

Advancing Microplastic Analysis in Complex Matrices Towards Harmonization: Insights from an Interlaboratory Study Supporting the Development of a Standardized Extraction Protocol

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Research Article

Keywords: Standardization, harmonization, microplastics, extraction, analysis, compost, interlaboratory comparison

Posted Date: October 9th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11293971/v1>

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Additional Declarations: The authors declare potential competing interests as follows: Some of the authors are employees of BASF SE / Corporation, a company producing and marketing polymers, including both conventional and biodegradable polymers.

Abstract

Reliable quantification of microplastics in complex environmental matrices remains challenging due to the lack of harmonized extraction procedures and matrix variability. To support standardization of a compost extraction protocol, we conducted an interlaboratory comparison across laboratories with diverse expertise and analytical capabilities. Compost samples were spiked with 33 mg of cryo-milled poly(butylene adipate-co-terephthalate) (PBAT) and 33 mg of low-density polyethylene (LDPE) particles (50–300 μm) and distributed with blank samples to participating laboratories. All laboratories applied an identical extraction protocol consisting of deagglomeration, Fenton oxidation, and calcium chloride density separation. Each laboratory then used their available technique, including μ -Raman, μ -Fourier Transform Infrared Spectroscopy (μ -FTIR), fluorescence microscopy, Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS), and Thermal Extraction Desorption-Gas Chromatography/Mass Spectrometry (TED-GC/MS) to identify and quantify the extracted microplastics. All laboratories detected and quantified both target polymers, demonstrating the applicability of the protocol. However, considerable inter- and intra-laboratory variability was observed for particles < 100 μm . Further investigations indicated that variability originated from compost subsampling, dispersion behavior of the particles, and non-harmonized analysis; rather than the extraction procedure. Despite differences in absolute particle counts, relative particle size distributions were comparable. Mass-based methods also identified and quantified both polymers, although recoveries varied across laboratories, reflecting differences in analytical workflows and quantification approaches. Feedback from participating laboratories enabled critical protocol improvements, including optimized Fenton conditions and modifications to the density separation procedure. Overall, this study shows that harmonized extraction of microplastics from compost is feasible and provides a foundation for the ongoing development of ISO/FDIS 24899 and future standardization of microplastic analysis in complex matrices.

INTRODUCTION

Recent studies estimate yearly global mismanaged plastic waste to be 52 million tons, with around 60 wt.-% being open-burned and 40 wt.-% ending up as debris in the environment, representing a yearly release of approximately 22 million tons.[1] The portion of the plastic waste that ends up in the environment has the potential to break down and form micro- and nanoplastics, contaminating natural environments with persistent small particles that are difficult to detect and remediate.[2], [3], [4], [5] The extraction and analysis of microplastics in environmental matrices represents a critical step towards understanding and ultimately addressing this contamination issue. Although the extraction of microplastics from water matrices (e.g. drinking water, surface water, ground water) is comparatively less complex[6], [7] than from compost and soil, it remains analytically challenging. In contrast, these matrices pose substantially greater difficulties due to diverse and high loads of non-plastic particles and organic matter.[6], [8], [9], [10]

Although there is some agreement on required organic matter removal and density separation steps for microplastic extraction from complex environmental matrices,[9], [11], [12] a consensus on a single workflow is still missing. This leads to ambiguous results that are not comparable. In general, the isolation of microplastics from complex environmental matrices comprises two main steps: removal of organic matter through oxidation or other digestion approaches, and separation of inorganic material via density separation.[8] The organic matter removal is usually done either using hydrogen peroxide only (H_2O_2)[13] or a Fenton reagent,[14] where Fenton is favoured due to the reduced reaction times. Density separations can be performed with different salt solutions and durations, [15] with the commonly used salt solution covering densities between 1.2 and 1.75 g/cm³ to extract a wide range of polymer types. [16], [17]

Following extraction, the recovered microplastics are analyzed to determine their chemical identity, quantity, and size. The analysis of extracted microplastics from any matrix can be done either by number-based methods (micro- infrared spectroscopy (μ -IR)[18], μ -Raman spectroscopy[19] and Fluorescence microscopy[20], [21]) or by mass-based methods like pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS)[22] and thermal extraction desorption-gas chromatography-mass spectrometry (TED-GC/MS).[23] Number-based methods report the size, shape, number and/or polymer type of the microplastic particles present in the sample. Currently, these methods are generally considered to provide reliable detection down to the lower micrometer range (approximately 20–50 μm) [24] and the measurement can be semi-automated, with the main limiting factor being the measurement time.[25] Mass-based methods identify the polymer type and quantify their total mass (without size information) present in the sample. They are limited by the minimum plastic mass in the sample, the instrument's detection limits, and/or the linear response range for each polymer,[26] with most recent developments trying to analyze trace amounts, including nanoplastics.[27], [28] While mass-based techniques have the potential to enable faster and automated measurements, matrix interferences can lead to false positives (especially for PE), requiring interpretation by highly experienced analysts. [26], [29]

Several interlaboratory comparisons (ILCs) on microplastic analysis have already been conducted. De Frond et. al [30], Ciornii et. al [31] and, Muller et. al, [32] evaluated the performance of different analytical approaches and laboratories using well-characterized test materials in aqueous matrices. These studies demonstrated substantial variability between laboratories and methods, particularly for particle quantification, while polymer identification generally showed better agreement. De Frond et. al [30] further reported markedly higher recoveries for larger particle size fractions (> 50 μm) than for the smallest particles. Cadiou et. al [33] investigated artificially prepared sediment and water samples and found that total particle counts were generally within $\pm 25\%$ of the reference values, although both under- and overestimations occurred. On the other hand, Gerigny et. al [34] evaluated spiked and real marine sediment samples and reported recovery rates ranging from 47 to 113 %, while similar spatial trends were observed across laboratories despite considerable variation in the reported absolute microplastic concentrations. Collectively, these studies highlight the need for standardized methods, well-

characterized reference materials, and robust quality assurance procedures to improve the comparability and reproducibility of microplastic measurements.

However, previous ILCs focused primarily on aqueous and sediment matrices. The present ILC study therefore investigates a substantially more complex, organic-rich compost matrix and includes both the conventional plastic LDPE, and the biodegradable polymer PBAT. Our study was designed to establish a basis for a standardized extraction method of microplastics from compost. Accordingly, its scope was limited to the extraction procedure and excluded both sampling at composting facilities and downstream analytical procedures. The extraction protocol evaluated here is based on the previous work of Wohlleben et al.[19] and Pfohl et al.[35]: Our adapted protocol comprises a deagglomeration step, followed by organic matter removal using a Fenton's reagent, and a final density separation with a calcium chloride (CaCl_2) solution. The Fenton protocol used in those studies was found to have insignificant effect on the polymer, maintaining their particle integrity, while removing most of the organic carbon material from compost.[36] We selected a CaCl_2 solution because it delivers the required density for most types of polymers, exhibits low reactivity and is readily accessible at reasonable cost. To assess its suitability for future standardization, the protocol was evaluated in an ILC using 33 mg of each LDPE and PBAT particles ranging from 50 to 300 μm in size.

Ten partner laboratories with varying levels of experience followed the same extraction procedure but used the analytical techniques available at their facilities for subsequent quantification. These included μ -Raman, μ -FTIR, Py-GC/MS, and TED-GC/MS. In addition to assessing the interlaboratory applicability of the extraction protocol, feedback from the participating laboratories was used to identify practical challenges and support further refinement of the extraction method.

MATERIALS

A 12- to 14-week-old compost (OWS, Belgium) was sieved to remove particles > 10 mm, then dried at 58 °C until no further weight loss was observed. The dried compost was sterilized by autoclaving (121 °C, 45 min, 100 kPa), after which 1 kg was ground manually with a mortar and sieved to < 1 mm. The compost was autoclaved to enable safe shipment and storage of the samples prior to distribution to participating laboratories. Since autoclaving may alter microplastics present in environmental samples, the autoclaving step is not recommended for the untargeted analysis of actual microplastics in compost.

Two polymer types were used to spike the compost: poly(butylene adipate-co-terephthalate) (PBAT) granules (BASF SE, $\rho = 1.26$ g/cm³) and low-density polyethylene (LDPE) granules (LyondellBasell, $\rho = 0.917$ g/cm³). Both materials were cryo-milled using a Retsch ZM 200 (18000 rpm) with liquid nitrogen and a 500 μm sieve insert. The resulting powders were subsequently size-fractionated for 1 hour with a Retsch AS 200 control sieving machine using 300 and 50 μm cut-offs. The final particle size distributions were determined via Fraunhofer light scattering, μ -Raman (Lab 1) and fluorescence microscopy (Lab 3) and are shown in Figure S1 and Table S1.

METHODS.

ILC organization.

The ILC was jointly organized by BASF Ludwigshafen and the University of Vienna in collaboration with international partners, including IMDEA Water Institute, the Joint Research Centre of the European Commission, ECSIN-ECAMRICERT SRL, Mérieux NutriSciences Italy, Nestlé, University of Applied Sciences Dresden, Umweltbundesamt GmbH, BASF Corporation and others. To harmonize method application across laboratories, hands-on in-person training sessions on sample extraction were conducted at BASF Ludwigshafen and at the University of Vienna in September 2024, complemented by video material and an online Q&A session on μ -Raman and μ -FTIR microscopy. As the ILC organizers had limited experience with mass-based analysis of complex environmental extracts, laboratories applying mass-based techniques relied on their own methodological expertise. The ILC was officially launched with two joint kick-off meetings in January 2025, during which study design, sample handling, and documentation requirements were aligned. Throughout the experimental phase, continuous bilateral exchange between the coordination team and participating laboratories was maintained, particularly to address analytical challenges and protocol deviations. Data evaluation and anonymized compilation of results were carried out during 2025. In December 2025, the results were jointly discussed with all partners, leading to the identification of methodological improvements and forming the basis for joint scientific publication as well as further development of the ISO standard.

Generation of test composts for ILC partners.

Two distinct ILC sample types were prepared based on the same compost (as described above): 1) unspiked matrix samples and 2) LDPE/PBAT-spiked samples. For unspiked matrix samples (1), no polymer particles were added to the compost. For (2) spiked samples (Figure S2), 300 g of autoclaved compost (<1 mm) and polymer powders were layered in a PP bottle, each layer consisting of 50 g compost plus 2.000 g LDPE and 2.000 g PBAT powders (until the full 300 g of compost and a total of 10 g of each polymer powder had been added). The polymer powders were weighed for each layer using an analytical balance. Weighing was performed outside a fume hood to minimize draft effects. The balance acceptance criterion was ± 0.15 mg (outside the fume hood). Both sample types 1 and 2 were transferred to separate polypropylene (PP) bottles and mixed for 1 h in a TurbulaSystem Schatz T2F, Willy A. Bachofen AG (without a fixed rotating axis), then 1.000-1.045 g subsamples were dispensed into 50 mL glass bottles. In total, 110 compost samples and 110 spiked compost samples were prepared. Aluminum foil was placed under the caps to minimize contact with PP cap material, and the bottles were additionally sealed for shipment. The use of aluminum foil beneath the caps is a general precaution commonly recommended for the storage and shipment of microplastic samples to minimize potential contamination from packaging materials. However, it should be noted that PP could not have been reliably quantified in these compost samples regardless, as the compost was homogenized in a PP bottle prior to distribution. The scope of this ILC focused exclusively on the targeted quantification of LDPE and PBAT.

Extraction protocol used in ILC.

Once the samples were distributed, the ILC participants were instructed to follow a well-defined extraction protocol (Figure 1), adapted from previous work.[19], [35], [36] A detailed description of the method and corresponding instructions are provided in the Excel file included in the Supporting Information (SI), which was supplied to the ILC partners.

In short, the compost sample was dispersed in ultrapure water and sonicated for 5 min with delivered acoustic power of 14.5-16.5 W (corresponding to a delivered energy of 4.4-5.0 kJ). In the next step, a mild organic matter removal protocol based on Fenton oxidation was applied while stirring the dispersion with a glass-encapsulated stir-bar or an overhead-stirrer. Fenton oxidation was carried out in five consecutive additions of 15 mL of 30% hydrogen peroxide and 5 mL of a 0.05 M iron(II) sulfate (FeSO_4) solution. After the last addition, 250 g of solid CaCl_2 dihydrate were directly added to the dispersion while continuously stirring. Afterwards, the dispersion was transferred to a pre-filled separation funnel containing 100 mL of a 5.25 M CaCl_2 solution (with a density of 1.4 g/cm^3). The beaker containing the dispersion was rinsed with 100 mL of the CaCl_2 solution. The density separation was carried out in two consecutive 24-hour stages. After the first stage, the high-density fraction was discarded, and 100 mL of fresh salt solution was added. To complete the extraction, the high-density fraction was discarded again until reaching a final volume of 100 mL. The remaining content of the separating funnel was transferred to a 250 mL glass bottle, filtered through a $25 \mu\text{m}$ stainless steel mesh (zivipf.com), and the retained particles were resuspended in 100 mL of ultrapure water. The resulting suspension was used for further sample preparation and analysis, with the specific analytical procedures depending on the techniques available at each participating laboratory.

Quality control/blank measurements.

Two different quality control samples were requested to be measured by ILC participants: a method blank and an unspiked matrix sample (as described above). To generate the method blank, participants had to filter all liquids used for the extraction and to analyze the particles retained on the filter, thereby assessing potential plastic contamination originating from the chemicals and laboratory surroundings. To generate data on the unspiked matrix sample, participants were provided with 1 g compost samples without added polymeric particles, which were extracted using the same protocol as the spiked samples. This analysis was used to assess background plastic contamination in the compost matrix and to enable comparison with the spiked compost samples. Participants analyzed the controls with the same analytical technique and parameters as the spiked samples. The detailed blank preparation description is provided in the Excel sheet included in the SI.

To verify the ability of each laboratory to analyze the spiked microplastics in the desired size range, participants were provided with pristine polymer powders of both LDPE and PBAT. After the initial round of data evaluation, it was decided to distribute an additional reference sample containing 33 mg of each polymer powder, corresponding to the theoretical mass of microplastics present in each spiked compost

sample. This reference sample was included to assess whether the observed variability originated from sample homogenization from extract subsampling or from the analytical procedures themselves. Participants were instructed to disperse the polymer powders in 100 mL of ultrapure water and to follow the same analytical workflow used for the sample analysis and measurement of the spiked samples.

Analysis and data collection.

The ILC was conducted to evaluate the applicability of the extraction protocol currently under ISO standardization (ISO/FDIS 24899). As the future standard is limited to extraction, subsequent analytical steps were not prescribed, allowing each participating laboratory to use the analytical equipment available at their facility. Five distinct analytical techniques were employed during this ILC: μ -Raman, μ -FTIR, fluorescence microscopy, TED-GC/MS and Py-GC/MS.

The ILC organizers (BASF Ludwigshafen and University of Vienna) provided some initial suggestions for the microscopy techniques on how to perform the analysis after the well-defined extraction, but each laboratory was encouraged to use their usual quantification protocols for conducting the measurements. For μ -Raman, the 100 mL extract should be subsampled, and filtered for analysis on a flat filter, which offers high contrast in the microscopy imaging. For μ -FTIR, the 100 mL extract should be subsampled and filtered on an IR inactive filter. Both measurement techniques require room-temperature dried filters, as moisture could interfere with the measurement. For both techniques it was highly recommended to employ an automated particle selection and analysis software. The participants were asked to provide the counts and sizes of the analyzed particles as equivalent circular diameter (ECD) and/or Feret max, preferably both metrics where possible. For mass-based techniques, only the total mass of LDPE/PBAT per g of compost was requested. Detailed descriptions of the analytical methods used by the partners are provided in the SI.

To report the results for the number-based methods the ILC organizers requested to have information about the exact volume of the analyzed subsample and the number of particles of each polymer (LDPE or PBAT) in defined size bins (see Excel file in the SI). For the mass-based methods the ILC organizers requested the volume of the analyzed subsample and the mass of detected polymer (LDPE or PBAT). Data reporting for polymer types other than the target materials detected in the spiked composts or blanks was not requested.

Participation and methodological diversity in the ILC.

In total, 29 laboratories were invited to participate in the interlaboratory comparison, of which 14 confirmed their participation. Ten laboratories submitted results from extraction procedures and associated blanks. Among these, four laboratories employed mass-based analytical techniques, with two additionally providing data for the water reference sample. Eight laboratories applied number-based approaches, including four reporting ECD, three reporting Feret max, and one reporting both ECD and Feret max. Seven of those also submitted results for the water reference sample. One laboratory

reported only total particle counts without size distribution. Furthermore, two laboratories combined both mass-based and microscopy techniques in their analyses.

Additional mass analysis for data interpretation – Soxhlet extraction

A set of eight ILC compost samples (six spiked samples and two unspiked matrix samples) was analyzed in Laboratory 1 using a two-stage Soxhlet extraction protocol (Figure S3). For each extraction step, 1 gram of compost was placed into a Soxhlet thimble (MN 649, 33 × 94 mm, Macherey-Nagel), covered with a 1 µm cellulose filter, and installed in the Soxhlet apparatus.

In the first stage, PBAT was extracted with hexafluoroisopropanol (HFIP, Apollo Scientific).[19] LDPE and the bulk of the compost are insoluble in HFIP and therefore remain in the thimble. The extraction flask was weighed, filled with 200 mL HFIP, and a stir bar was added. The HFIP extraction was carried out in an oil bath at 120 °C. After a 15-minute heat-up period, two complete Soxhlet cycles were permitted; cycle times were then recorded during cycles 3-5 to confirm operational consistency. Extraction continued for a total of 35 cycles. While the apparatus drained, the oil bath temperature was reduced. The thimble was not discarded after the HFIP extraction; instead, it was left to dry by evaporation overnight in a fume hood. The extract collection flask was removed from the apparatus, the stir bar withdrawn, and HFIP removed by rotary evaporation using a 45 °C water bath. The flask was first allowed to equilibrate to this temperature, after which the pressure was slowly reduced to 400 mbar. After 30 minutes, the pressure was lowered stepwise to 100 mbar and held until no visible liquid remained. Open flasks were then dried in a vacuum oven at 60 °C and 400 mbar, and the dried extract mass was determined by weighing the flask.

For the second stage, the dried thimble was reinstalled in the Soxhlet apparatus and again covered with a 1 µm cellulose filter. The extraction flask was weighed, filled with 200 mL xylene, and a stir bar was added. Xylene extraction was performed in an oil bath at 180 °C; used LDPE dissolves in hot xylene while the majority of the compost remains in the thimble.[37] The Soxhlet head was insulated with aluminum foil to maintain vapor temperature. Following a 15-minute heat-up, two full cycles were allowed to proceed and cycle times were recorded during cycles 3-5. Extraction was stopped after 35 cycles and the oil bath temperature was lowered during draining. After extraction, the stir bar was removed and xylene was evaporated by rotary evaporation with a 90 °C water bath. Pressure was gradually reduced to 130 mbar while the flask equilibrated; after 30 minutes the pressure was lowered stepwise to 90 mbar and maintained until no visible liquid remained. Flasks were then dried open in a vacuum oven at 60 °C and 400 mbar, and the final flask mass was recorded. Blank-corrected extract masses were obtained by subtracting the mean blank extract mass from the extract mass of the spiked sample.

Additional particle number analysis for data interpretation – Fluorescence microscopy

Additional analysis of the extracted ILC samples was conducted by Laboratory 3 using fluorescence microscopy. The applied sample preparation protocol was similar to the one for number-based analytical techniques. Subsamples from the final extracts were filtered through a 47 mm glass fiber filter with a

pore size of 1 μm and the particles on the filter were stained with a 10 μM 4-Dimethylamino-4'-nitrostilbene (DANS) in ethanol solution.[21] DANS is a solvatochromic dye that allows the differentiation of LDPE and PBAT (green and orange, respectively), given that emission wavelengths are distinct. The remaining compost matrix particles are only weakly stained by the dye and can be readily distinguished from polymer particles based on their lower fluorescence intensity and their appearance in bright-field microscopy (Figure S4). The stained filters were dried at 60 $^{\circ}\text{C}$ for 1h. Finally, the samples were analyzed with a Nikon SMZ1500 stereomicroscope, equipped with a Prior Scientific Lumen 200 fluorescence probe, reporting the same parameters as the spectroscopic techniques (particle number, identity and size with a sieve cut-off of 25 μm). Unspiked matrix samples were analyzed in parallel, and the corresponding values were subtracted from the results obtained for the spiked samples. The pristine polymer powders were analyzed as additional references. Eight extract replicates were analyzed (each from a different 1 g compost sample). Additionally, for the pristine powder analysis 16.5 mg of LDPE was dispersed in 50 mL of ethanol (to improve dispersibility) and 16.5 mg of PBAT in 50 mL of ultrapure water, maintaining the proportion corresponding to the final extract of the samples analyzed by number-based methods.

RESULTS AND DISCUSSION.

Qualitative feedback on the extraction protocol applied in the ILC.

Participating laboratories provided feedback on aspects requiring revision to further improve the extraction protocol for the future ISO standard (Figure S5). One key point identified was that the use of glass-encapsulated stir bars should be avoided, as the glass is prone to breakage, potentially releasing undesired glass particles during mixing and altering the shape of microplastic particles. Some participants raised the concern that particle fragmentation may have occurred during the more aggressive steps of the ILC protocol, particularly during sonication and Fenton oxidation. Although previous stability tests indicated that the selected LDPE and PBAT particles were stable under defined extraction conditions,[36] fragmentation under the exact ILC conditions cannot be fully excluded, especially for PBAT. This feedback was therefore considered in the subsequent optimization of the protocol for the future ISO standard. Another aspect was the formation of iron precipitate on the microplastics resulting from the Fenton reagent used during the organic matter removal step. This hinders the analysis with μ -Raman and necessitated an adjustment of the reagent amounts added to the solution. It was also stated that the direct addition of solid calcium chloride in the dispersion after the Fenton reaction generated heating and foam formation. This observed phenomenon could be due to the reaction of the CaCl_2 with remaining H_2O_2 in the solution to gaseous species, mainly CO_2 and O_2 .[38], [39] Heating could alter the shape of the microplastics, and foaming could push microplastics upward, reducing recovery rates. In addition, it was also observed that due to the incomplete solubility of the salt in the solution calcium chloride crystals were formed during the density separation phase. The observed issues related to calcium chloride solubility and reactivity were not consistent across laboratories, indicating that the difficulties encountered are likely powder handling, batch- or supplier-dependent. As calcium chloride is typically supplied in plastic containers, attention should be paid to the container

material, and the salt solution should be checked for potential microplastic contamination and, if needed, filtered in case of microplastic contamination prior to use.

Quantitative results from number-based analytical techniques.

For data interpretation, we followed a stepwise data reduction approach, moving from full particle size distributions toward simplified and more robust groupings, to identify the main sources of variability and enable meaningful interlaboratory comparison.

Figure 2 represents the reported particle counts for each polymer in the specified size ranges for six out of eight participating laboratories. Spiked compost data from all laboratories are included in Table S2, blank and unspiked matrix sample data are included in Tables S3 and S4. Blank correction was not applied, in accordance with the ISO 16094 series,[40], [41] which requires blank results to be evaluated and discussed separately from sample results. Inclusion of Laboratory 7 data in the same plot would require rescaling and thereby compromise the visual comparison of results among the other laboratories. However, it is noteworthy that a substantial number of LDPE particles were detected in both the method blanks and the unspiked matrix samples analyzed by Laboratory 7. Subtraction of these values would reduce the particle counts observed in the spiked samples. Laboratory 8 measured the samples with a μ -FTIR that did not have an integrated particle identification/selection software, therefore only total particle counts could be reported. Overall, all laboratories were able to count and detect, to some extent, LDPE and PBAT particles. However, the reported data show considerable intra- and interlaboratory variability, which may arise from multiple sources of experimental uncertainty (such as sample inhomogeneity, sample preparation, chemicals, operators and analytical procedures). To better compare and pinpoint the differences of the inter- and intralaboratory data variability in the raw reported results from the participants, the microplastic size distribution data was grouped in larger or smaller than 100 μ m (Figure 3). This distinction was made because the analysis of particles < 100 μ m is generally considered more challenging and may therefore contribute to increased variability in the reported results.[42], [43]

Overall, the reported data consistently indicate higher numbers of PBAT particles compared to LDPE in the distributed samples. With the exception of Laboratory 1, the results show higher particle counts for microplastics larger than 100 μ m than smaller 100 μ m. When comparing the ILC data and fluorescence results, particle counts for sizes > 100 μ m are generally comparable across all laboratories and analytical approaches (Figure 3). The only exception was the LDPE dataset from Laboratory 7, for which total counts >100 μ m remained approximately threefold higher than those reported by the other laboratories, even after subtraction of the unspiked matrix sample results. In the case of LDPE, however, lower particle counts were observed compared with measurements of pure LDPE powder dispersed in ethanol. This suggests that particle losses may have occurred during analytical sample preparation, potentially due to the hydrophobic nature of LDPE and its density being lower than that of water. To determine whether these losses predominantly occur during subsampling from the final extract or already during the extraction process, an additional experiment was conducted with μ -Raman comparing subsampling

from the aqueous extract (as applied in the ILC) with subsampling after redispersing the extracted and filtered particles in ethanol instead of water (Figure S6, Table S1). This approach resulted in higher recoveries for larger particles but simultaneously led to reduced recoveries of smaller particles (Figure S6a). The latter effect was attributed to ethanol-induced bleaching of the filter, leading to the transfer of black dye from the black polycarbonate (PC) filter used to LDPE particles. This reduced the contrast between the particles and the filter background, thereby impairing the reliable detection and selection of small particles prior to analysis (Figure S6b). Based on these findings, a final ethanol rinsing step was incorporated into the future ISO standard, given that the total recovery of LDPE is higher when using ethanol for dispersing. However, follow-up ISO standards addressing the analysis of extracts from complex environmental matrices should carefully consider this trade-off, as ethanol can enhance recovery of some hydrophobic polymers while simultaneously impairing the detectability of small particles due to the described artefact.

Particle counts < 100 μm for both LDPE and PBAT were only comparable between the fluorescence measurements and Laboratory 1, underlining the need for improved and harmonized quantification approaches for the reliable analysis of small microplastic particles. However, such comparisons should be interpreted with caution, as the lower size detection limits varied among participating laboratories. In particular, not all laboratories were able to analyze particles down to 25 μm , which may have contributed to differences in the reported particle counts below 100 μm . Labs 1, 2, 3 (Fluorescence microscopy), 4 and 5, reported a theoretical cut-off at 25 μm (ECD), Labs 5 and 7 had a cut-off at 25 μm (Feret max), Lab 6 reported a cut-off at 40 μm (Feret max), Lab 3 (μ -Raman) had a cut-off at 50 μm . Lab 8 reported that they used manual particle selection and spectra analysis. Laboratory 1 used μ -Raman for analysis, used black rather than dark grey filters and applied dark field imaging for particle selection. The combination of dark-field imaging and black filters may be particularly advantageous for the detection of smaller microplastic particles, as it enhances the contrast between particles and the filter substrate. [44] Laboratory 6 also used dark-field imaging; however, particle selection was performed on non-black filters, and a higher abundance of particles in the 200-300 μm size range was reported compared with the other laboratories. One possible explanation is that particle agglomeration on the filters led to multiple small particles being classified as fewer, larger particles during image analysis. Based on the reports from the participating laboratories, it became apparent that automated particle analysis at the lower extraction cutoff of 25 μm remains technically challenging for many laboratories. In some cases, the current analytical systems do not support automated measurements down to this lower size threshold. Alternatively, a substantial increase in detected particle numbers is observed when the cut-off size is reduced, resulting in analysis times that are not practically feasible.

To facilitate the analysis of particles < 100 μm , the abundance of non-plastic background particles remaining after the extraction protocol should be reduced, which might require an adaptation of the ISO/FDIS 24899 protocol or a separate dedicated extraction protocol for small microplastics. Future developments of the standard could focus on this aspect to decrease the time required for an automated analysis. Another possibility could be the development of better contrasting filters for a more accurate particle selection. The ILC organizers used and recommended PC filters for μ -Raman analysis.

While these filters have proven suitable for routine measurements, they are based on polymeric material and therefore do not allow the analysis of PC microplastics. In addition, surface inhomogeneities and irregularities on the filter can be falsely identified as particles during optical image analysis. To our knowledge, current developments are increasingly focusing on metal-coated filters and silicon wafer substrates, which may improve optical particle detection and reduce background interference. However, these alternatives remain comparatively expensive and are currently not well suited for high-throughput or large-scale analytical workflows.

With respect to the high intra-laboratory variability, several contributing sources can be identified. Variability may already arise at an early stage during compost subsampling. Mixing using the Turbula device was performed only once, followed by irregular manual mixing between subsampling steps, highlighting the need for a more clearly defined and standardized homogenization procedure. From the fluorescent analysis of the ILC extract samples (Figure 3), it can be approximated that the relative particle number deviation (from 8 replicates) is around 35% for both LDPE and PBAT particles. This is also generally reflected in the relative standard deviations observed for intra-laboratory replicates. Similar levels of variability observed for μ -Raman and μ -FTIR (20% for the size range of 100-500 μ m) were also reported in a separate ILC conducted in 2025.[31] In this context, it is important to note that sampling from composting facilities and subsampling will not be part of the future ISO standard, but only suggestions will be provided in its Annex. Considering that the future ISO standard could be used for the analysis of compost from industrial facilities operating at production scales of several tons, subsampling represents a critical step with the potential to introduce substantial additional variability. Accurate and representative subsampling from such large-scale systems is therefore particularly challenging.

In addition, subsampling from the final extract can substantially contribute to variability. For example, Laboratory 7, which reported substantially higher particle counts than the other participating laboratories, was the only laboratory that analyzed a 0.5 mL subsample instead of 2.0 mL. While the reason for this discrepancy remains unclear, differences in subsample volume cannot be ruled out as a contributing factor. Moreover, the subsampling effect is particularly evident for pristine polymer powders dispersed in ethanol or water and subsequently analyzed by fluorescence microscopy, where pronounced deviations between replicate subsamples were observed. It is therefore recommended to analyze multiple filters from the same extract to assess variability arising from subsampling or, alternatively, to analyze the entire extract, which is however not feasible in most cases due to the required analysis time or filter coverage and particle agglomeration on the filter.

Lastly, the microplastic test materials were produced by cryo-milling and inherently exhibit a broad particle size distribution (Figure S1, Table S1). As a result, total particle counts per 33 mg of powder, and consequently per gram of compost, are not precisely defined for this ILC. However, fluorescence analysis of the pristine powders (Table S1) indicates approximately 40800 ± 12100 LDPE particles and 51900 ± 14800 PBAT particles per 33 mg of powder. Comparing the mean particle counts reported by the individual laboratories with the corresponding fluorescence microscopy measurements of the pristine

powders yielded estimated LDPE recoveries of 15-169% for total particle counts, 4-112% for particles < 100 μm , and 15-208% for particles > 100 μm . For PBAT, the corresponding recoveries were estimated at 22-189% (total), 4-106% (< 100 μm), and 56-642% (> 100 μm), respectively. These estimates should, however, be interpreted with caution. The extraction protocol employed a 20 μm filter cut-off (50 μm for some labs), which may contribute to lower apparent recoveries in the < 100 μm fraction. Moreover, differences in particle dispersion and agglomeration between the extraction matrices and the water- or ethanol-based measurements of the pristine powders may shift particles between size fractions. Such effects could explain both lower recoveries of particles < 100 μm and apparent recoveries > 100% for particles > 100 μm , as observed for PBAT. To achieve more comparable inter- and intra-laboratory results, there is a need for standardized microplastic reference samples. These samples should have a well-defined particle number concentration and particle size distribution, enabling the use of an external non-measurement dependent comparison benchmark. Effort has been made in this direction, but there is still variability between specialized laboratories on the initial microplastic reference samples.[31]

Despite the observed variability in the data, the relative particle size distributions were comparable across laboratories (Figure 4). This indicates that neither the extraction protocol nor the analytical approach were biased toward specific size classes, meaning that no size class was systematically excluded. It must be noted that analysis performed at Lab 4 and fluorescence data from Lab 3 were performed analyzing a fraction of the filter. We acknowledge that data extrapolation might increase uncertainty, but there is not an observed trend or discrepancy between these two results and data reported from other laboratories. Comparing the ECD and Feret max results from Lab 5, it can be observed that Feret max increases the size approximation of the detected particles, as expected. This effect that can be clearly observed in Figure 3, where particles > 100 μm show higher numbers and particles < 100 μm show lower numbers for the data reported in Feret max compared to ECD. Therefore, relative particle size distributions between labs reporting ECD are comparable and also Feret max results from lab 5 and lab 7 are comparable, indicating that reported fragment values can be compared only if the measurand is the same. From the results of this ILC, it can be observed that the relative number of particles in the size range 100-300 μm is the most consistent between all participating laboratories, signifying that the confidence of reported particle counts in this size range is higher.

For the final comparison, the results were further condensed to total particle counts per gram of compost, now also including data from Laboratories 7 and 8 (Figure 5). The trends observed in Figure 3 were retained: PBAT counts were generally higher than LDPE counts, and agreement between spectroscopic and fluorescence measurements was better for PBAT. The lower LDPE counts are consistent with difficulties in obtaining representative subsamples from aqueous dispersions of this hydrophobic, low-density polymer, as discussed above.

As additional references, method blanks and unspiked matrix samples were analyzed to assess background LDPE and PBAT particle background and verify the validity of the analytical procedure (Tables S3 and S4). In the method blanks, four laboratories did not detect LDPE contamination, and two laboratories did not detect PBAT contamination, with all values remaining below the reporting limit

(particle number above the cut-off of the respective laboratory). In most cases, background contamination levels from the laboratory environment were substantially lower than the measured particle counts in the spiked samples, thereby fulfilling the validity criterion described in the future ISO standard. Laboratory 8 reported LDPE contamination in their CaCl_2 salt and consequently worked with filtered solutions for the extraction. Laboratory 7 also found a high LDPE background contamination in their method blanks despite filtering all reagents except CaCl_2 , which was added as a powder in the first preparation step. Comparable results were obtained for the unspiked matrix samples: Four laboratories reported LDPE numbers below the reporting limit, and two laboratories reported no detectable PBAT background. In contrast, laboratory 7 reported substantially higher LDPE particle counts than the other participants, while PBAT counts in one single replicate approached those observed for a spiked compost sample. Laboratory 5 reported the presence of PBAT particles larger than $100\ \mu\text{m}$ in a single unspiked matrix sample, corresponding to a concentration of 400 particles per gram of compost. The same laboratory also observed markedly elevated numbers of PBAT particles smaller than $100\ \mu\text{m}$ in the unspiked matrix samples (2067 ± 1857 particles per gram of compost) compared to the results reported by the other laboratories. However, it should be noted that the FTIR and Raman spectra of PET and PBAT share several characteristic bands and can be highly similar (Figure S7). Consequently, unambiguous discrimination between the two polymers cannot always be guaranteed, particularly when spectra are of low quality or when polymer assignment relies primarily on automated library matching.

Furthermore, participating laboratories were provided with a mixture of pristine microplastic powders, which were dispersed in water and analyzed with the original aim of enabling a direct comparison of particle counts and size distributions with those obtained from the extracted samples. However, the results (Figure S8) were strongly influenced by poor dispersion and subsampling. LDPE tended to float and adhere to vessel surfaces, whereas PBAT sedimented rapidly. Consequently, the position of the pipette tip and the use of a surfactant affected the reported particle counts. Three laboratories obtained comparable PBAT counts for the water reference and compost extract, while the remaining laboratories reported marked deviations that they attributed to these dispersion effects. The higher LDPE recoveries from compost extracts than from aqueous reference dispersions may reflect slight surface modification during Fenton oxidation or stabilization by residual organic matter, although the former was not detectable by ATR-FTIR analysis in previous work.[36] Overall, the water-reference approach was not suitable for direct comparison with extracted samples in its present form. A harmonized dispersion and subsampling procedure is required before a defined polymer mass can serve as a reliable number-based reference.

Results from mass-based analytical techniques.

In addition to microscopy analysis, mass-based quantification was performed by four participating laboratories using Py-GC/MS or TED-GC/MS following extraction. The results obtained with these techniques are presented in Figure 6. Soxhlet extraction data, highlighted as light blue bar representing the mean and standard deviation in Figure 6, were included as an orthogonal mass-based method with a

different extraction approach to compare extraction efficiencies and to illustrate the above-mentioned variability arising from subsampling of compost.

Overall, laboratory 2 was the only laboratory with recoveries exceeding 80 wt.-% for both polymer types. Laboratory 10 reported high recovery for PBAT, while recoveries for LDPE reached only approximately 60 wt.-%. Laboratories 4 and 9 recovered around 60 wt.-% of LDPE (Lab 4) and PBAT (Lab 9), respectively, but reported recoveries below 25 wt.-% for the other polymer. A possible cause of the low recoveries could be due to an inefficient homogenization of the particles in the final solid mixture or losses during cryo-milling for the sample preparation process (see method descriptions in the SI). However, it should be noted that the spiked compost samples themselves exhibited variability in polymer mass, as evidenced by the Soxhlet extraction data. Consequently, it cannot be excluded that Labs 4 and 9 received compost subsamples containing lower microplastic masses compared to those distributed to Labs 2 and 10. Concerning potential matrix effects, Lab 10 reported that no interferences were observed for the ions used for quantification in the residual compost matrix after extraction. However, a slight matrix effect was detected, resulting in signal enhancement, which was readily corrected during data evaluation. The participants also have reported that due to the high spiking amounts of microplastics in the samples they adapted the analysis protocol to only analyze 1 mL from the 100 mL final extract, which might lead to additional variability of the results. Another aspect that could play a role in the observed variability in recovery rates is the absence of a unified analytical protocol. The choice of the target degradation product for quantification can markedly influence the result. While literature and standards for PE do exist [45], information for PBAT is scarce. In addition, the sample-preparation steps were not consistent across laboratories: some participants added tetramethylammonium hydroxide (TMAH) to promote methylation of the degradation products, whereas others omitted it. These methodological differences may explain the variability in the results.

Regarding the reference samples, three out of the four laboratories applying mass-based techniques reported that polymer signals in their method blanks were below the instrument-specific limit of quantification (LOQ). Two laboratories detected LDPE in the unspiked matrix samples, with concentrations of $1.05 \pm 0.53 \text{ mg/g}_{\text{compost}}$ and $3.09 \pm 2.81 \text{ mg/g}_{\text{compost}}$, respectively, while one laboratory detected PBAT at a concentration of $0.09 \pm 0.08 \text{ mg/g}_{\text{compost}}$. The remaining reported concentrations of both polymers in the unspiked matrix samples were below the instrument-specific LOQ. These findings are consistent with the number-based analyses, which likewise showed a low presence of microplastics in the unspiked matrix samples, accounting for less than 10 % of the total particle counts in all cases.

For the water reference experiments (Figure S9), Laboratory 10 reported LDPE masses of 21 mg for the compost-spiked sample and 20 mg for the water-dispersed polymer, which is lower than the spiked amount of 33 mg but comparable between extract and water reference, indicating rather losses during subsampling than from the extraction itself, or during the cryo-milling prior to Py-GC/MS analysis. For PBAT, extracted masses of 38 mg and 55 mg were reported for the compost-spiked and water-dispersed samples, respectively, which might hint to PBAT overestimation, potentially due to the above-described effect of fast sedimentation of larger PBAT particles. In contrast, Laboratory 4 reported recoveries of less

than 10 % compared to those obtained from the compost-spiked and extracted samples. The reported difficulties were attributed to the use of pristine polymers in the water reference, which resulted in the rapid flotation of LDPE particles, rapid sedimentation of PBAT particles, and adhesion to the walls of the vessel. These effects prevented the establishment of a sufficiently homogeneous suspension and thereby compromised efficient recovery from the aqueous dispersion for subsequent analysis. These issues were not reported for the actual compost extracts.

Considerations for the ISO standard.

This interlaboratory comparison served to test a future ISO standard, which is currently under development (ISO/FDIS 24899). The ILC provided several important insights that contributed to the improvement and further development of the future ISO standard. The feedback received by the participants was considered in an updated version of the extraction protocol. Key modifications included restricting stirring to overhead stirrers equipped with glass or ceramic stirring bars, adjusting the proportions of the Fenton reagents to prevent iron precipitation on microplastic particles, and introducing an additional 20 µm filtration step after oxidation to avoid secondary reactions between the Fenton reagent and CaCl₂. A final rinsing step with ethanol ensures the successful recovery of low-density polymers, such as LDPE. Collectively, these improvements led to a substantial reduction of the undesired effects observed with the initial protocol evaluated in this ILC. In addition, more in-depth investigations on particle stability and extraction efficiency were performed with the new version of the extraction protocol in a smaller ILC between BASF Ludwigshafen and the University of Vienna. The results of those extra experiments (included in the Annex of the current version of ISO/FDIS 24899) demonstrate that the suggested improvements by the ILC partners led to higher microplastic recovery and ensured particle stability during extraction. The details will be published in the final version of the ISO standard.

CONCLUSION AND OUTLOOK

All participating laboratories were able to detect and quantify LDPE and PBAT after extraction from spiked compost. However, substantial inter- and intralaboratory variability remained, particularly for particles below 100 µm. The combined results indicate that insufficient compost homogenization, subsampling from the final extracts, particle dispersion, and non-harmonized analytical workflows were important contributors to this variability. The individual contributions of these factors could not be resolved conclusively in the present study. Number-based analysis would therefore benefit from harmonized extract preparation and measurement procedures, as well as reference materials with defined particle-number concentrations. Mass-based methods also identified both target polymers, but recoveries varied and their performance at environmentally relevant concentrations remains uncertain. The reliable quantification of polyethylene in environmental matrices can be critically influenced by sample-matrix composition, as organic-rich matrices are known to produce PE-like analytical signals and thereby bias mass-based estimates. Matrix-matched calibration, rigorous cleanup and blank correction, recovery testing, and careful selection of diagnostic markers are therefore essential for defensible PE

quantification. In general, the reporting limit, LOD and LOQ of the applied analytical techniques should always be carefully evaluated, as microplastic concentrations in environmental samples are expected to be lower than those in the spiked samples used in this ILC. Accordingly, validity criteria addressing statistical relevance as well as reporting limit, LOD and LOQ considerations were incorporated into the future ISO standard.

Based on the results of this study and the feedback received from all participants in the interlaboratory comparison, several key directions for future research can be identified. These include the development of sampling strategies for industrial composting facilities specifically aimed at microplastic analysis, compost homogenization, the use of well-defined standard microplastic reference materials, and the harmonized preparation of extracts for analytical purposes. The ISO standard currently under development focuses on the extraction of microplastics from compost but does not address either the initial sampling at composting facilities or the subsequent analytical steps. However, both are critical for generating comparable and reliable results across studies. Existing compost sampling approaches are generally not designed for microplastic analysis, which limits their suitability for this application. The implementation of tailored sampling and subsampling procedures would enable more reproducible sample preparation and significantly reduce variability between replicate measurements. As evident from the unspiked matrix sample analysis, the used compost for this ILC had a very low microplastic contamination. The amount of microplastics added to the compost in this ILC was comparable to the amounts typically used in standardized compost biodegradation tests, such as ISO 14855. The future standardized extraction protocol can therefore be used to monitor the fragmentation of biodegradable products over composting time, as reported by Wohlleben et al. (2023) and Pfohl et al. (2024), [19], [46] as well as to assess the transformation or persistence of non-biodegradable microplastics during composting. For untargeted analysis and quantification of microplastics in compost from industrial facilities – where microplastic concentrations are expected to be lower than in the spiked ILC samples – it may be necessary to extract multiple 1 g compost subsamples and subsequently up-concentrate the extracts, or alternatively to analyze multiple filters, in order to reliably quantify the microplastic content.

Another source of variability identified in this interlaboratory comparison was the inherent heterogeneity in particle size distribution and particle counts of the microplastic powders used. This observation highlights an urgent need for standardized, commercially available microplastic reference materials with well-defined particle number concentrations and size distributions. Ideally, such materials should be available in both pristine and aged forms, with aged plastics more closely representing microplastics encountered in environmental samples. The availability of standardized reference materials would provide a robust basis for method development and, in the context of this study, would be expected to substantially reduce interlaboratory variability. Furthermore, the present study demonstrated that currently available standardized methods for microplastic analysis are often not suitable for the analysis of extracts derived from compost samples, as they were primarily developed for water-based matrices. Residual particulate matter remaining after extraction introduces additional complexity, as it can prolong microscopy analysis and induce matrix effects in mass-based techniques. Consequently, each analytical

approach applied in this study is likely to require its own dedicated standard for sample preparation and analysis to ensure robust, reliable and comparable results in future assessments.

Abbreviations

ATR-FTIR Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy

CaCl₂ Calcium chloride

DANS 4-Dimethylamino-4'-nitrostilbene

ECD Equivalent Circular Diameter

FTIR Fourier Transform Infrared Spectroscopy

HFIP Hexafluoroisopropanol

ILC Interlaboratory Comparison

IR Infrared

ISO International Organization for Standardization

LDPE Low-Density Polyethylene

LOD Limit of Detection

LOQ Limit of Quantification

PBAT Poly(butylene adipate-co-terephthalate)

PC Polycarbonate

PE Polyethylene

PET Polyethylene Terephthalate

PP Polypropylene

Py-GC/MS Pyrolysis-Gas Chromatography/Mass Spectrometry

SI Supporting Information

TED-GC/MS Thermal Extraction Desorption-Gas Chromatography/Mass Spectrometry

TMAH Tetramethylammonium Hydroxide

μ-FTIR Micro-Fourier Transform Infrared Spectroscopy

μ-IR Micro-Infrared Spectroscopy

μ-Raman Micro-Raman Spectroscopy

Declarations

ASSOCIATED CONTENT

The following files are available free of charge: 1) Supporting information with additional information on original powder characterization, method descriptions/schematic representations for ILC sample preparation, Soxhlet extraction and analytical approaches used by all partners, representative images and illustrations of challenges encountered during extraction and analysis, tables including all results obtained by labs using microscopy techniques, IR spectra comparison, and results from water reference sample measurements (PDF); 2) the original excel spread sheet that was sent out to all ILC partners (excel).

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article (and its additional files).

Author Contributions

The manuscript was written and revised through contributions of all authors. All authors have given approval to the final version of the manuscript. DV, PH, MW, OH, CA, IA, LC, GB, OG, FB, MVdR, AP, AV, PACF, LL, PS, OK, FT, IL, AC, CS, EES, SK, KyA, SY, JH, KoA and YW carried out the experiments and data processing/reporting for this study. DV and PMS wrote the manuscript with contributions from all co-authors. AK, TH, FvdK and PMS supervised the project.

Competing Interests

Some of the authors are employees of BASF SE / Corporation, a company producing and marketing polymers, including both conventional and biodegradable polymers.

Funding

The work conducted by BASF SE, BASF Corporation, and the University of Vienna was supported by internal research resources from BASF SE and received no external funding. All other ILC partners contributed to the study in kind.

Acknowledgments

The authors are sincerely grateful to Spela Korez and Lars Peters for their support in the standardization process. The discussions within the ISO Working Group 4 on standard development were highly valuable in refining both the extraction protocol and its description in the ISO standard document. In this context, we would like to thank Stefanie Bierwirth and Ulrike Braun for moderating and organizing these discussions.

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Figures

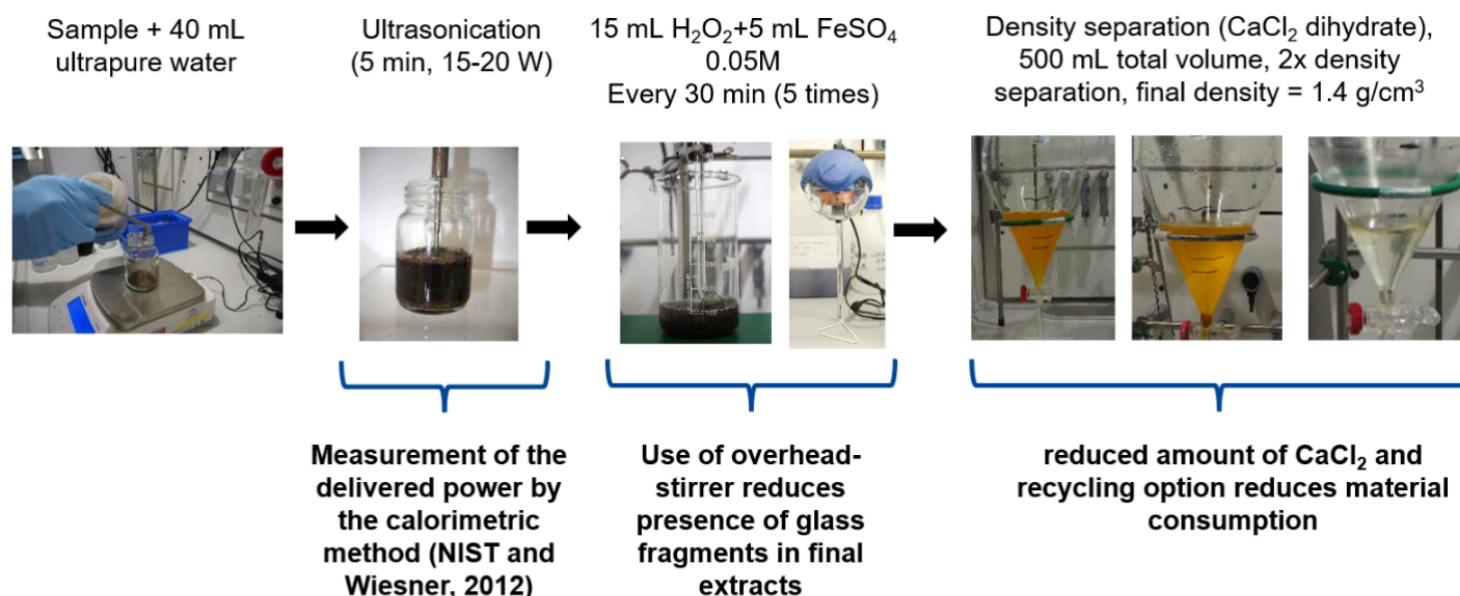


Figure 1

Schematic representation of the extraction protocol used in this ILC.

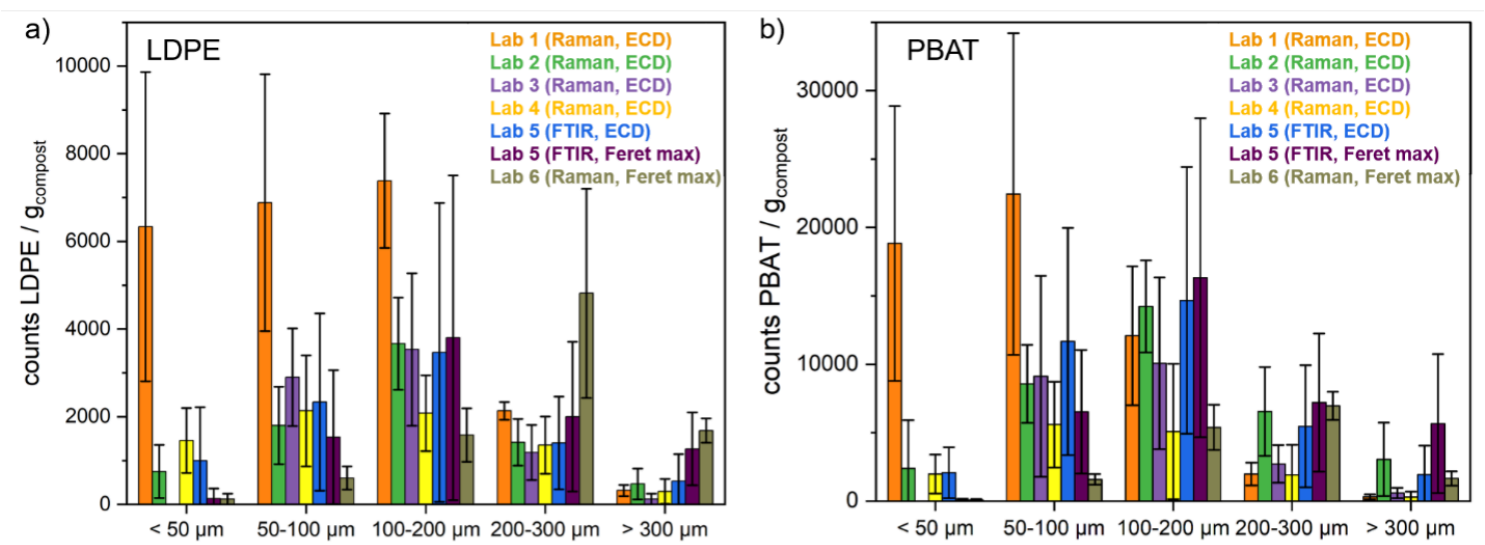


Figure 2

Particle size distributions and counts of a) LDPE and b) PBAT in spiked compost samples as reported by the ILC partners. Variability is expressed as the sample standard deviation. Blank measurements were not subtracted, in alignment with the ISO 16094 series.

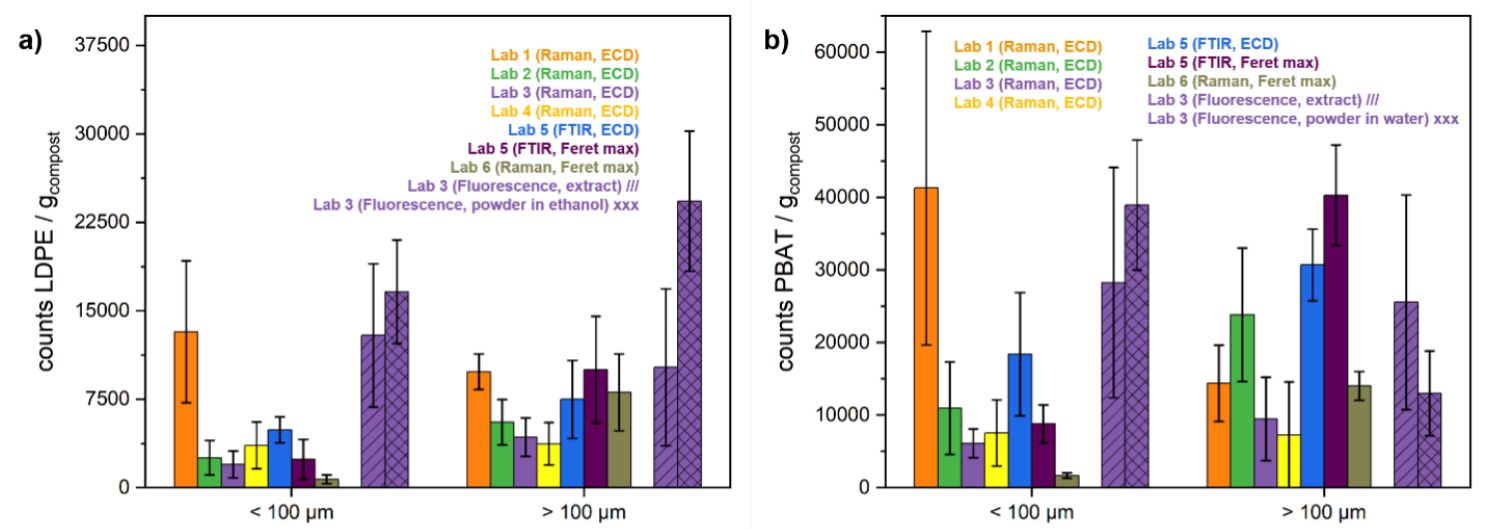


Figure 3

Particle counts of a) LDPE and b) PBAT determined by number-based analytical techniques. Replicate 3 reported by Lab 5 was excluded from this evaluation due to markedly lower counts detected. Particle size distributions were grouped into size classes < 100 μm and > 100 μm. Variability is expressed as the sample standard deviation. Blank measurements were not subtracted, in alignment with the ISO 16094 series.

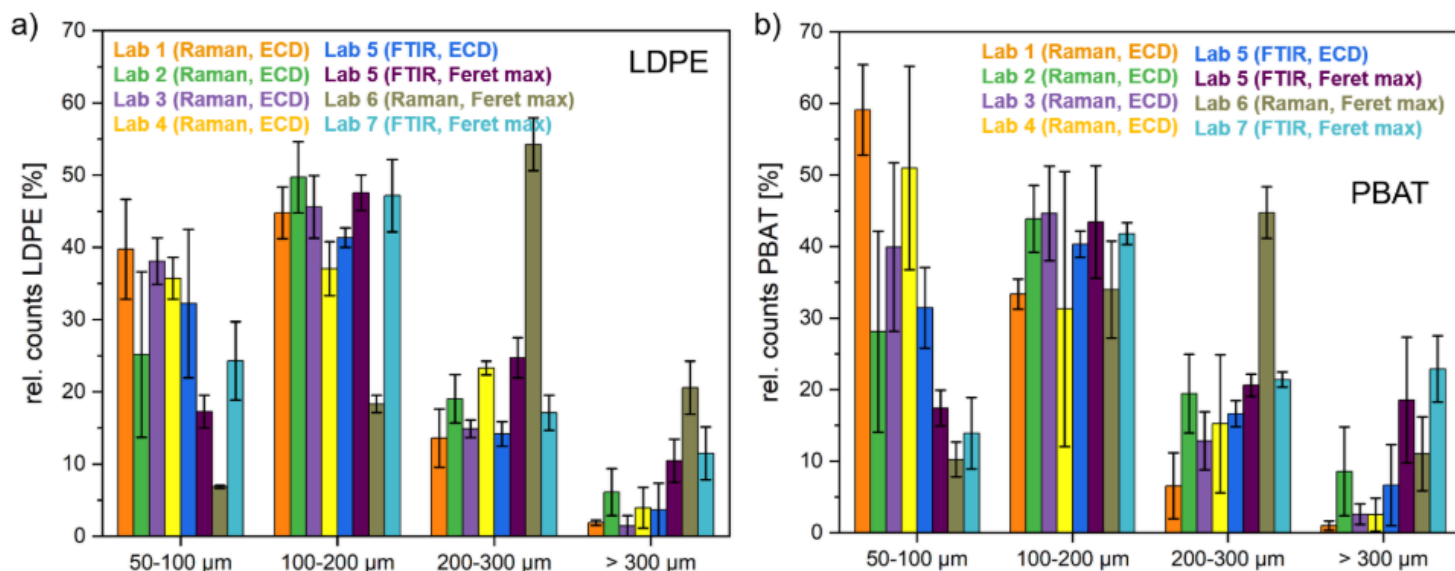


Figure 4

Relative particle size distributions calculated from the counts and sizes reported from the ILC partners for a) LDPE and b) PBAT. Reported data for particles smaller than 50 µm was not considered for these representations and analysis, given that some laboratories only reported particles larger than 40 or 50 µm. Relative size distributions were calculated independently for each replicate. Afterwards deviation was calculated using the sample standard deviation.

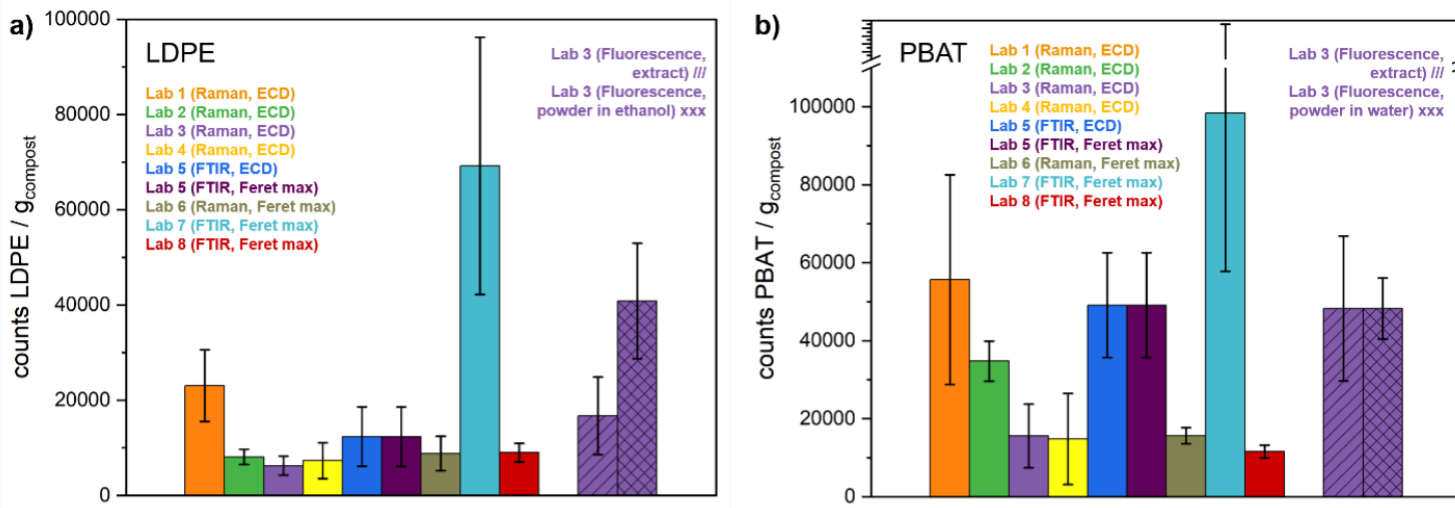


Figure 5

Total counts of particles per g compost for a) LDPE and b) PBAT reported from all laboratories. Replicate 3 reported by Lab 5 was excluded from this evaluation due to markedly lower counts detected. Lab 3 reported additional analysis using fluorescence microscopy: the value for the extract comprises 8 different extractions of the spiked ILC samples and as additional reference the MPs powders were directly dispersed and analyzed, in ethanol for LDPE and in water for PBAT. Variability is expressed as the

sample standard deviation. Blank measurements were not subtracted, in alignment with the ISO 16094 series.

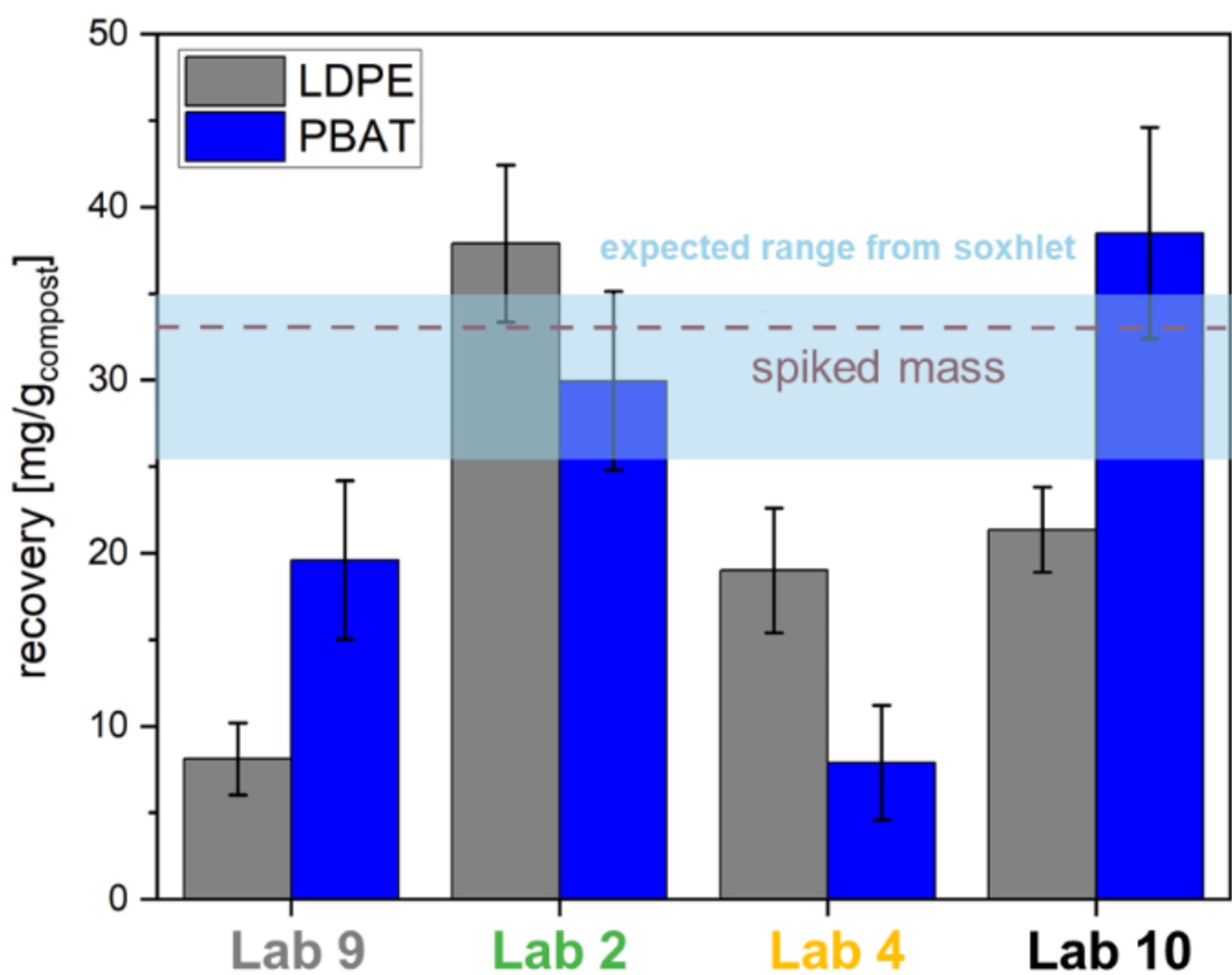


Figure 6

Results obtained from compost extracts analyzed by mass-based techniques. Lab 2, 4 and 10 used Py-GC/MS, Lab 9 used TED-GCMS for quantification. The light blue bar represents the variability between compost replicates determined via Soxhlet extraction as orthogonal technique.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryinformationILCCompostextractionfinal.docx](#)
- [ILCME1.xls](#)