Environmental Sciences Europe

RESEARCH

Open Access

Oil-based extraction as an efficient method for the quantification of microplastics in environmental samples

Nina Lekše[1*], Andreja Žgajnar Gotvajn[1], Marija Zupančič[1] and Tjaša Griessler Bulc[2,3]

Abstract

Background Wastewater treatment plant outlets are a major source of microplastics, with more than 90% retained in sewage sludge. No standardised method for the extraction, quantification, and characterisation of microplastics in sewage sludge or soil exists, and direct comparison of studies is often impossible. Our aim was to validate oil extraction efficiency with and without pre-treatment with Fenton's reagent of selected microplastics in various types of environmental samples (sewage sludge and organic-rich substrates).

Results Oxidation with Fenton's reagent removed up to 90% of organic material, which improves the recovery rate and made quantification and characterisation easier and more reliable, regardless of type, shape, size, or density of the selected microplastic particles used in this study. Pre-treatment, as a pre-step of the oil extraction method, was shown to be important in reducing organic matter in all environmental samples, including sewage sludge and organic-rich substrates. It also improved the reliability of the selected method, shortened its duration, and, by reducing organic matter, made extracted microplastics more visible. The recovery rate was better for particles 1–5 mm and lower for particles 0.1 < 1 mm.

Conclusions By achieving up to a 100% recovery rate for certain types of microplastics (polypropylene and polystyrene), the selected method proved to be a promising extraction method. It was also shown to be efficient in the organic-rich substrates, for which the characterisation of microplastic particles was done by Fourier transform infrared spectroscopy. The most commonly detected types of microplastics in organic-rich substrates were polyethylene, polypropylene, polystyrene and polyester.

Keywords Characterisation of microplastics, Oil extraction, Quantification, Sewage sludge, Organic-rich substrate

Background

In recent years, interest in microplastics (MPs) research has been growing, mostly because the concentration of plastics in the environment is increasing [1, 2]. MPs, with high specific surface area, can adsorb contaminants of emerging concerns (CEC), such as potentially toxic elements, pesticides, and bisphenols, among others, and at the same time also contain various pollutants added during production, mostly to improve the flexibility and other characteristics of plastic materials [3, 4]. Once in the environment, MPs can release CEC [5] into different matrixes, causing potential environmental and health risks. MPs impact the growth of

*Correspondence:
Nina Lekše
nina.lekse11@gmail.com
[1] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna Pot 113, 1000 Ljubljana, Slovenia
[2] Faculty of Health Sciences, University of Ljubljana, Zdravstvena Pot 5, 1000 Ljubljana, Slovenia
[3] Faculty of Civil and Geodetic Engineering, University of Ljubljana, Jamova 2, 1000 Ljubljana, Slovenia

plants [6] and animals [7], are ingested by animals, and reach humans through the food chain or inhalation, raising serious concerns since the possible impact on human health remains unknown.

Currently, there is no uniform definition of MP sizes, although most of the literature defines MPs as particles of plastic material with a diameter less than 5 mm, as proposed by the National Oceanic and Atmospheric Administration (NOAA) [8]. New definitions of size characterisation have been proposed by several researchers. For example, Hartmann et al. [9] suggested that plastic particles from 1 to < 1000 nm should be categorised as 'nanoplastics', from 1 to < 1000 µm as 'microplastics', from 1 to < 10 mm as 'mesoplastics', and 1 cm and larger as 'macroplastics'. Because no uniform size definition exists, the extraction and identification methods are sometimes questionable and non-comparable.

Many studies have concluded that communal wastewater treatment plants (WWTP) outlets are a major source of MPs released into the environment [10–12]. The most common shapes of MPs found in WWTPs are pellets, microbeads, fragments, films, and foams [10, 11]. Via sewage, MPs enter WWTPs and, after treatment, can end up in the environment [12, 13]. However, municipal WWTPs have been found to be efficient in removing MPs, with the removal efficiency of MPs increasing after each treatment phase [12, 14–16]. Most bigger MP particles are removed in the pre-treatment units using screens and meshes, while smaller MPs are settled down during primary or secondary sedimentation. Further removal efficiency can also be increased in the tertiary treatment. The average efficiency of WWTPs is 90% but can reach up to 99.9% with tertiary treatment [12–17]. Studies have also shown that more than 90% of MPs are retained in sewage sludge (SS) [18]. The removal efficiency of MPs in WWTPs depends on the type of WWTPs, the level of treatment (primary, secondary, or tertiary), season, rainfall, and characteristics of communal wastewater [12, 19, 20]. Different types of MPs were detected in SS and varied from 1000 to 24,000 particles/kg [21, 22], even up to 113,000 particles/kg_{dw} [23]. The most commonly detected MPs types in SS were polyethylene (PE), polypropylene (PP) and polyamide (PA), followed by polyethylene terephthalate (PET), polyester (PES) and polystyrene (PS) in various shapes (films, fragments, films, lines, microbeads) [24].

The new European Union (EU) Wastewater Treatment Directive [25] and European Green Deal [26] aims for energy neutrality and circular economy by improving the existing SS management [27] and thus its reuse based on quality defined with new standards for micropollutants, including MPs and by quaternary treatment for potential micropollutant removal from wastewater.

MP extractions made in rich organic material, including SS, remain challenging and, due to people's constantly changing lifestyles, are also poorly controlled. Therefore, an efficient, fast, and reliable method for identifying MPs at individual process stages in the SS treatment process is needed to facilitate a decision support system for efficient SS management.

The lack of standard sampling methods for detecting MPs requires attention since there is still no standardised method for the extraction, quantification, and characterisation of MPs in SS or soil, and direct comparison of studies is often impossible or highly difficult [28]. Methods of MP quantification can be summarised in the following steps: (1) sampling; (2) pre-treatment; (3) extraction; (4) quantification; and (5) identification [29]. According to research results [30, 31], different sample volumes, pre-treatment methods, extraction, quantification, and characterisation methods were used [32]; the size of extracted MPs remains a limitation of extraction methods. The standard MP quantification and identification protocol includes all types of MPs, regardless of their size or density. Organic material in environmental samples, microorganisms and extracellular polymeric substances may interfere with MP extraction and identification [30]. This is why the pre-treatment of those samples is necessary before further analysis. Most researchers focused on the pre-treatment of samples to remove organic material. Most methods take days or even weeks to digest organic material, which may also cause the degradation of polymers. Fenton's oxidation seems to be the most efficient time- and cost-effective pre-treatment method, without visual and chemical impact on MP properties [30, 33–35].

After removing organic material, an extraction method is followed. Numerous different extraction methods were performed by several researchers, using density separation, electrostatic separation [36], froth flotation [37], magnetic extraction [38] and others. Density separation is the most common method; a salt solution of a specific density is mixed with an SS sample to be settled down. Particles with lower densities tend to float to the surface and are later recovered by filtration. Oil extraction proved to be a cost-effective, easy, and efficient method due to the lipophilic characteristics of plastics [39]. Plastics, which are lipophilic, become hydrophobic and bound together in a water matrix. Scopetani et al. [40] used Fenton's reagent to remove organic material in soil and compost samples with an oil extraction method with custom-made polytetrafluoroethylene (PTFE) cylinders with removable caps and a piston. Cylinders, in which the mixture of sample, MPs and oil, after settling, were

frozen at -40 °C. After freezing, ice columns with oil layers were defrosted and filtered through glass microfibre filters. Crichton et al. [39] compared oil extraction and density separation using NaI and $CaCl_2$ to determine the recovery rate of MPs in aquatic sediments, for which oil extraction reached the highest recovery rate, up to 96.2% ± 2.2%. To our knowledge, the combination of Fenton's reagent and oil extraction method has not yet been used for SS samples.

We hypothesised that the combination of digestion with Fenton's reagent and oil extraction is an efficient method regardless of the shape and type of MPs in different SS samples (anaerobic sludge and sludge cake) and organic-rich substrates for agricultural application (peat substrate, treated sludge and mixture of both). Our aim was to validate the oil extraction efficiency of MPs with and without possible pre-treatment with Fenton's reagent in various types of environmental samples (anaerobic sludge and sludge cake). After validation to extract MPs with a previously validated method (oil extraction and Fenton's pre-treatment) in environmental samples (organic-rich substrates), the extracted MPs are characterised using the Fourier transform infrared (FTIR) method.

Materials and methods

Validation of pre-treatment and oil extraction recovery rate

The efficiency of the oil extraction procedure in combination with pre-treatment of SS samples was validated by comparing the recovery rate of the oil extraction procedure with and without pre-treatment of the samples spiked with MPs.

Spiking the sewage sludge with microplastics

For each experiment, MP particles, which differed in size, shape, type, and density (Additional file 1: Table S2, Fig. S1), were used to identify the impact on the extraction efficiency based on their characteristics. MPs were cut on smaller particles by hand and sieved on particles 0.1 < 1 mm and particles 1–5 mm by steel sieve. The densities of used MPs were determined using a certified glass pycnometer based on the principle of displaced liquid (by measuring the weight of an empty pycnometer, pycnometer filled with particles and pycnometer with added liquid of known density with and without particles).

The selected MPs were transferred into a 300 mL glass beaker with an SS sample (40 g of sludge cake and 150 mL of anaerobic sludge), 150 mL of deionised water was added to the sludge cake, and both samples were stirred at 600 rpm for 1 h until homogenisation with a magnetic stirrer (Velp Scientifica). Mixtures of larger and smaller MP particles with higher and lower densities were used, with average weights from 0.05 mg to 0.70 mg for particles from 0.1 mm to 1 mm and from 0.11 mg to 18.19 mg for particles sized from 1 to 5 mm (Additional file 1: Table S2); together 20 particles of each type and size of MP were used, from which 10 particles' sizes were 0.1 < 1 mm and 10 particles 1–5 mm. The MPs used were easily recognisable due to specific colour and shape and easily separated from other environmental particles already present in the SS samples (Additional file 1: Fig. S1).

Characteristics of sewage sludge (SS) samples

For each SS sample, we determined the moisture content and the organic material removal before and after digestion with Fenton's reagent according to APHA (2017) [41]. Three trials were tested for each sample, and average values of moisture content and the percentage of organic material removal were calculated (Additional file 1: Table S1).

Method's description for microplastics' extraction

Two different SS samples were used to determine the efficiency of the extraction method: (i) anaerobic sludge and (ii) sludge cake. MPs were spiked into SS samples to determine the recovery rate with and without pre-treatment.

Three repetitions were done for each type of SS sample in three trials.

Some researchers suggested drying samples before performing the extraction method [39, 42] to extract MPs from the soil easily. However, as investigated by Vermeiren et al. [43] and Lekše et al. [35], the drying of SS samples turned them into a solid agglomerate, which made it harder to extract MPs. Therefore, the SS samples were not dried before the extraction procedure.

To avoid contamination of the samples, nitrile gloves and 100% cotton clothes were used while treating the samples. Only glass and metal spoons were used, and the mixture was rinsed with deionised water before starting the experiment. Laboratory surfaces were wiped with cellulose tissue and ethanol each time after and prior to starting a new experiment.

Pre-treatment of sewage sludge (SS) samples SS samples contain a high amount of organic matter, microorganisms, and extracellular polymeric substances, which makes the extraction of MPs difficult due to high density and visibility [30]. To reduce high levels of organic material (Fig. 1) and to increase extraction efficiency, Fenton's reagent as pre-treatment of samples was used prior to the extraction procedure; 30 mL of Fenton's reagent (0.05 M) and 30 mL of 30% H_2O_2 was added to both SS samples of the same masses (see "Organic-rich substrates samples"

Fig. 1 Whatmann filter with (right) and without (left) pre-treatment with Fenton's reagent

section) and with spiked MPs. To avoid thermal reactions after adding Fenton's reagent, the temperature was regulated with an ice bath. An Fe(ll) solution was made of 3.6 g of iron (ll) sulphate heptahydrate ($FeSO_4{\cdot}7H_2O$), 250 mL of deionised water and 1 mL of sulfuric acid (H_2SO_4).

Oil extraction method Olive oil with a density of 912 kg m^{-3} was selected for the extraction of MPs according to the research results regarding the most appropriate oil for oil extraction (Fig. 2) [40].

After spiking of MPs with SS samples with a control addition of MPs, homogenisation, and digestion in the case of pre-treatment, samples were transferred from a glass beaker into 1 L separation glass funnel. Glass beaker was rinsed three times with deionized water to ensure that all content with MPs particles was transferred into glass funnel; 500 mL of deionised water was added and shaken by hand for 30 s. After mixing, 10 mL of olive oil was added, and to ensure homogenisation, the sample was again mixed with an aeration pump for 60 s. The funnel was sealed and shaken again by hand for 30 s to ensure contact between MPs and olive oil. The walls and lid of the funnel were rinsed with 200 mL of deionised water, and then the mixture was let to settle for 15–30 min. After settling, a lower layer with settled solids and supernatant was released from the funnel into another 1 L separation funnel. The upper oil layer was left in the funnel, and the procedure was repeated for the second and third times. All three oil layers, with captured MPs, were filtered through vacuum filtration with Whatmann filters GF/C, 47 mm. The lid and walls were rinsed with 100 mL of deionised water and 100 mL of ethanol (EtOH 96%) and filtered through Whatmann GF/C filters to remove oil layers from MPs particles. After adding ethanol, oil layer was easily filtered and only MPs particles remained on the filter. After filtration, the filters were carefully transferred to Petri dishes, half covered and left to dry at 37 ± 2 °C in an incubator. For each parallel, the oil extraction process was repeated three times to ensure a reliable recovery rate.

The effect of Fenton's reagent and ethanol used in vacuum filtration was investigated after the procedure, as described in Lekše et al. [35]. FTIR and micrographic examination of spiked MPs (0.1 < 1 mm) was performed to determine any changes on the surface and their impact on particle identification before and after pre-treatment of samples with Fenton's reagent and oil extraction.

Quantification and recovery rate determination

MP particles obtained by the oil extraction protocol were examined under an optical microscope (Olympus CX43 Biological Binocular LED Upright Microscope) under 4 × objectives (40 × magnification). The recovery rate was determined after counting the MP particles (mean ± SD).

Environmental samples without spiked microplastics

Organic-rich substrates samples

Four different environmental samples were used for the experiment: (i) peat substrate (raw sample, collected from the bag), (ii) peat substrate—control (peat fertilised with mineral fertilisers), and (iii) peat substrate—fertilised with treated sludge and (iv) treated sludge (activated sludge). For each organic-rich substrate sample, we determined the concentration of moisture content and the percentage of organic material removed before and after digestion with Fenton's reagent (Additional file 1: Table S1).

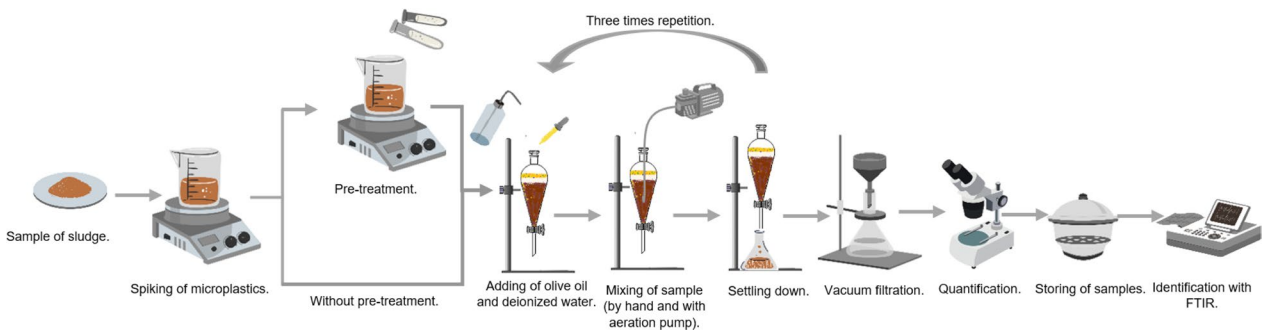


Fig. 2 Oil extraction method (FTIR means the Fourier transform infrared)

The description of the method for extraction of microplastics

Pre-treatment of organic-rich substrate samples (peat substrate, treated sludge and mixture of both) was done with Fenton's reagent (as described in "Pre-treatment of sewage sludge (SS) samples" section), followed by oil extraction method (described in "Oil extraction method" section).

Microplastic quantification

Whatmann filters GF/C, 47 mm were placed in Petri dishes and dried at 37 °C overnight in a Thermolyne (Type I42300) incubator and examined the following day under an Olympus CX43 Biological Binocular LED Upright microscope, which enabled identifying particles of size range between 0.1 < 1 mm.

Characterisation of environmental particles by Fourier transform infrared spectroscopy

After drying the MP particles and examination under a microscope, the extracted MP particles were saved in a desiccator until identification. The extracted particles were analysed using FTIR spectroscopy. Infrared spectra of the samples were recorded using a Perkin Elmer Spectrum Two FTIR Spectrometer equipped with Spectrum Two Universal ATR (Single Reflection Diamond). The wavenumber ranged from 4000 cm^{-1} to 400 cm^{-1} (resolution 2 cm^{-1}, 10 scans).

Results

Sewage sludge (SS) samples

Recovery rate of oil extraction procedure (samples with spiked addition of MPs)

The recovery rate was determined to validate the oil extraction procedure with and without pre-treatment with Fenton's reagent. Any other extracted particles not added by spiking and determined in the SS samples (anaerobic or sludge cake) were not included in the recovery rate calculation.

The anaerobic sludge When comparing spiked MP recovery rate with different spiked sizes (0.1 < 1 mm and 1–5 mm), densities, types, and shapes, with and without pre-treatment, it appears that the recovery rate was, on average, higher for MP particles with the size of 1–5 mm in comparison with MP particles with lower densities and with MP shapes of fragments.

The recovery rate of pre-treated samples with Fenton's reagent was up to 100.0% ± 0.0% (size 1–5 mm) for PP fragments, PS fragments and PA lines, while for other types of MPs, the recovery rates reached up to 95.0% ± 4.7% for PP pellets and up to 98.9% ± 3.3% for PET, PE, and HDPE fragments. It seems that the oil extraction method is not effective for polymers with higher densities, such as PVC and PA in the shape of pellets and microbeads, since their recovery rate was lower and reached up to 40.0% ± 11.2% for PA and 62.8% ± 4.3% for PVC. As shown in Fig. 3, the method's reliability increases after pre-treatment.

The recovery rate of particles 0.1 < 1 mm decreased compared to the recovery rate of particles of 1–5 mm. Pre-treatment of sludge samples increased the recovery rate and reached up to 100.0% ± 0.0% for fragments of PP and PS, while for other types reached up to 80.0% ± 7.4% (PP pellets) and up to 97.8% ± 4.3% for HDPE fragments. Particles of PA, with the highest density, shaped as microbeads with sizes 0.1 < 1 mm, reached a lower recovery rate, up to 50.6% ± 17.3% after pre-treatment. As shown in Fig. 4, the method's reliability also increases after the pre-treatment of sludge samples, except for PA microbeads, where reliability decreased by 4%.

The reliability of the oil extraction method generally increases for particles of 1–5 mm after pre-treatment and reaches up to 98.9% ± 3.3% for PP and PS fragments without and 100% ± 0.0% with pre-treatment. However, reliability is lower for particles 0.1 < 1 mm. It appears that the method is suitable for the determination of bigger particles of 1–5 mm, shaped as fragments, lines and PES fibres, while for particles 0.1 < 1 mm, the reliability of the method should be improved for polymers with higher densities and the shapes of microbeads and pellets.

The sludge cake The recovery rate significantly increased after pre-treatment with Fenton's reagent for particles of 1–5 mm for sludge cake (Fig. 5) for all types, densities, and shapes of spiked MPs. The recovery rate with included pre-treatment reached 92.3% ± 2.1% (for PET fragment) and up to 100.0% ± 0.0% (for PA lines) but reached low efficiency for polymers with higher densities of PVC (52.4% ± 6.4%) pellets and PA microbeads (55.2% ± 8.0%). The reliability of the combined method, with pre-treatment included, increased for all types of MPs.

As shown in Fig. 6, the efficiency of the combined procedure increases with pre-treatment included for all MP particle types, densities, and shapes of polymers with sizes 0.1 < 1 mm, although the recovery rate is lower than for particles 1–5 mm. The highest recovery rate was reached for all types of fragments, lines, and fibres, where the highest recovery rate was for PE (94.4% ± 6.8%), PA lines (93.7% ± 2.5%), and PES fibres (91.3% ± 5.3%). The reliability of the combined method with pre-treatment increased for all spiked MPs.

Recovery rate after each extraction phase

Since oil extraction was repeated three times (from the phase of shaking/aeration to the phase of vacuum filtration), calculations regarding each extraction phase were

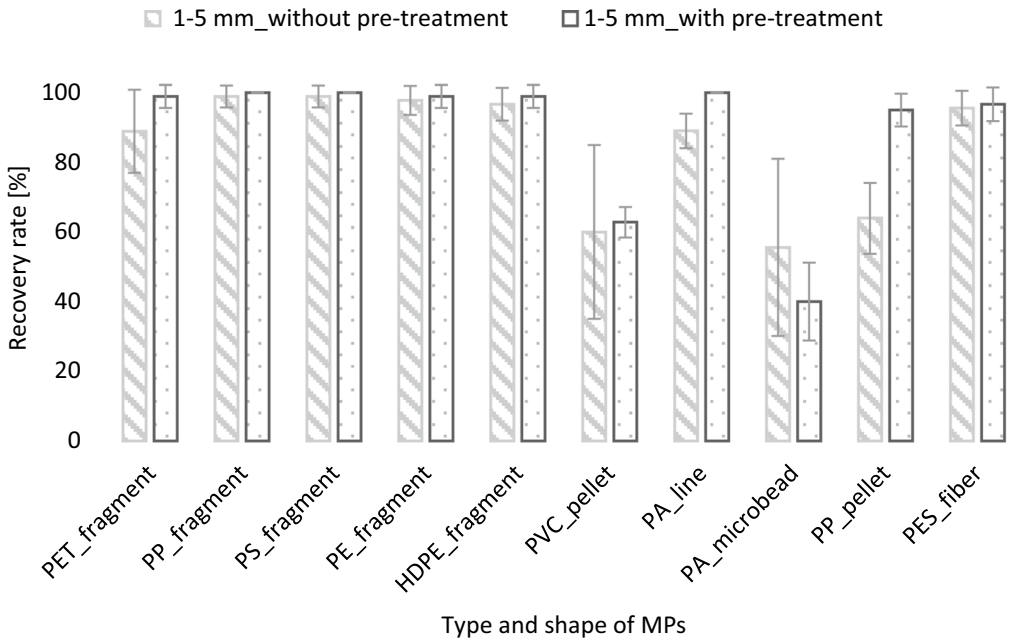


Fig. 3 Recovery rate (mean ± SD) of particles 1–5 mm without and with pre-treatment (No. of samples: 9)

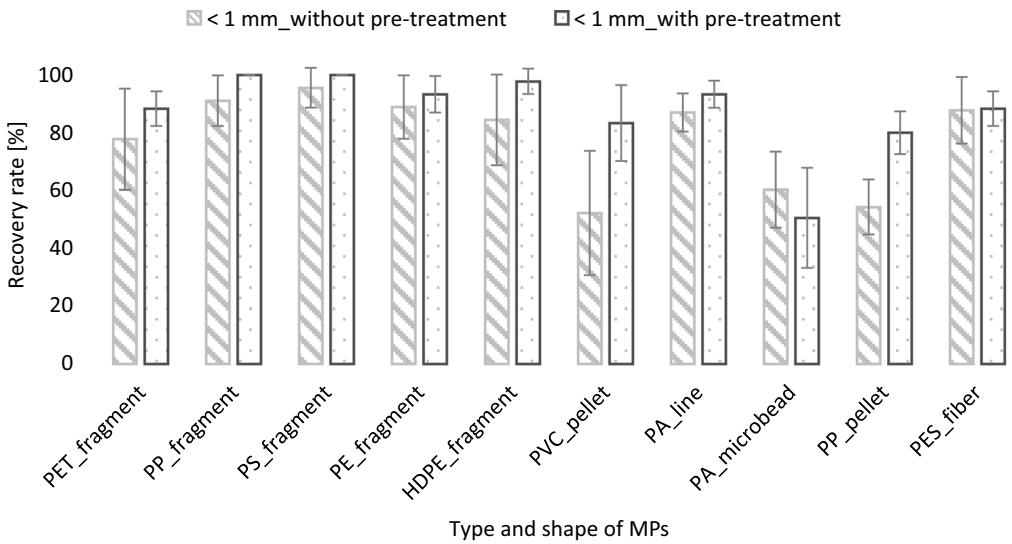


Fig. 4 Recovery rate (mean ± SD) of particles 0.1 < 1 mm without and with pre-treatment (No. of samples: 9)

made (Tables 1 and 2). The extraction efficiency after each phase increased, and it can be seen that extraction efficiency was higher for each phase at pre-treated samples, except for PA microbeads, for which the second and third phases of the extraction reached higher recovery rate without pre-treated samples for anaerobic sludge. The highest significant increase in the recovery rate of pre-treated samples in comparison to SS samples without pre-treatment was seen for PP pellets (up to 26% for particles 0.1 < 1 mm and 31% for particles of 1–5 mm for anaerobic sludge and 18% for particles 0.1 < 1 mm and 14% for particles of 1–5 mm), PVC pellets sizes of 0.1 < 1 mm (for up to 31% for anaerobic sludge and 21% for sludge cake) and PET fragments (for up to 39% for particles of 0.1 < 1 mm and 52% for particles of 1–5 mm for sludge cake).

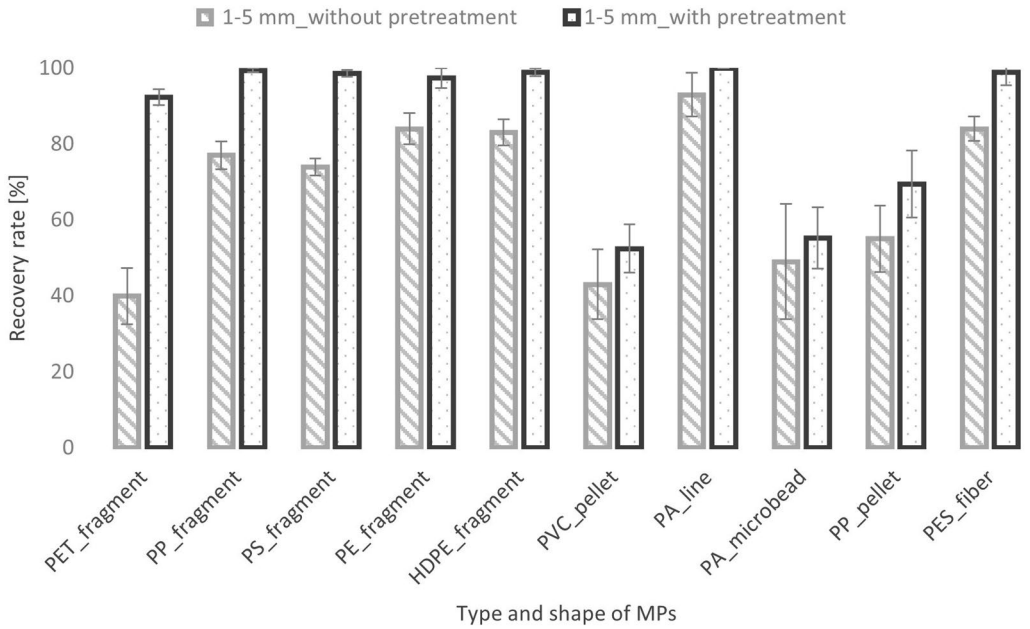


Fig. 5 - Wrong Figure, I attached the right Figure in Attachments. Recovery rate (mean ± SD) of particles 1–5 mm without and with pre-treatment (No. of samples: 9)

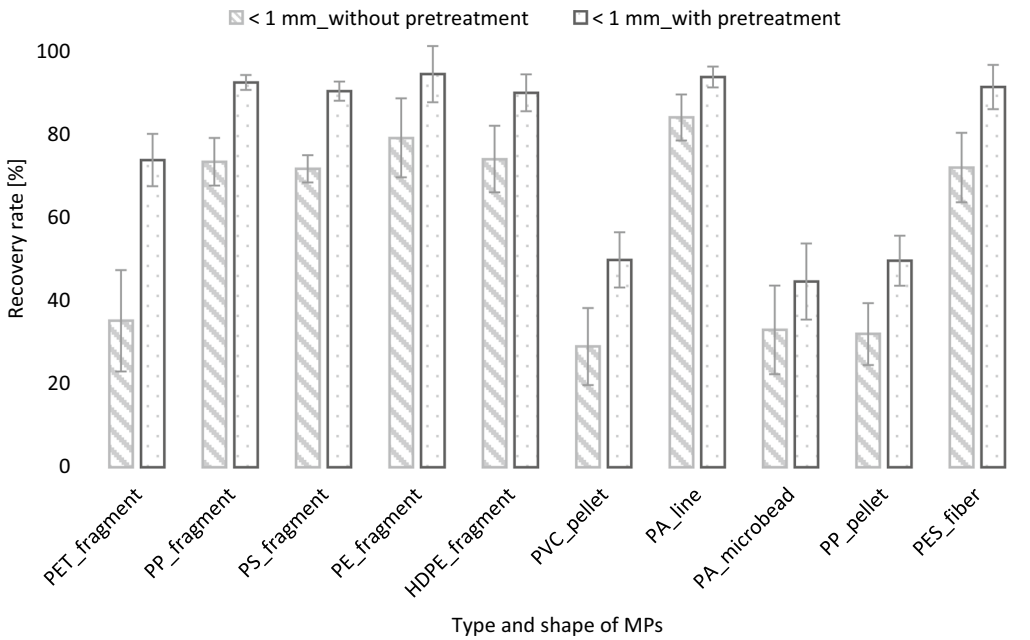


Fig. 6 Recovery rate (mean ± SD) of particles 0.1 < 1 mm without and with pre-treatment (No. of samples: 9)

After a comparison of oil extraction efficiency with and without pre-treatment, it seems that the recovery rate was mostly higher (up to 15% for PP pellets) for particles of 1–5 mm and particles spiked in SS samples that were pre-treated with Fenton's reagent prior the oil extraction. The recovery rate was lower after pre-treatment for PA microbeads in anaerobic sludge (lower by as much as 16%), with higher densities and average weight of PA particles. It can be assumed that pre-treatment reduces the density of SS and MP particles, which (due to their weight and density) sunk to

Table 1 Recovery rate [%] of MPs in anaerobic sludge after each extraction phase for particles 0.1 < 1 mm and 1–5 mm in anaerobic sludge

	With pre-treatment						Without pre-treatment					
Size	**0.1 < 1 mm**			**1–5 mm**			**0.1 < 1 mm**			**1–5 mm**		
Extraction phase	**1st**	**2nd**	**3rd**	**1st**	**2nd**	**3rd**	**1st**	**2nd**	**3rd**	**1st**	**2nd**	**3rd**
PET_fragment	69	74	88	59	89	99	61	77	78	60	81	89
PP_fragment	86	98	100	96	100	100	81	91	91	88	96	99
PS_fragment	81	97	100	43	65	100	79	93	96	74	95	99
PE_fragment	84	91	93	97	98	99	73	87	89	89	98	98
HDPE_fragment	93	96	98	96	99	99	74	82	84	91	97	97
PVC_pellet	70	79	83	28	53	63	41	51	52	31	51	60
PA_line	80	93	93	93	100	100	65	72	87	62	81	89
PA_microbead	25	45	51	21	34	40	19	50	60	26	43	56
PP_pellet	33	60	80	50	85	95	27	51	54	44	58	64
PES_fiber	68	72	88	46	76	97	70	80	88	63	87	96

Table 2 Recovery rate [%] of MPs in anaerobic sludge after each extraction phase for particles 0.1 < 1 mm and 1–5 mm in sludge cake

	With pre-treatment						Without pre-treatment					
Size	**0.1 < 1 mm**			**1–5 mm**			**0.1 < 1 mm**			**1–5 mm**		
Extraction phase	**1st**	**2nd**	**3rd**	**1st**	**2nd**	**3rd**	**1st**	**2nd**	**3rd**	**1st**	**2nd**	**3rd**
PET_fragment	49	69	74	83	88	92	14	25	35	17	35	40
PP_fragment	69	92	92	85	93	99	59	67	73	69	71	77
PS_fragment	72	88	90	90	94	99	54	68	72	62	74	74
PE_fragment	77	88	94	83	91	97	71	79	79	69	81	84
HDPE_fragment	74	81	90	88	96	99	59	69	74	71	80	83
PVC_pellet	33	46	50	40	52	52	16	27	29	31	39	43
PA_line	71	88	94	87	98	100	64	76	84	79	89	93
PA_microbead	34	45	45	47	51	55	12	28	33	32	49	49
PP_pellet	26	45	50	54	64	69	8	29	32	46	53	55
PES_fiber	64	84	91	84	93	99	56	67	72	69	81	84

the bottom of the mixture before coming in contact with oil, thus negatively impacting the recovery rate.

MP particles were quantified after the extraction, and the recovery rate was determined in anaerobic sludge and in sludge cake. The highest recovery rate was determined for types of MPs with densities from 825 kg m^{-3} to 1337 kg m^{-3}, shaped as fragments (PP, PS, PE, HDPE and PET), PES fibres with a density of 1332 kg m^{-3} and PA lines with a density of 1243 kg m^{-3}.

In addition to spiked MPs, 12 environmental MP particles were identified during the oil extraction procedure in the anaerobic sludge samples and 14 in the sludge cake samples, which originated from SS at WWTP and were not included in the determination of recovery rate.

Environmental samples without spiked microplastics

Quantification of microplastic particles in the organic-rich substrates

The collected and obtained MPs were quantified after pre-treatment, and the oil extraction procedure, where the highest number (25 particles) were quantified in treated sludge and 17 in peat substrate fertilised with the same treated sludge. The lowest number (2 particles) was obtained in peat substrate, while in peat substrate-control, fertilised with mineral fertilisers, 11 particles were collected (Table 3). Particles that were too small to be characterised by FTIR were destroyed (see "Characterisation of microplastic particles in the organic-rich substrates" section) at the beginning of the analysis, and we continued the characterisation with particles of > 0.1 mm, while particles < 0.1 mm were collected and saved for further analysis.

Table 3 Number obtained, characterised, and destroyed particles

	Number of obtained particles	Number of characterised samples	Number of destroyed samples[a]	Number of particles < 0.1 mm collected for further analysis
Treated sludge	25	16	4	5
Peat substrate	2	1	0	1
Peat substrate-fertilised	17	6	0	11
Peat substrate-control	11	4	0	7

[a] for an explanation, see "Organic-rich substrates samples" section

Characterisation of microplastic particles in the organic-rich substrates

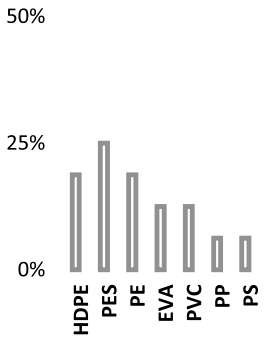
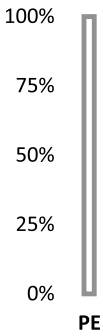
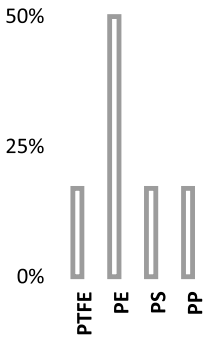
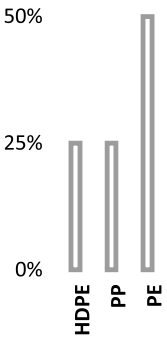
The research was carried out on only MP particles of > 0.1 mm because particles < 0.1 mm were destroyed during the characterisation with FTIR analysis and transformed into dust due to pressure. The most commonly detected types of MPs in organics-rich substrates were PE, PP, PS and PES in shapes of fragments, films, and fibres, where PE was detected and most abundant in almost all four samples, followed by PP and PS (Table 4). The treated sludge contained EVA type of MPs, which was also found in personal care products [44]. The most common shape, detected in organic-rich samples, were shapes of fragments and films. The sizes of extracted MPs ranged from 93.64 in peat substrate-control and up to 532.95 µm in treated sludge.

Micrograph images (40× magnification) of some of the selected characterised types of MPs, determined in environmental samples, are shown in Fig. 7a–r.

Discussion

Based on the results, we can conclude that pre-treatment with Fenton's reagent generally improves the recovery rate of the MP particles used in this study, regardless of their type, shape, size, or density. Pre-treatment, as a pre-step of the oil extraction method, was shown to be important in reducing organic matter in all environmental samples, such as SS and organic-rich substrates. Reducing organic material in environmental samples is vital for the easier quantification and later characterisation of collected particles, for example, as a basis for improved SS management in the context of the new EU Wastewater Treatment Directive [25], promoting nutrient recovery while managing health and environmental risks. It also improved the reliability of the selected method and made it quicker (lasting approximately 4 h) since it reduced organic matter, making extracted MPs more visible and reducing the probability of clogging the funnels with solids, thus slowing down the filtration of environmental samples. By achieving up to a 100% recovery rate for certain types of MPs (PP and PS), the method seems highly promising for particles bigger than 1 mm. The results of recovery rates are comparable with the results of other studies; Lares et al. [45] reached a total 88.3% ± 5.5% recovery rate with oil extraction procedure from sludge samples without pre-treatment for PS beads, PE fragments, PVC fragments and SBR

Table 4 Characteristics of microplastic particles in the organic-rich substrates

	Treated sludge	Peat substrate	Peat substrate—fertilised	Peat substrate—control
Images	Figure 7a–j	Figure 7k	Figure 7l–o	Figure 7p–r
Types of MPs				
Size of MPs [µm]	140.63–532.95	111.43	171.29–296.61	93.64–98.39
Shape of MPs	Fibres, fragments, films	Fragment	Fragments, films, fibers	Fragments, films

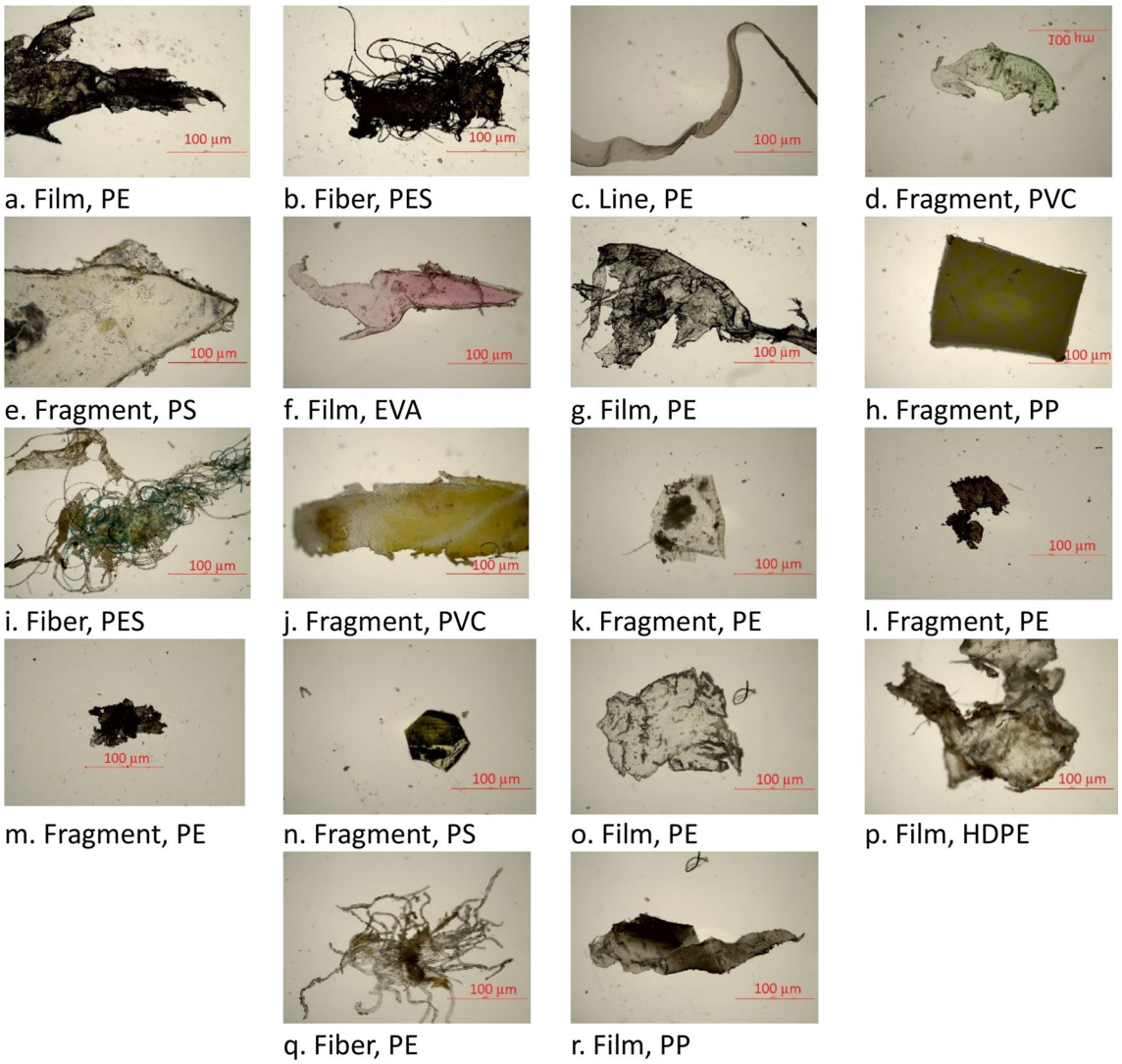


Fig. 7 Micrograph images at 40 × magnification of selected particles after pre-treatment and oil extraction protocol

(styrene–butadiene rubber) fragments with densities ranging 900–1600 kg m^{-3} and sizes 300–3400 µm. Mani et al. [46] achieved for 0.3–0.5 mm and 0.5–1 mm sized particles the highest recovery rate for PP and PS fragments (99% ± 3%), followed by PET-G (glycol modified PET) particles with a recovery rate of 98% ± 5% in environmental samples when using oil extraction procedure and H_2O_2 oxidation. Crichton et al. [39] used an oil extraction protocol in sediment beach samples, with up to 7.02% of organic matter (without removal of organic matter) and reached 92.7% ± 4.3% extraction efficiency for fibres (PES, nylon and stain-resistant polyester) and 98.0% ± 4.5% for PVC particles. In our study, SS samples contained up to 70% organic matter, reaching a recovery rate of up to 100% for PP and PS and PA lines.

Despite high recovery rates for PP, PS, PE, HDPE, PET fragments, PA lines, PP pellets and PES fibres, the method is unsuitable for extracting particles with higher densities and weights, such as PA and PVC, shaped as microbeads or pellets, since particles remained in SS and avoided contact with added oil. Rodina et al. [47] also attained lower efficiency of PA-6 MPs extraction efficiency, possibly due to the higher density of used polymers. Wang et al. [48] used PS beads to test extraction from biosolids and soil samples and discovered similar findings, specifically that pre-treatment reduced extraction efficiency for smaller types of PS beads, while pre-treatment improved extraction efficiency for bigger particles of PS beads. Their study also showed that smaller particles were partially affected by H_2O_2, which could impact extraction

efficiency, while larger particles were not negatively affected by H_2O_2 digestion; the influence of pre-treatment was suspected and not proven. The results of our study, and those of Rodina et al. [47] and Wang et al. [48] indicate that the size, shape, density and weight of specific polymers can influence extraction efficiency; *e.g.* in the study particles of PA, with the highest density, shaped as microbeads with sizes < 1 mm, reached a lower recovery rate, up to 50.6% ± 17.3% after pre-treatment. It can be assumed that as the size decreases, the particles could also be attached to any residue (organic or inorganic) material in the SS samples, avoiding oil contact and being due to gravity sedimented while density separation. After pre-treatment the particles in our study showed no visual changes on the surface under microscopic examination with 40 × magnification (Fig. S2). No changes in absorption spectra of studied MPs samples were also observed at ATR–FTIR analysis, except that in almost all IR spectra of MPparticles after oil extraction, despite the extensive washing with EtOH, vibrational bands characteristic of oil are also observed (Fig. S3). Hurley et al. [30] and Li et al. [34] also reported no impact of chemical pre-treatments by Fenton's reagent, while Maw et al. [49] discovered no changes in the surface area for PET, LDPE, PVC, PP and PS; however, minimal changes in the weight for LDPE and PP were observed, while FTIR spectra were not affected for observed polymers. The method was also shown to be efficient in the organic-rich substrates, for which characterisation of MP particles was done using FTIR. The types of extracted MPs are consistent with other studies, in which PE, PP, PS, and PES were the most commonly detected types of MPs [18, 50]. In the organic-rich substrates, particles shaped as fibres, fragments and films, with sizes from 93.64–532.95 µm, were extracted. Characteristics of extracted MPs coincide with the results of Liew et al. [51], who discovered different shapes and sizes of MPs particles in SS samples (from primary clarifier and activated sludge) and also observed that MP size reduces when going through different stages of wastewater treatment. Van der Berg et al. [51] confirmed PP and PVC as the most abundant types of MP particles with sizes between 150 and 250 µm, with shapes of fragments, fibres and films in olive and cereal farmland. Zhu et al. [52] discovered PE, PP and PS, with sizes 0.02–1 mm and fibres as the largest proportion of discovered particles, followed by films, fragments, and foams in farmland, while Liu et al. [53] found PP, PE, and PES with shapes of fibres, fragments, films and pellets as the most common types of MPs, sized between 0.03 and 5 mm in vegetable farmland. Discoveries of extracted MP particles in organic-rich samples confirm the validation of the oil extraction method with included pre-treatment. The highest recovery rate (up to 100%) was also reached for PP, PE, and PS fragments, as well as for PES fibres in SS. The results of extraction efficiency coincide with extracted MPs particles obtained from organic-rich substrates, where the most abundant types of MPs were PP, PE, PS, PES with shapes of fragments, films and fibers.

Benefits of the oil extraction method

The presented method is quick, reliable, and efficient, as well as being cost-effective and environmentally friendly. The oil extraction protocol took, on average, 4 h per sample, including pre-treatment, extraction, and characterisation of determined particles, making the method time-efficient compared to other pre-treatment and extraction methods that can take days [30, 45]. Pre-treatment with Fenton's reagent generally improves the recovery rate of the selected MP particles used in this study, regardless of their type, shape, size, or density.

To characterise obtained particles, the Fourier transform infrared (FTIR) spectroscopy method was identified as an effective method for characterising particles > 0.1 mm.

Limitations of the oil extraction method

The oil extraction method is not suitable for all types of fibres, as proven in the preliminary research with spiked polyacrylic and PA microfiber; during the oil extraction method, fibres degraded into many thinner fibres that were impossible to count. Another limitation of the proposed method is the size of the funnel outlet, which limits the size of MPs and could clog the funnel. To avoid or minimise clogging, the funnel outlet was physically enlarged. Further studies could be conducted using different glass devices to separate heterogeneous phases. Nakajima et al. used a small portable device provided by the Japan Agency for Marine-Earth Science and Technology (JAMSTEC), a microplastic–sediment separator (JAMSS) unit that separates sediment and supernatant by sliding two plates against each other [54].

MP particles with higher densities and masses (PA with a density of 1150 kg m^{-3} and an average weight of particles 0.1 < 1 mm 0.69 mg and PVC with a density of 1326 kg m^{-3} and average weight of particles 0.1 < 1 mm 0.36 mg) shaped as microbeads and pellets had lower efficiency since those particles remained in the SS and were not transferred to the upper oil phase. Some particles were also left on the glass surface of the separation funnel and could easily be missed if the separation funnel was not appropriately rinsed with deionized water and ethanol after each extraction.

While FTIR analysis, particles of < 0.1 mm were destroyed due to attachment pressure. Smaller MP particles (not destroyed during characterisation) that cannot

be detected with FTIR can be characterised with the micro-FTIR as planned for future experiments.

Conclusions

The combination of digestion of waste sludge using Fenton's reagent and oil extraction method is a cost-effective and reliable method for the quantification and characterisation of particles of > 0.1 mm due to efficient removal of organic material and non-destructibility for degradation of polymers. The oleophilic interaction between oil and polymers is strong enough to extract denser polymers to the surface of the oil layer, but it has lower recovery rates and reliability for certain types and shapes (PA microbeads and PVC pellets). The reliability of the oil extraction method is higher with pre-treatment of environmental samples of sludge for particles of 1–5 mm, regarding the type and shape of MP.

The most frequently detected type of MPs in the analysed organic-rich substrates were PE, PP, PS, and PES in the shape of films, fragments, and fibres, sized from 93.64 to 532.95 µm. For the characterisation of detected particles (> 0.1 mm), the Fourier transform infrared (FTIR) method was identified as effective. The time efficiency and reliability of the oil extraction protocol with pre-treatment included for organic-rich substrates enable the method to improve the decision support system for efficient SS management. Future experiments should focus on the oil extraction efficiency of particles smaller than 0.1 mm, investigate density and shape influence and possible mechanisms to improve oil extraction efficiency.

Abbreviations

MPs	Microplastic(s)
CEC	Contaminants of emerging concerns
NOAA	National Oceanic and Atmospheric Administration
WWTP	Wastewater treatment plants
SS	Sewage sludge
PE	Polyethylene
PP	Polypropylene
PA	Polyamide
PET	Polyethylene terephthalate
PES	Polyester
PS	Polystyrene
EU	European Union
H_2O_2	Hydrogen peroxide
PC	Polycarbonate
PMMA	Polymethylmethacrylate
PA-6,6	Polyamide 6,6
ABS	Acrylonitrile Butadiene Styrene
KOH	Potassium hydroxide
NaOH	Sodium Hydroxide
PTFE	Polytetrafluoroethylene
NaI	Sodium iodide
$CaCl_2$	Calcium chloride
HDPE	High-density polyethylene
PVC	Polyvinyl chloride
$FeSO_4 \cdot H_2O$	Iron (II) sulphate heptahydrate
H_2SO_4	Sulfuric acid
EtOH	Ethanol
FTIR	Fourier transform infrared spectroscopy
EVA	Ethylene-vinyl acetate
SBR	Styrene–butadiene rubber

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-024-00898-6.

Additional file 1: Table S1. Moisture content [%] and % removal of organic matter with Fenton's reagent. **Table S2.** Characteristics of microplastics. **Figure S1.** Used microplastics, sizes 1–5 mm. **Figure S2.** Visual changes of spiked particles under 40× magnification before and after pre-treatment with Fenton's reagent. **Figure S3.** FTIR spectra of spiked particles before and after digestion with Fenton's reagent and oil extraction protocol (**a** PES, **b** PET, **c** PP fragment, **d** PP pellet, **e** PS, **f** PE, **g** HDPE, **h** PA microbead, **i** PA line, **j** PVC pellet).

Acknowledgements

The authors are very grateful to the Faculty of Chemistry and Chemical Technology and to the Centre for Research infrastructure at the Faculty of Chemistry and Chemical Technology, where all analytical procedures were performed. The contribution and assistance of Asst. Dr. Ula Rozman from the Faculty of Chemistry and Chemical Technology, University of Ljubljana is greatly acknowledged.

Author contributions

NL analysed and interpreted analysed data. FTIR analysis was performed by MZ. TGB, MZ and AŽG helped with interpretation of analysed data and were major contributors in writing the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the Slovenian Research and Innovation Agency ARIS (Research Program Chemical Engineering P2-0191 and Infrastructure programme I0-0022 and Mechanisms of Health Maintenance P3-0388 and Research Projects J2-4427, L7-4422). The authors acknowledge the support of the Centre for Research Infrastructure at the University of Ljubljana, Faculty of Chemistry and Chemical Technology, which is part of the Network of Research and Infrastructural Centres UL (MRIC UL) and is financially supported by the Slovenian Research and Innovation Agency ARIS (Infrastructure programme No. I0-0022).

Availability of data and materials

The data sets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 11 January 2024 Accepted: 24 March 2024
Published online: 02 April 2024

References

1. Bertoldi C, Lara LZ, Mizushima FAdL, Martins FCG, Battisti MA, Hinrichs R, Fernandes AN (2021) First evidence of microplastic contamination in the freshwater of Lake Guaíba, Porto Alegre, Brazil. Sci Tot Environ 759:143503. https://doi.org/10.1016/j.scitotenv.2020.143503
2. Huang Y, He T, Yan M, Yang L, Gong H, Wang W, Qing X, Wang J (2021) Atmospheric transport and deposition of microplastics in a subtropical urban environment. J Hazard Mater 416:126168. https://doi.org/10.1016/j.jhazmat.2021.126168

3. Heath E (2020) Compounds of emerging concern during wastewater treatment and in the environment: a case study on bisphenols: [keynote lecture]. In: Conference proceedings, 2020
4. Laimou-Geraniou M, Heath DJ, Heath E (2023) Analytical methods for the determination of antidepressants, antipsychotics, benzodiazepines and their metabolites through wastewater-based epidemiology. Trends Environ Anal Chem 37(19):1–24. https://doi.org/10.1016/j.teac.2022.e00192
5. Lan T, Wang T, Cao F, Yu C, Chu Q, Wang F (2021) A comparative study on the adsorption behavior of pesticides by pristine and aged microplastics from agricultural polyethylene soil films. Ecotox Environ Safe 209(15):111781. https://doi.org/10.1016/j.ecoenv.2020.111781
6. Yu H, Qi W, Cao X, Hu J, Li Y, Peng J, Hu C, Qu J (2021) Microplastic residues in wetland ecosystems: do they truly threaten the plant-microbe-soil system? Environ Int 156:106708. https://doi.org/10.1016/j.envint.2021.106708
7. Luo T, Weng Y, Huang Z, Zhao Y, Jin Y (2021) Combined hepatotoxicity of imidacloprid and microplastics in adult zebrafish: endpoints at gene transcription. Comp Biochem Phys C 246:109043–109049. https://doi.org/10.1016/j.cbpc.2021.109043
8. Arthur C, Baker J, Bamford H (2019) Proceedings of the International Research Workshop on the Occurrence, Effects and Fate of Microplastic Marine Debris. National Oceanic and Atmospheric Administration Technical Memorandum NOS-OR&R-30. 2009
9. Hartmann NB, Hüffer T, Thompson RC, Hassellöv M, Verschoor A, Daugaard AE, Rist S, Karlsson T, Brennholt N, Cole M, Herrling MP, Hess MC, Ivleva NP, Lusher AL, Wagner M (2019) Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. Environ Sci Technol 53(3):1039–1047. https://doi.org/10.1021/acs.est.8b05297
10. Di Bella G, Corsino SF, De Marines F, Lopresti F, La Carrubba V, Torregrossa M, Viviani G (2022) Occurrence of microplastics in waste sludge of wastewater treatment plants: comparison between membrane bioreactor (MBR) and conventional activated sludge (CAS) technologies. Membranes (Basel). https://doi.org/10.3390/membranes12040371
11. Akarsu C, Kumbur H, Gökdağ K, Kıdeyş AE, Sanchez-Vidal A (2020) Microplastics composition and load from three wastewater treatment plants discharging into Mersin Bay, north eastern Mediterranean Sea. Mar Pollut Bull 150:110776. https://doi.org/10.1016/j.marpolbul.2019.110776
12. Sun J, Dai X, Wang Q, van Loosdrecht MCM, Ni BJ (2019) Microplastics in wastewater treatment plants: detection, occurrence and removal. Water Res 152:21–37. https://doi.org/10.1016/j.watres.2018.12.050
13. Talvitie J, Mikola A, Koistinen A, Setälä O (2017) Solutions to microplastic pollution—removal of microplastics from wastewater effluent with advanced wastewater treatment technologies. Water Res 123:401–407. https://doi.org/10.1016/j.watres.2017.07.005
14. Talvitie J, Heinonen M, Pääkkönen JP, Vahtera E, Mikola A, Setälä O, Vahala R (2015) Do wastewater treatment plants act as a potential point source of microplastics? Preliminary study in the coastal Gulf of Finland, Baltic Sea. Water Sci Technol 72(9):1495–1504. https://doi.org/10.2166/wst.2015.360
15. Elkhatib D, Oyanedel-Craver V, Carissimi E (2021) Electrocoagulation applied for the removal of microplastics from wastewater treatment facilities. Sep Purif Technol 276:118877–118885. https://doi.org/10.1016/j.seppur.2021.118877
16. Karimi Estahbanati MR, Kiendrebeogo M, Khosravanipour A, Drogui P, Tyagi RD (2021) Treatment processes for microplastics and nanoplastics in waters: state-of-the-art review. Mar Pollut Bull 168:112374. https://doi.org/10.1016/j.marpolbul.2021.112374
17. Talvitie J, Mikola A, Setälä O, Heinonen M, Koistinen A (2017) How well is microlitter purified from wastewater? A detailed study on the stepwise removal of microlitter in a tertiary level wastewater treatment plant. Water Res 109:164–172
18. Li X, Chen L, Mei Q, Dong B, Dai X, Ding G, Zeng EY (2018) Microplastics in sewage sludge from the wastewater treatment plants in China. Water Res 142:75–85. https://doi.org/10.1016/j.watres.2018.05.034
19. Dronjak L, Exposito N, Sierra J, Schuhmacher M, Florencio K, Corzo B, Rovira J (2023) Tracing the fate of microplastic in wastewater treatment plant: a multi-stage analysis of treatment units and sludge. Environ Pollut 333:122072. https://doi.org/10.1016/j.envpol.2023.122072
20. Yaseen A, Assad I, Sofi MS, Hashmi MZ, Bhat SU (2022) A global review of microplastics in wastewater treatment plants: Understanding their occurrence, fate and impact. Environ Res 212(Pt B):113258. https://doi.org/10.1016/j.envres.2022.113258
21. Mintenig SM, Int-Veen I, Löder MGJ, Primpke S, Gerdts G (2017) Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. Water Res 108:365–372. https://doi.org/10.1016/j.watres.2016.11.015
22. Mahon AM, O'Connell B, Healy MG, O'Connor I, Officer R, Nash R, Morrison L (2017) Microplastics in sewage sludge: effects of treatment. Environ Sci Technol 51(2):810–818. https://doi.org/10.1021/acs.est.6b04048
23. Magni S, Binelli A, Pittura L, Avio CG, Della Torre C, Parenti CC, Gorbi S, Regoli F (2019) The fate of microplastics in an Italian wastewater treatment plant. Sci Total Environ 652:602–610. https://doi.org/10.1016/j.scitotenv.2018.10.269
24. El Hayany B, Rumpel C, Hafidi M, El Fels L (2022) Occurrence, analysis of microplastics in sewage sludge and their fate during composting: a literature review. J Environ Manage 317:115364. https://doi.org/10.1016/j.jenvman.2022.115364
25. European Commission (2022) Proposal for a Directive of the European Parliament and of the council concerning urban wastewater treatment (recast). https://environment.ec.europa.eu/system/files/2022-10/Proposal%20for%20a%20Directive%20concerning%20urban%20wastewater%20treatment%20%28recast%29.pdf. Accessed 25 Aug 2023
26. European Commission (2022) European Green Deal: Commission proposes rules for cleaner air and water. https://ec.europa.eu/commission/presscorner/detail/en/ip_22_6278. Accessed 24 Aug 2023
27. European Environment Agency (1991) Urban Waste Water Treatment Directive. https://www.eea.europa.eu/policy-documents/urban-waste-water-treatment-directive. Accessed 30 Aug 2023
28. Bellasi A, Binda G, Pozzi A, Boldrocchi G, Bettinetti R (2021) The extraction of microplastics from sediments: an overview of existing methods and the proposal of a new and green alternative. Chemosphere (Oxford). 278:130357. https://doi.org/10.1016/j.chemosphere.2021.130357
29. Junhao C, Xining Z, Xiaodong G, Li Z, Qi H, Siddique KHM (2021) Extraction and identification methods of microplastics and nanoplastics in agricultural soil: a review. J Environ Manage 294:112997. https://doi.org/10.1016/j.jenvman.2021.112997
30. Hurley RR, Lusher AL, Olsen M, Nizzetto L (2018) Validation of a method for extracting microplastics from complex, organic-rich, environmental matrices. Environ Sci Technol 52(13):7409–7417. https://doi.org/10.1021/acs.est.8b01517
31. Gao D, Li XY, Liu HT (2020) Source, occurrence, migration and potential environmental risk of microplastics in sewage sludge and during sludge amendment to soil. Sci Total Environ 742:140355. https://doi.org/10.1016/j.scitotenv.2020.140355
32. Collivignarelli MC, Carnevale Miino M, Caccamo FM, Milanese C (2021) Microplastics in sewage sludge: a known but underrated pathway in wastewater treatment plants. Sustainability (Basel, Switzerland) 13(22):12591–12614. https://doi.org/10.3390/su132212591
33. Lusher A, Hurley R, Vogelsang C, Nizzetto L, Olsen M (2017) Mapping microplastics in sludge. Norwegian Inst Water Res. https://doi.org/10.13140/RG.2.2.25277.56804
34. Li X, Chen L, Ji Y, Li M, Dong B, Qian G, Zhou J, Dai X (2020) Effects of chemical pretreatments on microplastic extraction in sewage sludge and their physicochemical characteristics. Water Res 171:115379. https://doi.org/10.1016/j.watres.2019.115379
35. Lekše N, Griessler Bulc T, Žgajnar Gotvajn A (2021) Oil extraction of microplastics from communal dewatered sludge. Proc Socrat Lect. 6:49–55
36. Felsing S, Kochleus C, Buchinger S, Brennholt N, Stock F, Reifferscheid G (2017) A new approach in separating microplastics from environmental samples based on their electrostatic behavior. Environ Pollut (Barking, Essex : 1987) 234:20–28. https://doi.org/10.1016/j.envpol.2017.11.013
37. Jiang H, Zhang Y, Wang H (2020) Surface reactions in selective modification: the prerequisite for plastic flotation. Environ Sci Technol 54(16):9742–9756. https://doi.org/10.1021/acs.est.9b07861
38. Grbic J, Nguyen B, Guo E, You J, Sinton D, Rochman C (2019) Magnetic extraction of microplastics from environmental samples. Environ Sci Tech Lett. https://doi.org/10.1021/acs.estlett.8b00671
39. Crichton EM, Noel M, Gies EA, Ross PS (2017) A novel, density-independent and FTIR-compatible approach for the rapid extraction of microplastics from aquatic environment. Anal Methods. https://doi.org/10.1039/C6AY02733D

40. Scopetani C, Chelazzi D, Mikola J, Leiniö V, Heikkinen R, Cincinelli A, Pellinen J (2020) Olive oil-based method for the extraction, quantification and identification of microplastics in soil and compost samples. Sci Total Environ 733:139338. https://doi.org/10.1016/j.scitotenv.2020.139338
41. Standard Methods Committee of the American Public Health Association, American Water Works Association, and Water Environment Federation (2023) Preface. In: Lipps WC, Baxter TE, Braun-Howland E (eds) Standard Methods For the Examination of Water and Wastewater. APHA Press, Washington DC. https://doi.org/10.2105/SMWW.2882.001
42. Nuelle MT, Dekiff JH, Remy D, Fries E (2014) A new analytical approach for monitoring microplastics in marine sediments. Environ Pollut 184:161–169. https://doi.org/10.1016/j.envpol.2013.07.027
43. Vermeiren P, Muñoz C, Ikejima K (2020) Microplastic identification and quantification from organic rich sediments: a validated laboratory protocol. Environ Pollut 262:114298. https://doi.org/10.1016/j.envpol.2020.114298
44. Hisam S, Taneez M, Sagheer MZ, Dilshad A (2023) Microbeads in personal care products sold in Pakistan: extraction, quantification, characterization, and buoyancy analysis. Environ Monit Assess 196(1):50. https://doi.org/10.1007/s10661-023-12227-0
45. Lares M, Ncibi MC, Sillanpää M (2019) Intercomparison study on commonly used methods to determine microplastics in wastewater and sludge samples. Environ Sci Pollut Res Int 26(12):12109–12122. https://doi.org/10.1007/s11356-019-04584-6
46. Mani T, Frehland S, Kalberer A, Burkhardt-Holm P (2019) Using castor oil to separate microplastics from four different environmental matrices. Anal Methods. https://doi.org/10.1007/s11356-019-04585-6
47. Rodina D, Roth C, Wohlleben W, Pfohl P (2024) An innovative microplastic extraction technique: the switchable calcium chloride density separation column tested for biodegradable polymers, polyethylene, and polyamide. MethodsX 12:102560. https://doi.org/10.1016/j.mex.2024.102560
48. Wang Z, Taylor SE, Sharma P, Flury M (2018) Poor extraction efficiencies of polystyrene nano- and microplastics from biosolids and soil. PLoS ONE. https://doi.org/10.1371/journal.pone.0208009
49. Maw MM, Boontanon N, Fujii S, Boontanon SK (2022) Rapid and efficient removal of organic matter from sewage sludge for extraction of microplastics. Sci Total Environ 853:158642. https://doi.org/10.1016/j.scitotenv.2022.158642
50. Lares M, Ncibi MC, Sillanpää M (2018) Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. Water Res 133:236–246. https://doi.org/10.1016/j.watres.2018.01.049
51. van den Berg P, Huerta-Lwanga E, Corradini F, Geissen V (2020) Sewage sludge application as a vehicle for microplastics in eastern Spanish agricultural soils. Environ Pollut 261:114198. https://doi.org/10.1016/j.envpol.2020.114198
52. Zhu YE, Wen HX, Li THX, Li H, Wu C, Zhang GX, Yan J (2021) Distribution and sources of microplastics in farmland soil along the Fenhe river. Huan Jing Ke Xue. https://doi.org/10.13227/j.hjkx.202012072
53. Liu M, Lu S, Song Y, Lei L, Hu J, Lv W, Zhou W, Cao C, Shi H, Yang X, He D (2018) Microplastic and mesoplastic pollution in farmland soils in suburbs of Shanghai. China Environ Pollut 242(Pt A):855–862. https://doi.org/10.1016/j.envpol.2018.07.051
54. Nakajima R, Tsuchiya M, Lindsay DJ, Kitahashi T, Fujikura K, Fukushima T (2019) A new small device made of glass for separating microplastics from marine and freshwater sediments. PeerJ. https://doi.org/10.7717/peerj.7915

Publisher's Note