakers. (5) After the $CaCl_2$ had completely dissolved, samples were transferred to 1L separation funnels and allowed to settle under gravity while MPs floated due to their lower density. (6) Samples were then collected and pre-filtered with a 25 µm mesh. Finally, retained material was collected on glass fiber filters for further analysis.

4.5.2 OMR before DC (Density separation using centrifugation)

Same with the method in section 4.5.1, but for step 5, centrifugation was used for the density separation method instead of using funnels. Samples were transferred to centrifuge tubes and centrifuged at 6000 xg for 15 minutes. The process was repeated twice to maximize the recovery rate.

4.5.3 OMR after DF

The step by step procedure is as follows: (1) Soil samples were put in beakers, filled with 40 mL Milli-Q, then sonicated using an ultrasonicator at 40W for 5 minutes. (2) $CaCl_2$ is added directly to the beakers. (3) After the $CaCl_2$ had completely dissolved, samples were transferred to 1L separation funnels and allowed to settle under gravity while MPs floated due to their lower density. (4) After removing the sediment, the samples were collected and filtered with a 25 µm mesh to rinse with $CaCl_2$. (5) Samples were then transferred to 600 mL beakers, pH was adjusted to 3-4. (6) OMR was conducted through Fenton reaction. (7)

Samples were pre-filtered with a 25 µm mesh. Finally, Samples were collected on glass fiber filters.

4.5.4 OMR after DC

Similar to method in section 4.5.3, but for step 3, instead of transferring it to funnels, the samples were transferred to centrifuge tubes and centrifuged at 6000 xg for 15 mins, repeated twice to maximize the recovery rate.

4.5.5 Experiment with no pre-stain MPs

Based on the previous experiments, it was determined that PBAT had a high recovery rate and did not undergo degradation or possible fragmentation during the extraction process. Given this, it was considered logical to assume that the method could also be effective for other polymers and environmental soil samples. To test it, five different polymers: PP, LDPE, PS, PLA, and PBAT, 10 particles of each were added to 5g of agricultural soil from a test facility of the University of Natural Resources and Life Sciences (BOKU). The samples were then subjected to the most effective extraction method, OMR followed by DS by funnels (OMR-DF). Finally, the extracts were analyzed with fluorescence using DANS to assess recovery efficiency.

Table 4.1: Five different polymers and sizes

No.	**Polymer type**	**Size (µm)**
1	PLA	<500
2	PS	<500
3	LDPE	<500
4	PBAT	100 – 300
5	PP	<500

5 Results and Discussion

5.1 Recovery rate

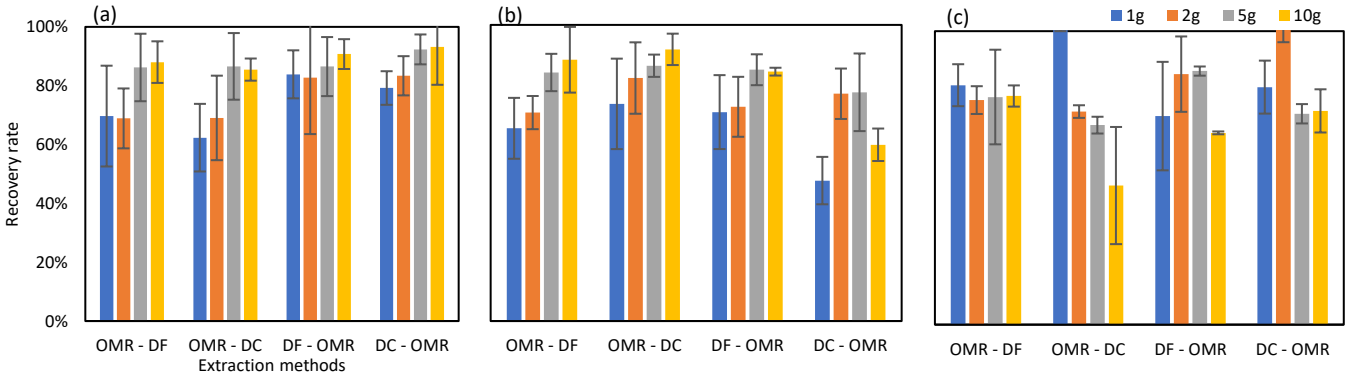

Figure 5.1: Recovery rate of PBAT in (a): LUFA 2.1, (b) LUFA 2.2, (c) 5M soil

To evaluate the recovery rate, four different methods and four different sample sizes were tested across three different soil types. LUFA 2.2 was tested first since in all three soil types, the % of sand, clay, and silt was in the middle and had the highest OM content. Glassware was used to prevent contamination from plastic objects.

From Figure 5.1, graph (b), which represents the extraction from LUFA 2.2, method OMR before DS (both funnels and centrifugation) showed that the recovery rate increased with the increase of sample size. The sequence with OMR-DC proved to be more efficient than the OMR-DF method because centrifugation provided a stronger separation force and better phase separation. The recovery rate in four sample sizes was higher and required a shorter time. This may help MPs adhere less to soil particles, achieving a better separation and higher recovery rate. Whereas for method DS before OMR, the 5g size had the highest recovery rate (85 ± 5% for DF-OMR and 77 ± 13% for DC-OMR). Low recovery and high variability were observed in all 1g samples. It was likely due to the small number of particles (around 10 to 15), which were easily lost during extraction. Also, after the OMR step, OM is decomposed, which helps to separate the MPs from the organic matrix or at least weakens the interaction between them, making it easier to separate by DS with $CaCl_2$. This explained the higher recovery rate of both methods OMR-DF and OMR-DC. Although OMR-DC showed higher recovery rates, its performance was inconsistent across all soil types. The final selection of the extraction method should be practical and able to apply to broader soil matrices, which will be discussed in the following sections.

Figure 5.1, graph (a), represented the LUFA 2.1 soil and showed a consistent trend of increasing recovery rate with increasing sample size. The overall higher recovery compared to the other two soils suggests a lower interaction between the soil and the MPs,

which is most likely due to the higher sand content (86.1 ± 0.7%) in the LUFA 2.1 and lower OM (<1%). Sand can interact with MPs through the Van der Waals force, which is a weak force, giving a lower interaction force. Additionally, sand had a relatively low adsorption because of its low specific surface area, and finally, sand can trap small MPs in its pores due to its surface morphology [59]. The first step of the extraction method was ultrasonication, which served to separate sand from MPs. The results in graph (a), Figure 5.1, indicated that performing the DS before OMR was beneficial for achieving a higher recovery rate. While MPs can be released after the ultrasonication step, if sand is not removed immediately afterward, there is a possibility that the free MPs could become re-trapped. In this sequence, sand was removed early in the process, reducing the chance of MPs being lost due to physical entrapment. Extra care is needed when collecting samples from centrifuge tubes, as sand may unintentionally slide out with the supernatant during decanting.

While LUFA 2.1 and 2.2 showed an overall increase in recovery rate with increasing sample sizes, the situation is different for 5M soil, as shown in Figure 5.1 (c). The high percentage of silt and clay means that if MPs are present in the soil increases the possibility for strong interaction between MPs and soil particles, which results in greater variability between methods and sample sizes. Those interactions occurred in all three soil types, but are highlighted here because the 5M soil had the highest % of clay and silt. Organic compounds with polar and non-polar ends can act as bridges that bind the nonpolar MPs to the polar clay, inhibiting the separation and isolation of MPs. LUFA 2.1 contained lower OM and a higher proportion of sand, making the interaction between sand and OM less critical, and it is mainly about the direct interaction between MPs and sand. With LUFA 2.2, although the OM content is higher, the % of clay and silt is lower, making the bridging interaction less noticeable than in 5M soil.

In the sequence OMR-DF of 5M soil, the recovery rate was nearly equal across all four sample sizes, 82 ± 7% for 1g, 77 ± 5% for 2g, 78 ± 16% for 5g, and 78 ± 4% for 10g. This could be because when OMR took place before DF, the OM was already removed, breaking some of the bonds between MPs and other soil particles, which allowed MPs to float during the density separation step. However, it was also possible that in the larger sample sizes (5g and 10g), the OM content was higher and may not have been removed completely. This resulted in a smaller proportion of OM being degraded compared to the 1 g and 2 g samples, which could affect MP release and recovery. The DF–OMR sequence showed variability and inconsistency across sample sizes. Clay and silt may attach to MPs through the ester linkages of PBAT and through bridges formed by OM, increasing the density of

MPs and causing them to sink during DS. This effect was observed in 10g samples, which showed a lower recovery rate than other sample sizes. On the other hand, the 2g and 5g samples produced high recovery, though 2g showed greater variability. This could be due to two possible reasons: first, the initial count may have been underestimated because some MPs were hidden by clay and silt, making them unobservable and not counted. They appeared after processing steps, which likely led to a higher apparent recovery across all samples. The second reason was that for the 1g sample, the loss of only a few MPs resulted in a significant drop in recovery percentage.

Unlike LUFA 2.1 and LUFA 2.2, the recovery rate of the OMR–DC sequence in 5M soil showed a decrease in recovery with increasing sample size. This may be because the upward movement of some plastic particles may have been hindered by the settling of sand and clay, given that we have more material, and even if we have removed the OM beforehand. Overall, reducing the recovery rate at the end of the centrifugation process [39]. For the DC–OMR sequence, a different pattern appeared, showing that smaller sample sizes of 1g and 2g yielded better results than 5g and 10g, with 2g giving the highest recovery but also a high variability (105 ± 8%), while 5g gave the lowest recovery but the smallest variability (72 ± 3%). Since OM had not yet been removed, and the samples were not thoroughly shaken before DS, leading to uneven mixing and distribution. As a result, larger sample sizes tended to trap MPs in the sediment. Meanwhile, the high variability observed in the 1g and 2g samples may be due to heterogeneity during sampling, some samples may have contained lower clay content than others, leading to higher recovery rates. Recovery rates exceeding 100% could be explained by undercounting during the initial sample preparation. The likelihood of MP fragmentation is relatively low. As shown in the thesis of Heinemann (2023), no significant particle size reduction was observed during extraction with a similar Fenton reaction, suggesting that fragmentation within the MP size range was limited [56]. Therefore, recovery rates exceeding 100% were likely due to undercounting during initial sample preparation rather than fragmentation of particles. The MPs were spiked, counted, and extracted within two weeks, so fragmentation or biodegradation from soil incubation is also unlikely. Another possible explanation for the low recovery rate of a 10g sample size lies in the experimental procedure itself, as shown in Figure 4.3. When centrifugation was used as the density separation method, the Fenton solution needed to be washed through a 25 µm mesh. This step could have caused clay, silt, sand, and MPs to reagglomerate after being deagglomerated during the sonication step.

A disadvantage during the centrifugation process in 5M soil was particle reagglomeration, occurring (1) after rinsing the Fenton solution step (Figure 4.3) and (2) after the first centrifugation round. To mitigate this issue, suggestions would be to apply sonication or use a vortex mixer before each centrifugation step. Since the centrifugation process is repeated twice, placing the centrifuge tubes in a sonication bath or on the vortex mixer between those steps could help deagglomerate the particles. However, caution is needed when using glass centrifuge tubes, as they may crack or break during sonication and vortexing if not appropriately handled. If plastic tubes are used instead, a blank sample must be included to check for potential cross contamination. A second solution could involve using a specific chemical agent as a deagglomeration solution, like sodium pyrophosphate ($Na_4P_2O_7$) [60], which would help reduce particle clustering without requiring additional mechanical disruption. Another option would be, after collecting the supernatant, instead of discarding the sediment part, to collect and redo the OMR steps with the DC–OMR method, like in Weber et al. (2025) [61].

Performing DS before OMR showed inadequate results when treating with a more complex soil matrix, like 5M soil. Silt and clay also have Van der Waals interactions with MPs and can adsorb onto MPs' surfaces, forming aggregates and trapping the MPs with high-density particles. Clay with a permanent negative surface charge can undergo electrostatic repulsion with MPs. But a complex matrix like soil might contain other minerals that can act as a binding agent to attach clay onto MPs and can even cover MPs in the soil. However, this sequence may still be appropriate for simpler matrices with low organic content.

In this study, the MPs' size range was from 125 to 500 µm, and each sample was mixed well before sampling to ensure homogeneity. It suggested that the size distribution of MPs has little or no influence on the sample size required for good representation, particularly in controlled experiment conditions. Since the analysis only considered total MPs without distinguishing particle sizes, no conclusions can be made about size dependent losses during extraction. In field sampling research, the vertical and horizontal distribution of MPs is more variable. Smaller MPs are often found in the deeper layer of soil. Therefore, it is necessary to take samples at different locations and depths for a representative result.

5.2 MPs lost

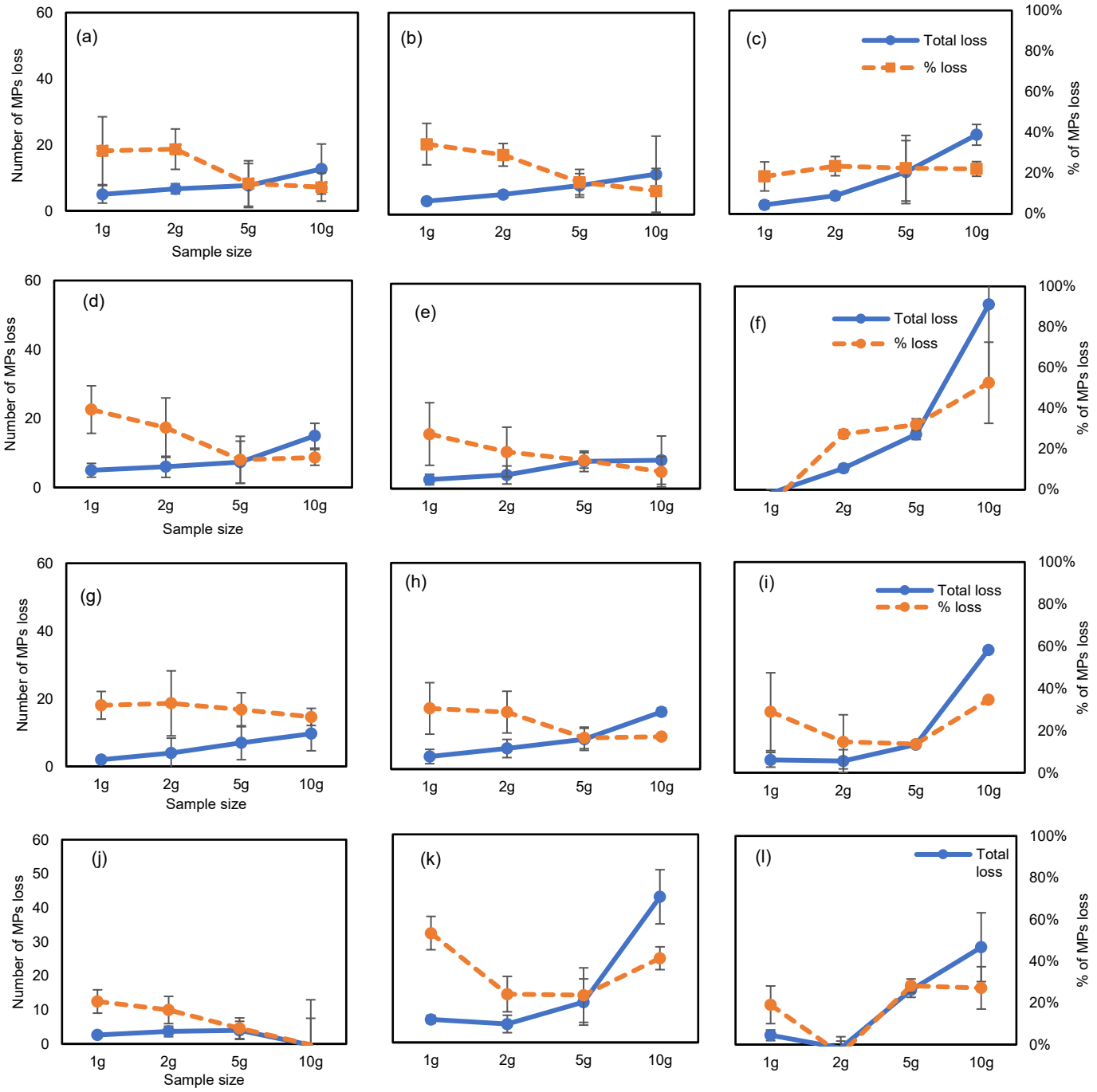

Figure 5.2: Total and percentage loss of microplastic particles during extraction from LUFA 2.1 (a, d, g, j), LUFA 2.2 (b, e, h, k), and 5M soil (c, f, i, l) using four different extraction methods: (a–c) OMR-DF, (d–f) OMR-DC, (g–i) DF-OMR, and (j–l) DC-OMR. The graphs display the average number of microplastic particles lost and the corresponding percentage loss across four sample sizes (1g, 2g, 5g, and 10g), enabling the observation and comparison of loss patterns across soil types and extraction procedures.

After obtaining the recovery rate in section 5.1, it was observed that the extraction efficiency depended most on the soil type. Therefore, knowing the number of MPs lost and the ratio of loss to total spiked MPs also needs further research. Method OMR-DF showed a consistent trend across three soil types (Figure 5.2), while MP loss increased with sample size, the percentage loss decreased. This indicates the high stability of the OMR-DF method when applied to different soil matrices. In contrast, figures 5.2 (d) – (l) showed the loss during the extraction of the three soil types and extraction methods, demonstrating considerable variability and a lack of consistency. The diverse and conflicting trends observed across these methods and soil types suggest that their application may be less suitable in contexts requiring high reliability and reproducibility.

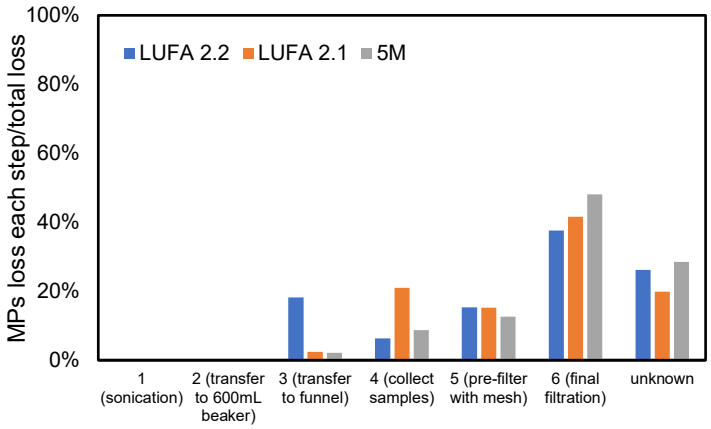

Figure 5.3: Percentage of MPs loss during each step and unknown loss within the total MPs loss across three soil types using method OMR–DF

To identify which step resulted in the highest loss of MPs, an overall count was conducted after each experiment step to detect and quantify the number of MPs lost. These values were then used to calculate the percentage of loss attributed to each step and the rate of unknown loss within the total MPs loss across the three soil types. Note that the unknown amount is the number of MPs lost during the extraction process, but it is unclear at which step they were lost. Figure 5.3 showed that with OMR–DF method, the percentage of unknown loss within the total MPs loss across the three soil types was both low and consistent, indicating a high level of traceability of MPs loss. The percentage at each step was calculated by dividing the number of MPs lost at each step by the total number of MPs lost. This extracting method (OMR-DF) differs from other methods (Figure S1) when applied to complex soils like 5M, where unknown loss is significantly higher, in some cases, reaching nearly 80% of the total loss, reflecting lower reliability under practical conditions. According to Figure S1, the percentage of MPs lost at each step relative to the total loss highlights the critical steps of the extraction procedure, allowing researchers to focus on

steps with higher loss rates and to explore strategies for minimizing MP loss at those stages. Among all steps, the filtration step was identified as the main cause for MPs' loss during extraction, more than 40% of the total loss across three soil types for the OMR–DF method. This loss was mainly because of the adhesion on the filtering funnel of the filtration system. Some improvements could be changing the upper part material to the less adhesion or redesigning the filtration system.

Based on the recovery rate of PBAT across three different soil types and the results of the total and percentage loss of MPs during extraction from LUFA 2.2, LUFA 2.1, and 5M soils in Figure 5.2, the OMR–DF method is recommended for analyzing MPs in a random soil sample. This recommendation assumes that the sample had a high percentage of OM, contained mainly clay and silt, had a low concentration of MPs, and lacked access to glass centrifuge tubes due to cost. The method OMR-DF with a 5g sample size is considered appropriate. Particularly, OMR step before DS helps eliminate or weaken interactions between OM and MPs. Using a funnel is practical and straightforward, and the 5g sample size demonstrated the most stability and a high recovery rate across all soils.

5.3 Application of the extraction protocol on agricultural soil samples with other polymer types

This experiment was conducted using an agricultural soil sample obtained from the agricultural test field of the BOKU University in Vienna with an OM content of 5 ± 0.18%, measured by LECO RC612. The OMR-DF method was selected to extract MPs, with five different types (PBAT, PLA, PP, LDPE, PS) spiked into the 5g sample with a total of 50 MP particles, exactly 10 particles of each type. Calcium chloride ($CaCl_2$) was used as the separation solution because the densities of these polymers are all less than 1.4 g/cm³. MPs were not pre-stained with fluorescence; instead, 4-dimethylamino-4'-nitrostilbene (DANS) was used for detection and quantification at the final step on filter paper. DANS was chosen over Raman spectroscopy because Raman requires that soil and plastic particles be clearly separated on the filter for accurate analysis. However, with this soil sample, even after a 3 day settling period, substantial amounts of wood debris remained. These residues were not fully removed by the applied extraction method, which was originally developed for Raman-based analysis, and the filter did not meet the requirement for Raman (see Figure S3 (a) showing the filter after filtration). A blank soil sample was also processed in triplicate to ensure accuracy.

After performing the extraction, the next step was the DANS incubation. The diluted dye was applied dropwise onto the sample filters and then incubated at 60 °C for 2 hours. Each polymer type was also pre-stained separately to confirm the color signature and thereby be able to recognize and quantify the number in the sample. The colors of each different polymer are as follows: PP: green, LDPE: green, PBAT: red-orange, PLA: orange-yellow, PS: yellow-white (Figure S2). In the blank samples, many particles absorbed dye and emitted a similar color to the targeted polymers under UV light, making the counting step challenging. Only the MPs within the 200 – 500 µm size range were considered during sample preparation and analysis, as this range corresponds to the size of the spiked MPs (see Table 4.1).

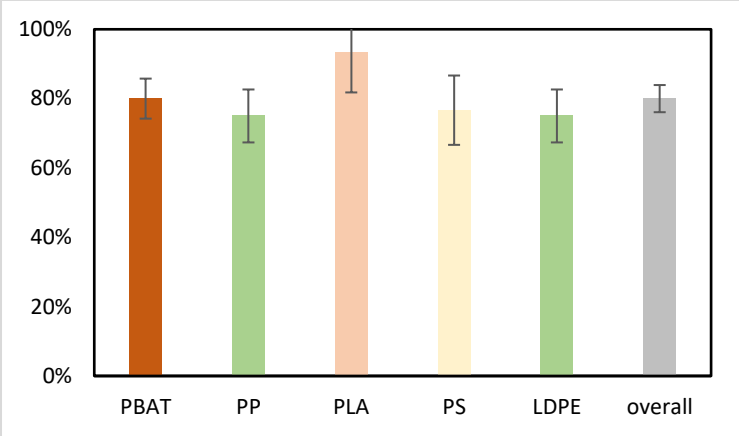

Figure 5.4: Recovery rate of different polymer types, and overall after extraction from the BOKU agricultural soil sample using method OMR-DF. The sample size was 5g, and each polymer type had 10 particles.

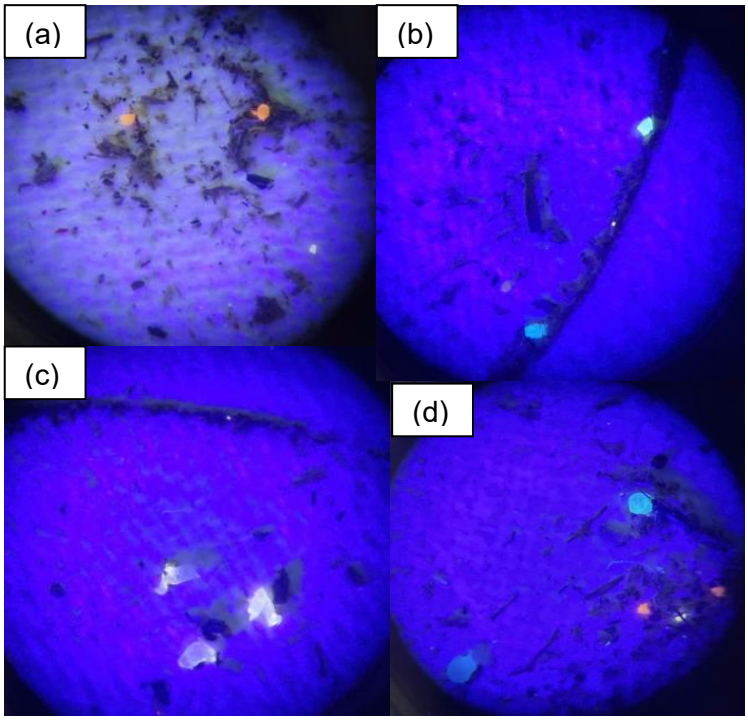

Figure 5.5: Fluorescent MPs observed under UV light after extraction from soil samples using method OMR - DF. Images show different polymer types stained with DANS dye, with distinct fluorescence colors observed on the filter paper. (a) is PLA with orange color, (b) PP or LDPE with green color, (c) PS with yellow color, and (d) PBAT with red color. The images are taken from an external camera, which is why it does not have a size bar.

Figure 5.4 shows the recovery rate of each polymer and overall performance. The overall recovery rate was 80 ± 4%, which matched the expected value from the recovery rate experiment above for a 5g sample size. PLA had the highest recovery rate, 93 ± 12%, which is understandable since PLA had the largest size among the five polymer types, causing it to generally float faster than the smaller ones, as its rising velocity increases with particle size, based on Stokes' law. PLA has the highest recovery rate because this polymer has a relatively high polarity due to the presence of ester groups in the structure, which helps reduce hydrophobic interactions with OM. This helps PLA remain an isolated state in the solution and is almost completely recovered after filtration. With the other four polymer samples, the difference in performance was minimal, with all recovery rates ranging from 75% to 80%. This confirms the initial assumption that if the method works with PBAT, it will also work with other polymers under the same extraction conditions.

Because of having the same fluorescence colors, the total number of green fluorescence particles counted was split equally between PP and LDPE, resulting in an equal recovery rate for both PP and LDPE in this case. This is also a disadvantage of DANS when several

polymers produce the same color, which can be difficult to distinguish and may require further examination using FTIR or Raman. Although Sancataldo et al. (2021) reported that PP typically produces blue fluorescence, it appeared green in this study. It is likely due to the similar dielectric constant (PP: 2.2 – 2.5, LDPE: 2.2 – 2.35), and both polymers had overlapping fluorescence peaks, making the visual differentiation challenging [58]. Compared to the other more commonly used Nile Red staining, DANS offered a distinct advantage in this study and proved to be a reasonable and effective alternative for analyzing MPs.

6 Conclusion and outlook

This study carried out the necessary experiments to test four hypotheses, which were using PBAT for four extraction methods across three different soil types in four sample sizes. In addition, after determining the most suitable method (OMR-DF) base on the recovery rate of PBAT, apply it to the real soil sample from the BOKU university site with an OM content of 5 ± 0.18%. The test was conducted with a polymer mixture containing PBAT, PP, LDPE, PS, and PLA, examining whether similarly high recovery rates could be achieved for other polymer types.

The results showed that the OMR-DF method at 5g sample size has high recovery efficiency, is easy to implement, and has low cost. Across all three soil types, the recovery rate is consistently around 80% or higher. During these experiments, it was possible to track some particles that were lost during the extraction, allowing for identifying critical steps where losses occur. Filtration was found to contribute the most to overall losses and needs to be further optimized in the future to reduce MP loss during the extraction. The recovery rate of OMR-DF method in this thesis ranges from 65 ± 10% (1g, LUFA 2.2) to 88 ± 7% (10g, LUFFA 2.1). When applied to the BOKU soil sample, the overall recovery rate of 80 ± 1% with individual recovery rates for each different polymer type was 93 ± 12% for PLA, 80 ± 6% for PBAT, 77 ± 10% for PS, and 75 ± 8% for both PP and LDPE.

Based on all experimental results and data, this thesis concludes that hypotheses 1 and 3 are confirmed, while hypotheses 2 and 4 are partially supported. Specifically, OMR before DS enhanced the recovery rate (Hypothesis 1), and performance differed across soil types (Hypothesis 3). Although centrifugation yields a higher recovery rate compared to the funnel-based method, this observation is limited to soil types LUFA 2.1 and LUFA 2.2. It does not apply to sample 5M (hypothesis 2). Similarly, the assumption that increasing sample size leads to higher recovery rates is generally valid. Nevertheless, this trend is not

consistent across all methods and soil types. For instance, the OMR-DC method applied to sample 5M demonstrates the opposite effect (hypothesis 4).

In conclusion, each method in this study yielded promising results under specific conditions. However, in cases where soil composition is unknown or variable, the OMR-DF method is recommended for MPs extraction based on its performance across soil types and sample sizes. In particular, 5g sample size is considered to be the most suitable for an unknown sample, as it demonstrated a high recovery rate for the OMR-DF protocol. Future research could focus on optimizing the filtration step, which accounted for the highest MPs loss, and improving deagglomeration techniques, and reducing MPs losses during centrifugation. Finally, the developed protocol could be expanded to a broader range of polymers and soil matrices.

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List of Abbreviations

AA: Adipic acid

BDO: Butanediol

BOKU: University of Natural Resources and Life Sciences

DANS: 4-dimethylamino-4’-nitrostilbene

DC: Density separation (centrifugation)

DF: Density separation (funnel)

DS: Density separation

HDPE: High-density polyethylene

LDPE: Low-density polyethylene

MP: Microplastic

OM: Organic matter

OMR: Organic matter removal

PA: Polyamide

PB: PFASPFA

PBAT: Polybutylene adipate-co-terephthalate

PC: Polycarbonate

PHA: Polyhydroxyalkanoates

PLA: Polylactic acid

PE: Polyethylene

PET: Polyethylene terephthalate

PP: Polypropylene

PS: Polystyrene

PTA: Terephthalic acid

PTFE: Polytetrafluoroethylene

PVC: Polyvinyl chloride

ROS: Reactive oxygen species

SDG: Sustainable Development Goal

UN: United Nations

WWTP: Wastewater treatment plant

Supplementary Information

Table S1: Summarization of various extraction methods used in different matrices

Matrices	**Polymers**	**Extraction methods**	**Recovery rate**	**Ref.**
Topsoil	PE, PS, PVC, PA, PES, PP	OMR by Fenton - DS (NaI)	95%	[49]
Biosolids, Compost, Soil	PET, PVC, HIPS, PP, ABS, HDPE, PA	OMR by Fenton - DS (NaI)	92% (compost); 95% (biosolids); 98% (soil)	[53]
Industrial compost	LDPE	OMR by Fenton - DS ($ZnCl_2$)	78.2 ± 3.5%	[47]
WWTP sludge	PP, PS, PET, PE, HDPE, PVC	OMR by Fenton - DS (oil)	100% (PP, PS); 95% (PET, PE, HDPE); <65% (PVC)	[62]
Agricultural soil	PE, PP, PS, PVC, PET	OMR by Fenton - DS ($CaCl_2$)	>95% (PE, PP, PS, fPVC); 94.9% (PET)	[39]
WWTP sludge	PE, PP, PS, PET, and PA	OMR by Fenton, KOH, H_2O_2 - DS	75–100%	[31]
Sandy and loamy soils		DS (centrifugation)	>90% across all soils tested	[19]
Simulated soils	PET, HDPE, PVC, LDPE, PP, and PS	OMR – DS	$ZnCl_2$: 80%; Canola Oil: 84%; NaCl: 59%.	[63]
soil and compost	PET, LDPE, PET, PE, PP, PS, PVC	DS - OMR by Fenton	>93% for all polymers except PET; PET (~98% in soil vs. 80% in compost).	[41]
Coastal sediment	PE, PP, PVC, PET	DS (centrifugation)	107% (PE); 83% (PP); 93% (PVC); 83% (PET)	[64]
Agricultural soil	LDPE, PP, PVC	DS (NaCl + oil) - OMR by H_2O_2	LDPE: 95.2–98.3% PP: 95.2–98.7% PVC: 76.0–80.5%	[55]

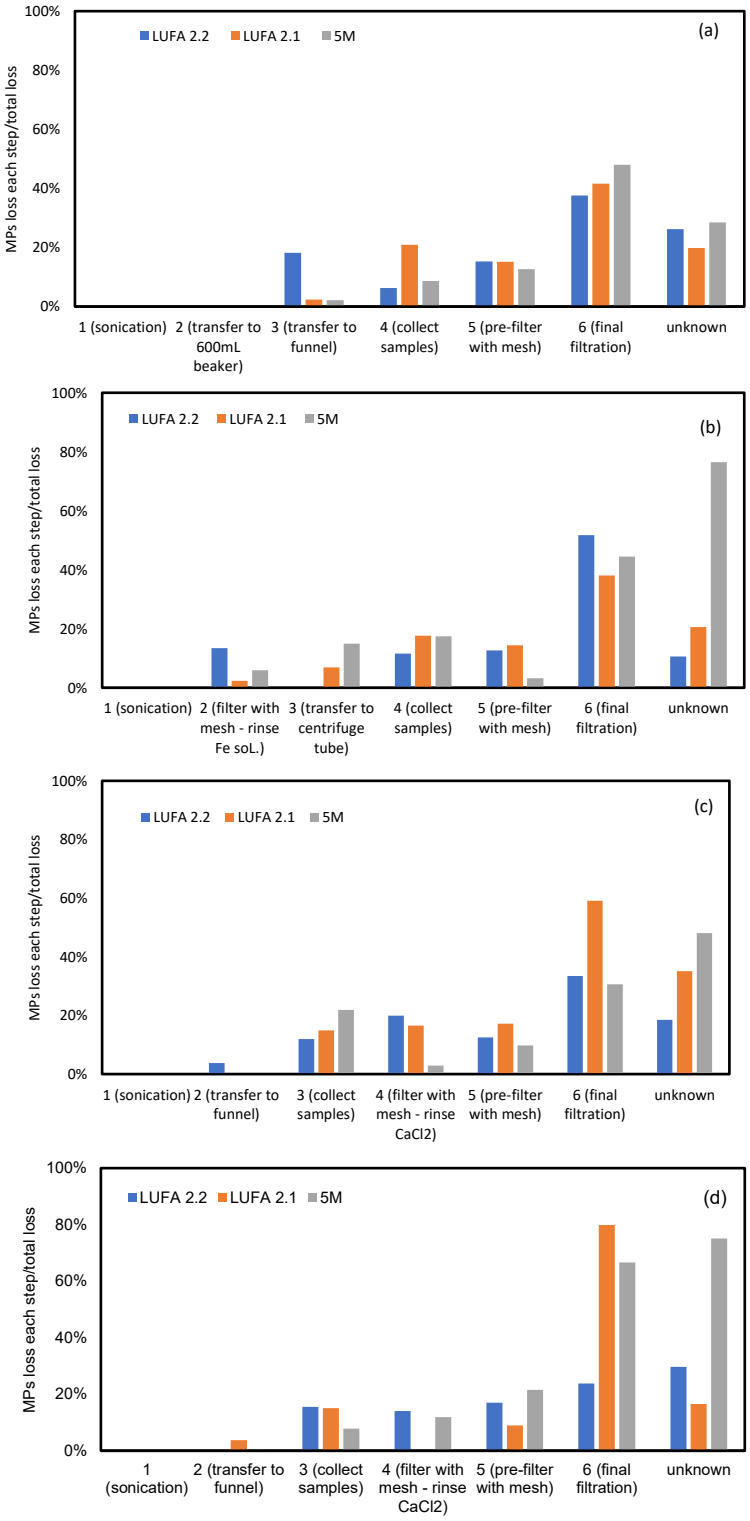

Figure S1: MPs' loss each step/total loss of four methods in three different soil types (a) OMR-DF, (b) OMR-DC, (c) DF-OMR and (d) DC-OMR

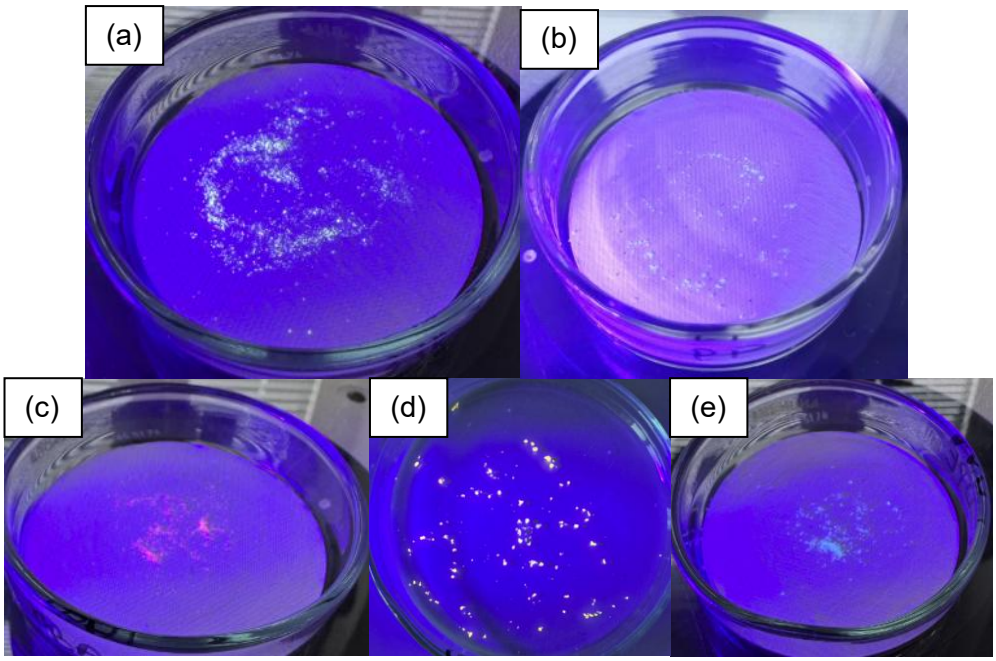

Figure S2: Polymers' color signature with DANS with (a): PS, (b): PP, (c): PBAT, (d): PLA, (e): LDPE

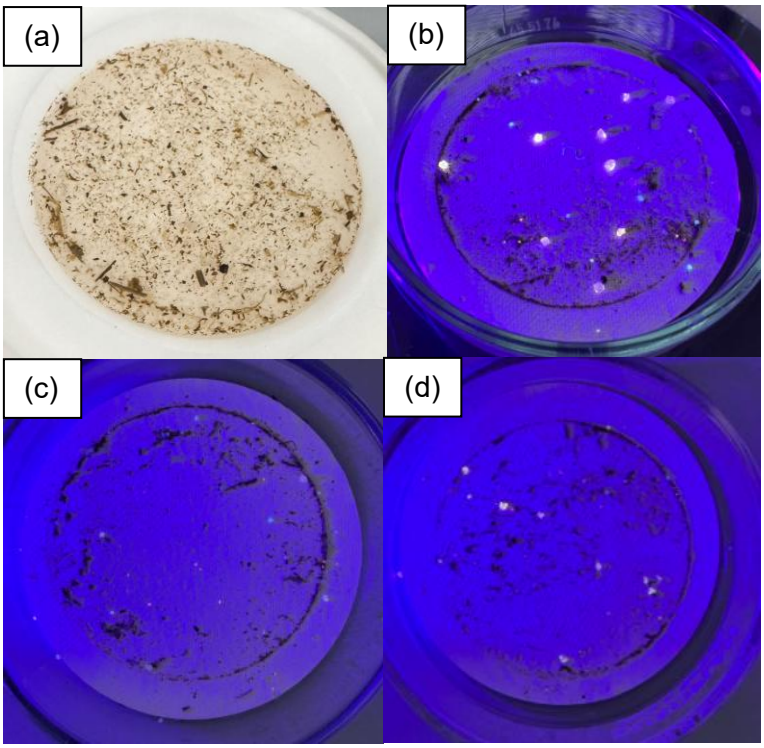

Figure S3: Polymers' color signature with DANS of agricultural soil sample with (a): filtered sample before incubating with DANS, (b),(c) and (d) are the triplicate after incubating with DANS and under UV light.

Table S2: Calculation from number-based concentration to mass-based concentration

Matrices	Size of MPs (µm)			Amount of MPs (MPs/kg)				Volume of MPs (cm^3)			Mass-based concentration (mg/kg)				Notes
	Min	Max	Mean	Min	Max	Mean	SD	Min	Max	Mean	Min	Max	Mean	SD	
farmland	32.70	3464.80	845.20	NA	NA	136.70	41.70	1.83×10^{-8}	0.022	3.16×10^{-4}	NA	NA	43.12	13.15	(a), (b)
Yellow brown soil	83.40	3606.20	794.70	NA	NA	155.00	95.20	3.04×10^{-7}	0.025	2.63×10^{-4}	NA	NA	40.64	24.96	
Paddy soil	172.70	4765.20	1574.40	NA	NA	190.00	31.20	2.70×10^{-6}	0.057	2.04×10^{-3}	NA	NA	387.36	63.61	
Floodplain soil	57.00	4617.20	1549.40	NA	NA	256.70	62.20	9.70×10^{-8}	0.052	1.95×10^{-3}	NA	NA	498.81	120.86	
Compost	140	4930	2535	2200	6400	NA	NA	1.44×10^{-6}	0.063	8.53×10^{-3}	20708.60	60243.20	NA	NA	(a), (c)
Compost-amended soils	990	2110	1550	191	248	NA	NA	5.08×10^{-4}	0.005	1.95×10^{-3}	410.98	533.63	NA	NA	
agricultural soil	NA	200	100	30500	74900	NA	NA	NA	NA	5.24×10^{-7}	19.16	47.06	NA	NA	(a), (d), (e)
Biowaste composting	1000	5000	3000	20	24	NA	NA	NA	NA	0.014	325.28	390.34	NA	NA	(a), (f)
Biowaste digestion	1000	5000	3000	14	895	NA	NA	NA	NA	0.014	227.70	14556.25	NA	NA	
Agricultural digestion	1000	5000	3000	0	11	NA	NA	NA	NA	0.014	0	178.90	NA	NA	
Compost from municipal organic waste (Netherlands)	30	2000	1015	NA	NA	2800	616	NA	NA	5.48×10^{-4}	NA	NA	1839.65	404.72	(a), (d)
Compost from garden and greenhouse waste (Netherlands)	30	2000	1015	NA	NA	1253	561	NA	NA	5.48×10^{-4}	NA	NA	823.24	368.59	
Agricultural soil (Netherlands)	30	2000	1015	NA	NA	888	500	NA	NA	5.48×10^{-4}	NA	NA	583.43	328.51	
Agricultural soil (Spain)	30	2000	1015	NA	NA	2242	984	NA	NA	5.48×10^{-4}	NA	NA	1473.04	646.51	

Notes:

(a) Assuming all the MP particles are in spherical shape

(b) Average polymer density: d = 0.998 g/cm^3 (40% PP, 35.5% PE, 15.6% Acrylic, 6.7% PET, 2.2% PA)

(c) Average polymer density: d = 1.104 g/cm^3 (15.7% PET, 10.4% PP/PE copolymer, 23.9% PE, 19.2% PP, 5.7% polyamide (PA), 5.4% rayon, 3.7% polyacrylonitrile (PAN), 3.4% styrene butadiene rubber (SBR), 3.2% polyacrylamide (PAM), 2.9% polyacrylic acid (PAA), 2.2% PP/PA, 2.2% polyvinyl acetate (PVAC), 1% PS, 1% PVC)

(d) If the content of polymers is not mentioned, assume average polymer density: d = 1.2 g/cm^3

(e) Mass-based concentration mentioned in this paper is from 5.4 ± 4.4 mg/kg to 20 ± 20 mg/kg

(f) Average polymer density: d = 1.15 g/cm^3 (44% PS, 25% PES, 2.3% PVC, 15.8% PE, 6.5% PP, 2.5% PET, 3.5% cellulose base polymer, 0.33% PA)