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D6.4 Report on presence of microplastics

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Abstract

The deliverable 6.4 (D6.4) investigates the presence and prevalence of microplastics (MPs) in organic fertilisers selected from the fertilising products generated in WP3 and WP4 (see D6.1). Modified extraction methods were developed and validated using spiked references, including primary and secondary MPs particles, selected based on the global plastic production and their environmental prevalence. Organic digestion is the key step in microplastic extraction, which effectively digested or faded organic components in BBFs, facilitating the observation and characterization of MPs. Oxidation digestion with hydrogen peroxide (H_2O_2) solution was applied due to complex varied matrix of the fertilizers. For liquid fertilizers—NPK solution with amino acids (FER3), hydrolysates with amino acids and peptides (UNI1) and chitin-rich fertilizer (UNI3)—H_2O_2 solution and surfactant treatments were applied to remove lipids sticked on the glassware to enhance organic digestion and MPs recovery. The solid fertilizer CAT1, derived from solely fish processing waste, was digested with H_2O_2 solution and then treated with nitric acid for organic and bone structure removal. Fish sludge-based pellet fertilizers, FSP and FMP, underwent with first density separation with NaCl (1.2 g/cm^3), followed by oxidation digestion. BP and OA1, produced from mixed waste streams, were first digested with H_2O_2 solution and then subjected to density separation (NaCl 1.2 g/cm^3). After filtration and oven-drying, the recovered residues were observed and sorted under microscope. The sorted MPs were appeared in various colours, such as black, blue, grey, green, and white. While the morphology of the extracted MPs were categorized in fragment, film and fiber, fragments and films were predominant. Using (μ)FTIR, MPs were identified as polyethylene (PE), polypropylene (PP), polystyrene (PS), polyamide (PA), polyurethane (PU) and polyvinyl chloride (PVC), as well as other thermoplastics. MP abundances showed variations across the fertilizers: FER3 approx. 670 items/kg, mainly PET,PE and PA; UNI1 approx. 1610 items/kg, mainly PE, PP, PA, PET and PU; CAT1 approx. 2750 items/kg, mainly thermoplastics: polyurethane-styrene-isoprene copolymers; FSP approx. 1670 items/kg, mainly PE and PP; FMP approx. 1170 items/kg, PE, PET and PU; BP approx. 23,000 items/kg, mainly thermoplastics polyoxymethylene (POM), PE and PVC; and OA1 approx. 3330 items/kg, mainly PE, PA and ethylene vinyl acetate. These findings highlight that organic fertilizers derived from fishery waste contain significant MPs loads, underscoring the need for mitigation measures to minimize the occurrence of MPs in fertilising products and further attention to MPs analysis in organic fertilizers.

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Disclaimer:

Abbreviations

µFTIR: Micro-Fourier transform infrared spectrometry

BBF: Biobased fertiliser

BP: Bokashi pellet

CAT1: Protein fraction

D: Deliverable

DM: Dry matter

EVA: ethylene vinyl acetate

FER3: NPK solution with amino acids

FMP: Fish mix pelleted fertiliser

FSP: Fish sludge pelleted fertiliser

H_2O_2: Hydrogen peroxide

MPs: Microplastics

OA1: Organic amendment

PA: Polyamide

PE: Polyethylene

PET: Polyethylene terephthalate

PLA: Polylactic acid

PP: Polypropylene

PS: Polystyrene

PU: Polyurethane

PVC: Polyvinylchloride

UNI1: Hydrolysates with amino acids and peptides

UNI3: Chitin-rich fertiliser

WP: Work package

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1 Introduction

Microplastics (MPs) are tiny plastic particles with size range between 1μm and 5 mm, comprised of primary and secondary sources. Primary MPs are considered micro-size plastics generated directly from plastic production, while secondary MPs are originated from fragmented large plastic objects. It is a growing environmental concern globally due to the durable nature, pervasive occurrence and massive amount. Approximately 1.5 million of primary MPs are released into the marine environment every year with an estimated load of 100-250 million tons by 2025 (Habib and Thiemann, 2022). Bioavailability and ecotoxicity potential of MPs present significant concerns, making it a crucial issue that warrants thorough investigation (Pittura et al., 2023).

Fishery waste can contain significant amount of MPs due to the current plastic contamination of marine environment. Similarly, fertilisers are also considered one of the major sources of MPs in soil agroecosystems and can negatively affect soil organisms' fitness, soil fertility and texture, thus decreasing crop yield (Gui et al., 2021; Li et al., 2019; Okeke et al., 2022; Weithmann et al., 2018; Zhang et al., 2022). Although MPs are not (yet) included in regulations as a decisive/regulative parameter, their vast presence in aquatic and terrestrial environments is already a remarkable sign to monitor their presence and prevalence in the fishery waste-derived fertilising products.

Despite the end products and their application, approximately 80% of global plastic production consists of polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polyurethane (PU), among which more than three quarters is comprised of PP, PE and PS (Hurley et al., 2018; Weithmann et al., 2018). Additionally, biodegradable plastics, such as PLA, faces an increasing production and application trend, leading to enhanced MPs formation due to the aging and fragmentation process. Previous studies have revealed a wide variety of MPs typologies, including broad size ranges, diverse shape categories, and variations in color and physiochemical composition. The most common MPs involve PE, PP, PS, PVC, polymethyl methacrylate (PMMA), PET, and polycarbonate (PC). These MPs can take various shapes, such as fragment (hard particles with irregular outlines), fiber (long fibrous materials that have a length substantially longer than their width), and film (flat, flexible particles with smooth or angular edges) (Pittura et al., 2023). In addition to the chemical composition, the density of these microplastics plays a crucial role in their recovery during extraction processes. The typical density ranges for these plastics are as follows: PE (0.89–0.98 g/cm^3), PP (0.83–0.92 g/cm^3), PS (1.04–1.1 g/cm^3), PET (0.96–1.45 g/cm^3), PVC (1.16–1.58 g/cm^3), PC (1.20–1.22 g/cm^3), and PMMA (1.09–1.20 g/cm^3) (Duis and Coors, 2016).

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Many studies have focused on determining the quantity, type and shapes of the MPs in various fertilizing products using different extraction methods, highlighting that MPs are a significant concern in fertilizing products. For example, Weithmann et al. (2018) identified 11–895 items of MPs in per kg dry compost, including 11 polymer types, such as PS, PE, PP PET,PU, PVC, with shapes including fragments, fibers and spheres. Zhang et al. (2020) reported that the abundance of MPs in the raw sewage sludge compost ranged between 200 and 420 items/kg, with flakes and fibers being the predominant morphotypes. Zhang et al. (2022) summarized findings from 102 organic fertilizer samples, noting that MPs in most organic fertilizers were primarily film-shaped with highly varied abundances ranging from ND (not detected) to 2550 items/kg.

To gain a deeper understanding of microplastics (MPs) in the selected fertilizers, this report presents optimized protocols and findings on the presence and prevalence of MPs in organic fertilizers derived from fishery waste. Considering the complex matrices of these fertilizers, the results mainly focus on identifying key characteristics such as polymer types, morphotypes, colors, and size of the MPs using modified extraction methods.

2 Materials and methods

2.1 Characteristics of the selected biobased fertilizers

Eight organic fertilizers were pre-selected out of 26 fertilizing products for further assessment as previously reported in D6.1. These biobased fertilizers (BBFs) (**Figure 1**) include three semi-liquid and five solid products. Three liquid BBFs were produced in slurry forms, in which UNI1 and UNI3 having viscous nature. CAT1 was produced from pure fish processing waste, including fish head, bones and viscera. Two pellet products, FSP and FMP, were generated from dried fish sludge, in which FMP were produced with the combination of blood meal, bone meal and sodium sulphate. BP and OA1 are in granular forms and plant components. The characterization and selection of the fertilizing products are reported in **D6.1**. Feedstock materials, product forms and their OM and DM are summarized in **Table 1**.

Eight BBFs have various characteristics, with a wide range of organic matter contents. Based on the feedstock, forms and components of the end products, eight BBFs were categorized into three subgroups for easier MPs investigation, 1) BBFs derived from purely fish processing waste: UNI1, UNI3, FER3 and CAT1; 2) BBFs derived from fish sludge (and mixture): FSP and FMP, and 3) BBFs generated from mixed waste including plant materials, showing complex nature in the end products: BP and OA1.

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Bokashi Pellet (BP) | **NPK solution with amino acid (FER3)** | **Amino acids and peptides (UNI1)** | **N fertilizer with chitin (UNI3)**

Protein fraction (CAT1) | **Fish sludge Pellet (FSP)** | **Fish mix Pellet (FMP)** | **Organic Amendment (OA1)**

Figure 1 Eight selected biobased fertilizers (BBFs) for microplastic analysis

Table 1 Fishery-waste derived potential organic fertilizers for microplastic analysis

BBFs	Form		Feedstock					Dry matter	Organic matter
			Fish processing waste	Fish sludge	Plant waste	Mineral	Other waste	%	%DM
BP	Solid	Granular	✓		✓	✓	✓	91	62
FER3	Semi-liquid	Slurry	✓					34	79
UNI1	Semi-liquid	Slurry	✓					42	82
UNI3	Semi-liquid	Slurry	✓					42	83
FSP	Solid	Pellet		✓				94	82
FMP	Solid	Pellet		✓		✓	✓	95	71
CAT1	Solid	Granular	✓					98	84
OA1	Solid	Granular		✓	✓	✓		46	42

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2.2 Extraction of microplastic particles

The MPs analysis consists of four main procedures: sample collection, MPs extraction, visual observation and characterization (Prata et al., 2024). Extraction of MPs from above-mentioned organic-rich BBFs is the most complex and challenging step. Based on previous studies, the selection of extraction methods depends on sample matrix. In summary, the extraction method involves density separation, organic digestion and filtration, which can be adapted and combined depending on the characteristics of BBFs. **Figure 2** shows the schematic workflow related to the extraction of MPs from BBFs. The BBFs included in this study were processed following extraction methods tailored to their specific characteristics.

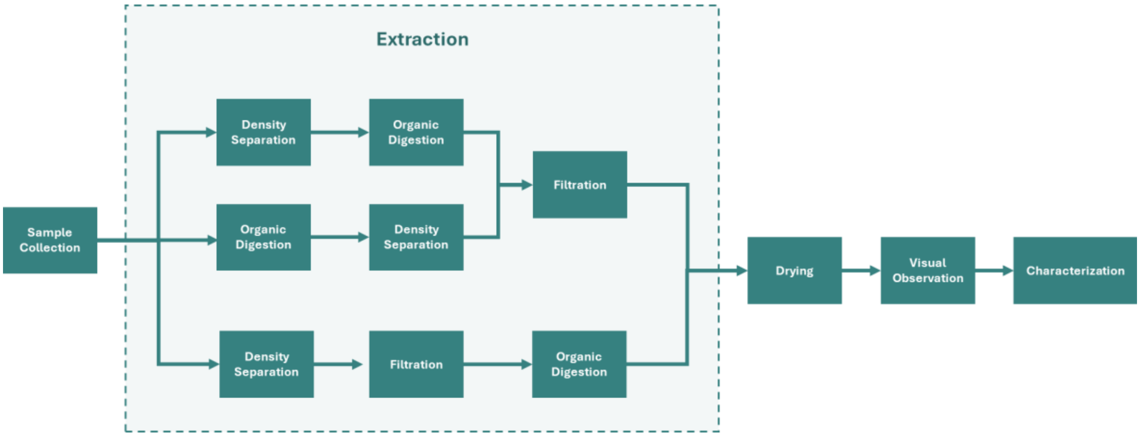

Figure 2 Schematic workflow of the microplastic analysis in BBFs

2.2.1 BBFs derived from fish processing waste: FER3, UNI1, UNI3 and CAT1

2 g samples of the liquid BBFs — FER3 and UNI1 — were digested with 50 mL of 15% H_2O_2 solution in a 200 ml glass bottle. To prevent lipid components from sticking to the glassware, 0.5 ml of Tween 20 (or Triton X-100) was added to each sample on the second day of digestion. Sonication was conducted for 5 min to accelerate the removal of the lipid residues from the glassware surface and to ensure better dispersity of the sample in the H_2O_2 solution. Triplicates (6 g) were involved for each BBF. All mixtures were agitated at 150 rpm for around 15 min, then digested at 40°C in the oven for 7 days. The glassware was covered with aluminium foil to prevent contamination during the digestion. After 7-day digestion, the digested samples were filtrated with 8 μm nitrocellulose membrane using vacuum filtration unit. The membranes were then transferred to the glass petri dishes and oven-dried at 40°C.

The liquid UNI3, containing recalcitrant chitin, was digested with 50 mL of 15% H_2O_2 solution and 0.5 mL of Triton X-100 in a 100 ml glass bottle. The digestion lasted 7 days in

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the water bath unit under fume hood. The samples were prepared by rinsing the glass holder and membrane thoroughly with ultrapure water. It should be noted that, due to the high ecotoxicity of Triton X-100, it must be used under a fume hood, and any used solution must be collected and disposed of in designated hazardous waste containers.

2 g of CAT1 was digested with 20 ml 30% H_2O_2 solution and 0.2 ml of Tween 20 in a petri dish. During the digestion, few drops from the 30% H_2O_2 solution were added to the petri dish after daily check of the digestion. Duplicate samples (4 g in total) were prepared for the digestion of CAT1. After one week of digestion, the bone structure residues were treated with 10-20 ml of 50% (*v/v*) nitric acid for 15 min. After the treatment, the residues left in the petri-dish was transferred and diluted with 10% Tween 20 solution prepared with ultrapure water into a beaker. It was followed by vacuum filtration using 8 µm nitrocellulose membrane. The membranes were then transferred to the glass petri dishes and oven-dried at 40°C.

2.2.2 BBFs derived from fish sludge: FSP and FMP

The pelletized sludge products — FSP and FMP — were gently ground using a pestle and mortal. 2 g of the ground samples were added to a 50 ml centrifuge tube containing 40-45 ml NaCl solution with a density of 1.2 g/cm^3. Triplicate samples (6 g in total) were utilized for each BBF. All prepared samples were shaken at 150 rpm for 20 min, followed by 20 min of centrifuge at 4000 rpm. Approximately 10-15 ml of the supernatant was collected in a glass beaker. The shaking and centrifuge of the samples, and collection of the supernatant were repeated three times to recover all MPs lighter than 1.2 g/cm^3. Afterwards, the collected supernatant was filtrated through a nitrocellulose membrane (pore size: 8 µm) with vacuum pump unit. Subsequently, the membrane was carefully transferred with metal tweezers to a glass petri dish, and then digested with 10 ml of 30% H_2O_2 solution at 40°C in the oven for two days. During the digestion, few drops from the 30% H_2O_2 solution were added to the petri dish after check. After two days of digestion, the petri dishes were left open for drying. Potential MPs were selected and sorted from the dried petri dishes under microscope and transferred to a trimmed nitrate acetate membrane (0.45 µm) sticked on a glass slide for further characterization. **Figure 3** shows the extraction steps of the FSP and FMP.

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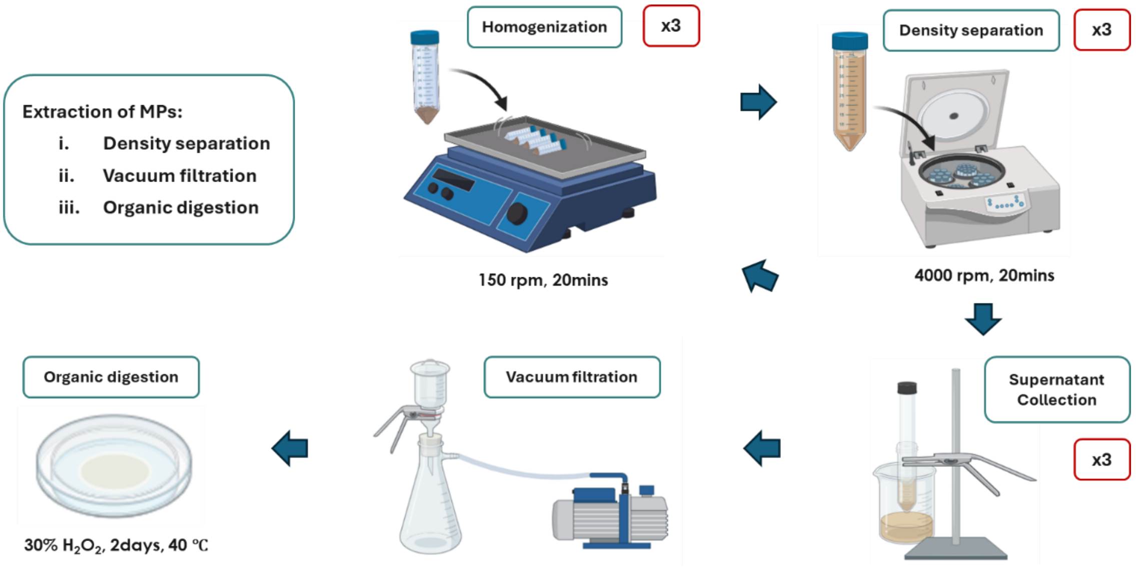

Figure 3 Schematic workflow of MPs extraction from FSP and FMP

2.2.3 BBFs derived from mixed waste stream: BP and OA1

The BP was gently ground using mortar and pestle before digestion. 2 g of the well-mixed ground BP sample was placed in a petri-dish (diameter: 20 cm) for digestion. Because of the mineral component within BP, intensive foaming was observed after adding few drops of 15% H_2O_2 solution in the pretrial. It showed that the more H_2O_2 solution and the higher concentration of the solution added, the more intensive reaction was observed. To avoid intensive reaction and risk of the sample loss due to the foam reaction, 5 ml of 15% H_2O_2 solution was added incrementally into the petri-dish. Samples were agitated at 150 rpm during the addition of H_2O_2 solution. H_2O_2 solution was only added when the intensive foaming reaction got mild. After adding 15% H_2O_2 solution for 4-5 times, the intensive reaction stopped, then 30-50 ml of 30% H_2O_2 solution was added into each petri-dish. The digestion lasted 5 days in the oven at 40°C. At the 4th day of the digestion, the petri dishes were left half-open to allow H_2O_2 solution to evaporate, minimizing its volume to less than 5 ml. The digested samples were then transferred to a glass beaker, and the petri dishes were thoroughly rinsed using NaCl solution (1.2 g/cm^3) to ensure complete sample recovery. The collected digested sample and NaCl solution were transferred to a 50 ml centrifuge tube, and centrifuged for 20 min at 4000 rpm. Approximately 10-15 ml of the supernatant was transferred to a clean glass beaker. The centrifuge and collection of supernatant procedures were repeated at least three times to recover all MPs lighter than 1.2 g/cm^3. Afterwards, MPs in the collected supernatant was extracted by sequential filtration. The filter stack was composed of four stainless steel filters and one nitrocellulose membrane. The pore sizes of the four stainless steel filters were 1 mm, 0.5 mm, 0.25 mm, and 0.15 mm, and the nitrocellulose membrane was with pore size at 8 μm, respectively. The filtration through the stainless steel filter with pore size at 0.15 mm and the nitrocellulose membrane were conducted via vacuum filtration unit. Afterwards, the residues on each stainless steel filter were flushed with ultrapure water and collected in separated petri dishes. The membrane was transferred to a clean petri dish. Duplicate samples (4 g on total) were prepared for MPs extraction from BP. For each sample, five petri-dishes were oven-dried at 40°C. Potential MPs in each petri dish were observed and sorted under microscope and transferred to a trimmed nitrate acetate membrane (0.45 μm) sticked on the glass slide for further characterization.

Finally, 2 g of OA1 was treated with 15% and 30% H_2O_2 solution as previously done for BP. After density separation with NaCl solution (1.2 g/cm^3), the collected supernatant was directly vacuum-filtrated with a nitrocellulose membrane (pore size: 8 μm). Then the membranes were carefully transferred to clean petri-dishes and oven-dried at 40°C. Potential MPs were observed and sorted under microscope for further characterization. Triplicate samples (6 g on total) were prepared for MPs extraction from OA1.

2.3 Characterization of extracted microplastic

2.3.1 Visual observation

Residual microliters on the membranes and in the petri dish were observed via stereomicroscope (GZ808-Optech) equipped with a digital camera (OPTIKAMB5) with maximum magnification of 65×. The suspected MPs were manually collected using a tweezer with fine tips and a mounted needle, then transferred to a trimmed cellulose acetate membrane (0.45 μm) glued on a microscope slide for further identification and quantification. All items on the slide were categorized based on their colors and shapes. The shape of the MPs was classified for fiber, film, and fragment. The size of the suspected MPs were measured on the basis of their largest dimension (Pittura et al., 2021).

2.3.2 Microplastic characterization

All suspected MPs collected on the cellulose acetate membrane were characterized through micro Fourier Transform Infrared Spectrometry (μFTIR) in attenuated total reflectance mode, using a Spotlight 200i FT-IR microscope system (PerkinElmer) equipped with Spectrum Two and driven by Spectrum 10 software. Big resembling MPs (> 1mm) were identified with FTIR using the same software as μFTIR. Each sample spectrum was recorded by performing 32 accumulations after background scan. The spectral resolution was at 4 cm^{-1} and the spectrum was in range of 450-4000 cm^{-1}. The output spectra were then analyzed through a spectral search against the reference library of polymer spectra from the modified PerkinElmer database (Pittura et al., 2021). Following the comparison with the library, the suspected MPs were identified as plastic polymers

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when the match score exceeded 0.7 with the reference spectra. For spectra with lower matches (0.6-0.7), careful and detailed interpretation and examination of the peaks characteristics were conducted. The MPs abundance was confirmed and calculated by summing all MPs sorted from the total digested samples for each BBF after the identification of MPs.

2.4 Quality assurance and quality control

Before the experiments, the experimental benches were thoroughly cleaned with ethanol and deionized water. Additionally, cotton lab coats and nitrile gloves were used throughout the experiments. Glassware or stainless-steel materials were used for sampling, processing and storage to avoid external MPs contamination. All open apparatuses were covered with aluminium foil. All glassware and other experimental materials were rinsed with deionized water and dried before commencement of the experiment. The prepared NaCl solution was pre-filtered thorough nitrate acetate membrane (0.45 μm) and stored in glass bottles. Procedural blanks were prepared by adding all used materials without sample to monitor potential contamination. Air blanks were added when processing each step and checked consequently to eliminate the potential contamination due to MPs deposition from air. When transferring, glassware and metal materials were rinsed with ultrapure water or corresponding solutions at least three times to ensure all MPs were collected.

This study focused on the most abundant plastic types in the market, using both primary and secondary PP, PE and PS microplastic particles (MPs) as spiked reference for quality control. In this study, secondary PLA MPs were also included in the extraction process as reference due to the increasing PLA production and application. For extraction without density separation, seven pieces of MPs shaped as fragments were spiked into each replicate to assess the impacts of digestion, including three primary MPs (PP, PS and PE) from commercial plastic production, and four secondary MPs (PP, PS, PE, and PLA) from environmental plastic waste for quality control. For the extraction requiring density separation, 10 pieces of MPs were included as reference, comprising 7 pieces of PP, PS, PE and PLA prepared as described before, along with one film-shaped PE, one film-shaped PP, and 1 PET fragment (**Figure 4**). The size of the MPs ranged from 0.5 to 1.5 mm. All secondary MPs were characterized using FTIR and selected as reference materials only when the match score exceeded 0.8 before being spiking to the samples.

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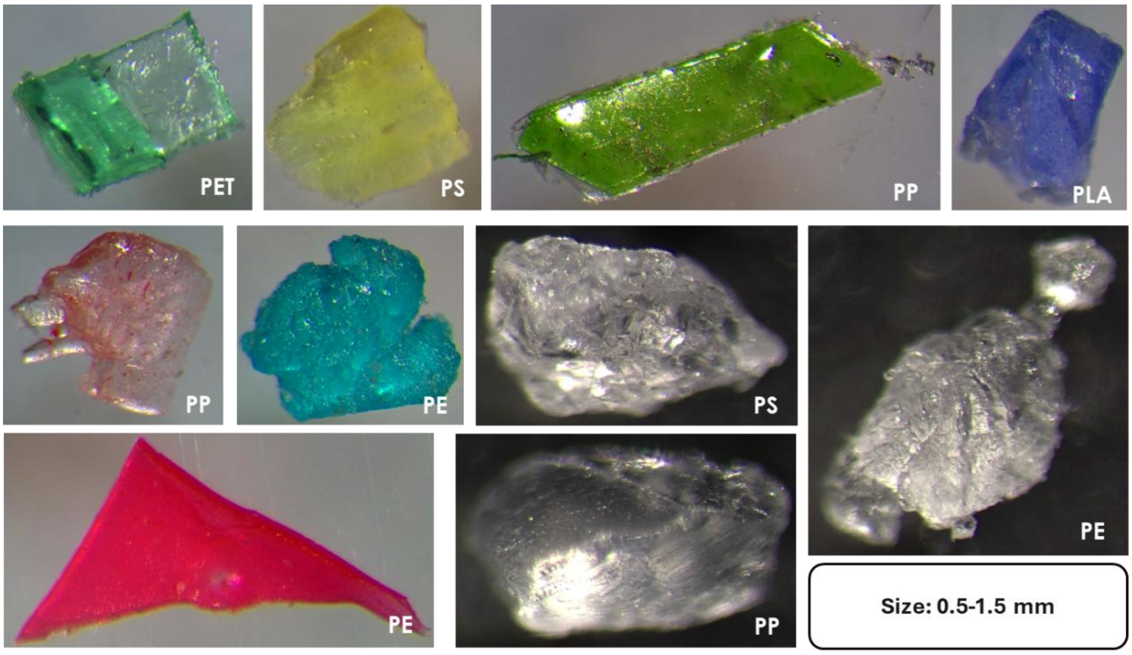

Figure 4 Examples of the spiked reference and size range

2.5 Calculations

The spiked MPs references were to establish a recovery experiment to verify the reliability of the MPs extraction method from samples. The calculation of the recovery of the spiked MPs is listed as follow:

$$R(\%) = \left(\frac{Recovered\ number\ of\ spiked\ MPs}{Total\ number\ of\ spiked\ MPs}\right) \times 100\%$$

The abundance of the identified MPs in each BBF were calculated by summing all MPs sorted from the total digested samples for each BBF and converted to MPs per kg of BBF:

$$MPs\ abudance\ of\ the\ BBF\ (MPs/kg) = \frac{Sum\ items\ of\ the\ identified\ MPs}{Total\ mass\ of\ the\ BBF\ (g)} \times \frac{1000g}{kg}$$

3 Results and discussion

3.1 Recovery of the spiked references

The spiked references were sorted out from the dried samples under microscope. For liquid BBFs — FER3, UNI1, UNI3 — as well as CAT1, 7 spiked reference were applied for quality control, while the other BBFs with density separation step 10 spiked references were added to each sample (For more details, see section 2.4). For solid CAT1, around 75% of the spiked MPs were recovered, most probably due to the abundant lipid residues. For liquid BBFs, FER3 and UNI1, processed with surfactant Tween 20 in organic digestion,

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the recovery of spiked reference ranged from 93%-100%. It indicated that the usage of Tween 20 was an efficient method for some lipid-rich BBFs, which can not only remove the lipid residues from the glass surface but also ensure the recovery of the MPs within the sample.

However, liquid BBFs derived from animal by-products via different technologies have complex components, which can influence the digestion efficiency and filtration process. For UNI3, using Tween 20 as surfactant was unsuccessful. The filtration process took a long time (> 3h). A thick and whiteish layer remained on the membrane made visual observation and sorting impossible. Instead, Triton-X 100 was used during the digestion, which is known for its better performance in lipid emulsification. After one week of digestion, the filtration of the UNI3 samples were faster (<1h), leaving a thinner layer of residues remained on the membrane. The recovery ratio of spiked references was around 71%, which may be attributed to the complexity of the UNI3's components, e.g., recalcitrant chitin, making the recovery process more challenging. Further investigation could focus on enhancing the digestion method by incorporating new surfactants or using new identification techniques, like pyrolysis GCMS, to address this issue.

Table 2 Recovery of the spiked references

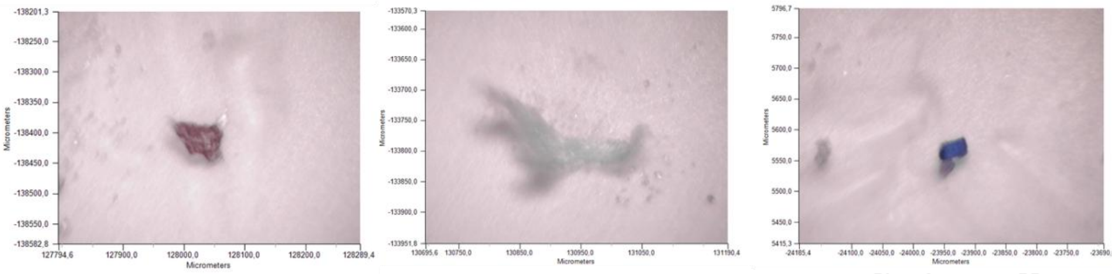
BBF	Extraction method	Recovery/%
FER3	Organic digestion + Vacuum filtration	96%
UNI1	Organic digestion + Vacuum filtration	93%
UNI3	Organic digestion + Vacuum filtration	71%
CAT1	Organic digestion + Acid addition + Vacuum filtration	75%
FSP	Density separation + Vacuum filtration + Organic digestion	78%
FMP	Density separation + Vacuum filtration + Organic digestion	70%
BP	Organic digestion + Density separation + Sequential filtration	70%
OA1	Organic digestion + Density separation + Vacuum filtration	73%

For the fish sludge-derived BBFs, FSP resulted in 78% spiked reference recovered, while FMP had 75% recovery. Theoretically, NaCl solution (1.2 g/cm^3) used for density separation was only able to recover MPs lighter than 1.2 g/cm^3. In this case, PET with heavier density (typical range: 0.96-1.45 g/cm^3) and PLA with typical density around 1.24 g/cm^3 cannot be recovered from the density separation. Apart from PET and PLA, the recovery of MPs < 1.2 g/cm^3 turned to be 97% and 88% for FSP and FMP, respectively. It indicated that the recovery of MPs < 1.2 g/cm³ from FSP and FMP was successfully done by using saturated NaCl solution.

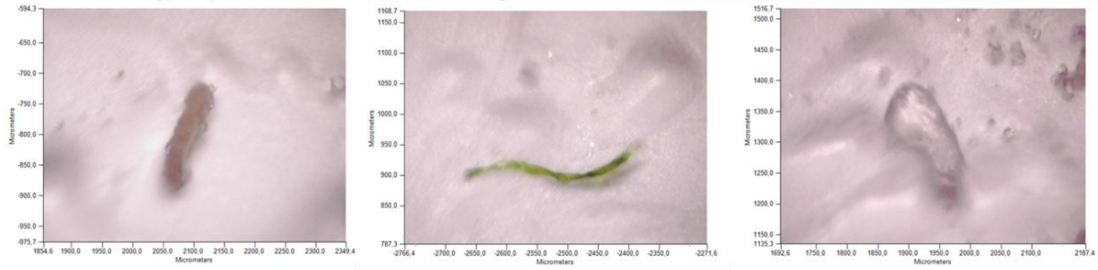
For BP, the spiked reference MPs were recovered from the filter stack. For the procedural blank, the spiked reference was 80% recovered except for PET and PLA, which were not presented in the supernatant after density separation with NaCl solution (1.2 g/cm³). While for the extraction, the recovery of the spiked reference resulted in around 7 out 8 being recovered, indicating the validation of the selected extraction method for MPs recovery from BP using saturated NaCl solution. For OA1, the recovery of the spiked MPs was around 73%. Similarly, apart from the heavier spiked reference PLA and PET, the recovery ratio was a good indicator for the extraction method.

3.2 Characterization of microplastic particles in BBFs derived from fish processing waste

The viscous UNI1 was mostly digested, leaving dried membranes with a whiteish color, which facilitated observation and sorting. The resembling MPs were mostly film-shaped, followed by fragment and fiber shapes, and appeared in various colors including black, transparent, light blue, green and brownish (**Figure 5**). Many fiber-shaped items turned out to be cellulose. To validate the digestion methodology, three digestions were performed, testing a total of 18 g of UNI1 sample. All recovered subsamples were sorted and characterized. The size range of the MPs were between 0.03 and 0.30 mm. Overall, the extracted MPs included 23 items of PE, 1 item of PET, 2 items of PP, 2 items of PA and 1 item of PU. The MPs abundance in UNI1 was calculated as approximately 1610 items/kg.



Grey, film, PE **Light blue, film, PE** **Blue, fragment, PP**



Brown, fragment, PU **Green, fiber, PA** **Transparent, fragment, PET**

Figure 5 Typical MPs extracted from UNI1

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After the extraction and drying processes for FER3, almost no sample residues were observed on the membranes with bare eyes, indicating high digestion efficiency of the organic matter in FER3. The MPs sorted from the membranes were primarily fiber-shaped, followed by film and fragment. All fibers sorted from the procedural blank were identified as cellulose. Only one brownish fiber was characterized as PET from the sample, while the other fibers were identified as cellulose. Additionally, one black fragment was characterized as PET, along with one transparent PA fragment, one grey PE film, and one transparent PP fragment. To eliminate the influence caused by the added spiked reference, the transparent PP fragment was excluded for MPs abundance in FER3. The size range of the MPs were approximately between 0.05 and 0.13 mm. In summary, 4 MPs were found in the tested FER3 sample, corresponding to a MPs abundance of around 670 items/kg.

For CAT1, the white-yellow lipid residues were carefully removed for easier observation and sorting after drying. Many fragments colored in dark green, green and yellow (**Figure 6**) were sorted for characterization. 10 dark green and green fragments were identified as polyurethane-styrene-isoprene copolymers, which are a type of thermoplastic elastomer. While one yellow fragment was identified as Polycyclohexylenedimethylene Terephthalate (PCT), which is a thermoplastic polyester with similar chemical structure to PET. Overall, 11 items of thermoplastic (size range: 0.23–1.24 mm) were detected in CAT1, corresponding to the MPs abundance of around 2750 items/kg.

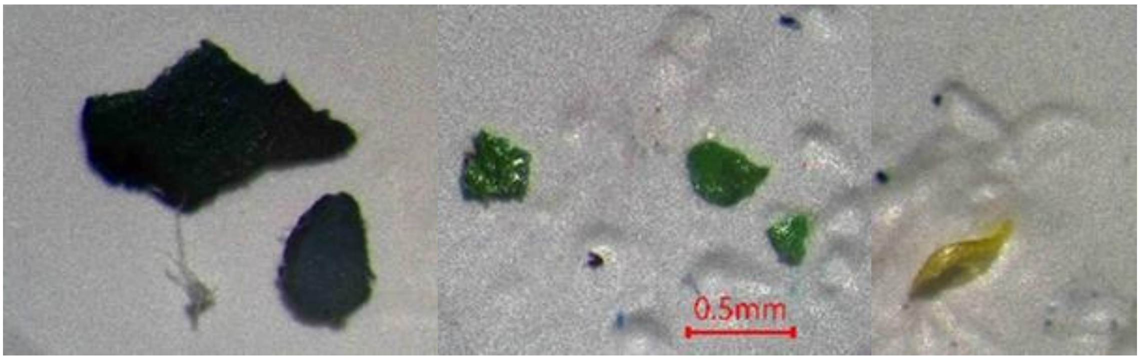

Figure 6 Dark green, green and yellow fragments sorted from digested CAT1samples.

3.3 Characterization of microplastic particles in BBFs derived from fish sludge

The ground FSP showed finer particles under microscope, while the ground FMP displayed coarser particles under microscope. Abundant white-transparent, line-shaped fragments were observed in ground FMP (**Figure 7**, A). At this stage, MPs with a density below 1.2 g/cm^3 were analyzed in both FSP and FMP (see **Figure 7**, B).

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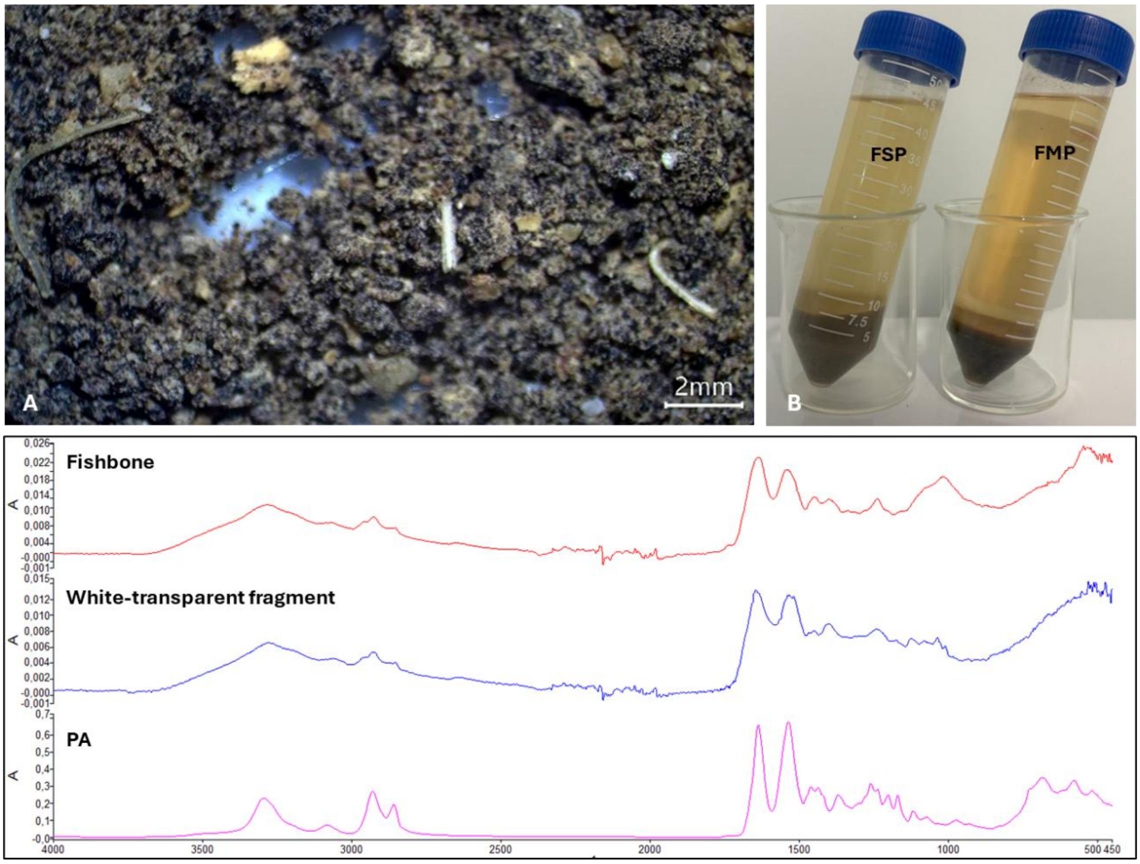

Figure 7 Fishbones in ground FMP (a), and (b): transparent supernatants of FSP and FMP after shaking and density separation in the NaCl Solution (1.2 g/cm^3); the spectra of fishbone, PA and white-transparent fragment sorted out from the digested FMP sample (c).

During the microscopic observation of ground FMP, a fragment emitted a strong odor when burned with a heated mounted needle. It occurred at a temperature considerably lower than the typical burning temperature of PA (400-500°C). The FTIR spectrum of the fragment closely resembled that of fishbone. Combining these, these fragments were identified as fishbones. Further confirmation came from the spectrum of a sorted white-transparent fragment after digestion, which was identical to the spectrum of the big fragment (**Figure 7**, C). Although fragment's spectrum showed a high match to PA, it also exhibited strong similarity to that of fishbone. After careful comparison, the fragment was characterized as fishbone, primarily due to the high abundance of fishbones in the FMP sample. The sorted MPs included one transparent film identified as PET, one blue fragment and three black film identified as PE, one orange-black PP fragment and one water-blue fragment characterized as PU. The size of the MPs ranged approximately between 0.05 mm and 0.87 mm. Overall, the tested FMP sample contained 7 items of MPs with a density below 1.2 g/cm^3, corresponding to the MPs abundance of approximately 1170 items/kg.

From the sorted resembling MPs from FSP, eight items of black MPs shaped in film or fragment were identified as PE. Additionally, two MPs in light blue and yellow, and shaped in film and fragment were identified as PP. The size of the sorted MPs ranged approximately between 90 µm and 1.04 mm. Overall, 10 items of MPs were detected in the tested FSP sample, corresponding to approximately 1670 items/kg.

3.4 Characterization of microplastic particles in BBFs derived from mixed waste streams

The MPs analysis of BP was much more challenging due to its mixed input and ash component. After density separation using NaCl solution (1.2 g/cm^3), mineral components, like quartz and heavy ash, retained from the waste streams were partially removed. The recalcitrant plant materials was not fully digested but their colors were faded for easier observation (**Figure 8**, A-C). The black particles were char-like materials with light density and fragile nature.

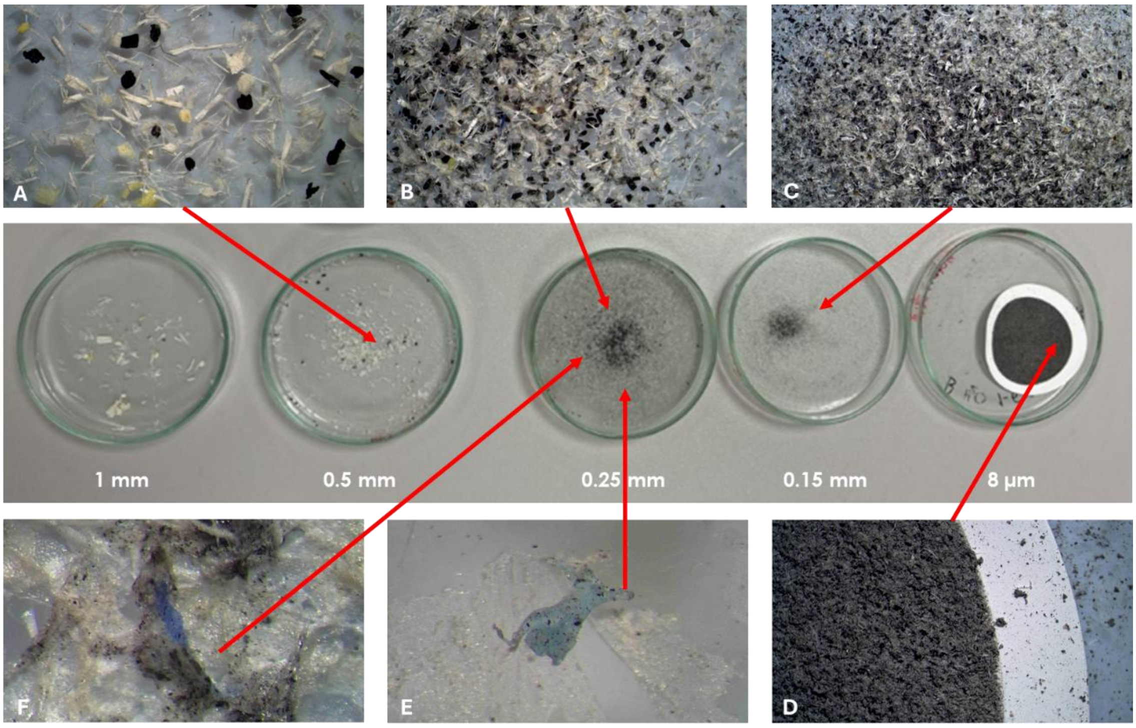

Figure 8 Organic residues retained on the stainless steel filters and membrane after digestion and sequential digestion. From left to right, petri dishes display organic residues collected on stainless steel filters with pore sizes of 1 mm, 0.5 mm, 0.25 mm, and 1 mm, as well as on an nitrocellulose membrane with an 8 µm pore size. Images A–C at the top are zoomed-in views with a magnification of 8×, while image D is a zoomed-in photo with a magnification of 10×. Images E and F provide examples of detected blue and light blue MPs observed within the collected organic residues on the 0.25 mm stainless steel filter.

When sorting the MPs, these particles were carefully removed for a better view and easier recognition of the MPs. However, the residues on the nitrocellulose membrane

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(pore size: 8 μm) showed as a dark-grey ash layer (**Figure 8**, D), which was not feasible for MPs sorting. In this case, the MPs determination was conducted on residues recovered from filters with pore size larger than 0.15 mm. Most of the resembling MPs were blue and shaped in fragment and film under the microscope (**Figure 8**, E and F). Eighty-eight darker blue fragments and films were characterized as Polyoxymethylene (POM), one light blue film was identified as PE, and three bright orange fragment-shaped MPs as PVC. The approximate size range of the MPs was 0.08–2.48 mm. In total, 92 MPs were detected in BP, corresponding to an approximate MPs abundance of 23,000 items/kg.

To enhance the sorting process in OA1, dried plant tissues with pale white and yellowish hues were carefully removed. The microplastics (MPs) recovered from the NaCl solution (1.2 g/cm^3) were predominantly film-shaped, followed by fragments and fibers. Two big pink fragments (size: 0.82 mm and 1.42 mm) were sorted out and characterized by FTIR, being identified as PE. Additionally, 14 MPs were identified as PE via μFTIR, colored in black, yellow and pink, and shaped in film and fragment. Two black fibers were identified as PA and two green fragments were identified as ethylene vinyl acetate (EVA). Additionally, two light brown sticky fragments were characterized as styrene vinyl toluene butyl acrylate copolymers. It is a synthetic polymer composed of different monomers, which gives it specific physical properties like flexibility, durability, and chemical resistance. The size range of smaller MPs analyzed by μFTIR were between 0.05 mm and 0.41 mm. In total, 16 items of MPs were detected in the tested OA1 sample, corresponding to the MPs abundance of approximately 3330 items/kg.

4 Conclusion

This study developed modified extraction methods tailored to the specific characteristics of bio-based fertilizers (BBFs). For lipid-rich liquid BBFs, using a small amount of surfactants during digestion can shorten treatment time and simplify the recovery of microplastics (MPs). However, further investigation is recommended to enhance its broader usability. The systematic integration of extraction steps—digestion, density separation, and filtration—facilitates an easier pre-treatment process before characterizing MPs. For solid samples containing mineral components, density separation effectively reduces the mass needing treatment. In samples with plant tissue, oxidation digestion can decolour lignin, making observation more straightforward. BBFs derived from complex waste materials require tailor-made extraction methods for effective MPs analysis.

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The analyzed BBFs exhibited high abundance and diverse types of MPs, ranging from approximately 670 to 23,000 items/kg sample, mainly composed of PE, PA and PET. These findings highlight the variability in MPs contamination across different fertilizers, highlighting the need for better waste separation in source and for further investigation of MPs in organic fertilizers derived from fishery waste.

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