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The power of centrifugation: How to extract microplastics from soil with high recovery and matrix removal efficiency

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A R T I C L E I N F O

Method name:
Centrifugation-based microplastic extraction technique from soil

Keywords:
Microplastics
Extraction
Density separation
Centrifugation
Environment
Complex matrices
Soil

A B S T R A C T

Understanding the occurrence and transformation of microplastics when released into the environment is essential for risk assessment. The use of biodegradable polymers in agriculture can help to reduce microplastic accumulation in soil, since released fragments of such materials are not persistent and are further transformed into CO_2 and biomass (Wohlleben et al., 2023). To be able to monitor the fragmentation and biodegradation of these materials in soil, a validated extraction protocol is needed, which does not induce changes in the chemical and particle properties, additionally it should show high recoveries and matrix removal efficiency. A density-based extraction method in the centrifuge has the potential to remove a high amount of the soil matrix and is very selective for the polymer at the same time. Here we developed an efficient and non-destructive extraction protocol for biodegradable fragments from different soils using sequential centrifugation steps with varying densities and a freezing approach for sample collection. Although the focus of the present study was on biodegradable fragments, the technique can also be used for other types of microplastics with similar or lower density than the one tested for the method validation, but additional recovery tests for the target analyte are recommended.

- A density-based extraction method for microplastics from soil, validated by recovery and stability tests using biodegradable polymers
- Vessel changes and harsh chemical treatments are kept to a minimum

Specifications table

Subject area:	Environmental Science
More specific subject area:	Biodegradable fragment extraction from soil
Name of your method:	Centrifugation-based microplastic extraction technique from soil
Name and reference of original method:	The extraction method described here was newly developed and has not been mentioned in literature before. Density separation techniques are commonly used for microplastic extractions, e.g. by Pfohl et al. [2]. and Claessens et al. [3].
Resource availability:	Ultracentrifuge (Beckman Coulter): https://www.beckman.de/centrifuges/ultracentrifuges Ultrasonication tip (Branson Digital Sonifier SFX 550): https://www.emerson.com/de-de/catalog/branson-sfx550-de-de Sodium polytungstate (TC-Tungsten Compounds): https://www.heavy-liquid.com/natriumpolywolframat/

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Method details

Materials and reagents

For protocol development a prelabeled biodegradable polyester blend (ecovio® PS1606, BASF SE, $\rho = 1.25$ g/cm^3) was used for visual inspection and for recovery tests with the final protocol. As described in our previous publication, the prelabeled blend was synthesized by compounding 3 kg of the biodegradable polyester with 3 g of the fluorescence dye Lumogen F Yellow 083 (BASF SE, 0.1 % in weight) at 180 °C on a mini-extruder Rheomex CTW 100 [1]. In the next step, the polymer granules were cryomilled using a Retsch ZM 200 ultra centrifugal mill. Cryomilling was performed using liquid nitrogen and a sieve size of 500 µm. The obtained powder was dried for 1 h at 40 °C.

For method validation 4 different types of standardized soils were tested: 3 sandy soils (LiHof C11, Lufa 2.2, Refesol 01-A) and 1 loamy soil (Lufa F6S). Table 1 lists relevant properties of the soils used in this study. The soils were selected to represent common agricultural soils. The focus of this study was on a comparison of sandy soils regarding matrix removal efficiency and microplastic recovery, but also a more complex and challenging loamy soil was in the scope.

Table 1
Information on soils used for method development.

Soil	Source	Soil type	pH	N [g/kg]	C_{org} [%]	Organic matter[a] [wt.-%]
LiHof C11	BASF Limburgerhof	Sandy loam	6.97	0.59	0.52	1.2
Lufa 2.2	LUFA Speyer	Sandy loam	5.60	0.18	1.61	3.4
Refesol 01-A	Fraunhofer IME	Sandy loam	5.61	0.97	0.93	2.1
Lufa F6S	LUFA Speyer	Clayey loam	7.21	0.16	1.75	6.1

[a] Determined using Thermogravimetric Analysis 20–550 °C, 20 K/min.

The final protocol requires the use of sodium polytungstate (SPT, here acquired from TC-Tungsten Compounds with a density of 3 g/cm^3), but also the use of sodium iodide (NaI, reinst, acquired from Bernd Kraft) was tested for the use in the presented protocol. With both salts densities of 1.8 g/cm^3 or higher can be achieved. To improve dispersibility of microplastic and soil particles the surfactant Lutensol TO7 (BASF SE) was added to the solution.

The presented extraction technique was developed using a Beckman Coulter ultracentrifuge Optima XL-I with swing-out rotor (6 × 38 mL capacity for open top tubes) and 70k rpm maximum.

Preparatory

The final microplastic extraction protocol consists of several sequential steps and concerns homogenization and deagglomeration, as well as density separation. Due to the low amount of organics contained in the tested soils, and the successful homogenization and density separation leading to a high matrix removal efficiency, an additional oxidation step was not needed. The total volume of the centrifuge vials (polypropylene, Beckman Coulter, 89 mm height, 24 mm inner diameter) used for this protocol is 38 mL and each vial can extract microplastics from 2 g of soil. The centrifuge rotor used can hold 6 buckets containing each one vial. This means that per centrifuge run microplastics from 12 g of soil can be extracted.

In the following the extraction steps (Fig. 1) are listed and explained in detail:

(1) To each 38 mL PP centrifuge vial 2 g of soil is added (either with or without microplastics), as well as one droplet of the surfactant. A pre-prepared stock solution of SPT ($\rho = 1.8$ g/cm^3) is used to fill the vial up to 15 mL total volume. The high density was chosen because it was found, that the microplastic particle loss in the sediment was lower with higher densities (see method validation section). Then the sample is treated with ultrasonication for 1 min, 40 %, directly in the centrifuge vial (see graphical abstract: a holder for the centrifuge vial was 3D printed at BASF SE, ice water in a glass beaker is used to cool the sample during ultrasonication). Since the sonication process may generate fragments from the PP centrifuge vial, the present method is not advised for the analysis of PP microplastics [4].

(2) After ultrasonication, the vials are filled up with the 1.8 g/cm^3 SPT stock solution to a total volume of 35 mL and are then centrifuged for 1 h at 30k rpm (222k rcf), 25 °C. The relatively short time does not allow for the formation of a density gradient. The centrifuged vials are carefully transferred into the freezer (−20 °C) where they are stored and frozen overnight.

(3) The next day the frozen vials can be cut to separate the sediment from the microplastics. To avoid contamination of the final sample by PP microplastics, that might be formed during cutting of the PP vial, a special cutting technique was developed (Fig. 1): (a) A small part of the vial bottom is cut, (b) a glass push rod is used to push the frozen column out of the vial, (c) the upper 2 cm of the frozen column (~ 8 mL) can be cut without touching the PP vial and are, with the aid of a metal funnel, transferred into a Schottky bottle and set aside for the next purification steps. The metal funnel is flushed with additional 20 mL of SPT ($\rho = 1.8$ g/cm^3), (d) the middle part of the column is pushed out of the vial, cut and discarded, (e) the sediment is pushed out of the vial, and is retained for a second centrifugation (step 4), whereas the lowest layer that was in touch with the bottom of the centrifuge vial (approximately 5 mm of height) where the hole for introduction of the glass push rod was drilled, is discarded.

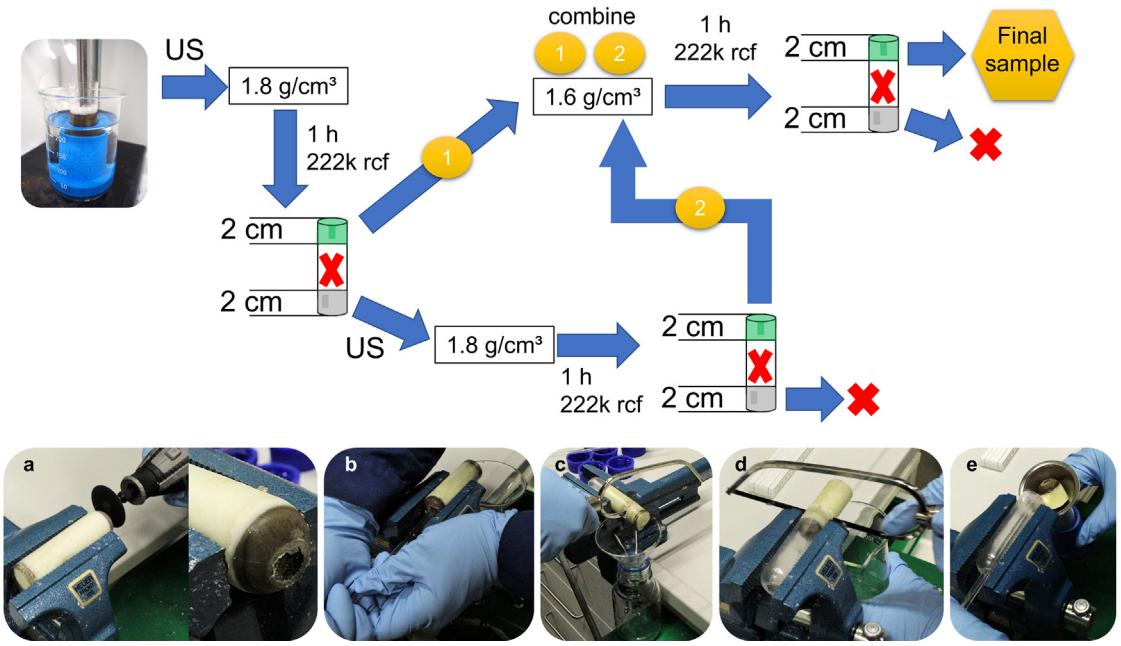

Fig. 1. Schematic overview of the centrifugation-based extraction. A-e: Steps applied in the cutting technique for the soil extraction method. Steps a-e are described in detail in the preparatory section.

(4) The sediment from the first centrifugation step is added to a new centrifugation vial, filled up again with 1.8 g/cm^3 SPT stock solution, ultrasonicated for 1 min, 40 %, centrifuged for 1 h at 30k rpm (222k rcf), frozen and cut. This time the entire sediment is discarded and the upper 2 cm containing microplastics are set aside for the next step. This second centrifugation step of the sediment was needed to extract remaining microplastics that have been trapped in the sediment in the first centrifugation step due to the fast settlement of soil inorganics.

(5) The supernatants from both centrifugation steps are combined (~56 mL) and additional 20 mL of DI water are added, resulting in a final density of 1.6 g/cm^3. The solution is transferred into two new centrifugation vials and the glass vessel in which the collected supernatants were contained is set aside to be used as final glass vessel after extraction is completed (to avoid particle loss due to sample transfer).

(6) After another centrifugation step (1 h, 30k rpm, 222k rcf) and freezing overnight, the supernatants are cut and combined to form the final sample for microplastic analysis. The centrifugation step with a lower SPT density is needed to remove part of the soil components that are floating with the microplastics using the 1.8 g/cm^3 SPT solution.

Method validation

The presented protocol was developed with focus on biodegradable polymer fragments, which are usually prone to chemical degradation [5]. However, any type of microplastic with density below 1.25 g/cm^3 (hence including a range of environmentally relevant plastics) should show a similar recovery for the particle sizes investigated here. Within the scope of this study, several tests were carried out to validate the presented extraction protocol for biodegradable fragments from soil:

(1) *Particle and polymer stability tests in NaI and SPT salt solutions*:
Any microplastic extraction protocol should be non-destructive to polymer and particle properties [5]. The presented protocol works with mild conditions, since no harsh chemical treatments are included. Still, it is important to check the polymer and particle stability in the high-density salt solutions. During method development, SPT and NaI were tested to be used in the centrifuge, which is why stability tests were conducted for both solutions. However, it was found that freezing of NaI solutions after centrifugation is not possible and consequently SPT was chosen for the final protocol. For the stability tests the biodegradable polyester blend was incubated for 2 h in the salt solutions (SPT: 1.8 g/cm^3, NaI: 1.6 g/cm^3), then filtered, washed with ultrapure water, dried at 58 °C and analyzed by gel permeation chromatography (GPC; molar mass distribution) and Fraunhofer light scattering (particle size distribution). The results were compared to the pristine material. Method descriptions are provided in our previous study [5]. Depending on the research question (e.g., analysis of specific chemical properties of environmental microplastics) the use of additional analytical techniques is recommended. More suggestions are provided in our previous publication on microplastic stability testing [5]. Fig. 2 clearly shows that the treatment did not induce any changes in the molecular weight and particle size distribution of the tested biodegradable polymer blend. Stability tests for other types of microplastics were out of scope for this study but should be performed for each target analyte of interest.

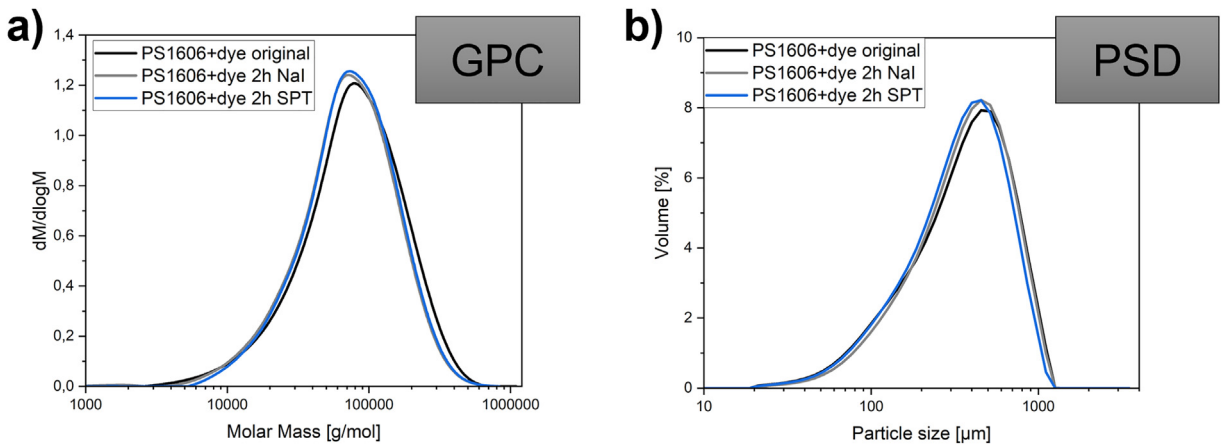

Fig. 2. Results from stability tests of biodegradable fragments in different salt solutions; (a) molar mass distributions and (b) particle size distributions before and after incubation.

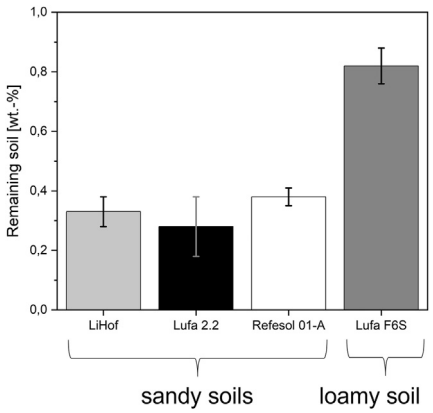

Fig. 3. Matrix removal efficiency with the presented extraction protocol for three different types of sandy soils and one loamy soil. Experiments were carried out in duplicates.

(2) *Matrix removal efficiency*:
The matrix removal efficiency of this protocol was tested for three different sandy soils and one loamy soil to explore its applicability to different soils. Matrix removal efficiency was assessed by determining the blank soil mass before and after applying the extraction protocol. Experiments were carried out in duplicates. Fig. 3 shows the results of the assessment: The matrix removal efficiency was comparable for the sandy soils with less than 0.4 wt.-% of initial soil mass remaining after extraction. For the loamy soil a slightly lower matrix removal efficiency was found with around 0.8 wt.-% of initial soil mass remaining.

(3) *Fragment recovery tests using a biodegradable polymer powder*:
For method development, it was first checked, if any microplastic loss in the sediment would occur during centrifugation. For this purpose, we compared centrifugation with 1.6 g/cm^3 SPT solution to centrifugation with 1.8 g/cm^3 SPT solution and found 168 (1.6 g/cm^3) and 51 (1.8 g/cm^3) microplastic particles per gram LiHof C11 soil in the sediment using fluorescence microscopy for the analysis (the method description can be found in our previous study [1]). The original spiking was 50 mg biodegradable fragments in 2 g of LiHof C11 soil, corresponding to about 4500–5000 particles by using the known size distribution and polymer density for calculation, assuming spherical particles. This assessment, showing losses on the order of only 2.0–2.3 % (1.8 g/cm^3), shows that the centrifugation is efficient, especially with the higher SPT density, but still single particles are lost in the sediment, which is why the second purification step for the first sediment was included in the protocol. To check if a specific size class would likely get lost during extraction, we also analyzed the particle size distribution of the biodegradable fragments before and after extraction with the aid of fluorescence microscopy (Fig. 4). The particle size distribution was comparable to the pristine material before extraction, but we also found that difficulties in homogeneous sampling for the fluorescence analysis led to large standard deviations in the smallest size bins.

To determine the microplastic recovery, the cryomilled polymer powder was added to 2 g of soil and intensively mixed into the soil by using a spatula. For real world samples it is recommended to use a mixing device (e.g., TurbulaSystem Schatz T2F [1])

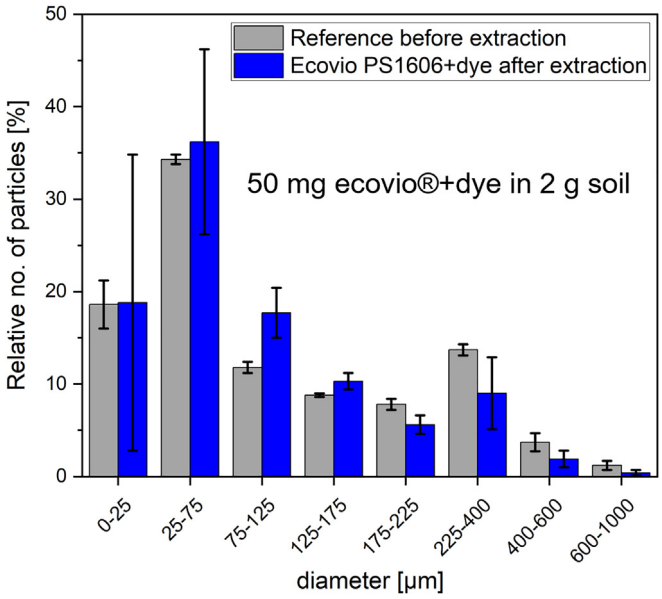

Fig. 4. Particle size distribution of biodegradable fragments before and after extraction from soil using the presented protocol. Experiments were carried out in duplicates.

for homogenization and subsampling. Soil agglomerates are broken apart during the ultrasonication step. Two different approaches were used for spike recovery tests:

(a) In the first approach 50 mg of the blend was spiked into 2 g of soil and extracted. The final dispersion was filtered over a 1 µm glass fiber filter and the mass was determined. This mass includes soil residue and microplastics, which is why additionally, each soil blank was extracted without intentionally added microplastics and its mass was subtracted from the mass of the spiked and extracted soil sample to determine the recovery. Potentially, single microplastic fragments could be contained in the blank soils. By extracting the blanks, we could also show, that the mass of spiked polymer was approximately 3 (Lufa F6S) to 8 (Lufa 2.2) times higher than the blank mass of extracted soil (compare to Fig. 3). This approach was done for all soils investigated in duplicates.

(b) In the second approach (trace analysis) 20 fluorescent, biodegradable polymer particles > 350 µm and 20 fluorescent, biodegradable polymer particles < 350 µm were counted using a light microscope and a UV-LED flashlight (NITECORE CHAMELEON CU6) and spiked into 2 g of soil (LiHof C11). Due to the unique color and fluorescence of the spiked fragments they could easily be distinguished from soil residue or from potentially contained microplastic fragments in the blank soils. After extraction the whole extract was filtered over 3 filters (PTFE, 0.45 µm, 13 mm diameter) and the particles were counted again with the aid of light microscopy and the UV-LED flashlights. Pictures of the filters showing the fluorescent particles were taken (Fig. 5b). The fluorescent label enabled a distinct visual identification of the spiked and extracted fragments.

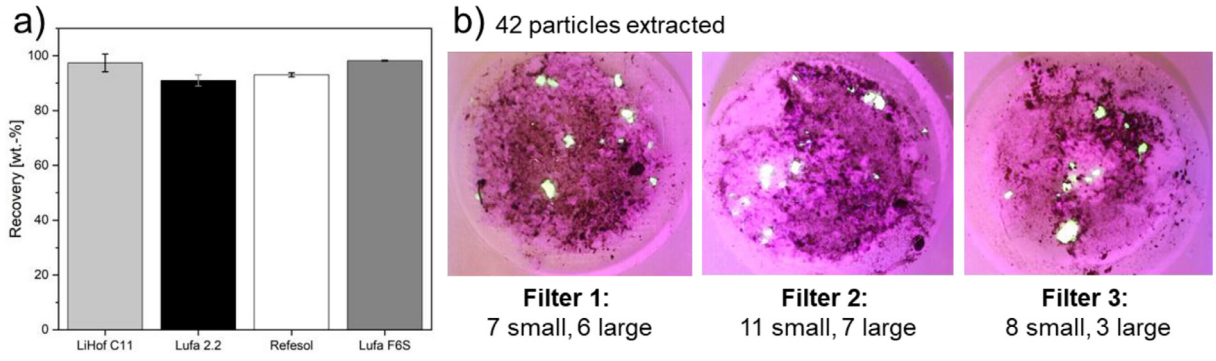

Fig. 5. Recovery test results for biodegradable fragments from soil; (a) mass-based approach applied to different soils, (b) counting approach applied to LiHof C11.

The results for the mass-based analysis are shown in Fig. 5a: For all the soils tested the microplastic recovery was higher than 90 wt.-%, which is in the upper range of recoveries commonly reported in literature [6–8]. The results for the counting approach are shown in Fig. 5b: 42 particles were counted after extraction, which is more than the originally spiked 40 particles. More smaller particles than originally spiked were counted, but at the same time less larger particles than originally spiked. However, it must be mentioned that during the first counting it was not always clear if one large particle might also consist of two or more small particles attached to each other. Overall, these results show the high potential of the presented extraction technique for microplastic extraction from soil yielding high microplastic recoveries without substantial losses. Assuming an average particle size of 100 µm for the smaller particles, the limit of detection would be 10 particles per gram soil and, after conversion into mass (assuming a particle volume of $V = \frac{1}{6} \cdot \pi \cdot d^3 \cdot \frac{1}{\sqrt{3}}$), 3.8 µg per gram soil, which is comparable to the lower detection limit determined by Li et al. via pyr-GCMS [8]. In contrast to pyr-GCMS, the analysis by extraction and microscopy provides not only the polymer concentration but also the particle size distribution.

Ethics statements

Not relevant to this work.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

All authors are employees of BASF, a company producing and marketing polymers, including biodegradable plastics.

CRediT authorship contribution statement

Patrizia Pfohl: Supervision, Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Christian Roth:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Wendel Wohlleben:** Supervision, Conceptualization, Methodology, Project administration, Writing – review & editing.

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