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Patterns of microparticles in blank samples: A study to inform best practices for microplastic analysis

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Abstract

Quality assurance and quality control (QA/QC) techniques are critical to analytical chemistry, and thus the analysis of microplastics. Procedural blanks are a key component of QA/QC

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Author contributions

All authors conceptualized the questions and methodologies together in a working group. KM, KH, ECM, CTL performed data analysis and KM created figures for the manuscript. The writing was led by KM. All authors contributed to the editing. CR and CW led project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online.

for quantifying and characterizing background contamination. Although procedural blanks are becoming increasingly common in microplastics research, how researchers acquire a blank and report and/or use blank contamination data varies. Here, we use the results of laboratory procedural blanks from a method evaluation study to inform QA/QC procedures for microplastics quantification and characterization. Suspected microplastic contamination in the procedural blanks, collected by 12 participating laboratories, had between 7 and 511 particles, with a mean of 80 particles per sample (±SD 134). The most common color and morphology reported were black fibers, and the most common size fraction reported was 20–212 μm. The lack of even smaller particles is likely due to limits of detection versus lack of contamination, as very few labs reported particles <20 μm. Participating labs used a range of QA/QC techniques, including air filtration, filtered water, and working in contained/'enclosed' environments. Our analyses showed that these procedures did not significantly affect blank contamination. To inform blank subtraction, several subtraction methods were tested. No clear pattern based on total recovery was observed. Despite our results, we recommend commonly accepted procedures such as thorough training and cleaning procedures, air filtration, filtered water (e.g., MilliQ, deionized or reverse osmosis), non-synthetic clothing policies and 'enclosed' air flow systems (e.g., clean cabinet). We also recommend blank subtracting by a combination of particle characteristics (color, morphology and size fraction), as it likely provides final microplastic particle characteristics that are most representative of the sample. Further work should be done to assess other QA/QC parameters, such as the use of other types of blanks (e.g., field blanks, matrix blanks) and limits of detection and quantification.

Graphical Abstract

Keywords

Plastic; Methods; Controls; QA/QC; Cross contamination; Procedural contamination

1. Introduction

The quality of laboratory measurement data is of critical importance to the investigation of environmental pollutants (Valcárcel and Ríos, 1994; Taverniers et al., 2004a), including microplastics (Brander et al., 2020), because the accuracy and uncertainty of laboratory results determine our ability to evaluate and compare the magnitude and character of pollution (Andersen, 2014). A major limiting factor of measurement quality is the

magnitude and uncertainty of background values of the analyte, which determine the limits of detection (LOD) and quantification (LOQ) for a given analytical procedure (Taverniers et al., 2004b). In microplastic research (i.e., particles <5 mm in size), background contamination is of particular concern because of the ubiquitous nature of microplastics (Song et al., 2021) and other interfering particulates in dust, and the large amount of effort (i.e., number of procedural steps and amount of time) required to analyze microplastic samples (Miller et al., 2021), which together can result in high blank particulate levels relative to sample measurement values (Shim et al., 2017).

There are several inherent challenges in isolating and accurately detecting microplastics in environmental samples (Brander et al., 2020). These include the detection and handling of small particles, extracting them from complex matrices, and accurately identifying them by material type. Because small particles are ubiquitous, a key step in microplastic analysis is to quantify the levels of background or procedural contamination in field and/or laboratory blanks. Microparticles, including microplastics, may be introduced to samples or equipment from air deposition, working with plastic equipment and tools, unfiltered water or reagents used during cleaning and processing, and synthetic clothing worn by researchers. Critical reviews in the relevant literature have recommended laboratory practices to minimize background contamination (Koelmans et al., 2019; Brander et al., 2020; Prata et al., 2021; Martin et al., 2022; Primpke et al., 2022). Commonly recommended practices include working in contained/enclosed and filtered air environments (e.g., fume hood, laminar flow cabinets, HEPA filters in room) and adopting clothing policies to reduce the shedding of synthetic fibers, limiting the use of plastic containers and equipment, filtering water and solutions, covering samples to limit air exposure, and cleaning surfaces with water and other solutions (e.g., acid, 70% ethanol). Although in progress by standardization bodies (e.g., ASTM, NIST, State of California, ISO/CEN), there are currently no standardized QA/QC practices for limiting background contamination in microplastics research.

As standardized and/or harmonized methods are brought online for microplastics (e.g., GESAMP, OSPAR, State of California), QA/QC procedures to reduce background contamination need to be standardized as well (Cowger et al., 2020; Schymansky et al., 2021). Standard practices should guide the types of blanks (e.g., field, laboratory, matrix) necessary to measure background contamination and recovery, the most appropriate laboratory materials, and equipment to use (e.g., air filtration systems), methods for washing equipment, procedures that limit exposure to laboratory air and/or contaminated water/reagents, and methods for reporting contamination. Moreover, standard methods should guide whether background contamination and/or recoveries measured in the blanks should be used for the correction of final data, and to inform LODs and LOQs.

These gaps and challenges are best addressed by interlaboratory comparison studies designed to evaluate the efficacy of methodologies (Mesley et al., 1991). Interlaboratory comparison studies have catalyzed the advancement of metrology and analytical quality assurance and are essential to the standardization and harmonization of methods (e.g., Brandsma et al., 2013). In this study, laboratories from Canada, the United States, Norway, Germany, Australia, and China participated in a method validation study implemented by the Southern California Coastal Water Research Project (SCCWRP) to assess the accuracy and

precision in recovering microplastics from simulated drinking water samples (De Frond et al., 2022). Participating laboratories were provided a protocol for extraction, visual identification, quantification, and chemical identification. They were also provided with one procedural blank sample. In general, results from participating laboratories are collectively informing recommendations for microplastic identification and quantification for future monitoring by the State of California and beyond. For this manuscript, we investigate the background contamination reported by 12 participating laboratories from laboratory (or procedural) blanks. Note that this method evaluation study was not designed to diagnose reasons for blank contamination or to inform methods for blank correction specifically. Still, to take full advantage of all the data collected in this method evaluation study, we use this data to assess patterns relevant to laboratory practices and inform future studies and best practices relevant to QA/QC including the incorporation of background contamination into measurement magnitudes and uncertainty. Specific recommendations on quantitative assessment of LODs and LOQs are beyond the scope of this manuscript and are discussed in Lao et al. (2023; *this issue*).

2. Methods

2.1. The interlaboratory study

Participants from 22 laboratories were supplied with three simulated drinking water samples spiked with microplastics and a strict protocol for extraction, visual identification, quantification, and chemical identification of the spiked samples (more information can be found in the Supplementary Materials of De Frond et al., 2022 *this issue*). Spiked samples were created by adding gelatin capsules (Martínez-Francés et al., 2023) containing 15 types of plastic with different shapes, colors, and sizes, as well as natural materials (i.e., false positives: cellulose fibers, animal fur and shell fragments) to 405 mL of 1 μm filtered deionized (DI) water with 15 mL of 1 μm filtered 10% Alcojet. Participants were instructed to filter the spiked samples, separate them into four size fractions (<20 μm, 20–212 μm, 212–500 μm, and >500 μm), and quantify the observed microplastics using visual identification based on a morphology and color key. The quantified particles, or a subset of the particles, were imaged, measured, and chemically identified using Fourier-transform infrared (FTIR) spectroscopy or Raman spectroscopy. Study participants had the option to attend training sessions conducted by SCCWRP. Training sessions included microplastic identification and categorization by morphology and color, as well as microplastic extraction following the standard operating procedures (SOPs) provided for the study (SOPs provided for the study can be found in the Supplementary Materials of De Frond et al., 2022 ; *this issue*). Study participants were also provided with instructional guides and videos detailing the material covered in the training sessions.

Each laboratory also received one procedural (or laboratory) blank sample that was prepared by SCCWRP, hereinafter referred to as a ‘blank’, to run in parallel with the three spiked samples. Blanks consisted of empty gelatin capsules dissolved in 450 mL of 1 μm filtered DI water in containers identical to the spiked samples. No particles were intentionally added to the blanks. The purpose of these samples was to assess procedural contamination from the laboratories that the samples were prepared and processed in. Blanks were to be extracted

and quantified using the same procedure as spiked samples. We report blank data from only 12 laboratories. Laboratories that did not acquire or quantify a blank were excluded, as were laboratories that did not quantify and characterize suspected microplastic particles in their blanks using the same procedure as the spiked samples (e.g., counting particles suspected as non-anthropogenic, using different sieve mesh sizes).

In addition to reporting summary statistics of microparticles reported in blanks, we used this data to inform various QA/QC procedures. To assess how blank contamination should be used and reported, we compared various methods for blank subtraction. We explored how different subtraction methods affected the overall recovery, including subtraction by the total quantity of particles, subtraction by single particle characteristics (size fraction, color or morphology), and by a combination of size fraction, color and morphology of particles identified in the blanks. For example, when subtracting by size fraction, the numbers of particles reported in each size fraction within the blank were subtracted from the total number of particles reported in each size fraction within the spiked samples. The subtraction was performed by applying the various subtraction methods to the spiked samples from each participating laboratory based on the corresponding blank. For example, the blank from Lab U was subtracted from each spiked sample extracted and quantified by Lab U. Due to limited data available, chemical identity of the particles detected in the blanks was not considered as a characteristic and method for blank subtraction. Blank-subtracted data was compared to the quantity and characteristics of the particles spiked initially and recovered after sample extraction and quantification.

In addition to reporting the quantification and characterization of suspected microplastics in samples, participating laboratories were instructed to report on the QA/QC procedures used to reduce the potential for procedural contamination in both spiked samples and blanks. Data was obtained by a survey of laboratory participants (See Supplementary Materials). These procedures included triple rinsing all equipment before use, keeping containers and equipment covered/closed whenever possible and wearing clean cotton lab coats (and sometimes only non-synthetic clothing). In addition, some laboratories performed work in a contained/enclosed environment with reduced air flow (e.g., clean cabinet or constructed enclosure) and used air filtration systems (e.g., HEPA filters). The QA/QC data collected from the surveys and used to evaluate blank contamination included: water type (1 μm filtered tap water, MilliQ, DI, RO and Nanopure), air filtration systems (presence/absence and type), whether the manipulations were performed in a sealed environment, clothing policy, additional cleaning procedures (e. g., sieve cleaning), time spent on processing (filtering, counting), training (at SCCWRP, or in-laboratory), number of sample transfers and number of fractions processed.

2.2. Data analysis and statistics

Summary descriptive statistics (mean, standard deviation, median and interquartile range (IQR)) were used to describe blank contamination across all labs and for each of the single microparticle characteristics (color, morphology, and size fraction). To evaluate the effects of various QA/QC procedures (number of sample transfers, and fractions processed) and varying levels of training on microparticle contamination, nonparametric

rank-based Kruskal-Wallis tests were performed. Non-parametric tests were used due to the uneven sample sizes. Nonparametric Mann-Whitney U tests were performed to evaluate whether there was a difference in mean microparticle contamination among laboratories employing, or not employing, several other QA/QC procedures. The additional QA/QC procedures include extra sieve cleaning procedures, the use of air filtration systems, and sealed/closed working environments with limited air flow. Spearman correlations (ρ) were used to evaluate potential associations between microparticle contamination and time spent processing samples, with and without outliers. All statistical analyses were performed using R Statistical Software (version 4.2.1 R Core Team, 2021). Plots were generated with the ggplot 2 (version 3.3.6; Wickham, 2016).

3. Results and discussion

3.1. How contaminated were the blanks?

Across all laboratories, suspected microplastics reported in the blanks ranged from 7 to 511 particles (Table S1; see Supplementary Materials for raw data). The mean (±SD) number of particles detected in the blanks is 80 (±134; median = 45, IQR = 71). The mean number of microparticles in the blanks is likely driven by one laboratory with nearly five times the total number of microparticles relative to the next most contaminated blank (i.e., 107 particles). This laboratory was contaminated due to construction as reported by the researchers. Instead, if we consider the median (45 particles), the value is more similar to a typical blank for microplastics samples ranging up to 36 particles per blank (as reviewed by Prata et al., 2021). The procedural contamination reported in this study is higher than most other reported values (Prata et al., 2021), though prior studies vary in particle size ranges and methodology making it difficult to directly compare.

Overall, the distribution of suspected microplastics among size fractions was relatively similar (26–39% of the overall blank contamination represented by each size fraction), except for the smallest size fraction (Fig. 1A). The mean number of particles (±SD) is greatest for the 20–212 μm size fraction (34 ± 50 particles), and least for the 0–20 μm size fraction (16 ± 27 particles) (Table S1). However, the number of laboratories that included the smallest size fraction was limited (n = 5 laboratories). The variation in procedural contamination across size fractions in our study deviates from trends in literature, where greater contamination is observed in smaller size classes (Prata et al., 2021). This is likely due to the small sample size of blanks from the smallest size fraction in our study and challenges associated with quantifying particles visually within the smallest size fraction. Laboratories may have deviated from the SOP slightly to mitigate the challenges of quantifying the smallest size fraction. For example, researchers were encouraged to assess particle resistance to breakage using forceps as an indicator of synthetic origin but likely are not able to do so for small sizes. As such, some particles in the smallest size fraction may be overlooked or discounted as natural.

The majority of suspected microplastics in the blanks were described as fibers (55%; mean = 44 ± 52 particles), followed by fragments (38%; mean = 31 ± 86 particles) (Fig. 1B; Table S1). This is consistent with reports of airborne procedural contamination in literature (Liu et al., 2019; Prata et al. 2020, 2021; Song et al., 2021). Contamination from airborne

deposition consists of predominantly fibers (Prata et al. 2020, 2021), likely consisting of cellulosic fibers from paper towels and cotton lab coats (Prata et al., 2020) and other clothing fibers.

Black was the most common color used to describe the suspected microplastics in the blanks (49%; mean = 39 ± 85 particles), followed by clear (11%; mean = 9 ± 12 particles) and blue (10%; mean = 8 ± 9 particles) (Fig. 1C; Table S1). Blue and black fibers are frequently observed in airborne microplastic contamination (Liu et al., 2019), and aerial deposition likely contributes to procedural contamination (Song et al., 2021). Also, researchers rely on visual identification under various microscopes. Distinguishing clear or lightly colored microparticles is challenging, which likely contributes to the disproportionate identification of dark or black microparticles in the blanks.

3.2. How should blank contamination be reported and used?

Blank contamination should be reported in particle counts, but also particle characteristics such as morphology, color, size, and/or material type. Because microplastics are so diverse (Rochman et al., 2019), microparticles reported in the blank may vary in color and morphology from particles in the sample. These counts and characteristics should be taken into consideration for both reporting blanks and using them to blank correct (or blank subtract). This is similar to quantifying, reporting, and/or subtracting individual analyte concentrations found in a blank from samples in an analysis of a chemical mixture (e.g., polychlorinated biphenyls).

In this study, raw counts in the spiked samples were generally less than the spiked value, meaning that recoveries tended to be less than 100% (De Frond et al., 2022). This suggests that after blank correction, the samples' total measured values will become even less accurate compared to the spiked samples. Before blank correction, microplastic raw counts were 271 ± 177 particles. After blank correction, totals went down to 197 ± 132 particles when correcting by size fraction, 195 ± 124 particles when correcting by morphology and 201 ± 118 particles when correcting by color. When correcting using the combined method (i.e., by color and morphology within size fractions) the total particle count was 230 ± 136 particles (Fig. 2, Table S2). Because the results among all blank subtraction methods are below the spiked particle counts, no method of blank subtraction appears most correct. Still, correction by specific characteristics using the combined methods is more precise, and the total corrected value subtracts the lowest number of particles.

In general, when researchers choose to blank correct their data, we suggest blank subtraction by combined characteristics. Blank subtraction by combined characteristics should remove particles from samples that are more characteristic of contamination. For example, lab CC detected a pink fiber in both the blank and in an actual sample. Pink fibers were not spiked into the sample. Blank subtraction by color and morphology has been performed in prior studies (e.g., Catarino et al., 2018; Grbíc et al., 2020; Felismino et al., 2021), and thus there is a precedent to suggest this method. Microplastics are ubiquitous and diverse (Rochman et al., 2019), and it is inevitable that some degree of procedural contamination will occur. However, procedural contamination in the laboratory may not produce the same types of particles as those in a sample. This suggestion aligns with methods used for the

analyses of chemical mixtures, whereby particle characteristics are used in the same way as specific components of the mixture. Applying blank subtraction by all characteristics combined also prevents researchers from eliminating rare particles detected in environmental samples by chance. The remaining particles following blank subtraction should be most representative of the actual microparticles in the sample. Hermsen et al. (2017) assessed potential airborne contamination and determined no particles were of a similar appearance to particles in environmental samples. Blank correcting by particle count would have thus led to an incorrect final sample number. In future studies, when all particles are chemically identified (e.g., via automated Raman spectroscopy or FTIR), blank subtraction by characteristics that include chemical identity of the microparticles in the blanks should also be considered. To further inform how contamination in blank samples should be used to correct samples, *in silico* studies could be used to generate "samples" with diverse particle characteristics, including blank samples. This data can then be used to test the different blank correction methods tested here and see which methods lead to the most accurate counts and characteristics in final sample data.

3.3. How did laboratory QA/QC procedures to reduce background contamination affect the blanks?

Below we use the quantity and characteristics of suspected microplastics detected in blanks combined with QA/QC survey data to inform recommendations for best laboratory practices to reduce background procedural contamination. The aim of the recommendations below is to acquire the most accurate and representative assessment of microplastic contamination in environmental samples. Similar recommendations have been proposed in previous studies (Koelmans et al., 2019; Brander et al., 2020; Prata et al., 2021) based on reviews of procedural contamination reported in literature.

4. Recommendations

4.1. Recommendations based on findings

1. We recommend processing samples in a controlled air environment to reduce procedural contamination (e.g., sealed environments, air filtration systems).—We did not observe differences in the number of blank particles among laboratories with and without air filtration (Fig. S1, $p > 0.05$, $W = 16.5$), or whether manipulations were performed in a sealed/closed environment (Fig. S2, $p > 0.05$, $W = 17$). This may be due to the instructions in the protocols, which told researchers to ensure that samples were exposed to ambient air for only the minimum time necessary (e.g., covering all equipment and samples with covers or aluminum foil). This suggests that efforts to physically block samples from atmospheric deposition may be more important for the reduction of procedural contamination than efforts to treat atmospheric contamination. When available, we still suggest air filtration and sealed environments based on experience and guidance in the literature (Prata et al., 2019; Brander et al., 2020). Controlled air environments have been shown to reduce airborne fiber contamination by 50–97% (Prata et al., 2019), with laminar flow hoods having the greatest reduction. Prata et al. (2021) determined that only 38% of studies reviewed were performed in a controlled air environment (e.g., laminar flow hood, HEPA filter in room). Currently, controlled

air environments are not commonplace amongst researchers, but we suggest laboratories implement these more broadly to increase data reliability. When these are not accessible, care should be taken to reduce the amount of time a sample is exposed to ambient air. The severity of airborne contamination may be monitored by acquiring air blanks alongside processed samples.

2. We recommend additional measures to reduce deposition of airborne contamination and cross-contamination of samples (e.g., cleaning equipment and workspaces thoroughly, covering samples).—Cleaning procedures (e.g., thorough cleaning of equipment before use and triple rinsing between samples) were implemented in the SOP for this study making it difficult to evaluate the effects of cleaning procedures on blank contamination when protocols were followed. Some laboratories introduced additional sieve cleaning and rinsing protocols; however, sieve cleaning measures and additional rinsing protocols appeared to have no effect statistically (Fig. S3, $p > 0.05$, $W = 23.5$) though blank contamination appears slightly lower for laboratories using additional cleaning. The lack of statistical significance is likely due to the cleaning measures already dictated in the SOP followed by all participating laboratories. In prior studies, only 16% reported cleaning between samples to reduce cross-contamination (Prata et al., 2021). Such cleaning should be considered standard for microplastics analysis.

In our study, there was a strong, significant correlation ($\rho = 0.61$, $p < 0.05$) between total time processing (extraction and counting) a sample and the number of particles detected. Furthermore, laboratories that reported blank particle counts that were close to the actual number of blank particles detected in samples from the production laboratory (SCCWRP; 15–20 particles) generally spent less than 10 h/sample (Fig. S4A). Removing outliers from the data for the longest time and the highest log particle count (Fig. S4B) slightly strengthened the correlation ($\rho = 0.70$, $p < 0.05$). Covering samples as much as possible is a means of mitigating the potential contamination from airborne microparticle deposition while samples are being processed. Covering samples thoroughly should reduce the effect of time. The significant positive association between blank contamination and time of sample processing along with the reports in literature of contamination resulting from airborne microparticle deposition (Liu et al., 2019; Prata et al. 2020, 2021) support the recommendation to limit the amount of time samples are exposed as much as possible.

3. We recommend implementing a clothing policy to reduce procedural contamination of synthetic particles.—In our study, all laboratories implemented a clothing policy as is common among microplastics researchers (Koelmans et al., 2019; Brander et al., 2020; Song et al., 2021) and was discussed during our training session. Textiles are a major contributor to airborne microplastic contamination (Liu et al., 2019), and synthetic fibers make up a large proportion of airborne fiber contamination. One study reported no procedural contamination following the implementation of a 100% cotton clothing policy (Hermsen et al., 2017). While clothing (including lab coats) is prone to shedding, reducing shedding of synthetic microparticles is preferable as natural-looking microparticles are less likely to be characterized as suspected microplastics. Lightly colored, cellulose-like fibers likely originating from paper towels and cotton lab coats have

been observed frequently in procedural blanks (Prata et al., 2020), unlike fibers of other characteristics. These lightly colored cellulose fibers are not often counted as microplastic as these fibers would not be indicated as plastics using a hot needle test (Vandermeersch et al., 2015), have visual characteristics of natural fibers (Lusher et al., 2020), and produce spectra that are different from microplastic particles using Raman spectroscopy (Cabernard et al., 2018; Munno et al., 2020) and FTIR (Primpke et al., 2018; De Frond et al., 2021).

4. We recommend using filtered solutions to process samples—Due to the lack of replication among water types, we were not able to assess the statistical significance of water type on blank contamination. However, no obvious pattern in the number of blank particles is apparent among water types in our study (Fig. S5). However, all laboratories used some form of filtered water to process samples (1 μm filtered tap water, DI, RO, MilliQ, Nanopure) because it was written in our protocols. The samples and blanks were also prepared using filtered DI water. As such, we cannot properly test how filtering of solutions reduces background contamination. Still, we recommend using filtered solutions, consistent with recommendations in literature (Koelmans et al., 2019; Prata et al., 2021; Schymanski et al., 2021; Shruti et al., 2021).

5. We recommend implementing training procedures for researchers to ensure samples and procedural blanks are processed and quantified consistently among samples and researchers.—Training (at SCCWRP or in-laboratory) did not have a significant effect (Fig. S6, $p > 0.05$ for all KW analyses) on the number of particles in the blanks. Still, blanks from laboratories with training from SCCWRP and within their laboratories appeared to have reduced background contamination (Fig. S6). Moreover, we did see a positive effect of training on overall recovery (De Frond et al., 2022, *this issue*; Kotar et al., 2022, *this issue*). We suspect that training aided researchers in following the SOPs provided. The implementation of training programs ensures researchers learn how to follow steps in standard protocols and helps them understand the value of implementing QA/QC procedures.

6. We recommend minimizing the handling of samples and blanks to reduce potential for procedural contamination and crosscontamination.—Differences in blank contamination among laboratories processing varying numbers of size fractions (e.g., one fraction means all particle sizes were kept together) was not significant (Fig. S7, $p > 0.05$). However, blank contamination decreased with an increased number of fractions. This trend may be related to the time the sample was exposed during processing under the microscope (relevant to recommendation two above). If the sample was not divided into fractions, more particles remain within the single fraction and may make visual microscopy more challenging and time consuming. While this may not always be the case for microplastic samples, this likely applies to samples where particle counts are relatively high, or the matrix requires more labour and handling to process. Thus, we recommend splitting samples into at least two fractions as needed if particle counts are high or the matrix poses challenges in processing.

There was not a significant difference in blank contamination on the basis of the number of sample transfers (Fig. S8, $p > 0.05$). Typically, we might expect blank contamination

to increase with increasing sample transfers because there is an increased chance for contamination due to increased risk of airborne microparticle contamination or cross contamination. Here, because of a strict SOP, we did not have the variability required to really answer this question statistically. Still, we recommend minimizing the handling of samples to reduce the potential for procedural and cross-contamination while still enabling researchers to process and count samples within a reasonable amount of time.

Overall, in this study, many of the factors we hypothesized would affect particle counts in the blanks neither decreased nor increased blank contamination. We believe there are several reasons for this outcome, which include the small sample size of laboratories that submitted data that could be used for this study. Moreover, each laboratory acquired a single blank, so there was no repetition within laboratories. Another factor may be the implementation of strict protocols for extraction and quantification which included steps to reduce procedural contamination when followed. This limited the variability among practices. As noted in the introduction, some of the challenges relevant to testing our hypotheses are due to the use of a study not designed to answer these questions. Due to this constraint, we were left to assessing relevant patterns using the data we had. A more accurate way to test the effects of these practices may be to test the factors within one laboratory and manipulate one QA/QC protocol at a time. For example, one single laboratory (and perhaps including multiple personnel to account for differences among humans) could test the effect of manipulating a sample in a sealed environment compared to open laboratory space while holding all other variables constant. Another study could deploy many laboratories using an ANOVA design, where replicate laboratories are each given different strict protocols prescribing the type of QA/QC to use. This study could measure the significant differences in total blank contamination between treatments – informing how different practices affect contamination. Using these types of experimental design with replication could better determine which QA/QC protocols are effective in reducing contamination.

4.2. Moving forward: field blanks, matrix spikes, LODs and LOQs

Here, we report how the data in laboratory blanks from a method evaluation study can be used to inform QA/QC. We use our data to assess patterns in blank contamination across laboratories, how blanks vary with laboratory procedures, and ways in which they can be reported and used to correct for sample contamination. Future studies could use method evaluation studies to consider other types of reference samples (e.g., field blanks taken during sampling, matrix spikes) that should be used in microplastics studies, and whether the data from these blank samples should be used to correct raw data (e.g., blank- or recovery-correct) and/or inform LODs and LOQs.

In analytical chemistry, laboratory, field, and matrix blanks are often used. Here, we report on laboratory blanks because no field sampling occurred in this method evaluation study. Laboratory blanks account for any procedural- and cross-contamination from the laboratory and during laboratory processing. Many studies also use field blanks (Hung et al., 2021; Brander et al., 2020). Field blanks are taken in the field, at the time of sampling, using the same methods and equipment that are used for sampling. They account for contamination starting at the point of sampling, and then are carried through the full procedure. As such,

they operate as both a field and laboratory blank and may be viewed as more holistic. Both are recommended, especially if a researcher is interested in understanding where the contamination occurs in their sampling and laboratory processing. If enough field blanks are taken to be representative (e.g., one per every twenty samples, or one per sample collection period), and the objective is not to know where/how contamination occurs, they can operate as both a field and laboratory blank.

To date, matrix spikes are less common in microplastics research but very common in analytical chemistry. A matrix spike is generated by spiking a relevant concentration of target analytes into a similar environment, or matrix, to your field, or real-world sample of interest. The value of a matrix spike is it increases the relevance of quantitative analysis results in the laboratory to real-world environments. The recovery of target analytes from a model sample matrix can be calculated and the analyte recovery can be used to complement quantitative results for samples of interest. For microplastics, a matrix spike would consist of a relevant matrix (e.g., soil, water, or fish tissue) spiked with microplastics that are representative of the desired targeted microplastics. The relevancy of microplastic spikes should take into consideration size, density, morphology, and polymer type. This is because these parameters may affect recoveries during extraction, microscopy, and chemical identification via spectroscopy or spectrometry. Until standard reference materials for microplastics are available, representative matrix spikes will be difficult to make and non-uniform across the field of study. This is likely one reason why these are generally not yet used in microplastic studies. At present, studies usually test the recoveries of their methods for proof of concept. We recommend the creation of standard reference materials to facilitate reference spikes and standard protocols for using them. Once reference materials are available, we suggest matrix spikes be used in every study. As mentioned above, laboratory blanks and field blanks can be used to blank-correct a sample. Similarly, once matrix spikes are common, they can be used to recovery-correct a sample if deemed appropriate.

Another metric that is often reported in analytical chemistry, but still missing from most microplastics studies, is the reporting of LODs and/or LOQs. Although these have been discussed and/or used in a few studies (e.g., Hung et al., 2021; Bråte et al., 2018; Brander et al., 2020), they remain very rare, and have not been rigorously evaluated to date. In traditional analytical chemistry, LODs and LOQs can be determined based on the detection capabilities of the instrument in conjunction with the magnitude and variability of blank contamination and/or low-level spiked matrix samples. These values are such that studies can confidently report that a measurement is distinguishable from background contamination. LODs are typically set as being some multiple of the blank level or at the level of the blank plus some multiple of the standard deviation of the sample or a low-concentration standard (Harris, 2010). However, only one blank was processed from each laboratory in this method evaluation study and the spiked sample was at only one concentration. Accordingly, calculations based on multiple blanks in a given laboratory cannot be achieved, nor can a standard deviation for a low-concentration standard be obtained. Lao and Wong (2023; *this issue*) suggests best practices for using LODs and LOQs based on results from this study.

5. Conclusion

Procedural contamination of simulated drinking water samples as indicated by blanks was highly variable among laboratories. For microplastics data, we acknowledge that systematic correction for secondary contamination of microplastic samples is necessary to generate robust data. However, the most accurate procedure for such a correction is still under development. For now, we recommend reporting counts of samples without blank correction, as well as counts of particles detected in the blanks. If blank corrections are performed, a clear description of the method of blank correction must be provided. Blank corrections by all characteristics are recommended to lend to a sample with the most representative particles (similar to analytes in analytical chemistry of chemicals). We also recommend several QA/QC procedures for reducing procedural contamination. We recommend efforts to physically block atmospheric contamination from entering samples, working within controlled air environments, with thorough cleaning procedures in place, and policies that bar synthetic clothing from the laboratory. We also recommend the use of filtered water for cleaning and in samples, thorough training regarding cleaning, sample processing and counting, and minimal sample handling when possible, to avoid procedural and cross contamination. The use of blanks is critical to the analysis, and future work should conduct studies that enable us to recommend best practices for field blanks, matrix spikes and the use of LODs and LOQs in microplastic research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

All data is freely available in the Supplementary Materials section and in De Frond et al. 2022; *this issue*.

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HIGHLIGHTS

- Results from a method evaluation study inform QA/QC.
- Procedural contamination is common in microplastics samples.
- Laboratory and/or field blanks are essential to microplastics research.
- Future work should inform methods to reduce and report contamination.

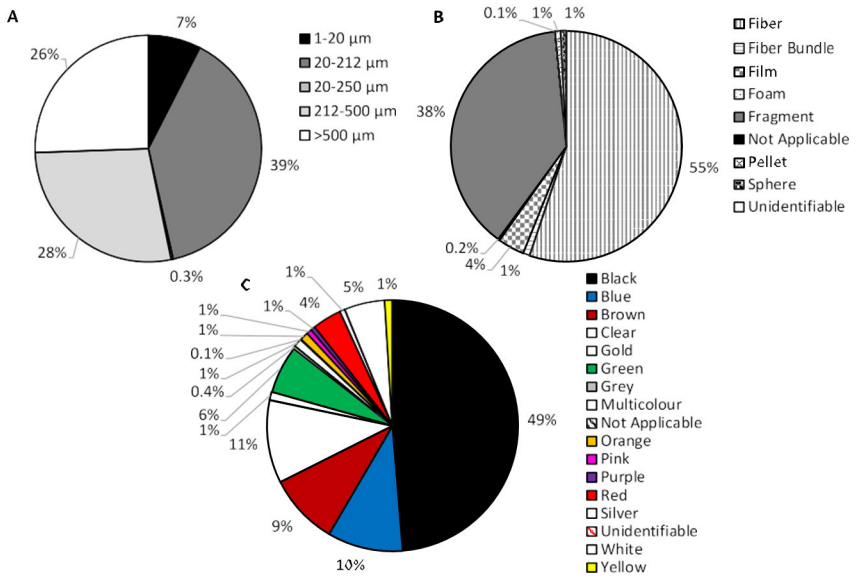

Fig. 1.

Composition of the particles detected in the blanks across all labs (n = 1037) by (A) size fraction, (B) morphology, and (C) color.

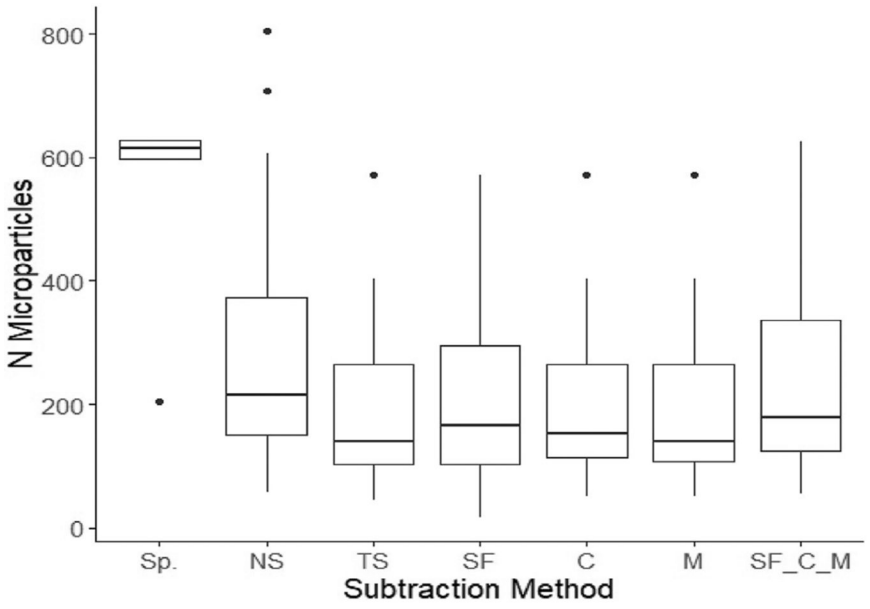

Fig. 2.

The total number of microparticles spiked in the samples (Sp) as well as the raw counts of microparticles detected in the samples with no subtraction (NS), counts of microparticles detected in the samples after subtraction by the total number of particles detected in the corresponding blanks (TS) and by single characteristics (size fraction [SF], color [C] and morphology [M]), and combined characteristics (color and morphology within size fractions [SF_C_M]).