

# MICROPLASTICS IDENTIFICATION IN LANDFILL LEACHATES BY DIFFERENT SPECTROSCOPIC TECHNIQUES

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## ABSTRACT

Discovered more than 40 years ago, microplastics have become a major environmental issue. With increasing global plastic production, microplastics are of growing concern. Landfills have been pinpointed as primary sources of microplastics to surface waters and they have, in fact, been identified and quantified as such. Due to their small size, different polymers and interfering non-plastic materials, microplastics are difficult to analyse in a complex matrix such as leachate. To elucidate the impact of pre-treatment on the performance of the most common microspectroscopical analytical methods employed, i.e., FT-IR and Raman, we re-examined previously pre-treated and analysed leachate samples. Additionally, we subjected duplicates of previously analysed samples to different concentrations of H<sub>2</sub>O<sub>2</sub> with varied reaction times to digest and remove non-plastic organic matter. The pre-treated samples were subjected density separation and (re-)analysed by means of FT-IR and Raman microspectroscopy. Larger particles were also analysed by near-infrared (NIR) hyperspectral imaging. We found the concentration of H<sub>2</sub>O<sub>2</sub> to impact the possibility of identifying and quantifying PET particles, with Raman scattering microspectroscopy enabling more particles to be counted than with FT-IR. This is likely due to the increased detectable particle size range, from around 50 µm for FT-IR to 1 µm for Raman scattering microspectroscopy. Optimized H<sub>2</sub>O<sub>2</sub> concentration with subsequent density separation enabled to clearly identify numerous PE particles, but also PP, PS, and PET particles and carbon compounds with Raman scattering microspectroscopy. Hyperspectral imaging performed well for particles larger than 30 µm.

## 1. INTRODUCTION

Plastic pollution is one of the most pressing environmental issues. Plastic production has been increasing year by year and is projected to double in the next 20 years (World Economic Forum et al., 2016). It is estimated that at least 10% of plastic produced ends up in the environment; through storm water, wastewater treatment plants, waste management and littering, as well as by air deposition, it reaches the oceans. Microplastic particles (MP) are defined as plastic particles below 5 mm in size (Arthur, 2009; Thompson et al., 2004), of mixed shape, size, colour, and chemical composition that are present in air, soil, freshwater, seas, biota, and in several components of our diet (Science Advice for Policy by European Academics -SAPEA-, 2019). Microplastic pollution is estimated to account for 60–80% of marine litter (Derraik, 2002). Storm water

from road run off and wastewater are regarded as the main transport routes for microplastics to surface waters (Bläsing and Amelung, 2018, Eriksen et al., 2013; Barnes et al., 2009; Horton et al., 2017). Landfilling and even leachate has been suspected to be a significant source of microplastics to surface waters as well (Sundt et al., 2014, Magnusson et al., 2016).

Leachate composition widely varies depending on the age of the landfill, the type of accepted waste, operational praxis, whether the landfill is capped, etc. Generally, landfill leachates contain nitrogen, salts, and inorganic and organic micropollutants such as metals and persistent organic substances (Kjeldsen et al., 2002; Haglund et al., 2015; Modin et al., 2011).

Comprehensive information or data on the amount of microplastic particles in leachate or their potential environmental impacts is still scarce. The Nordic Waste Group

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and the Marine Group under the Nordic Council of Ministers, therefore, commissioned the design and conduction of a study on the occurrence of microplastics in landfill leachates in Norway, Iceland and Finland, in order to elucidate whether landfill leachates are potentially significant sources of microplastics to surface waters (van Praagh et al., 2018).

Isolation of microplastic particles from the complex environmental matrix that constitutes landfill leachates is crucial. It typically includes sieving/filtration followed by chemical treatments to remove non-plastic organic materials that may obstruct further analysis (Zarfl, 2019). Identification of microplastics can be done by visual inspection of the isolates. It allows estimating the amount of the microplastic particles, but misses information on the specific polymers comprising them, which can be important for determining the microplastics source and environmental impact. Therefore, spectroscopy-based methods have been utilized to characterize microplastic particles in various environmental samples.

The most common methods include infrared absorption (IR) and Raman scattering microspectroscopy (Käppler et al., 2016), as well as near-infrared (NIR) hyperspectral imaging (Shan et al., 2018). IR spectroscopy and NIR hyperspectral imaging have been found to be suitable to characterize larger (>50 µm) particles, but spectral analysis can be hindered by contributions of contaminants on microplastic particles' surfaces. Furthermore, only thin (<10 µm) particles can be analysed in transmission mode of IR spectroscopy, and the reflection spectra can be affected by baseline artefacts resulting from scattering from wavelength-scale sized, irregular shaped particles (Rasskazov et al., 2019). Meanwhile, Raman microspectroscopy provides higher spatial resolution (down to 1 µm), but it is more sensitive to sample pre-treatment as organic contaminants can yield fluorescence masking the Raman signal. Therefore, further comprehensive studies are necessary to identify the most suitable sample pre-treatment and analytical techniques for microplastic identification and characterization in landfill leachates.

The work presented here aims at providing guidelines in support of the identification of microplastic particles in

landfill leachate, paving the way for scalable applications aimed at better understanding the typology and size of microplastic particles emitted by landfill leachates. This is a fundamental aspect for dealing with plastic-waste management in any context, including the minimization of plastic-waste production at the source. This scope is pursued through the further analysis of samples collected and pre-treated in the aforementioned project (van Praagh et al., 2018), by comparing three different techniques for microplastic analysis while studying the effects of sample preparation on analytical results.

## 2. METHODS AND MATERIAL

### 2.1 Leachate samples

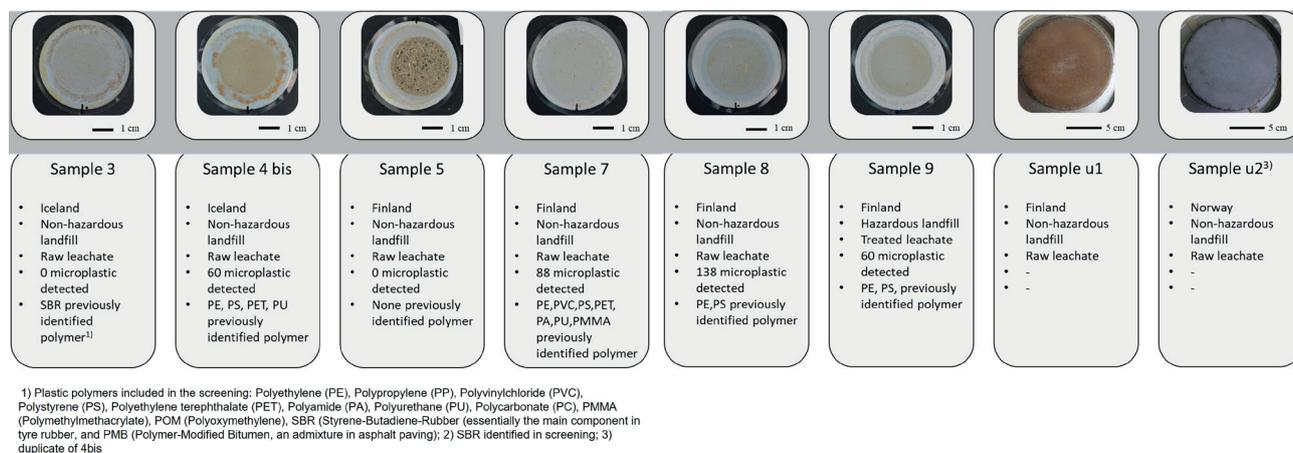
As part of the study commissioned by the Nordic Waste Group and the Marine Group under the Nordic Council of Ministers, leachate samples were collected by pumping leachate through three different stainless-steel filters of different mesh size (5000, 411, 47 µm). Subsequently, filters were rinsed with deionized water, re-filtered and subjected to a hydrogen peroxide solution (15% H<sub>2</sub>O<sub>2</sub>) in order to remove organic and non-organic materials. Screening of split samples (particles of >500 and >50 µm), showed no or few particles >500 µm (see van Praagh and Liebmann, 2019, for details).

For this study, we chose those samples available from the aforementioned investigation that exhibited the highest number of MP particles with relatively low particle density for Raman scattering microspectroscopy analysis (see Figure 1). All samples, except for u1 and u2, had been previously treated and analysed at the Austrian EPA's laboratory in Vienna by means of µ-FT-IR (Fourier transform infrared) microspectroscopy and imaging (see van Praagh and Liebmann, 2019).

### 2.2 Sample treatment

#### 2.2.1 Hydrogen Peroxide Treatment

Selected samples were treated with 15 or 30% H<sub>2</sub>O<sub>2</sub> solution, see Table 2. Sample 9, already treated with a 15% solution of H<sub>2</sub>O<sub>2</sub> at the laboratory in Vienna, underwent treatment in 30% solution of H<sub>2</sub>O<sub>2</sub> for 6 days. Nuelle et al.,



**FIGURE 1:** Samples background information: type and location of landfills covered in this study, microplastics (MP) count and polymers detected previously by FT-IR microspectroscopy (see van Praagh and Liebmann, 2019).

2013, found that this removes as much as 50% of non-plastic organic matter.

In order to test potential impact of H<sub>2</sub>O<sub>2</sub> on microplastic particles, PET and PP particles from reference materials were treated with a 30% solution of H<sub>2</sub>O<sub>2</sub> for 3, 8 and 15 days.

### 2.2.2 Density separation

Particles were separated by dispersion in a NaCl solution (35 g of NaCl in 100 ml of *milliQ* deionized water), see for example Quinn et al., 2017. The solution was mixed with a magnetic stirrer for at least 5 minutes, after which NaCl grains were undissolved on the container bottom, indicating saturation. The solution was left to rest, allowing density separation. To remove the floating particles (plastic particles, except for PET and PVC, exhibit a lower density than a saturated NaCl solution), two different methods were used, see Table 1. Subsequently, particles were deposited onto Anodisc® (Whatman) aluminium oxide filters with a polypropylene support ring (25 mm, pore size 0.2 µm)

## 2.3 Sample analysis

### 2.3.1 Raman microspectroscopy

Raman microspectroscopy measurements on all samples were performed using a LabRAM HR Evolution Raman system (Horiba Scientific), equipped with a front illuminated thermoelectrically deep cooled Charge-Coupled Device (CCD), called Syncerity (Horiba Scientific). Raman scattering spectra were recorded with a 50/0.5 LWD (Long Working Distance) objective and a diffraction grating of 600 gr/mm.

Microplastic identification in leachate samples requires the setting of specific optical measurement parameters to successfully apply Raman spectroscopy. Thus, initial analysis was carried out on single particles within the samples, chosen randomly and with a diameter larger than 10 µm. In addition, several spectral maps of multiple particles were recorded, showing areas with a size of 1 cm x 0.5 cm, easily discoverable for later analyses.

The defined measurement parameters used were as follows: excitation wavelength was 632.8 nm (He-Ne laser), with an acquisition time of 5-18 seconds; 5-8 scans were averaged for a single spectrum, a wavenumber range

1800 to 800 cm<sup>-1</sup>; excitation power set to 5-10% of the total laser power (17 mW).

In case of fluorescence when using the 632.8 nm excitation, the spectra were recorded with 785 nm (diode solid-state laser) excitation wavelength; acquisition time of 60 seconds, 12 scans for a single spectrum and an excitation power set to 100% of the total laser power (100 mW). Spectral analysis was performed by comparing the recorded Raman spectra of particles in the samples with spectra of references (microplastics of PE, PP, PS, etc.).

### 2.3.2 FT-IR Microspectroscopy

FT-IR microspectroscopy measurements on samples 7 and 8 were performed with a Hyperion 3000 microscope connected to a Tensor 27 IR spectrometer (*Bruker*) in reflection mode. Either a single channel mercury cadmium telluride (MCT) detector was used to obtain a single spectrum, or a focal plane array (FPA) detector, with a 64x64 array size, was used to obtain an IR multispectral image. The field-of-view of the x15/0.4 Cassegrain objective that was used in these measurements is 250x250 µm. The spectra were recorded with 4 cm<sup>-1</sup> spectral resolution. 64 interferograms were averaged and the result was Fourier-transformed into a spectrum applying the Blackmann-Harris 3 apodization function and zero filling factor 2.

Additionally, a x20 Attenuated Total Reflection (ATR) objective (field-of-view 100x100 µm) combined with the single channel MCT detector was used to record IR spectra of single particles. Spectral analysis was performed by comparing the recorded IR spectra of particles in the samples with spectra of microplastic (PE, PP, PS, etc.) references.

### 2.3.3 Hyperspectral Imaging

Hyperspectral imaging was performed on sample 8, by using the SISUChema XLTM Chemical Imaging Workstation (Specim, Finland), equipped with ImSpectorTM N25E imaging spectrograph. It works in the short-wave infrared (SWIR) range (1000-2500 nm). The device has been equipped with a macro lens, that has a field-of-view of 1 cm, since the particles in these samples are very small. One pixel corresponds to about 30 µm. The spectral resolution was 6.3 nm.

**TABLE 1:** Sample and reference treatment type and duration prior to spectroscopic analyses.

ID code	Previous treatment Vienna	Treatment Solution	Duration	Density separation	Floating particles removal method	Comment
Unit	H <sub>2</sub> O <sub>2</sub> (%)	H <sub>2</sub> O <sub>2</sub> (%)	d	Yes/no	-	-
3	15	-	-	Yes	Vacuum pump	-
5	15	-	-	Yes	Syringe filter	Particles moved by tweezers
9	15	30	6	No	-	-
u1	-	30	6	Yes	Vacuum pump	-
u2	-	15	6	Yes	Vacuum pump	-
PET	-	30	3/8/15	No	-	Reference material
PP	-	30	3/8/15	No	-	Reference material
PP	-	30	3/8/15	No	-	Reference material

The analysis of environmental samples with hyperspectral imaging requires building a hierarchical model based on pre-defined plastic polymers. Six polymers were selected - PA, PET, PE, PP, PS, PVC – their raw spectra acquired and carefully analysed to observe and compare characteristics, due to the different absorption of light of molecules in the SWIR range. Applying the procedure described by Bonifazi et al. (2018) a hierarchical Partial Least-Squares Discriminant Analysis (PLS-DA) model was built and represented by a dendrogram.

PLS-DA is a supervised classification technique that requires a prior knowledge of the data. In order to provide a better discrimination of each variable (wavelength), samples are classified with PLS-DA into predefined groups, by forming discriminant functions from input variables (wavelengths) to yield a new set of transformed values providing a more accurate discrimination than any single variable (wavelength) (Ballabio and Consonni, 2013). A discriminant function is then built using reference samples to be later utilized to classify samples belonging to an unknown set. Once the model is obtained, it can be applied to an entire hypercube and for the classification of new hypercubes (Bonifazi et al., 2018).

To create a hierarchical model, objects are progressively divided into a first and successive levels of subsets, until each subset contains a single object (Monakhova et al., 2016), with the main objective of isolating the most different object, for each step.

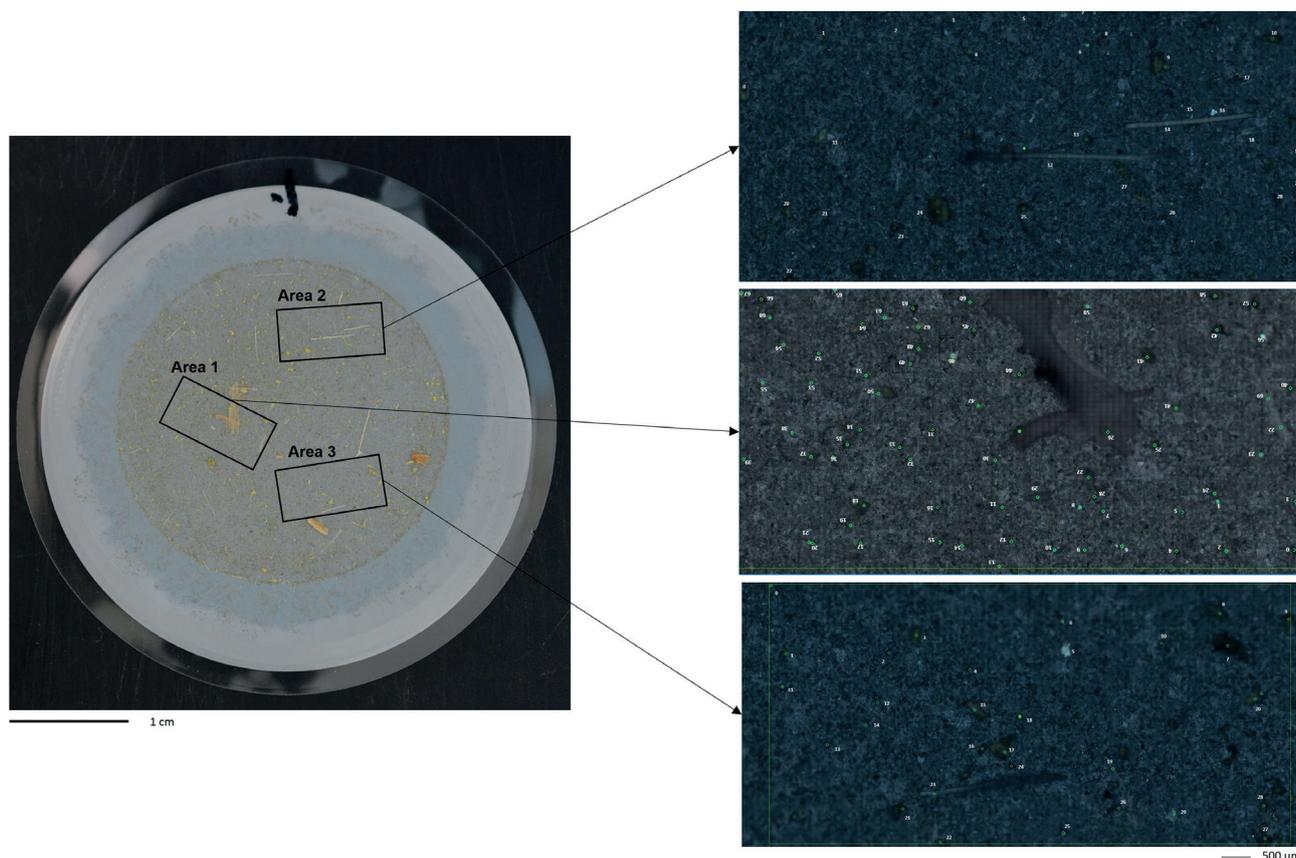
### 3. RESULTS AND DISCUSSION

#### 3.1 Raman Scattering and FT-IR microspectroscopy results

Raman scattering spectra of particles within randomly chosen areas (see example in Figure 2) of five samples were acquired. A total of 465 points were analysed within 2 to 3 areas per sample. Distribution and spectral analysis results are shown in Table 2.

From the results displayed in Table 2, the following can be derived:

- Many spectra identified as stemming from PET exhibited two unidentified bands at 1370-1399.3 cm<sup>-1</sup> in the spectrum (see Figure 2), which are potentially due to external contamination, as they are not observed after treatment with 30% H<sub>2</sub>O<sub>2</sub> and density separation (see Table 2 and Section 3.4 for more details).
- 26% of all detected particles are made of PET ("PET" and "PET + unidentified spectra"), 78% of which show the unidentified spectral bands at 1370-1399.3 cm<sup>-1</sup>.
- Sample 8 appears to have the highest number of PET particles ("PET" and "PET + unidentified spectra") related to the total number of analysed particles within the sample itself (about 41% of all the detected particles of the sample).
- 36% of particles are made of undefined materials; for 37% of the particles' spectra were too noisy or obscured by fluorescence to analyse.



**FIGURE 2:** Example of mapping areas. Mapping areas within sample 8 with measurement points on randomly selected particles highlighted on the right (70 points in total).

**TABLE 2:** Type and number of particles detected with Raman scattering microspectroscopy within the mapped areas.

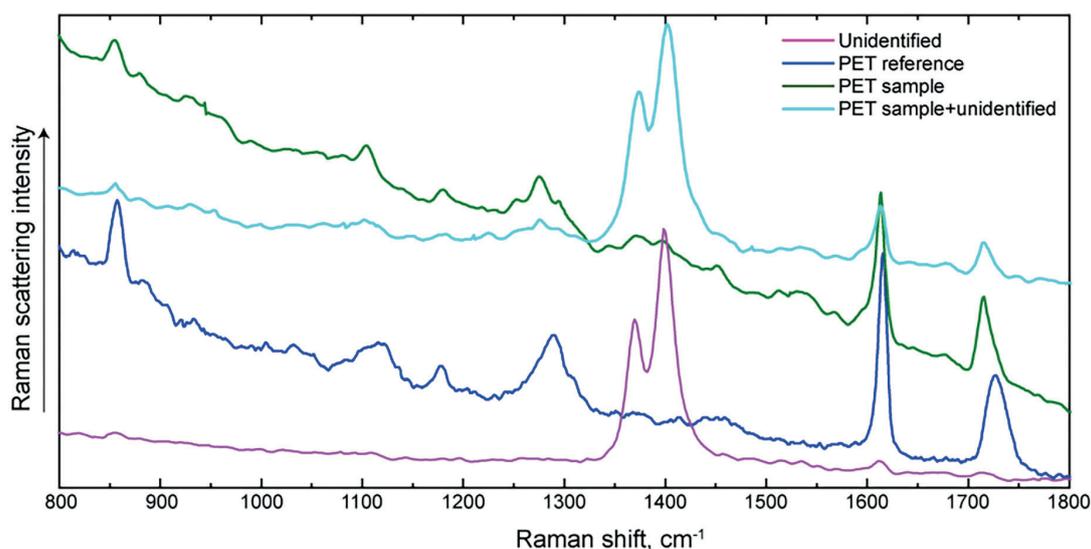
Sample	Area ID	No signal <sup>1)</sup>	PET	PET+ unident. <sup>2)</sup>	Unidentified spectra	Undefined materials <sup>3)</sup>	Particles analysed	Plastics ident. / Particles analysed (%)
3	1	20	7	16	3	19	65	35
	2	39	5	9	6	6	65	22
4bis	1 <sup>4)</sup>	4	-	-	-	26	30	-
	2	11	-	-	4	15	30	-
7	1	16	3	1	5	5	30	13
	2	9	1	2	2	11	25	12
	3	9	1	4	1	14	25	20
	4 <sup>3)</sup>	3	4	-	-	23	30	13
8	1	21	2	23	15	9	70	36
	2	5	0	12	6	7	30	40
	3	4	1	15	3	7	30	53
9	1 <sup>5)</sup>	9	0	4	0	7	20	20
	2	11	1	6	3	9	30	23
	3 <sup>5)</sup>	9	1	2	4	9	25	12
Sum	14	170	26	94	52	167	465	26

- No other plastic polymers have been detected. This result is unexpected, since the analysis carried out during the previous study by using FT-IR microspectroscopy (see Figure 1) showed the presence of several microplastic polymers.

In Figure 3, an example of identifying PET from Raman spectra is shown. Most of the spectral bands of the analysed particle in the sample ("PET sample") coincide with the ones observed in the spectrum from the "PET reference" particle. Two unidentified bands at 1370-1399.3 cm<sup>-1</sup> in the spectrum of the particle do not correspond to any of other plastic materials; we tentatively associated the occurrence of these bands with contaminations resulting from sample treatment. This is discussed more in detail in section 3.4 of this work.

For comparison and clarification of Raman microspectroscopy analysis results, sample 8 was re-analysed via FT-IR microspectroscopy. The main results from this analysis are as follows.

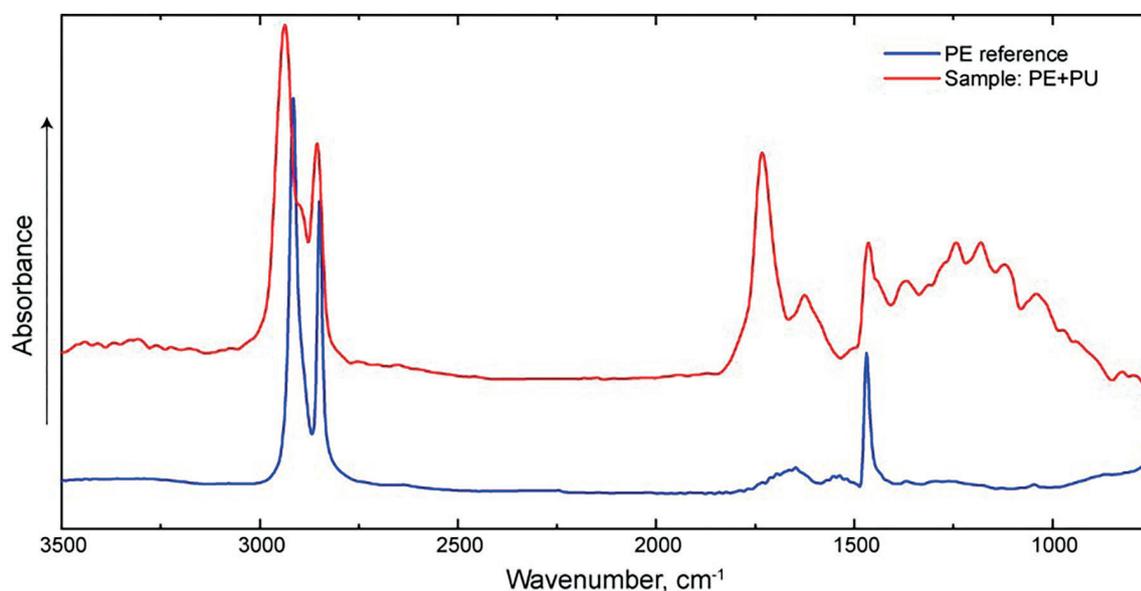
- A visible particle in sample 8 shown in Figure 4 was clearly identified as PE (see Figure 5).
- Spectra from other particles were recorded; however, they were distorted by the effects such as refractive index dispersion (see for example Korte, 1990). This phenomenon distorts the shape of the absorption bands, thus complicating or hindering the identification of materials from smaller particles.
- For the aforementioned reasons, only the PE largest particle was clearly identified through FT-IR analysis.



**FIGURE 3:** Raman scattering spectral analysis results. Results for PET reference material (blue); undefined spectrum (pink), PET particle in the sample (green), and PET with undefined spectra (cyan); spectra are shifted on the y-axis for clarity.



**FIGURE 4:** PE particle in sample 8. The PE particle image acquired with stereomicroscope Lecia M205 C with x1.25 magnification.



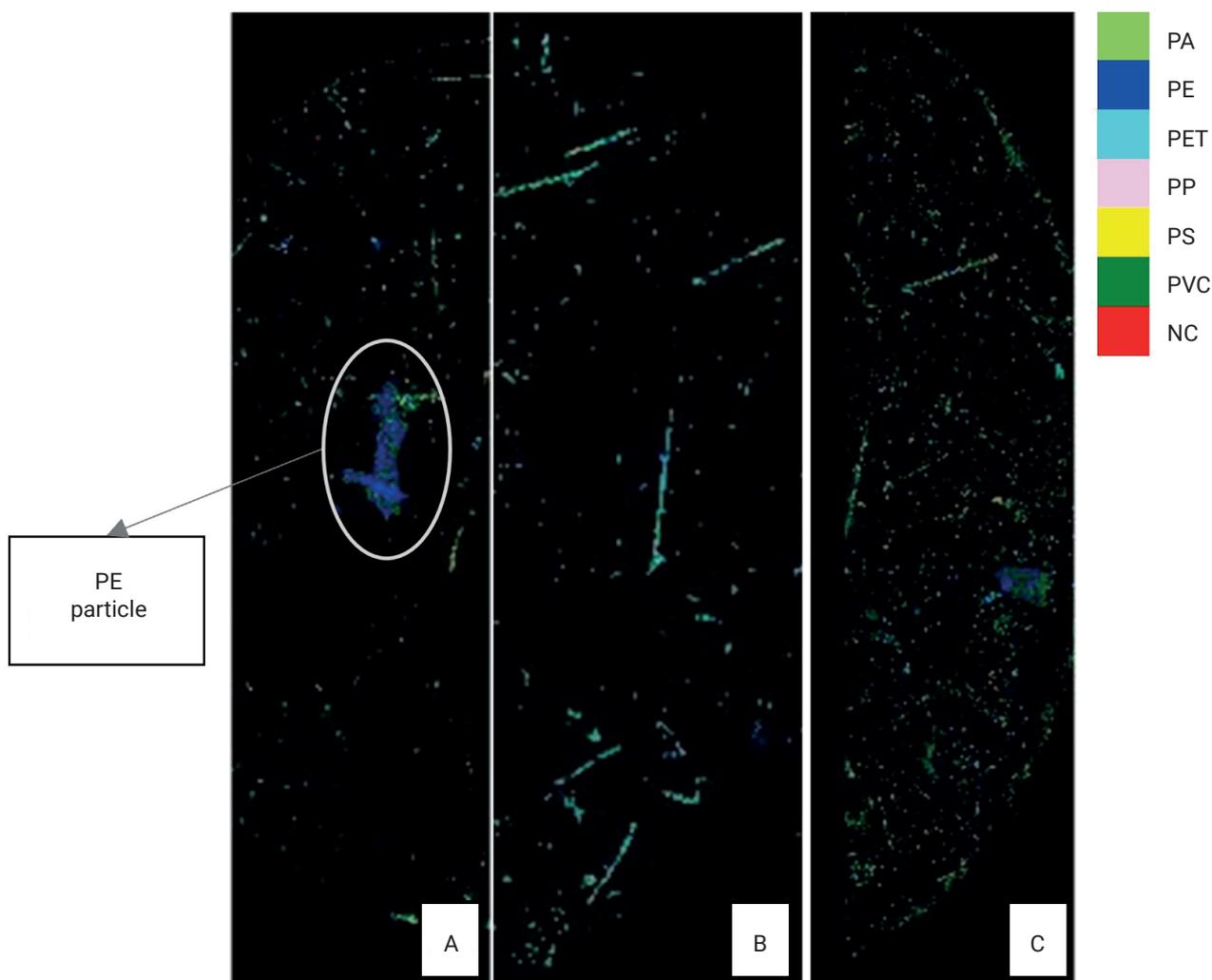
**FIGURE 5:** PE spectra. Spectrum of a reference material (blue) and PE particle spectrum acquired with FT-IR microspectrometer (red); spectra are shifted on the y axis for clarity.

After identifying the chemical composition of the large particle in sample 8 by using FTIR microspectroscopy, the same particle was re-analysed with the Raman scattering spectrometer and used to determine optimal parameters for recording its spectrum. The identified parameters were 785 nm excitation laser, acquisition time of 60 seconds, and 12 accumulations (see paragraph Materials and Methods). The newly identified spectral acquisition parameters were then used to collect spectra using Raman microspectroscopy from other particles on the sample. However, that signal was still overwhelmed by broad spectral bands of unidentified material(s) that we assign to contaminations that have not been removed by

the initial treatment. Therefore, new samples (ID u1 and u2) were re-treated with higher concentration of  $H_2O_2$  and density separation.

### 3.2 Hyperspectral Imaging results

The results of hierarchical PLS-DA applied to landfill leachate sample 8 are shown in Figure 6 as prediction maps. Sample 8 was selected for the larger size of some particles. In fact, particles larger than  $30\ \mu m$  in size are the key aspect for the correct implementation of hyperspectral imaging method, which includes also the removal of the sample background (i.e. the aluminium oxide filter) needed to highlight and better detect the particles.



**FIGURE 6:** Hyperspectral imaging results. Results shown in terms of prediction maps for sample 8 with the largest particle highlighted.

The sample was divided into 3 areas (named A, B and C from left to right) for a better acquisition, as shown in Figure 6.

Results derived from this analysis show that the largest particle (in area A in Figure 6), was clearly identified as a PE particle (as with Raman and FT-IR microspectroscopy previously), confirming the validity of the technique and the possibility to obtain successful results also on smaller particles changing the optics of the hyperspectral device. Hyperspectral imaging results suggest that most of the particles in this sample should be made of PE. However, these results are not reliable because the particles are significantly smaller than 30  $\mu\text{m}$ .

### 3.3 Effect of sample treatment on results and new treatment protocol

Results from the analyses with Raman scattering microspectroscopy of two retreated samples are reported in Table 3. As can be derived from Table 3, retreating samples does not have a clear, discernible positive effect on identifying PET with Raman scattering microspectroscopy, apart from that PS particles could be identified.

Thus, samples u1 and u2 were treated. They are shown in Figure 7 from which it can be derived that still a considerable amount of recalcitrant material is on the filter plates, indicating that digestion with  $\text{H}_2\text{O}_2$  (respectively 30% and 15%) and density separation was not sufficient. A multi-step pre-treatment approach, as for example developed to analyse microplastics in sewage sludge and described by Simon et al., 2018, is at hand. After the treatments, both samples were analysed with Raman scattering microspectroscopy, and results are shown in Table 3.

The following can be derived from the results shown in Table 3:

- Unlike the results of the analyses on the previous samples, in sample u1 it was possible to identify PS, PP, PE and carbon particles and not only PET particles.
- The most abundant identified polymer was PE (23% of particles in sample u1) whereas not a single PE particle had been detected in all previous observations.
- Only the floating particles from sample u1 (top) contained microplastics, thus confirming the need of a density separation treatment.

**TABLE 3:** Type and count of particles detected with Raman microspectroscopy within the mapped areas, of samples 3 and 9 before and after the treatment and of samples u1 and u2 treated with the new protocol.

Sample	Area ID	Noise <sup>1)</sup>	PE	PP	PET	PS	PET + uni-ident. <sup>2)</sup>	Unidentified spectrum	Unde-fined materi-al <sup>3)</sup>	Carbon	Particles analysed	Plastics ident. / Particles analysed %
3 previous	2	59	-	-	12	-	25	9	25	-	130	28
3 retreated	1	9	-	-	2	0	10	10	14	-	45	27
	2	7	-	-	1	0	21	8	18	-	55	40
3 retreated Subtotal	2	16	0	0	3	0	31	18	32	0	100	34
9 previous	3	29	-	-	2	0	12	7	25	-	75	19
9 retreated	1	10	-	-	4	0	-	13	23	-	50	8
	2	17	-	-	2	2	2	16	11	-	50	12
9 retreated Subtotal	2	27	0	0	6	2	2	29	34	0	100	10
<b>Samples with new treatment</b>												
u1 (top)	1 <sup>4)</sup>	6	16	-	-	1	-	-	5	2	30	57
	2 <sup>4)</sup>	1	-	-	-	4	-	-	25	-	30	13
	3	22	24	-	-	-	-	-	11	-	57	42
	4	52	23	2	-	1	-	-	21	-	99	26
	5	86	19	-	-	1	-	-	32	-	138	14
u1 (bottom)	1	20	-	-	-	-	-	-	10	-	30	-
	2	2	-	-	-	-	-	-	28	-	30	-
u2	1	24	-	-	7	-	2	-	12	-	45	16
	2	30	-	-	5	-	4	1	30	-	70	7
Total	9	243	82	2	13	6	6	1	174	2	529	21

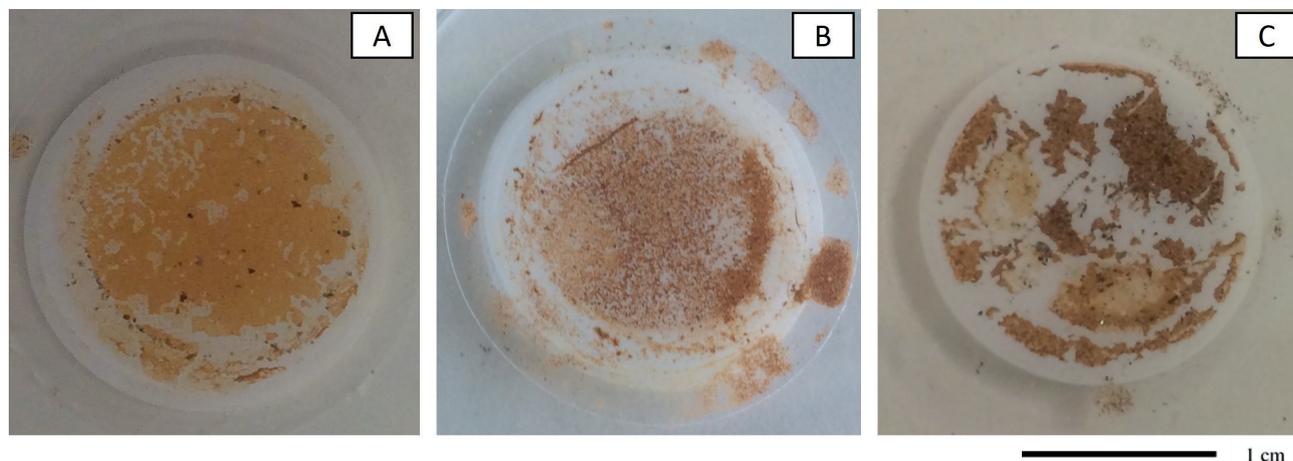
- Sample u1 did not show any kind of contamination in terms of the two bands in the spectra observed previously.
- Results from analysis of sample u2 showed the same result as for the samples pre-treated (i.d 7,8,9,3,4bis): only spectra of PET particles and unidentified contaminations could be recorded.

An example of identifying PP and PE with reference spectra is shown in Figures 8 and 9 for particles in sample u1. The PP spectrum of the sample was strictly matching

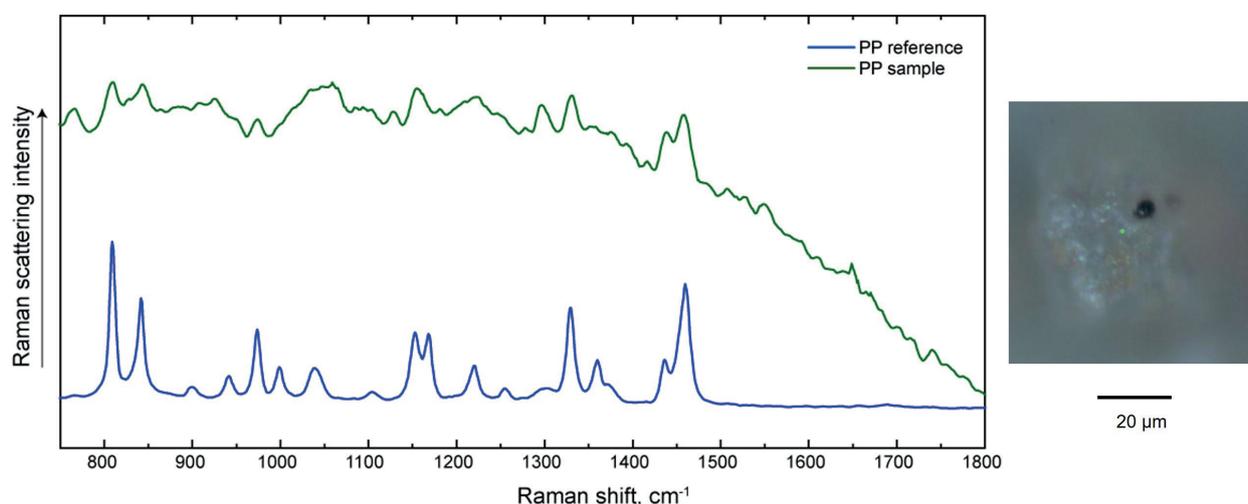
with the reference materials, the PE spectrum exhibited a contribution from several unidentified spectral bands as compared to the reference.

### 3.4 Comparison of spectroscopic methods

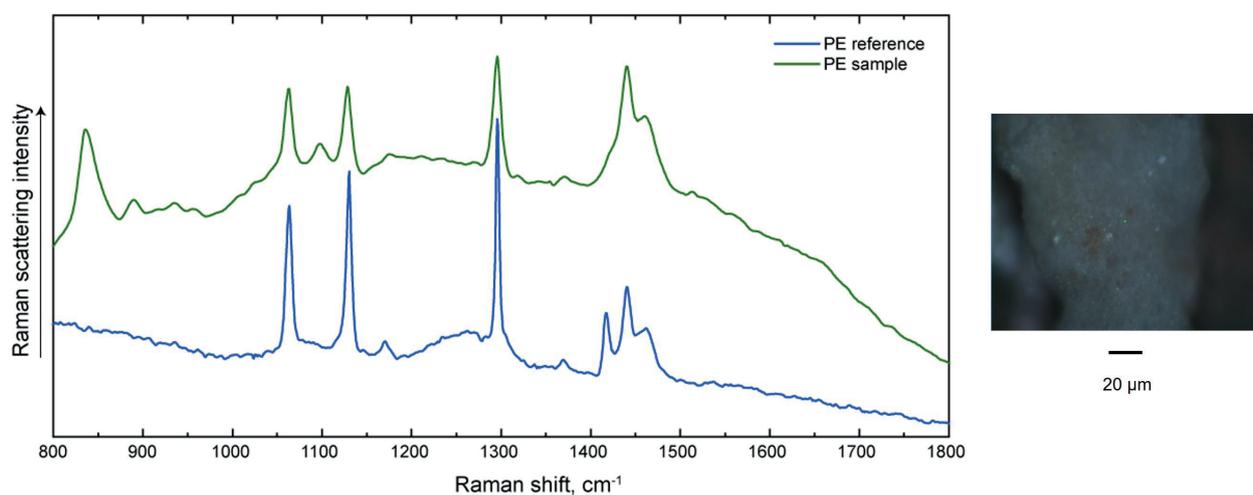
The use of the three different methods for microplastic analysis in landfill leachate samples clearly showed that all of them are suitable to identify microplastics, but that they all have their limitations. When it comes to a direct com-



**FIGURE 7:** Samples u1 and u2. Samples after density separation treatment (A: u1 top, B: u1 bottom), and without treatment (C: u2).



**FIGURE 8:** Example of a PP particle found in the sample u1 and its Raman spectrum. PP spectrum of a reference material (blue) and PP particle spectrum (green).



**FIGURE 9:** Example of PE particles found in the sample u1 and its Raman spectrum. PE spectrum of a reference material (pink) and PE particle spectrum (red).

parison between analysing the same filtered landfill leachate samples with Raman microspectroscopy and FT-IR microspectroscopy, it appeared that, even though Raman microspectroscopy was clearly more effective for particles smaller than 20  $\mu\text{m}$ , FT-IR was the more suitable solution to identify the spectra of some types of polymers (i.e. PE), affected by environmental or treatment distortion.

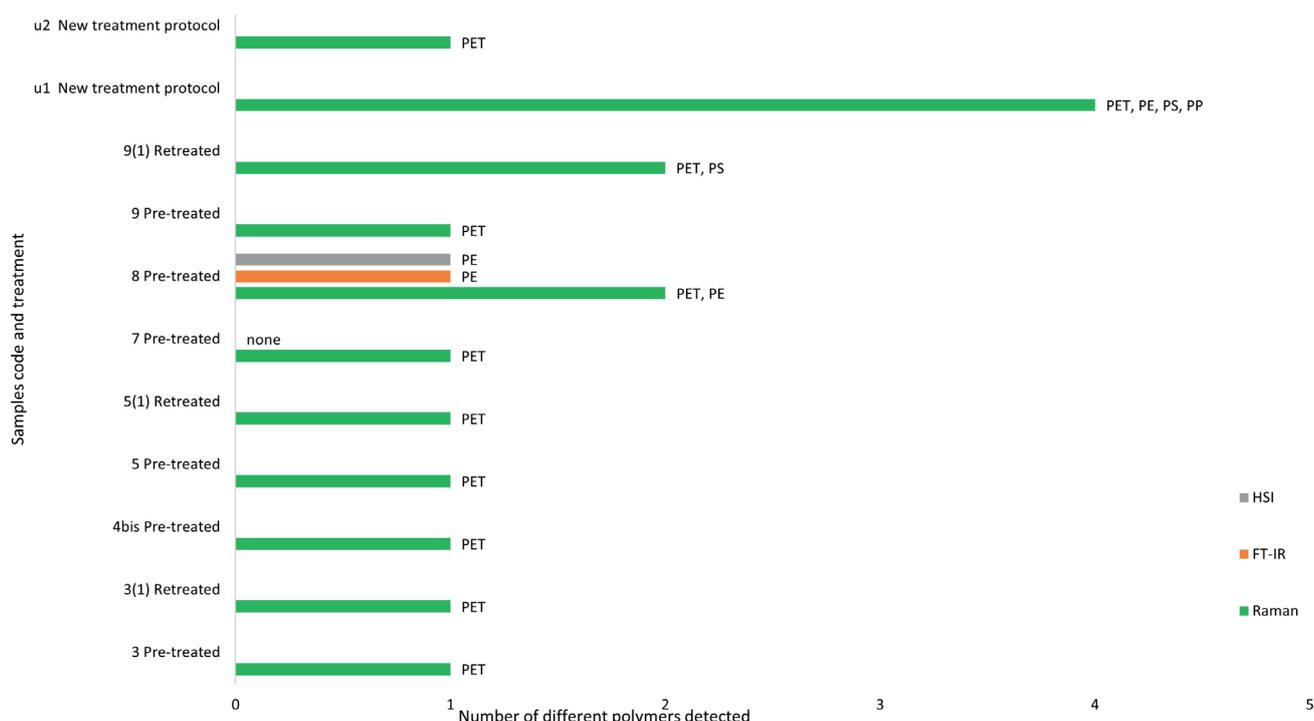
He et al., 2019, and Xu et al., 2020, found that FT-IR microspectroscopy worked well for analysing microplastics in landfill leachates with particles larger than 25 and 20  $\mu\text{m}$ , respectively, after sample treatment with 30%  $\text{H}_2\text{O}_2$  and Fe (II) followed by density separation, and treatment with 30%  $\text{H}_2\text{O}_2$  ( $\geq 48\text{h}$ ) and filtration, respectively. Su et al., 2021, applied Raman microspectroscopy on treated leachate samples (30%  $\text{H}_2\text{O}_2$  for 72h, and NaCl density separation) to analyse microplastic particles down to a size of 20  $\mu\text{m}$ . However, microplastics with much smaller sizes down to nanoscale have been observed in the environment (Gigault et al., 2016). In order to cover the full scale

of microplastics in landfill leachates, particles below 20  $\mu\text{m}$  have to be targeted, as well. Considering that, by using high magnification objectives, Raman microspectroscopy can work down to 1  $\mu\text{m}$ , and it appears to be promising for identifying these smaller microplastic particles in landfill leachate.

Raman scattering microspectroscopy in our study enabled to identify more microplastic particles than FT-IR but appeared to be very sensitive to sample preparation. This evidence is clearly shown in Figure 10, in which a synthesis of samples, treatment procedure and spectroscopic methods applied is described, as well as the results of analysis related to the identification of plastic polymers.

### 3.5 Recommended procedure for sample preparation and microplastic analysis

During this study, samples from landfill leachate were analysed and, in some cases, treated. It has been evident that environmental samples, especially leachate ones, re-



(1) retreated samples with the same name as the pre-treated ones

**FIGURE 10:** Sample treatment, spectroscopic method applied to each sample and results in terms of detected polymers.

quire an accurate treatment to avoid any kind of contamination that can influence the spectra. Thus, recommendations for future studies are suggested below, only related to sample treatment and techniques for the analysis.

Firstly, it is strongly suggested to pay much attention for potential sources of plastic contamination during the samples management (e.g., clothes, plastic equipment, gloves etc) and especially during the samples treatment. Moreover, the following procedure for sample preparation is recommended:

- Treatment: Selected samples should be treated with 30% H<sub>2</sub>O<sub>2</sub> solution.
- Density separation: Particles should be separated by dispersion using saturated salt solutions, e.g. NaCl (35 g of NaCl in 100 ml of *milliQ* deionized water). The solution should be mixed with a magnetic stirrer for at least 5 minutes. Saturation is indicated by NaCl grains undissolved on the container bottom. An adequate period of rest should be allowed for a correct density separation.
- Particle's collection: The most suitable method for the specific case should be selected to remove the majority of floating plastic particles. Both vacuum pump and syringe filter appeared to be effective for the collection. Eventually, particles should be properly deposited onto aluminium oxide filters with a polypropylene support ring, of adequate size and pore size.

Analysis of environmental samples often states challenges, especially if the analytical targets are of microscopic (or smaller) sizes. All three proposed methods are the-

oretically suitable for microplastic analysis in leachate or other effluents. However, due to the variable and unknown characteristics of the case-specific microplastics, a combination of techniques is recommended.

In addition, the following settings of instruments are recommended:

- Raman microspectroscopy: it is recommended to define the measurement parameters on a smaller section of the sample before starting the overall acquisition of the spectra. This allows to identify the most suitable and context-tailored set of parameters. However, this work highlighted that the following parameters are likely to be suitable for this kind of leachate sample studied:
  - excitation wavelength of 632.8 nm (He-Ne laser), with an acquisition time of 5-18 seconds; 5-8 scans in average for a single spectrum, a wavenumber range 1800 to 800 cm<sup>-1</sup>; excitation power set to 5-10% of the total laser power (17 mW).
  - In case of fluorescence, excitation wavelength of 785 nm (diode solid-state laser); acquisition time of 60 seconds, 12 scans for a single spectrum and an excitation power set to 100% of the total laser power (100 mW).
- FT-IR microspectroscopy: Either a single channel mercury cadmium telluride (MCT) detector may be used to obtain a single spectrum, or a focal plane array (FPA) detector, with a 64×64 array size, to obtain an IR multispectral image. A good response has been experienced by using a 250×250 μm field-of-view of the

×15/0.4 Cassegrain objective. The spatial resolution for recording the spectra was 4 cm<sup>-1</sup>, with 64 interferograms averaged. The result was Fourier-transformed into a spectrum applying the Blackmann–Harris 3 apodization function and zero filling factor 2. Furthermore, a ×20 Attenuated Total Reflection (ATR) objective (field-of-view 100×100 μm) combined with the single channel MCT detector showed to be suitable to record IR spectra of single particles but paying particular attention to the physical contact that could damage the sample.

- Hyperspectral Imaging: this analytical technique should be further explored in future studies. It is suggested to build a broader hierarchical model in order to detect a wider range of particles in the short-wave infrared (SWIR) range (1000-2500 nm). During this study, a satisfactory response has been experienced by equipping the device with a macro lens of a field-of-view of 1 cm, and with spectral resolution of 6.3 nm.

In Table 4, the advantages and disadvantages of samples treatment and analysis are listed, according and limited to the direct experience and findings of this study.

#### 4. CONCLUSIONS

During this study, more than 1000 particles from landfill leachate samples were analysed with Raman microspectroscopy and partially re-analysed with FT-IR microspectroscopy and Hyperspectral Imaging, to determine the best approach for analysis. Results indicate that with the application of the three methods it was possible to identify microplastic particles of different polymers. However, the sample treatment is fundamental to avoid contamination that can influence the spectra, especially in leachate samples. Both digestion with H<sub>2</sub>O<sub>2</sub> and density separation are invaluable sample treatments, but they should be optimized and complemented to remove more recalcitrant organic matter without impacting the plastic polymers. In fact, replicating

a H<sub>2</sub>O<sub>2</sub> treatment (with either the same or higher concentration) on a pre-treated sample did not improve at all the quality of results, supporting the hypothesis that the treatment itself could cause the undefined bands in the spectra.

In addition, the efficacy of pre-treatment has to be taken into regard for finding the optimal adjustment of key parameters for the FT-IR and Raman spectroscopic methods in order to avoid unwanted fluorescence or other spectral distortion phenomena. It is confirmed that microplastics in environmental samples are extremely difficult to detect since the spectra of particles are strongly affected by environment and treatments, which strengthen the need of a clear and efficient treatment protocol.

As a conclusion, the 30% H<sub>2</sub>O<sub>2</sub> treatment with a density separation is the best one to identify plastic particles with an acceptable time of acquisition.

Eventually, hyperspectral imaging, a fast and promising analytical technique, should be developed for particles smaller than 30 μm, so that a direct comparison to FT-IR and Raman scattering microspectroscopy can be carried out.

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**TABLE 4:** Summary of advantages and disadvantages of the recommended sample preparation and analytical techniques, based on actual and direct findings of this study.

Method	Advantages	Disadvantages
<b>Sample preparation</b>		
H <sub>2</sub> O <sub>2</sub> Treatment	Efficient removal of non-plastic organic materials	Risk of external contamination
Density separation	Efficient isolation of floating plastic particles	Risk of external contamination; risk of losing nano/micro particles
<b>Analytical technique</b>		
Raman microspectroscopy	Detection of particles up to 1 μm in size; possibility to detect particles in large areas of the sample; flexibility of measurement parameters; non-contact analysis	Sensitive to sample preparation; difficulties in identifying some types of polymers affected by environmental or treatment distortion
FT-IR microspectroscopy	More suitable solution to identify the spectra of some types of polymers (i.e. PE), affected by environmental or treatment distortion; flexibility of measurement parameters	Contact analysis (ATR); non suitable for particles smaller than 10-20 μm
Hyperspectral imaging	Extremely precise technique; context-tailored analysis technique	Non suitable for particles smaller than 30 μm; need to build the hierarchical model before the analysis; need to correctly remove the sample background for a better acquisition

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