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The influence of complex matrices on method performance in extracting and monitoring for microplastics

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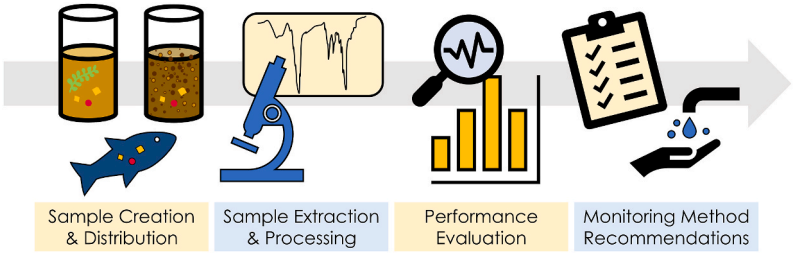
HIGHLIGHTS

- Microplastic method performance was evaluated in complex matrices.
- Tissue, sediment, and surface water were assessed relative to drinking water.
- Particle recovery was decreased in all matrices.
- Extraction procedures greatly increased sample processing times.
- Particle characterization and spectroscopy were largely unaffected.

GRAPHICAL ABSTRACT



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ABSTRACT

Previous studies have evaluated method performance for quantifying and characterizing microplastics in clean water, but little is known about the efficacy of procedures used to extract microplastics from complex matrices. Here we provided 15 laboratories with samples representing four matrices (i.e., drinking water, fish tissue, sediment, and surface water) each spiked with a known number of microplastic particles spanning a variety of polymers, morphologies, colors, and sizes. Percent recovery (i.e., accuracy) in complex matrices was particle size dependent, with ~60–70% recovery for particles >212 μm, but as little as 2% recovery for particles <20 μm. Extraction from sediment was most problematic, with recoveries reduced by at least one-third relative to drinking water. Though accuracy was low, the extraction procedures had no observed effect on precision or chemical identification using spectroscopy. Extraction procedures greatly increased sample processing times for all matrices with the extraction of sediment, tissue, and surface water taking approximately 16, 9, and 4 times longer than drinking water, respectively. Overall, our findings indicate that increasing accuracy and reducing sample processing times present the greatest opportunities for method improvement rather than particle identification and characterization.

1. Introduction

Standardized methods to consistently extract, measure, and characterize a given analyte are fundamental to any environmental monitoring program. As monitoring programs become more widespread, methods proliferate and provide practitioners with a range of choices that should be informed by an understanding of performance. Methods to extract, measure, and characterize microplastics in simulated drinking water were recently evaluated in an interlaboratory method evaluation study (De Frond et al., 2022a), and as a direct result, standardized operating procedures for the measurement of microplastics in drinking water were adopted for the state of California (California State Water Resources Control Board, 2021a, 2021b).

While the adoption of methods to measure microplastics in drinking water represents a major milestone toward characterizing microplastic contamination, there is also a need to understand the occurrence of microplastics in other environmental matrices such as biota, sediment, and surface water. However, standardized methods to extract, measure, and/or characterize microplastics in these more complex matrices are extremely limited. Only ASTM International has released a standardized method for the extraction of microplastics from water samples that contain a range of suspended solid concentrations (ASTM International, 2020), which was published during the later stages of this study. There are no standardized methods for microplastic analysis in biological tissues or sediment samples.

Standardization and evaluation of methods used for complex matrices is needed for microplastic monitoring as techniques remain highly variable, making it difficult to compare results across studies or evaluate method performance (Cowger et al., 2020). Harmonization of approaches and methods for microplastic monitoring have been called for repeatedly (Lusher et al., 2020; Primpke et al., 2020), and several entities have published documents detailing extensive recommendations for microplastic monitoring (GESAMP, 2019; AMAP, 2021). Yet, methods for the extraction and analysis of microplastics in complex matrices have not been quantitatively evaluated to assess method performance. Most environmental samples require additional processing steps to remove organic matter (e.g., chemical digestion) or separate plastic and non-plastic particles (e.g., density separation) (Brander et al., 2020). While it is reasonable to assume that these additional steps would require additional sample processing time and possibly contribute to particle loss, the impact of these specialized extraction procedures on method performance has yet to be evaluated.

Given the critical need to standardize, or at least harmonize, methods for analyzing microplastics in complex environmental matrices prior to the initiation of monitoring efforts, the goal of the current study was to evaluate how additional procedures required for the extraction of microplastics from complex matrices affect method performance. To achieve this goal, an international group of researchers, comprising a wide range of expertise and experience in microplastics analysis, were invited to participate in a multi-laboratory validation study. Laboratories were sent simulated fish tissue, sediment, and surface water samples spiked with a known composition of microplastic polymers (i.e., polystyrene (PS), polyethylene (PE), polyethylene terephthalate (PET), polyvinyl chloride (PVC)), morphologies (i.e., fragments, fibers, spheres), colors (i.e., orange, blue, white, green, clear) and sizes (i.e., 3–7000 μm). In addition, "false positive" particles that may be easily mistaken for microplastics (e.g., cotton fibers) were also added. Participating laboratories were provided with protocols to extract and analyze suspected microplastics from spiked samples based on commonly accepted practices in the microplastic literature (see section 2.3). Method performance (i.e., accuracy and precision, described in section 2.7) was determined among and between laboratories for results reported for visual microscopy, Fourier-transform infrared (FTIR) spectroscopy, and Raman spectroscopy. The time requirements for sample processing and analysis were also quantified to evaluate effort and labor costs associated with each method. The results of this study

are presented alongside results for drinking water samples to illustrate the impact of the additional extraction procedures. This study will contribute essential information when selecting methods appropriate for microplastic monitoring, including an understanding of critical method limitations to be marked for improvement in future studies.

2. Materials and methods

As an extension of the single-blind simulated drinking water method evaluation study comprised of 25 laboratories from the United States, Australia, Canada, China, Germany, and Norway (De Frond et al., 2022a), 12 laboratories continued their participation, and three laboratories were new participants. These 15 labs analyzed spiked simulated surface water, fish tissue, and sediment samples. Participating laboratories included a broad range of experience levels from novice (<1 year) to expert (≥3 years). This study followed methods outlined in De Frond et al. (2022a) regarding strict adherence to protocols for sample processing and extraction, particle counting (e.g., microscopy), chemical identification (e.g., FTIR and/or Raman spectroscopy), and data submission (e.g., experience level, time, cost, and quality assurance and control (QA/QC) procedures).

2.1. Creation of spiked samples

Drinking water, surface water, tissue, and sediment samples were created at Southern California Coastal Water Research Project (SCCWRP) using methods modified from De Frond et al. (2022a). In brief, glass sample jars were thoroughly cleaned using soap and water, then pre-ashed at 450 °C to destroy organic contaminants. Each sample jar and lid was triple-rinsed with 1 μm filtered (PCTE, Sterlitech) deionized water, hereafter referred to as microplastics analysis grade (MAG) water. A pre-determined volume of MAG water was added to each jar before spiking with a combination of natural and microplastic particles (Table S1, De Frond et al., 2022a) and adding matrix as described below. Each participating laboratory received three spiked samples and one matrix blank. Particles had a variety of sizes (i.e., 3–7000 μm), morphologies (i.e., fragments, fibers, spheres), colors (i.e., green, blue, orange, red, white, and clear), and materials (i.e., PS, PE, PET, PVC) including natural materials as false positives (i.e., cellulose, cotton, shells, and animal fur). While we attempted to use realistic matrices, we note that real-world environmental samples vary widely with regard to organic matter content, sediment grain size and composition, fat content of tissue, and plant or algal material in water. Detailed descriptions of sample creation for each matrix can be found in the SI.

2.2. Background contamination

Specific precautions and laboratory practices were put into place during sample preparation to minimize background contamination. All work was performed in a positive-pressure laboratory outfitted with high efficiency particulate air (HEPA) filtration. Technicians wore cotton laboratory coats and worked inside a clean cabinet whenever possible. All matrix materials (e.g., tissue, sediment, plant detritus) were analyzed for particle contamination prior to use. Each batch of samples was also prepared alongside a "batch blank" to determine background contamination levels during sample preparation.

2.3. Sample extraction

Sample extraction methods chosen for each sample type were evaluated by experts within the field of microplastics research during an in-person study planning meeting at SCCWRP in 2019. Methods were selected based on typical approaches published in the literature (Table S2), ease of use, safety (i.e., avoiding or minimizing the use of toxic or hazardous chemicals as appropriate), and cost of chemicals and equipment. Study participants were instructed to practice QA/QC measures to minimize laboratory contamination as well as to follow standard operating procedures (SOPs) for each sample type (Appendix A), briefly described below.

2.3.1. Drinking water

Drinking water methods are described in detail in De Frond et al. (2022a). Briefly, samples were filtered via sieving and vacuum filtration into four size fractions: 1–20 μm, 20–212 μm, 212–500 μm, and >500 μm. Participants were allowed either to rinse particles from each sieve into glass jars, or vacuum-filter each size fraction onto filter paper prior to analysis.

2.3.2. Fish tissue

Tissues were digested in 20% KOH in 500 mL polypropylene jars at 45 °C for up to 48 h. Following digestion, samples were soaked in detergent (10% Alcojet®) to remove excess fatty residue before proceeding with sieving and vacuum filtration into the same four size fractions as for drinking water analysis.

2.3.3. Sediment

Sediment samples were density-separated for 12–24 h using a $CaCl_2$ solution (1.4 g/mL). Samples were stirred vigorously with the $CaCl_2$ solution and allowed to settle before floating particles were removed with a metal spoon from the surface of the solution and transferred to a sieve stack. Samples were then sieved and vacuum-filtered into the same four size fractions as for the drinking water analysis.

2.3.4. Surface water

Surface water samples were sieved into two size fractions: 212–500 μm, and >500 μm. Sieve contents were collected, then digested via wet peroxide oxidation using Fenton's reagent ($FeSO_4$ + H_2O_2) before soaking in a detergent solution (10% Alcojet). Samples were then sieved again to release smaller particles that may have been previously trapped in organic matter. Sample digestion was repeated until all organic matter was removed before proceeding with analysis.

2.4. Microscopy

Following extraction, microscopy was used to count, measure (i.e., length and width), and categorize suspected microplastic particles by morphology and color (De Frond et al., 2022a; Appendix A). Following microscopy, up to 30 particles of each color and morphology combination (e.g., white fragment, green sphere) within each size fraction were randomly subsampled for chemical characterization via spectroscopy.

2.5. Spectroscopy

Laboratories were instructed to chemically identify all subsampled particles using μFTIR (or FTIR spectroscopy, hereafter collectively called FTIR) and/or Raman spectroscopy (De Frond et al., 2022a). Specific methods for chemical identification analyses were not specified within the SOP, but information on instruments and settings used were collected during data submission. Due to time limitations, some laboratories analyzed a percentage (25–30%) of the extracted particles.

2.6. Data submission

Participating laboratories submitted data via an online template modified from De Frond et al. (2022a, Appendix B). The full dataset is publicly available for download via the SCCWRP webpage: microplastics.sccwrp.org.

2.7. Data synthesis and analysis

Prior to data analysis, participating laboratories were anonymized. All data were analyzed using R (Version 4.0.3) analogous to the

procedure outlined in De Frond et al. (2022a). The total number of suspected microplastics reported via microscopy was enumerated for each spiked sample and each blank sample. This includes spiked plastic particles, as well as false positive particles misidentified as plastics, and suspected plastic particles that were introduced via background contamination (i.e., samples were not corrected based on blank contamination or spectroscopy results).

Given large differences in sample sizes, differences amongst matrices for particle counts in blank samples were determined using a Kruskal-Wallis test followed by a Steel-Dwass post-hoc multiple comparison test. Statistical significance (α) was set at 0.05. Accuracy was measured by comparing the total number of recovered particles to the total number of spiked particles for each sample. Generalized linear mixed models (GLMM) were conducted to determine if the deviated amount of microplastics recovered across labs (i.e., accuracy) compared to known spiked microplastic abundance in samples was influenced by microplastic particle size fraction, color, and morphology, and matrix type (details in SI). In some cases, there were slight variations in the number or type of spiked particles, but this was accounted for during recovery calculations. Precision was measured by calculating the standard error of recovery among samples and further explored with nonmetric multidimensional scaling (NMDS; details in SI). Particle counts, accuracy, and precision were also calculated based on reported particle characteristics (i.e., size fraction, color, and morphology). For drinking water, tissue, and sediment matrices, results were aggregated into two size classes: above and below 20 μm. When comparing across all four matrices (i.e., drinking water, tissue, sediment, and surface water) only size fractions >212 μm were included as one aggregated size class. The standard error of recovery within each lab was used to compare the precision within individual laboratories.

Spectroscopic results were analyzed by first filtering data so that only confirmed spiked particles were included. This was achieved by visually inspecting the submitted image for each measured particle to ensure that it was consistent with the specific spiked particle characteristics (e.g., blue fragment >500 μm) (Fig. S1). The number of spectroscopically analyzed particles varied widely amongst samples and laboratories. Thus, spectroscopic data from all laboratories were pooled together within each matrix. To determine the accuracy of FTIR and Raman analyses, each reported chemical identification was compared to the known chemical identification per particle. For instance, an orange PET fiber ≥500 μm reported as PET would be considered a correct match with Particle 1 (Table S1) whereas a green PE sphere detected in the 212–500 μm size fraction reported as polystyrene would be considered an incorrect match with Particle 7 (Table S1). Some particle types had a similar visible appearance (e.g., Particles 14 and 15), therefore, it was not possible to determine the true polymer type prior to spectroscopic analysis. In these cases, if the chemical identification result was one of the possible polymer types (e.g., PET or PS) the result was considered correct.

For each sample type, the average time per sample and per particle was determined for each analytical step, except for extraction time for which only the average time per sample was calculated. Differences amongst matrices in time per sample and time per particle for each analytical step were determined using individual one-factor ANOVAs, followed by a Tukey's post-hoc multiple comparison test as appropriate, after checking for normality and homogeneity of variance using a Shapiro-Wilk tests and Bartlett's tests, respectively. In cases where data were unable to be transformed to fit the assumptions for ANOVA, a Kruskal-Wallis test was used followed by a Steel-Dwass post-hoc multiple comparison test. Statistical significance (α) was set at 0.05. R-scripts used for all statistical analyses, summary results, and figures are available at microplastics.sccwrp.org.

3. Results

Drinking water results were previously reported in De Frond et al. (2022a) but are also summarized here to contextualize extraction effects on particle recovery and characterization in surface water, tissue, and sediment.

3.1. Background contamination

Background contamination during sample preparation was minimal as all matrix materials and batch blanks from sample preparation at SCCWRP contained ~0–20 particles. Average (± standard deviation) particle counts in blank samples as analyzed by participating laboratories were 73 ± 71, 28 ± 46, and 15 ± 20 for tissue (n = 6), sediment (n = 9), and surface water (n = 11) samples, respectively. Results for all matrices were less than the average of 91 ± 141 for drinking water (n = 17; Fig. S2). Significant differences in particle count were detected amongst matrices for blank samples (Kruskal-Wallis, p = 0.03) but post-hoc analysis was unable to detect which groups were statistically significant from one another.

3.2. Particle recovery

Generalized linear mixed models revealed that sample matrix and microplastic size, color, and morphology were significant explanatory variables for microplastic accuracy (Tables S4–S6). Reported deviance of microplastic abundance from spiked known values were significantly less for the clean water matrix compared to all other matrices (Fig. S3A). Accuracy was significantly reduced in a size dependent manner where smaller size fractions were less accurate than larger size fractions (Fig. S3B). Deviations in reported particle colors were significantly different across all colors with the largest deviations from spiked values for clear and white particles compared to blue and green particles (Fig. S3C). The abundance of fragments reported by labs deviated the most from known spiked values regardless of whether fibers were excluded (Fig. S3D) or included in analyses (Fig. S4).

As some laboratories did not submit results for the smallest size fraction, results for the drinking water, tissue, and sediment are aggregated into two size classes: <20 μm (n = 9 drinking water, 3 tissue, 4 sediment) and >20 μm (n = 22 drinking water, 6 tissue, 10 sediment). Surface water samples are excluded from these analyses as they were only spiked with particles >212 μm. Accuracy (i.e., particle recovery) was reduced in fish tissue and sediment relative to drinking water across all size fractions though precision increased.

For the <20 μm size class, mean recoveries for tissue and sediment were decreased by 23% and 30% compared to that in drinking water, and standard error which was 16% in drinking water decreased to 3% and 1% in tissue and sediment, indicating an increase in precision (Fig. 1A, S5A). For the >20 μm size class, mean recoveries were decreased in tissue and sediment by 30% and 47% compared to that in drinking water, respectively, and standard error was decreased from 12% in drinking water to 11% and 7% in tissue and sediment (Fig. 1B, S5B). In addition, microplastic abundance based on morphology, color, and size combination reported by participant labs were significantly different across matrices ($F = 5.8366$, $df = 3$, $P = 0.0001$). Fish tissue had greater variation in microplastic abundance reported across labs compared to all other matrices, while clean water samples had the least variation in reported microplastics (Fig. S6).

Precision amongst samples within each laboratory also improved in tissue and sediment, samples for both size aggregates. For the <20 μm size class, the standard error of recovery within each laboratory ranged from 1 to 3% (mean = 2%) and 0.1–1% (mean = 1%) for tissue and sediment samples whereas drinking water samples were 1–120% (mean = 19%). For particles >20 μm, the standard error of recovery for tissue and sediment samples ranged from 1 to 20% (mean = 5%) and 1–17% (mean = 6%) within each laboratory whereas drinking water samples ranged from 1 to 35% (mean = 11%). Particle counts, accuracy, and precision based on reported particle characteristics (i.e., size fraction, color, and morphology) are reported in the SI (Figs. S7–S9;

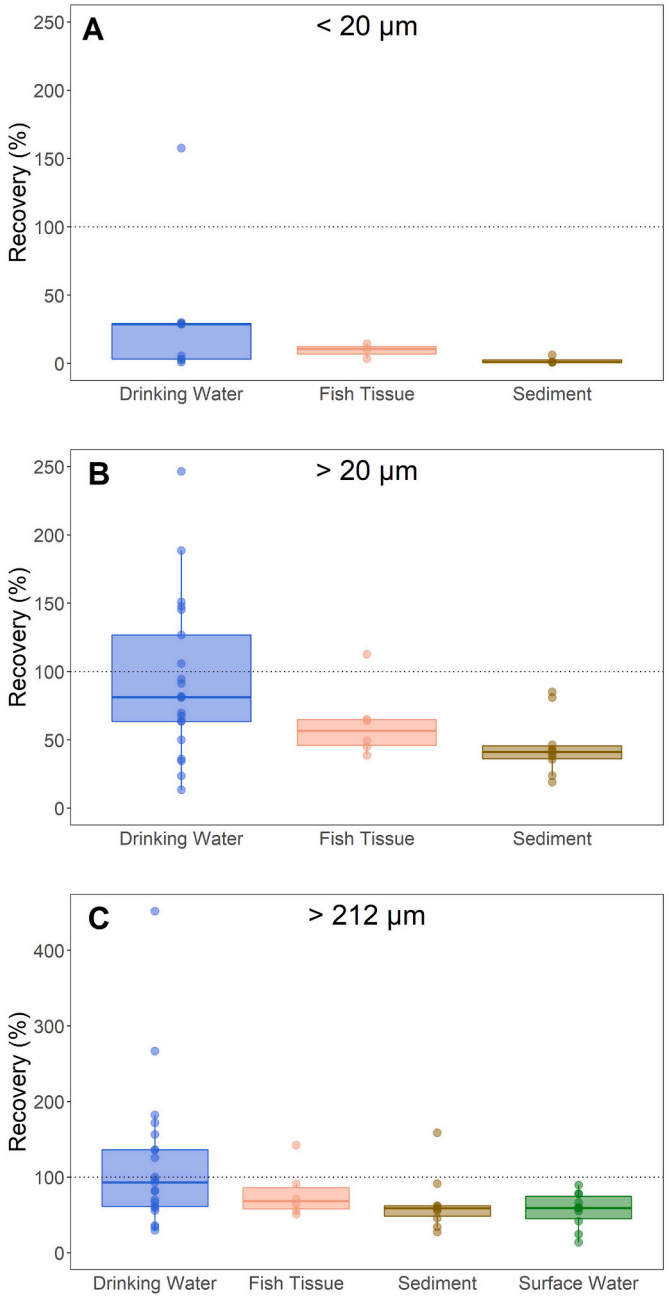

Fig. 1. Total particle recovery in drinking water, fish tissue, sediment, and surface water samples reported for visual microscopy as reported by each laboratory. Results are shown as A) recovery of size fractions <20 µm (n = 9 drinking water, 3 fish tissue, 4 sediment), B) recovery of size fractions >20 µm (n = 22 drinking water, 6 fish tissue, 10 sediment), and C) recovery of size fractions >212 µm (n = 22 drinking water, 6 fish tissue, 10 sediment, 10 surface water). Each point represents the mean of each laboratory (n = 1–3 samples). The dotted line represents 100% recovery of spiked plastic particles. Each box represents the interquartile range, the center line represents the median, whiskers represent the minimum and maximum values excluding outliers.

Tables S7–S9).

Comparisons with surface water were only made for size fractions >212 µm. Accuracy decreased in all matrices, as indicated by the mean recoveries for tissue, sediment, and surface water which were 39%, 52%, and 61% of the recoveries seen in drinking water. However, precision was increased as standard error decreased from 20% in drinking water to 14%, 12%, and 8% for tissue, sediment, and surface water samples (Fig. 1C, S10). As before, precision within laboratories amongst sets of samples was increased relative to drinking water as the standard error of recovery ranged from 1 to 25% (mean = 8%), 1–33% (mean = 8%), 0.3–29% (mean = 8%), and 2–48% (mean = 16%) tissue, sediment, surface water, and drinking water respectively. Particle counts, accuracy, and precision within categories based on reported particle characteristics (i.e., size fraction, color, and morphology) are reported in SI (Figs. S11–S13; Tables S10–S12).

3.3. Spectroscopy

Spectroscopy performance was largely unaffected by the additional extraction procedures required by complex matrices. Of all the known particles (both spiked plastics and false positives) analyzed via FTIR, accuracy was increased by 3%, 5%, and 5% in tissue (n = 619), sediment (n = 583), and surface water (n = 969) samples compared to that in drinking water (Fig. 2, Table S13). The percentage of particles correctly identified via Raman was increased by 15%, 9%, and 10% in tissue (n = 102), sediment (n = 238), and surface water (n = 109) in comparison to drinking water (Fig. 2, Table S14). Accuracy by plastic and non-plastic particles, size fraction, color, and morphology for both spectroscopic techniques are presented in the SI (Tables S13 and S14).

3.4. Time

Extraction procedures had the greatest impact on sample processing times for all matrices relative to drinking water (Table 1). Significant differences in extraction time were detected across matrices (Kruskal-Wallis, $p < 0.01$) where extraction time for drinking water required significantly less time compared to all other matrices. Estimated extraction times are as follows: drinking water at 15 ± 22 (mean ± standard deviation) hours per sample followed by surface water at 62 ± 60 h, tissue at 135 ± 156 h, and sediment at 238 ± 547 h.

Given the large differences in the number of particles recovered from each matrix, time results for microscopy, images and measurements, and spectroscopy are also presented on a per particle basis. There were no significant differences amongst matrices for microscopy time needed per sample or per particle (Samples: Kruskal-Wallis, p-value = 0.17; Particles: ANOVA, p-value = 0.50) or the time required for images and measurements (Kruskal-Wallis, p-value ≥0.08). There were no significant differences amongst matrices for the time required for Raman spectroscopy (Kruskal-Wallis, p-value ≥0.60), but for FTIR spectroscopy, the analysis time required for drinking water was significantly greater than that for sediment on both a per sample and per particle basis (ANOVA, p-values ≤0.04). Raman spectroscopy and some FTIR spectroscopy of fish tissue was done by individual analyses, hence the time required for spectral analyses was significantly longer than would be expected for automated analysis.

4. Discussion

The goal of the current study was to evaluate how additional procedures required for the extraction of microplastics from complex matrices affect method performance. Overall particle recovery, particularly for the smallest size fractions, and sample processing times were the most severely impacted by extraction whereas other aspects of method performance were unaffected (i.e., background contamination, spectroscopy) or enhanced (i.e., precision). As expected, sample extraction and processing times for the complex matrices were greater than that of drinking water given the nature of the matrices. In order to optimize the extraction/processing times for the complex matrices one needs to revisit the extraction and processing methods to determine if there are opportunities for further improvement. This may include modifications to the methods presented here or completely new approaches to quantify and characterize microplastics in complex matrices.

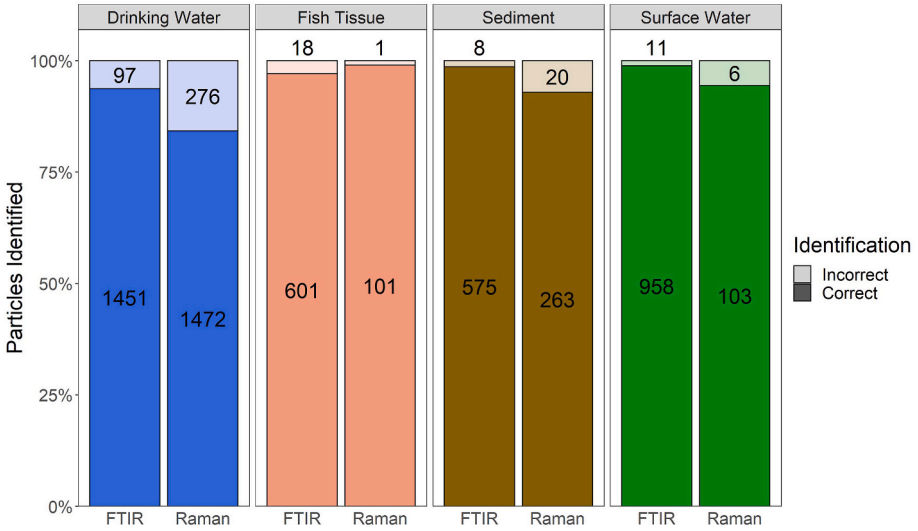

Fig. 2. The percent of particles (i.e., spiked plastic particles and false positives) correctly identified using FTIR or Raman in drinking water, fish tissue, sediment, and surface water samples. Data labels represent the number of particles correctly identified (dark shading) and incorrectly identified (light shading).

Table 1
Time per sample (hours) or per particle (minutes) for each step of processing and analysis. Results are calculated as the mean ± standard deviation of the mean among labs. The median is shown in parentheses. Not all laboratories included in summary statistics extracted and analyzed all size fractions. No standard deviation is reported for Raman spectroscopy for fish tissue as only one laboratory reported time data. Different letters indicate statistically significant differences between matrices.

	Sample Type	Extraction	Microscopy	Images & Measurements	FTIR Spectroscopy	Raman Spectroscopy
Sample	Drinking Water	15 ± 22^{a} (7)	16 ± 26 (7)	9 ± 13 (4)	10 ± 9^{a} (8)	19 ± 21 (12)
	Fish Tissue	135 ± 156^{b} (24)	13 ± 12 (7)	10 ± 10 (3)	7 ± 5^{ab} (5)	22
	Sediment	238 ± 547^{b} (54)	6 ± 6 (5)	6 ± 7 (3)	3 ± 1^{b} (2)	23 ± 17 (23)
	Surface Water	62 ± 60^{b} (50)	6 ± 7 (4)	6 ± 8 (4)	4 ± 1^{ab} (4)	12 ± 6 (12)
Particle	Drinking Water	–	4 ± 5	7 ± 17	11 ± 9^{a}	11 ± 10
	Fish Tissue	–	5 ± 5	16 ± 25	8 ± 9^{ab}	10
	Sediment	–	4 ± 3	21 ± 27	3 ± 1^{b}	15 ± 5
	Surface Water	–	6 ± 7	30 ± 37	7 ± 10^{ab}	15 ± 19

4.1. Background contamination

Blank sample particle counts in tissue, sediment, and surface water matrices were less than or comparable to drinking water, demonstrating that longer and more complex extraction procedures did not cause increases in background contamination. In fact, it is likely that density separation and chemical digestion procedures contributed to the separation and/or destruction of natural particles that might otherwise contribute to background contamination. While mitigating and monitoring background contamination is an essential component of any microplastic study, the results of this study do not indicate that it is a high priority area for method improvement in complex matrices. Further discussion on blanks is found in Munno et al. and Lao and Wong (both from this issue).

4.2. Particle recovery

Previous microplastic intercalibration exercises have reported overall particle recovery between 42% and 78% for sediment samples (Cadiou et al., 2020; Piccardo et al., 2022) and 97% for fish tissue samples (Tsangaris et al., 2021). While these recoveries are much greater than those reported in the current study, it is important to note that sediment and fish tissue samples in the previously reported studies were spiked with a minimum particle size of 300 μm and 3 mm, respectively. In contrast, all matrices except surface water samples were spiked with particles down to 3 μm in the current study. If only the >212 μm size fraction is considered, average particle recoveries are more consistent with previous studies as average particle recovery (± standard error) was 66 ± 12% and 79 ± 14% for sediment and fish tissue, respectively, suggesting that method performance in terms of accuracy was similar for larger size fractions for these matrices.

Unlike the drinking water samples, tissue, sediment, and surface water samples require additional processing steps to remove excess organic and non-plastic particulate matter, so it is reasonable to presume that this additional work may lead to increased particle loss or change particle characteristics as has been observed in previous studies (Ghosal et al., 2018). For instance, microplastics were extracted from sediment samples by performing multiple density separations. Samples were vigorously stirred for several minutes which led to particle breakage observed by several study participants. This may have decreased overall recovery and represents an opportunity for improvement in future studies. Particles may be lost during sample transfers, or when floating particles cannot be visually observed during density separation. For tissue and surface water, chemical digestion steps did alter the appearance of some spiked microplastics, making visual identification challenging. For example, some white and green PE spheres appear to have fragmented and/or changed shape during chemical extraction procedures (Figs. S14 and S15). However, given that only a small number of particles were affected (~10 particles/sample or ~ 1.5% of total particles), it is unlikely that changes in particle appearance affected the overall results of the current study.

For smaller size fractions (i.e., <212 μm), particle recovery dramatically decreased with decreasing particle size. While there are no comparable data sets for particles in these size ranges in complex matrices, results from method evaluations in drinking water samples have also reported lower recoveries for small particles (Michida et al., 2019; Isobe et al., 2019; Müller et al., 2020; De Frond et al., 2022a). The results presented here demonstrate that challenges with small particle

recovery are exacerbated in complex matrices which may occur in both processing and analysis. It is reasonable to conclude that smaller particles may be more likely to be lost during extraction procedures and/or sample transfers. They also may be more difficult to detect visually in a sample that is less clean. Though this study demonstrates that existing methods are limited in their ability to extract and characterize small particles, microplastic concentrations in real-world samples exponentially increase with decreasing particle size as larger microplastics break down (Lindeque et al., 2020), and particle size is a critical determinant of health impacts with smaller particles having a greater propensity to translocate (Thornton Hampton et al., 2022). Thus, it is critical that methods are developed to improve particle recovery and quantify the smallest microplastic size fractions, particularly for complex matrices.

While accuracy (i.e., particle recovery) was negatively impacted by the additional extraction procedures required for complex matrices, precision was improved in tissue, sediment, and surface water samples in comparison to drinking water. Most labs reported having extracted and processed drinking water samples prior to other matrices. It is therefore likely that participants were able to improve their precision as they gained experience in identifying and characterizing the spiked particle types, which were the same across sample types, thereby increasing precision (Kotar et al., 2022). This finding is consistent with results from other studies where researchers with more microplastic analysis experience outperformed more novice researchers in analyzing sediment samples (Piccardo et al., 2022).

4.3. Spectroscopy

Accuracy in using spectroscopy for chemical identification of spiked particles was unaffected by matrix or sample processing procedures, as the results for both FTIR and Raman were comparable across sample types. This observation is consistent with spectroscopic performance correlating most strongly with the type of particle being analyzed (e.g., size, color, morphology, material) rather than the approach (i.e., FTIR or Raman) or matrix (De Frond et al., 2022b). These results are consistent with previous studies that have also found minimal impacts on spectroscopy performance following extraction from complex matrices (Tsangaris et al., 2021; Piccardo et al., 2022). However, because so few particles <20 μm were recovered from tissue and sediment, only a small number of particles were available for spectroscopic analysis. Specifically, no particles <20 μm were analyzed using FTIR, and only six and two particles were analyzed using Raman for tissue and sediment samples, respectively. Though these particles were correctly identified via Raman, this data set is insufficient to evaluate spectroscopy performance for particles <20 μm, since the number of laboratories taking part in Raman analyses were 1 and 2 for fish tissue and sediment samples, respectively.

4.4. Time

Sample processing times were considerably impacted by additional extraction procedures. Surface water, tissue, and sediment samples took four, nine, and sixteen times more time to extract than drinking water samples, respectively. This represents a major obstacle as time, and therefore cost, requirements will quickly become a limiting factor for any monitoring program. Quantification of sample processing and analysis time is essential to accurately budget labor efforts and monetary costs. By quantifying sample processing and analysis time here, a baseline is set for developing faster, more efficient methods for microplastics analysis. It is important to note that the sample processing times shown here (Table 1) may be shorter in practice as some participating laboratories may have reported hands-off incubation times within extraction times (e.g., 48 h required for tissue digestion), and multiple samples may have been processed concurrently. Particle counts are also likely to be lower in real-world samples. Regardless, reducing extraction and sample processing time requirements should be a major priority for method development moving forward. Until then, monitoring programs should carefully consider the time and effort required to process each sample during the design phase to balance feasibility and costs with study robustness.

Though other analytical procedures did not have as profound an impact as extraction, some differences were observed amongst matrices. Most differences may be attributed to the number of particles successfully extracted from samples as processing times were comparable amongst matrices when reported on a per particle basis. For example, though significant differences were not detected, drinking water and tissue samples required 16 and 13 h to analyze, compared to sediment and surface water, which required only 6 h each. However, surface water samples were only spiked with particles >212 μm, and most laboratories did not attempt to analyze the smallest size fractions in sediment samples. It is not clear why FTIR took more time for drinking water than for sediment, though this result may be at least partially driven by particle size as more small particles were analyzed in drinking water via FTIR than the other matrices. However, laboratories did find that accurate results for both spectroscopic techniques were achievable when spending <10 min per particle (De Frond et al., 2022b), a time period similar to the averages generally observed.

4.5. Study limitations

There are some limitations that should be noted when interpreting the results of this study. First, most laboratories reported processing drinking water samples prior to tissue, sediment, or surface water samples. This order of sample processing may have provided researchers with some foreknowledge regarding the spiking materials as all samples were spiked with a similar composition of plastic and non-plastic particles to allow for comparisons across matrices. This may have contributed to the increases in precision that were observed in the other matrices in comparison to drinking water, but the effects of pattern recognition are expected to be small as this is likely limited to easily recognizable particles that stood out (e.g., colored spheres) and were also easy to count (i.e., particles spiked in small numbers). To counter this, future method comparison studies would benefit from randomizing the order of sample processing by matrix.

A second possible limitation of this study would be the wide range of experience amongst the participating laboratories, which may have led to high variation amongst laboratories. However, all participating laboratories received matrix-specific SOPs for sample extraction and analysis whereas many previous microplastic interlaboratory studies have not provided SOPs (Van Mourik et al., 2021; Cadiou et al., 2020; Müller et al., 2020; Isobe et al., 2019; Michida et al., 2019). It is reasonable to conclude that the use of the same SOP would greatly reduce variation amongst laboratories. The inclusion of novice, intermediate, and highly experienced laboratories likely provides a more realistic representation of method performance for a large-scale microplastic monitoring program with many contributing laboratories.

Finally, it is important to acknowledge that the samples in the current study were artificially created and spiked with a known amount and composition of microplastics. Matrices used here are not necessarily nor completely representative of the variability and diversity that researchers may encounter in real-world samples. For example, sediment grain size or the amount of organic matter within a sample will vary based on site location and habitat type, and method performance may vary as a result. However, artificial sample creation was necessary to 1) ensure starting matrices were microplastic-free and 2) spike samples with a known amount and composition of microplastics for quantification of accuracy.

4.6. Conclusions and recommendations for method development

Thus far, most microplastic intercalibration studies have focused on drinking water samples (Van Mourik et al., 2021; Cadiou et al., 2020;

Müller et al., 2020; Isobe et al., 2019; Michida et al., 2019; De Frond et al., 2022a). Some have assessed methods in complex matrices such as sediment (Cadiou et al., 2020; Constant et al., 2021; Piccardo et al., 2022; Tophinke et al., 2022) and biota (Ghosal et al., 2018; Wagner et al., 2019; Tsangaris et al., 2021). The current study is the first designed to evaluate the impacts of additional extraction procedures used for complex matrices. The results of this study highlight potential areas for further improvement: 1) overall particle extraction and recovery from complex matrices, 2) small (e.g., <50 μm) particle extraction and recovery from complex matrices, and 3) time requirements for the additional extraction methods for complex matrices. Some of these are already being addressed by improving existing methods such as those utilized here (Langknecht et al., 2023). It will also be worthwhile to explore new approaches to extract, quantify, and characterize microplastics from complex matrices. Modelling approaches such as the use of probabilistic density distributions to estimate microplastic densities may also be useful, particularly for small particles (Kooi and Koelmans, 2019).

Author contributions

Leah M. Thornton Hampton: Methodology, Formal analysis, Investigation, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Hannah De Frond: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing – Review and Editing, Chelsea Rochman: Conceptualization, Methodology, Writing – Review & Editing, Supervision, Project Administration, Kristine Gesulga: Methodology, Validation, Investigation, Writing – Original Draft, Writing – Review & Editing, Visualization, Syd Kotar: Methodology, Validation, Investigation, Data Curation, Writing – Review and Editing, Wenjian Lao: Methodology, Validation, Investigation, Data Curation, Writing – Review and Editing, Supervision, Cindy Matuch: Methodology, Validation, Investigation, Stephen Weisberg: Funding Acquisition, Resources, Conceptualization, Methodology, Writing – Review & Editing, Supervision, Project Administration, Charles Wong: Conceptualization, Methodology, Writing – Original Draft, Writing-Review and Editing, Resources, Supervision, Project Administration, Susanne Brander: Investigation, Writing – Review and Editing, Silke Christansen: Investigation, Writing – Review and Editing, Cayla Cook: Investigation, Writing – Review and Editing, Fangni Du: Investigation, Writing – Review and Editing, Sutapa Ghosal: Investigation, Writing – Review and Editing, Andrew Gray: Investigation, Writing – Review and Editing, Jeanne Hankett: Investigation, Writing – Review and Editing, Paul Helm: Investigation, Writing – Review and Editing, Kay Ho: Investigation, Writing – Review and Editing, Timnit Kefela: Investigation, Writing – Review and Editing, Gwendolyn Lattin: Formal Analysis, Investigation, Visualization, Amy Lusher: Investigation, Writing – Review and Editing, Lei Mai: Investigation, Writing – Review and Editing, Rachel E. McNeish: Methodology, Formal Analysis, Investigation, Writing – Original Draft, Writing – Review & Editing, Visualization, Odette Mina: Investigation, Writing – Review and Editing, Elizabeth Minor: Investigation, Writing – Review and Editing, Sebastian Primpke: Investigation, Writing – Review and Editing, Keith Rickabaugh: Investigation, Methodology, Writing – Review & Editing, Violet Renick: Investigation, Writing – Review and Editing, Samiksha Singh: Investigation, Writing – Review and Editing, Florian Vollnhals: Investigation, Writing - Review & Editing.

Declaration of competing interest

Leah M. Thornton Hampton, Hannah De Frond, Kristine Gesulga, Syd Kotar, Wenjian Lao, Cindy Matuch, Stephen B. Weisberg, Charles S. Wong, Susanne Brander, Silke Christansen, Cayla R. Cook, Fangni Du, Sutapa Ghosal, Andrew B. Gray, Jeanne Hankett, Paul A. Helm, Kay T. Ho, Timnit Kefela, Gwendolyn Lattin, Amy Lusher, Lei Mai, Rachel E. McNeish, Odette Mina, Elizabeth C. Minor, Sebastian Primpke, Keith Rickabaugh, Violet C. Renick, Samiksha Singh, Bert van Bavel, Florian Vollnhals, and Chelsea M. Rochman declare having no known competing financial interests or professional relationships that could have appeared to influence the work reported in this manuscript.

Data availability

Link to data is included in the Manuscript text.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.138875.

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