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Assessment of Filter Subsampling and Extrapolation for Quantifying Microplastics in Environmental Samples using Raman Spectroscopy

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Abstract

A common method for characterizing microplastics (MPs) involves capturing the plastic particles on a filter after extraction and isolation from the sediment particles. Microplastics captured on the filter are then scanned with Raman spectroscopy for polymer identification and quantification. However, scanning the whole filter manually using Raman analysis is a labor-intensive and time-consuming process. This study investigates a subsampling method for Raman spectroscopic analysis of microplastics (defined here as 45 −1000 μm in size) present in sediments and isolated onto laboratory filters. The method was evaluated using spiked MPs in deionized water and two environmentally contaminated sediments. Based on statistical analyses, we found quantification of a sub-fraction of 12.5% of the filter in a wedge form was optimal, efficient, and accurate for estimating the entire filter count. The extrapolation method was then used to assess microplastic contamination in sediments from different marine regions of the United States.

Keywords

Microplastics; Sediments; Raman spectroscopy; Subsampling; Marine

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Introduction

Microplastics (MP) are ubiquitous in many environments[1–4]. Based on guidance adopted by the State of California in 2020, MPs are defined as particles ranging in size from five mm to 1 nm[5] and derived from either primary or secondary sources[6,7]. Primary MPs are manufactured in the micro-size range[6,8], while secondary MPs are generated by the breakdown of larger plastic particles due to biological, chemical, and physical degradation[9–12]. MPs are mainly transported into marine and estuarine environments by hydrologic and aeolian processes[8,13]. Despite the low density of many MPs, they can sink and accumulate in sediments over time due to various mechanisms including weathering, agglomeration, biofouling, bioturbation, incorporation into phytoplankton aggregates, and discharge within the fecal pellets of aquatic organisms after consumption[14–18].

Assessing adverse effects associated with MPs is an active area of scientific investigation. For example, sediments contaminated with MPs may cause adverse impacts on the marine benthos due to their ingestion and accumulation[19,20]. Along with the polymers that comprise the MPs, manufacturers amend additives into plastic formulations to reduce degradation by microbes and sunlight, as well as to enhance the polymer’s plasticity and color. These additives include phthalates, bisphenol A (BPA), nonylphenols, and brominated flame retardants which are known toxicants and endocrine disruptors[21–23]. For these reasons, the identification and quantification of MPs in sediments is crucial to further understanding the potential exposure and effects on benthic life.

Previous studies have identified the presence of MPs in marine[24–28] and estuarine sediments[29–36]. However, the extraction, identification, and quantification methods of MPs in sediments vary greatly among studies. A common methodology for determining MPs in sediments involves capturing the plastic particles on a filter after isolation and extraction from the sediment particles. The MPs captured on the filter are then scanned using analytical techniques for polymer identification and quantification. One frequently applied identification method is Raman spectroscopy, a vibrational spectroscopic method applied for identifying specific polymers through characterization of their unique spectral fingerprints[37–39]. When coupled with microscopy, Raman spectroscopy provides chemical, morphological and quantitative (e.g., shape, size, abundance) characteristics of the particles[40–42]. An important advantage of Raman spectroscopy is its ability to detect and identify particles in smaller size ranges (e.g., 1–10 μm)[37, 43–45] compared to other identification techniques (e.g., Fourier transform infrared (FTIR) spectrometry). However, few studies report these smaller size ranges because the analysis is a tedious process requiring longer measurement times depending on the sample composition, particle isolation, extraction, counting methodologies, particles numbers, and instrument parameters[45,46]. For example, spectral identification of just a single particle can require between 0.5 s to five minutes as reported by previous studies[40]. Consequently, manually scanning an entire filter (e.g., 47 mm diameter) using Raman is a labor-intensive and time-consuming process, taking several days to analyze a single filter[46,47].

As a result of those challenges with Raman spectroscopy, automated maps are usually generated, and a subsample of the particles are examined to reduce the scanning time required to analyze all the particles. Subsampling methods vary from a few particles (e.g., 10 particles) on a filter to a sub-fraction of 50% of the total filter[40]. While, to the best of our knowledge, a limited number of studies have been performed assessing subsampling[48–50], a rigorous statistical analysis selecting an appropriate filter subsample has not been conducted. Recently, De Frond et al.[50] recommended a random particle counting strategy balancing quantifying fewer particles to assess basic proportions of types of particles (e.g., anthropogenic, natural) versus more thorough quantification to characterize the diversity of particles within each type. Because of the range of possible subsampling approaches, choosing the most representative sub-fraction of the filter is uncertain and remains an open question[51].

The main goal of this study was to determine the most appropriate sub-fraction of the filter to analyze using Raman spectroscopy, which can then be extrapolated to determine the total number of MP on the whole filter while minimizing time spent performing the analysis. After initial evaluation of the ‘optimal’ representative fraction using MPs spiked into deionized water and two environmentally-contaminated sediments, the method was used to assess microplastic contamination in several marine and estuarine sediments along the coast of the United States including the Northeast, Mid-Atlantic, and southern California collected from the U.S. EPA’s ten geographic regions (https://www.epa.gov/aboutepa/regional-and-geographic-offices).

Methods and Materials

Filter Sub-fractioning Study

Overview—To evaluate subsampling of the filters, we analyzed multiple sections of a filter using Raman spectroscopy and subsequent extrapolation to the whole filter to determine total MP particles in several validation samples (i.e., four DI water solutions, two environmentally-contaminated sediments). To determine the ‘optimal’ sub-fraction of a given filter to count, we evaluated (1) the accuracy of extrapolation to estimate the total number of MP particles on the filter, (2) the time required to analyze a given sub-fraction of the filter, and (3) the precision of the replicated count estimates. For this investigation, ‘optimal’ was a relative balancing of the maintenance of accuracy and precision while reducing counting time in comparison to the analysis of the entire filter. This analysis was performed on deionized (DI) water spiked with selected plastics (50 to 3000 μm). In addition, as a confirmation step, two environmental sediments from the U.S. EPA regions sampled in this investigation were also analyzed. DI water samples were performed using analytical grade Milli-Q water to avoid any particle contamination. More detail on the collection and preparation of both types of samples is provided below. Specific sediment samples used in this sub-fractioning study were from the Northeast (Narragansett Bay, RI – Region 1 station 4) and the West Coast (Long Beach, CA – Region 9 station 5) of the United States. Polymer particle target sizes ranged between 45–1000 μm. Statistical analyses were used to identify the filter sub-fraction which was optimal for estimating the entire filter count (see description below).

MPs in DI Water—Four solutions were each prepared in 100 mL of DI water spiked with polymer particles. One solution contained polyethylene (PE) microbeads, another polyvinyl chloride (PVC) fragments, a third polyethylene terephthalate (PET) fibers, and lastly, a combination of all three polymers (Table 1). Surfactant (Tween 80 Biocompatible surfactant, Cospheric, CA, USA) with a concentration of 1ml/L was added to the solution to disperse the plastic particles. Each solution included five replicates (n = 5) and was mixed using a magnetic stirrer to ensure homogeneous dispersion of the particles. An aliquot of the mixture (10 ml) was filtered on a polycarbonate track-etched (PCTE) membrane filter (47 mm diameter, 0.45μm pore size, GVS) using vacuum filtration. Particles were counted by placing a 5 mm × 5 mm grid sheet under the filter and scanning the filter with Raman spectroscopy square by square within defined fractions. Fractions were chosen in wedge forms to include the dispersion of particles from the center of the filter to the outer edge. Using Raman, we scanned the following sub-fraction sections of each of the filters: whole filter area (100%), three-quarter of the filter (75%), half of the filter (50%), one quarter of the filter (25%), a sector of 45° of the filter (12.5%), and a sector of 22° of the filter (6.25%) (Figure 1). Along with the quantification information, the processing time needed to scan each filter section using the Raman microscope was recorded. As noted above, these studies were performed with each MP solution using five replicates allowing the calculation of precision estimates including the relative standard deviation (RSDs).

MPs in Environmentally Contaminated Sediments—Following isolation and extraction of MPs from the sediments, they were placed on a filter and treated as described above for the DI water samples. Unlike the DI water study described above, the MPs in these sediments were from environmental contamination and not from laboratory spiking. The section below describes how the sediments were collected and the MPs isolated and extracted from the samples used in this part of the study. Specifically, the two sediment samples were from Region 1 station 4 (n = 1) and Region 9 station 5 (n = 1) (Figure 2).

MPs in Regional Sediments

Surficial sediment samples (top 5 cm) were collected from several marine and estuarine locations in three different U.S. EPA regions across the United States: (a) New York Harbor (New York and New Jersey - Region 2), (b) Delaware River (Delaware, Pennsylvania and New Jersey - Region 3), and (c) Long and Seal Beaches (California - Region 9). Sampling stations within each Region were chosen based on their proximal land use patterns (e.g., wastewater facilities, combined sewage overflows (CSOs)) to ensure that a wide range of sediment environments and potential plastic sources were captured (Figure 2). In total, 21 locations were sampled (SM Table S1). The number of stations sampled differed per region; for example, Regions 3 and 9 had the smallest number of stations (5 stations) whereas Region 2 had the greatest number of stations (11 stations). Samples from Region 1 in Narragansett Bay (Rhode Island, USA) were also used in the current study for comparison purposes. Region 1 samples were part of a previously published study from this laboratory that developed a hybridized method to extract MPs from sediments, which is used in the present study[52].

Samples were stored in clean glass jars at 4°C in the dark until used. Upon retrieval, a stainless-steel spatula was used to homogenize the sediment samples and divide them into 100 g subsamples. The composition of sediments was a mix of primarily silty samples (e.g., NY Harbor, Delaware River, Narragansett Bay) and dominantly sandy (e.g., Long and Seal Beaches). The grain size distribution of each sediment sample was determined with triplicate analysis using a MasterSizer 3000 instrument (Malvern Palanytical LTD, Malvern, UK) (SM Table S2).

Sediment samples were processed to isolate and extract the MPs following an optimized hybrid extraction method[52]. Briefly, sample processing consisted of three main steps: (1) sieving the 100 g (wet sediment) samples into two class sizes (45 to <250 μm and 250 to 1000 μm), (2) separating with high- and low-density sodium bromide solutions with densities of 1.3 and 1.5 g/cm^3 for each size class, and (3) filtering the supernatant onto 20 μm PCTE filters using a vacuum pump. After processing, each sample was separated into four treatment filters by size class and solution density. Filters were oxidized using 30% hydrogen peroxide to reduce natural organic matter interferences. Particles in the upper size range (250 to 1000 μm) were picked with metal forceps using a 10x magnification dissecting stereoscope (Nikon SMZ745-T), enumerated, categorized according to their shape (e.g., fragment, pellet, sphere, fiber, film), color, and sizes and then placed on double-sided sticky tape on glass slides for later Raman spectroscopic identification. Smaller particle sizes (45 to <250 μm) were retained on the filters for Raman scanning and stored in pre-cleaned petri dishes.

Quality Assurance/Quality Control

For Regional sediments, samples were collected minimizing exposure to ambient plastic contamination whenever possible. In addition, collection personnel wore brightly colored cotton clothing so that any contamination from this source could be readily identified. Collection blanks consisted of air blanks (i.e., clean filters in a petri dish) used during sample collections, and water blanks with DI water performed during sample laboratory processing. For the laboratory activity, samples were processed in a facility dedicated to MP research. The facility has minimum efficiency reporting value (MERV-13) filtration. MERV-13 filters remove 90% of particles in the 3–10 μm range, 90% of particles in the 1–3 μm range, and 75% of particles in the 0.3–1 μm range. The facility is also equipped with a HEPA filtered laminar flow cabinet where all samples were processed to avoid airborne particle contamination. As with the field collections, cotton laboratory coats dyed with bright pink and purple colors were used during all processing to assist in identifying contamination. Milli-Q water operational blanks and air blanks were analyzed alongside the environmental samples to assess potential laboratory contamination. To correct for procedural contamination in our samples, we subtracted the average number of MP particles found in the blanks from each sample type by matching the polymer type and morphology (e.g., PET fiber, PP fragment). Finally, to assess extraction recovery of MPs from environmental sediment samples, MPs of known polymer type, shape and size were added to a subset of samples as internal standards (SM Table S3). These MPs were added to the samples at the beginning of the sediment extraction[52].

Raman Spectroscopy

MP polymer types were identified using an inVia Qontor Raman Microscope (Renishaw plc., Gloucestershire, UK). The analyses were performed with a 785 nm excitation laser, 10s integration time, 1 accumulation, a spectral range of 200 to 3600 cm^{-1}, laser power of 1%, and an objective with 20x, 50x and 100x magnifications. For the Regional sediment samples, single point analysis was used for the upper size range particles (i.e., 250 to 1000 µm) where spectra were recorded at the center of each particle. For the small size range particles (i.e., 45 to <250 µm) retained on filters, a sub-fraction of the filter was analyzed as determined in the previously mentioned sub-fraction studies.

Based on the spectroscopy, montage pictures were acquired to capture particles across the filter surface and spectra were collected by running automated maps. Spectra were processed using Wire software (WIRE 5.4) to remove fluorescence background, perform baseline subtraction, noise filtration, and other commonly applied clean-up techniques. Identification of the polymers was achieved using built-in and external library database searches (e.g., polymer, inorganic, Slopp, Slopp-E, OpenSpecy)[53,54]. For this investigation, the following common plastic polymers were in the library: polypropylene (PP), polyester (PES), PE, polystyrene (PS), polyurethane (PU), PVC, polyamide (PA), polybutadiene (PB), PET, in addition to a collection of copolymers, additives, and inorganic substances. Spectral matches with High Quality Index (HQI) above 70% were accepted as a good polymer identification. Matches with HQIs between 40 and 70% were considered individually based on visual confirmation of the spectra for certain functional groups that were present. The remaining matches with HQIs below 40% were disregarded. For this investigation, the percentages of particles fitting in each matching category were 33% for HQI above 70%; 65% for HQI between 40 and 70%, and 2% for HQIs below 40%. These criteria were chosen considering the same particle may exhibit different Raman spectral intensity and shifts in peak because of environmental weathering, the presence of additives, or occurrence of other chemicals in the particle’s composition. Additionally, factors such as the instrument type, spectral measurements, and signal to noise ratio can cause different Raman spectral intensities which lowers the comparability to the Raman databases[55].

Statistical Analysis

For the analysis of the DI water solutions spiked with selected plastics, five replicate MP counts in each filter fraction (i.e., 6.25% to 100%) were performed. Extrapolation involved multiplying a given sub-fraction count by a factor to estimate the count at 100% filter. For example, the extrapolation for the 6.25% sub-fraction counts involved multiplication by 16. Following extrapolation, a multi-factor ANOVA (with replicate as a blocking factor) was performed comparing mean MP counts. If the ANOVA F-test detected significant differences at $p \leq 0.05$, a Dunnett’s multiple comparison test was performed to identify which filter sub-fractions were significantly different from the entire filter (100%). In addition to the hypothesis testing with ANOVA, the RSD of the extrapolated MP counts for each DI water sample sub-fraction was evaluated. The RSDs were used to assess if their magnitude could be explained simply by imprecision resulting from random counting error (described by a Poisson distribution) or if the sub-fractionation itself introduced additional error[56].

For filter sub-fractions generated from the environmental sediments selected from Regions 1 and 9 (which were not replicated; n = 1 for each Region), statistical analyses were performed using the non-parametric Kruskal-Wallis test to determine if any sub-fraction MP count extrapolations were significantly different from the actual count for the entire filter. If differences were detected, the post-hoc Dwass, Steel, Critchlow-Fligner pairwise comparison method was used[57] to identify specific sub-fraction extrapolations significantly different from the entire filter (100%). Two endpoints were used to assess differences between counts for the entire filter and extrapolated counts: relative accuracy and absolute bias. Results of these statistical analyses were used to select a filter sub-fraction to analyze the remaining Regional sediments in order to estimate total filter counts. In addition to the statistical analyses of the filter sub-fractions, one-factor ANOVA ($p \leq 0.05$) was used to determine if significant differences existed in MP abundances between Regions.

Results and Discussion

Filter Sub-fractioning

DI water samples—For the analysis of the DI water solutions, the numbers of MPs and polymer types found in each sub-fraction are summarized in SM Table S4. Figure 3a shows the extrapolated count values of the particles including individual polymer types and total polymers. For two of the MP DI solutions, statistical analyses of the total counts found no significant differences between the 100% treatment and 75%, 50%, 25%, 12.5% or 6.25% fractions (Table 2). However, for two of MP DI solutions, extrapolation of the 6% fraction was statistically greater than the 100% treatment (and the other fractions were not significantly different). The results illustrate that extrapolating from the smaller filter fractions leads to counting inaccuracy. For example, at the 6.25% measured sub-fraction, the extrapolation resulted in an error of 53% for the PET count and 36% for the total number of particles. However, at a larger sub-fraction (e.g., 12.5% and higher), the extrapolation error decreases with the increasing measured fraction (0 to 13% error). Based on this finding, we concluded the 12.5% fraction of the filter could be counted to estimate the entire filter. Table 3 shows the time consumption for scanning each sub-fraction; as expected, less time was required for analyzing the smaller fractions (e.g., 12.5% and 25%). The total time calculated includes the minutes required for Raman sample preparation, acquiring montage pictures, and scanning the particles. The scanning time depends mainly on the number of the particles retained in each filter fraction.

Results of the analysis of the sub-fraction RSDs found the sub-fractionation process did increase expected error above simple imprecision resulting from random counting error (Figure S1). The ratio of observed RSDs to the expected Poisson error ranged from 1.0 to 1.7, 3.1 to 7.9, and 3.6 to 6.3 for fragments, microbeads and fibers, respectively. The fragments showed the smallest additional error while the microbeads demonstrated the largest. This trend correlated with the number of particles counted on the filter sub-fractions, with the microbeads having approximately seven times more particles counted than the fragments for any given filter sub-fraction. As expected, the ratio also increased (i.e., the additional error increased) as the sub-fraction of the filter being counted became smaller (SM Figure S1). However, compared to the ratios determined when counting the entire filter (100%), the increase in error for the 12.5 sub-fraction (selected based on the ANOVA described above), increased by 42% to 71% for the fibers and microbeads while decreasing by 27% for the fragments. The increased error resulting from counting the 12.5% sub-fraction is clearly not desirable but the advantage gained in time and labor savings must be considered in balancing the practicality of using the 12.5% sub-fraction versus optimal accuracy (i.e., counting 100% of each filter). More specifically, counting the 12.5% sub-fraction saved 2 hr, 5 hr, 7 hr and 8 hr of counting when compared to the 25%, 50%, 75% and 100% sub-fractions, respectively. In addition, the accuracy of counts in the 12.5% and larger sub-fractions were of a similar magnitude (Figure 3a). This suggests counting a larger fraction does not necessarily result in greater accuracy.

Environmental sediments—Extrapolated fractional results for the two environmental sediment samples from a silty particle sediment (Region 1: Station 4) and a beach sand (Region 9: Station 5) are shown in Figure 3b and 3c. For both sediments, like the DI water study, statistical analysis found that extrapolation from the 6.25% fraction was significantly greater than extrapolations based on the other filter fractions (Table 2). Specifically, for the Region 1 sediment, the relative accuracy endpoint did not find significant differences, but the absolute bias endpoint did identify the 6.25% sub-fraction as significantly greater than the 75% filter fraction. Similarly, for the Region 9 sediment, the relative bias endpoint did not find any differences while the absolute bias found the 6.25% filter fraction was significantly greater than the 12.5%, 50% and 75% sub-fractions. These results also demonstrate that extrapolating from the smaller filter fractions leads to unacceptable counting inaccuracy. For example, at the 6.25% sub-fraction, the extrapolation of plastic polymers resulted in large range of errors between 10 and 230%. Additionally, the highest extrapolation errors occurred in a few polymer types such as PA (140% in Region 9 and 230% in Region 1), PP (36% in Region 1 and 255% in Region 9), and PVC (28% Region 1 and 185% in Region 9).

Both the DI water and environmental sediment data suggest using the 6.25% sub-fraction to estimate the entire filter counts of MPs would result in statistically significant over-estimations. Based on the analysis of the statistical differences between sub-fractions and the time consumed in the analysis of the DI water and two sediments, we adopted the 12.5% sub-fraction of the filters to count for the Regional sediment samples discussed below. Using this sub-fraction, the 12.5% count results were multiplied by 8 to extrapolate to the entire filter.

Environmental Regional Sediments

Overview—Spiked MP particles were successfully recovered from sediment samples at greater than 70%[52]. For the operation and air blanks, an average of 14 particles (range: 9 to 18 particles) were found and subtracted from the average MP concentrations for each Regional sediment sample. Microplastic particles were quantified in sediment samples collected from three of the four regions (i.e., Region 2, 3 and 9). Region 1 sediments were analyzed as part of an earlier study from our laboratory[52] and included here for comparison of differences among regions of the country. Results for all samples include concentrations for each particle size fraction, particle shape, size, color, and polymers identified in the 12.5% filter subset of particles (SM Table S5). On average, a total of 40 and 1200 particles per 100 g wet sediment were chemically analyzed in the upper (250 to 1000 μm) and lower (45 to <250 μm) size ranges per sediment sample, respectively. The overall microplastic abundances do not include MP concentrations from three samples in Regions 1 and 2 (Region 1 station 4, Region 2 stations 8 and 9). These samples had very large numbers of cellulose acetate (CA) particles (e.g., estimated millions of particles) which would bias the conclusions for the behavior of the other polymers. These three samples are discussed below and in a separate publication[52]. Figure 4a shows MP abundances (number of particles/100 g wet sediment sample) averaged (± standard deviation) for all stations within each of the four regions.

Overall microplastic abundances—Results showed MP abundances ranging from 5 to 400 particles/100 g wet sediment in the different regions with the average MP concentrations of 1160 ± 960, 1218 ± 1260, 570 ± 180, and 796 ± 483 MP particles/Kg wet sample for Regions 1, 2, 3 and 9, respectively. This result is consistent with previous studies of sediments where MP concentrations ranged from 11 to 12,000 MP/Kg sample[58–64]. The wide range of MP abundances reflects differences among sediments as well as variations in particle size and sampling techniques, isolation and extraction methods, and identification approaches.

The highest number of MPs were found in Region 2 (Station 7) with a concentration of 3970 particles/Kg wet weight, while the minimum also occurred in Region 2 (Station 12) with a concentration of 50 particles/Kg wet weight representing a range of up to two orders of magnitude among the different stations. Samples 7 to 12 in Region 2 were taken outside the Jamaica Bay Wildlife Refuge. This area is subject to a variety of sources of MPs such as wastewater treatment plants (WWTPs) effluent, tire wear particles and other road runoff, as well as recreational fishing and boating. The circulation is generally tidally controlled, and while there may be some local deposition at Station 7 from longshore transport of sand, we cannot determine that the differences result from circulation patterns. Region 2 stations 1 to 6 are located in Raritan Bay, New Jersey are more industrialized in nature. The Raritan Bay, Raritan River and the Arthur Kill are home to numerous industries and more than 20 U.S. EPA Superfund sites. Both areas have high-density human populations and receive treated municipal effluent as well as storm-water runoff which is a major-sources of MPs[65]. The wide range of MP concentrations are likely due to river flow, marine and estuarine currents and tidal action, and proximity to sources. These factors are likely to contribute to the overall heterogeneity and lack of obvious spatial distribution patterns within the Region.

While no statistically significant differences in MP abundances were found among the regions, Region 3 generally had the lowest levels of MPs. This was unexpected as this region includes numerous WWTPs, combined sewer overflows (CSOs) (Figure 2), and several marinas (SM Figure S2). Interestingly, a recent study observed that MP abundance, measured in surficial sediments, decreases exponentially with increasing sediment grain size[66]. These observations were also reported in beach sand[67]. This may explain our observations for low MP abundance in Regions 3 and 9 (Figure 4a) where the sediments are mostly sand with coarser particle distributions than the finer sediments from Regions 1 and 2 (SM Table S2). In addition, the Region 3 sediments were collected from the only riverine system in this investigation and may have higher flow rates and flushing of the MPs. Recently, Samandra et al.[68] noted similar complexities in understanding MP behavior in dynamic urban riverine systems in Australia. Region 9 had the second lowest MP abundances among the regions and the least number of WWTPs with a lower human population served in comparison with the other regions. However, Region 9 has a high number of marinas which can be point sources of MPs[69]. In addition, a recent study on the West Coast pointed to storm water runoff as a major source of MP pollution as compared to WWTPs[70].

Types of polymers measured—Raman analysis of particles from regional sediment samples showed a mixture of polymers (Figure 4a, b, SM Figure S3). The most abundant polymers across all regions were in descending order: PP, PES, PA, PE, PS, PU, PVC, polybutadiene, PET, and others (SI Table S3). These polymers in sediments have been reported in a review detailing the magnitude of plastic pollution in different parts of the world[58]. Knowledge of the presence of these polymers can be critical with determining their source attribution. For example, PP is frequently used in fishing nets and plastic bottles, two common sources of plastic pollution in sediments due to fragmentation. In contrast, PES is used in clothing, a major source of microfiber release, PE is used in manufacturing of food packaging and plastic bags, and PB is commonly found in tire wear particles an indicator of road run-off[71].

Polymer shapes, colors and sizes—In addition to the abundance and types of polymers, MPs morphology (e.g., shape, color and size) was also recorded in our study since the deposition of MPs in sediments can be affected by these variables[72]. Further, morphology can also influence ecological effects of MPs[73]. MPs particle shapes were similar among the four Regional environments and categorized into seven major shapes: fragment, fiber, film, flake, sphere, pellet and foam. Our results showed fragments were the most abundant shape in the sediment samples followed by fibers, accounting for 61.3% and 15.6%, respectively, with the minor presence of the remaining shapes (Figure 5a). This result is consistent with the findings of previous studies where both fragments and fibers were the two most common microplastic shapes[58]. As noted earlier, MP fragments are usually the result of physical, chemical and biological degradation of large plastic pieces through weathering processes, while fibers can be released from textiles, ropes, and fishing nets[74,75]. Additionally, ongoing research in our laboratory and others (e.g., Southern California Coastal Water Research Project, Costa Mesa, CA, USA)[76] indicate fibers may be under-reported in environmental samples given their relatively small diameters allowing passage through isolation method sieves and filters that impede larger particles.

Interestingly, there was a high abundance of CA fibers (approximately 6,000,000) in both Regions 1 and 2 at multiples stations (e.g., R1 (station 4) and R2 (stations 8 and 9)). These fibers are transparent with diameters of 5 µm and lengths of 300 µm and occurred in small bundles forming a fragment or mat shape (SM Figure S4). As noted above, the CA fibers were not accounted for within our polymer counts because of their unusual shape and extremely high abundances in comparison with the other polymer particles (at these stations). Not including them provides a better representation of the general distribution of all of the polymers present. The presence and origin of these CA fibers was discussed thoroughly in a previous paper[52]. Cashman et al.[52] speculated that in Region 1 (Narraganset Bay, RI) this polymer may have originated from manufacturing of CA textiles used in marine industries. While the same may be true of CA fiber masses found in Region 2 (NY/NJ Harbor) where recreational boating occurs, we cannot be certain of the source of this large number of CA fibers.

Like the shapes, findings for the MP colors showed a broad spectrum (Figure 5b) with black, brown and white being the most abundant colors averaging 26%, 15% and 11%, respectively. The numbers of these achromatic colors can be attributed to the difficulty of observing the exact color, especially for the smaller size range particles that are identified by Raman mapping as colors are difficult to determine with this type of spectroscopy. In addition, oxidation of sediment samples (performed to remove natural organic matter that can interfere with analysis) can “bleach” many of the brighter colors. Variability in MP colors is also reported in previous studies[77,78].

Similar to shape and color, MPs sizes were recorded and classified into four categories: 45 to 100, 100 to 200, 200 to 250, and 250 to 1000 μm. All sediment samples from the four Regions were found to have more smaller-sized MPs (e.g., 45 to 200 μm); for example, 74.8% of all particles were dominated by this size category (Figure 5c). This is consistent with several previous studies[79,80]. The high abundance of smaller MPs is most likely due to the breakdown by degradation processes of larger plastic particles resulting in their increased accumulation in the sediment.

Conclusions

In this study, we describe a subsampling method for the Raman-based spectroscopic measurement of microplastics present in sediments. The subsampling method is intended to ensure an accurate representation of all particles when using a sub-fraction of the filter to extrapolate to the entire filter (i.e., 100%). Statistical analyses showed a 12.5% fraction of the filter in a wedge shape provides an appropriate representation of the filter. This was demonstrated using a variety of the most common plastic polymers (i.e., PE, PVC, PET) in different sizes and shapes ranging between 50 to 3000 μm in several DI water solutions. A comparison of the time necessary to quantify 100% of a filter for MPs versus counting only a sub-fraction of the filter to estimate total MPs demonstrated how practical and useful the extrapolation approach can be for efficiently quantifying and identifying MPs in environmental samples (e.g., as would be performed in a monitoring program). For example, we found counting only 12.5% of a filter reduced the time necessary to characterize a sediment sample by 69%. This decreased time per sample increases the ability of monitoring programs to consider including the presence of MP in sediments as a routine measurement. We also validated the subsampling method using two environmentally contaminated sediments arriving at results that agreed with the findings from the DI water study. Beyond this, replicate analysis of the DI water amended with known MPs is useful for quantifying imprecision and understanding the relative error of environmental measurements. Finally, applying the subsampling method, 28 environmentally contaminated sediments from four coastal Regions of the United States were assessed for the presence of MPs. Results from that analysis agreed largely with similar studies performed on other coastal sediments. This component of the investigation contributes to our growing understanding of MP distributions, abundances and types in coastal areas and continues to reveal information necessary to identify sources of MP contamination.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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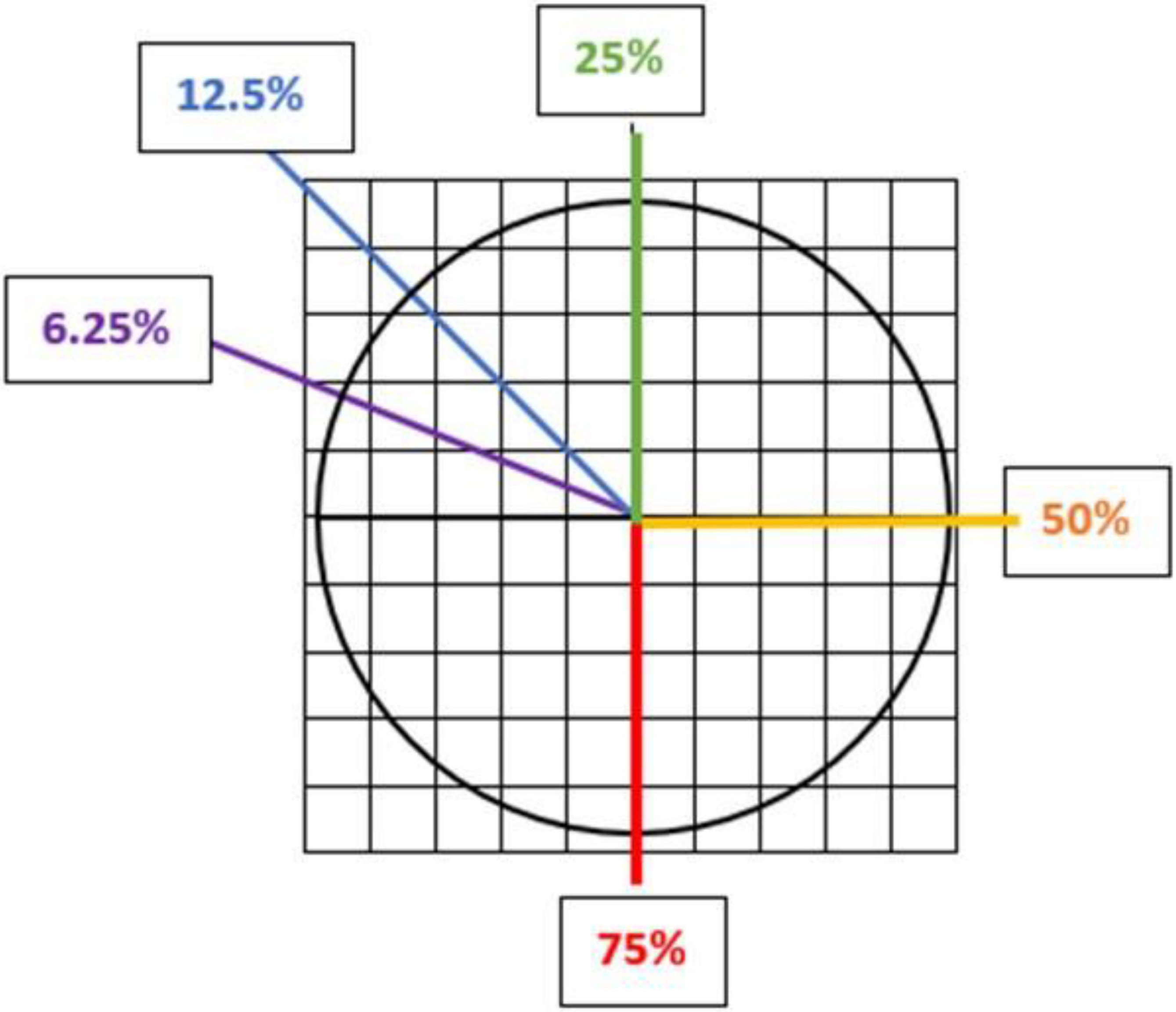
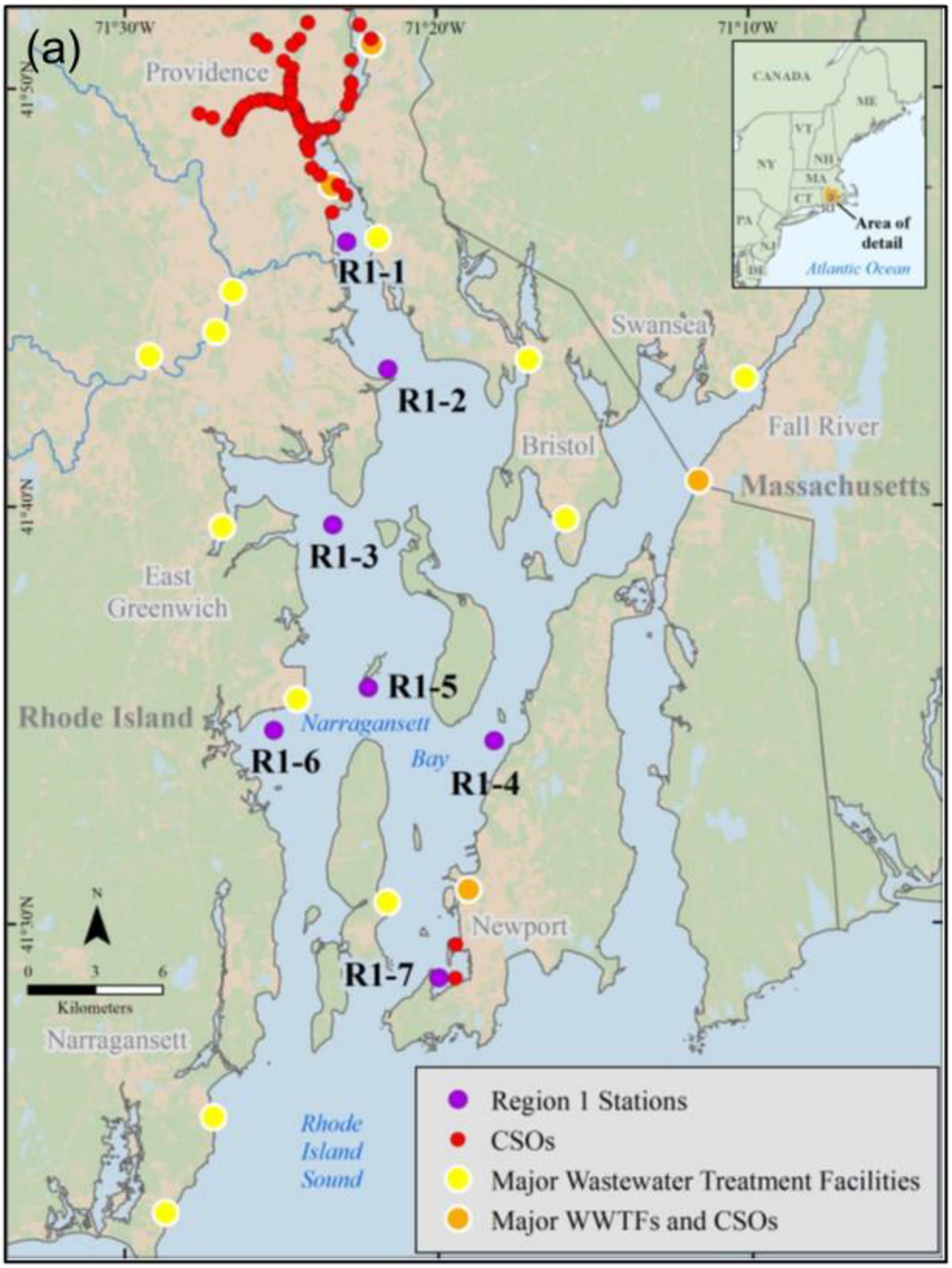
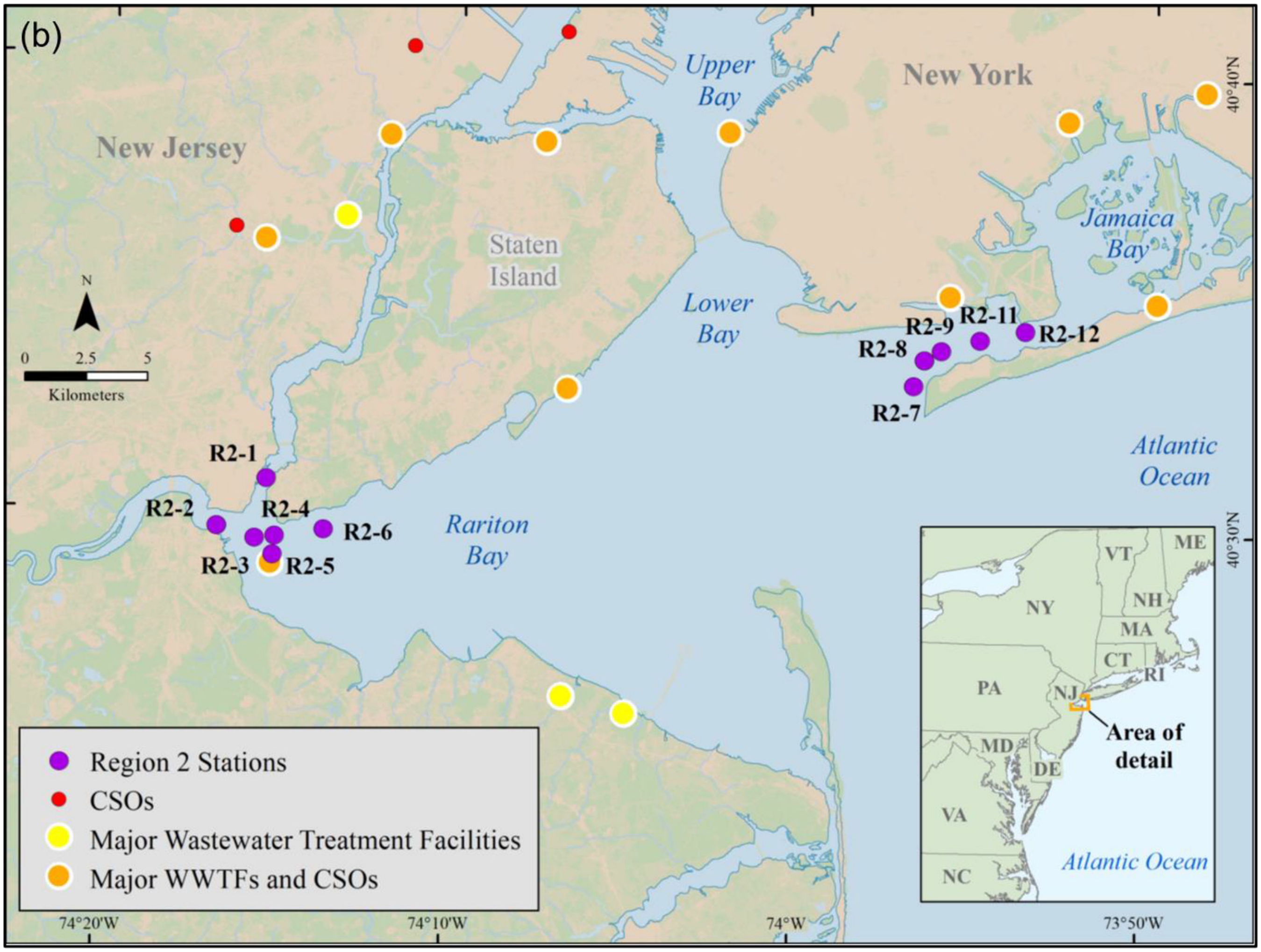
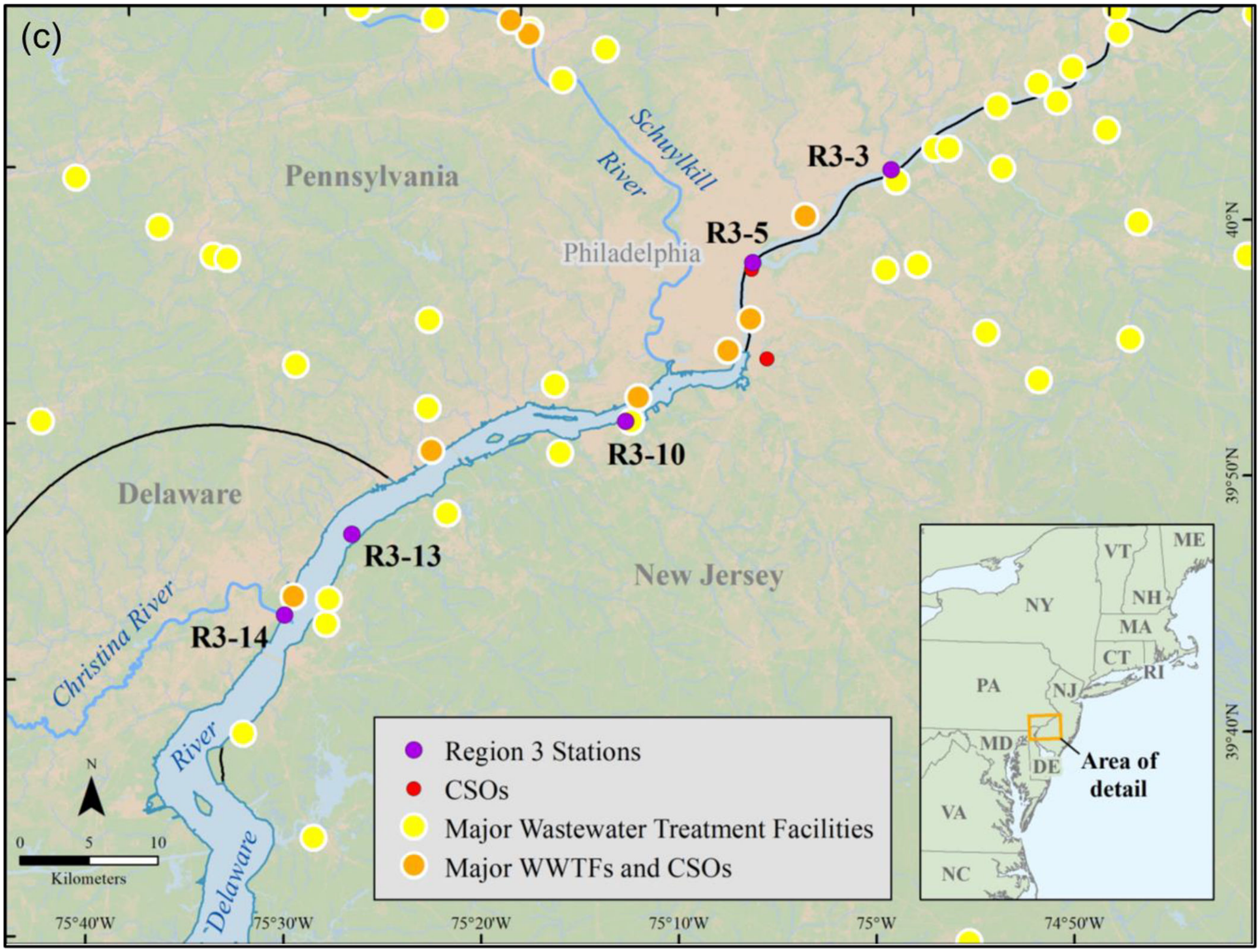


Figure (1).
Illustration of the subsections of filters scanned by Raman spectroscopy for the subsampling component of this study.


(a)
71°30'W
71°20'W
71°10'W
41°50'N
41°40'N
41°30'N
Providence
R1-1
R1-2
R1-3
R1-4
R1-5
R1-6
R1-7
Swansea
Fall River
Bristol
Massachusetts
East Greenwich
Rhode Island
Narragansett Bay
Newport
Narragansett
Rhode Island Sound
N
0 3 6
Kilometers
CANADA
ME
VT
NH
NY
MA
CT
RI
PA
NJ
DE
Area of detail
Atlantic Ocean
● Region 1 Stations
● CSOs
● Major Wastewater Treatment Facilities
● Major WWTFs and CSOs


(b)
New Jersey
Staten Island
Upper Bay
New York
Jamaica Bay
Lower Bay
Rariton Bay
Atlantic Ocean
N
0 2.5 5
Kilometers
R2-1
R2-2
R2-3
R2-4
R2-5
R2-6
R2-7
R2-8
R2-9
R2-11
R2-12
Region 2 Stations
CSOs
Major Wastewater Treatment Facilities
Major WWTFs and CSOs
74°20'W
74°10'W
74°W
73°50'W
40°40'N
40°30'N
NY
VT
ME
NH
MA
CT
RI
PA
NJ
MD
DE
VA
NC
Area of detail
Atlantic Ocean


(c)
Pennsylvania
Schuylkill River
Philadelphia
R3-3
R3-5
R3-10
R3-13
R3-14
Delaware
New Jersey
Christina River
Delaware River
N
0 5 10
Kilometers
Region 3 Stations
CSOs
Major Wastewater Treatment Facilities
Major WWTFs and CSOs
75°40'W
75°30'W
75°20'W
75°10'W
75°W
74°50'W
40°N
39°50'N
39°40'N
VT
ME
NY
NH
MA
CT
RI
PA
NJ
MD
DE
VA
NC
Area of detail

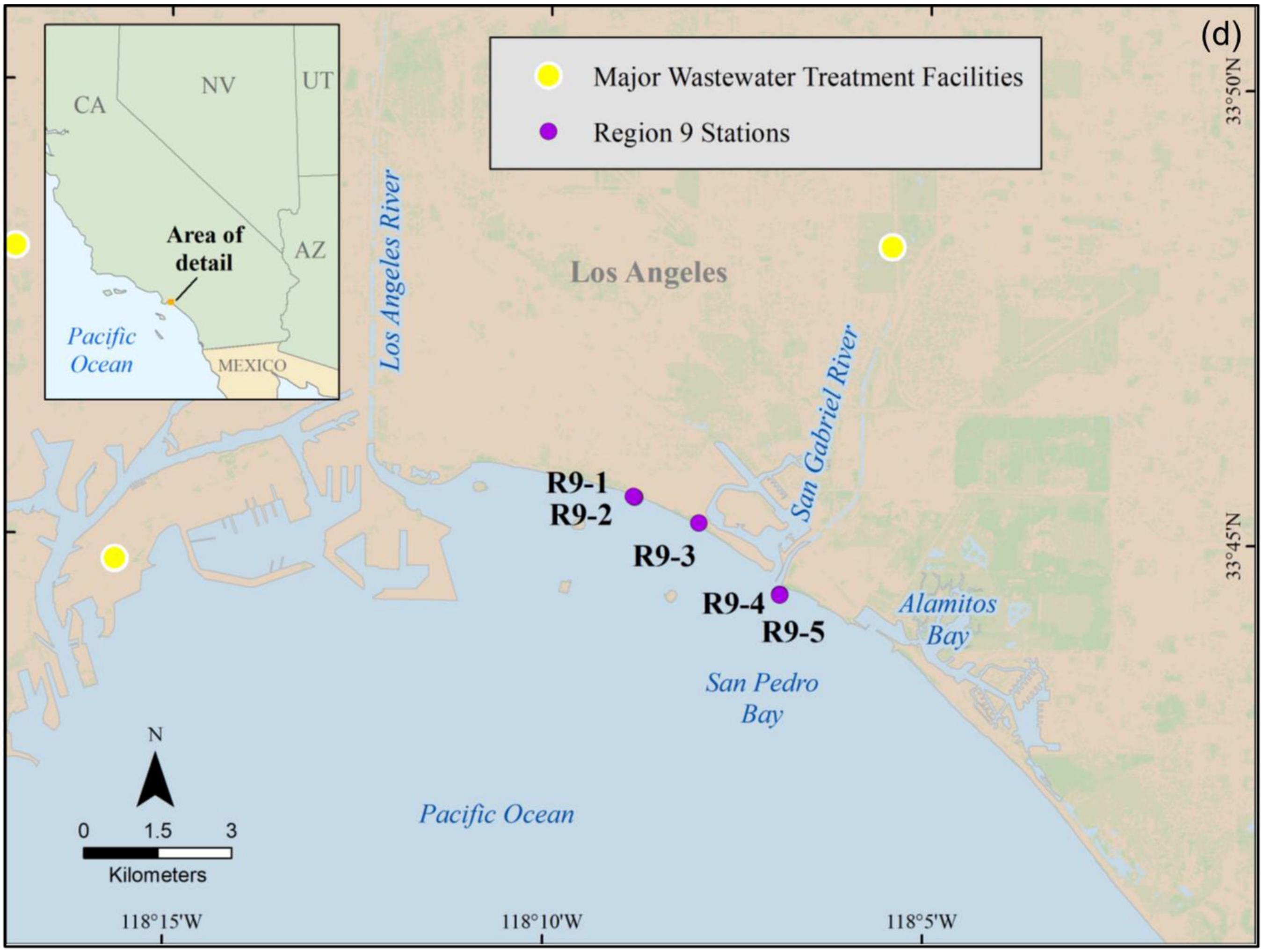
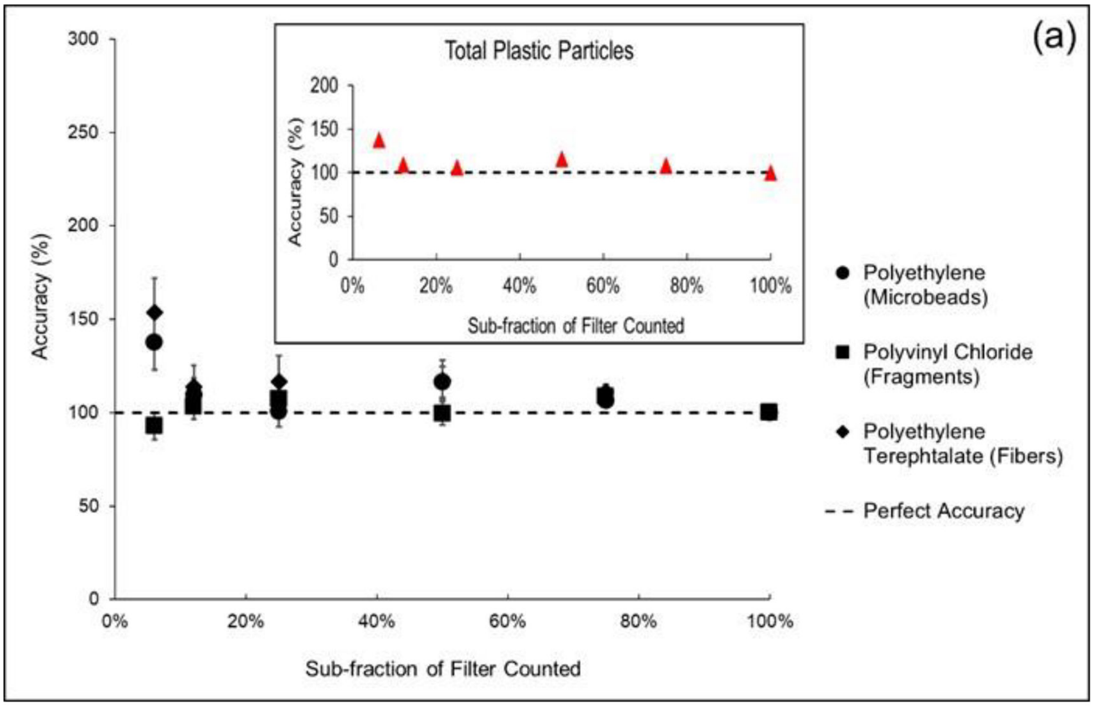
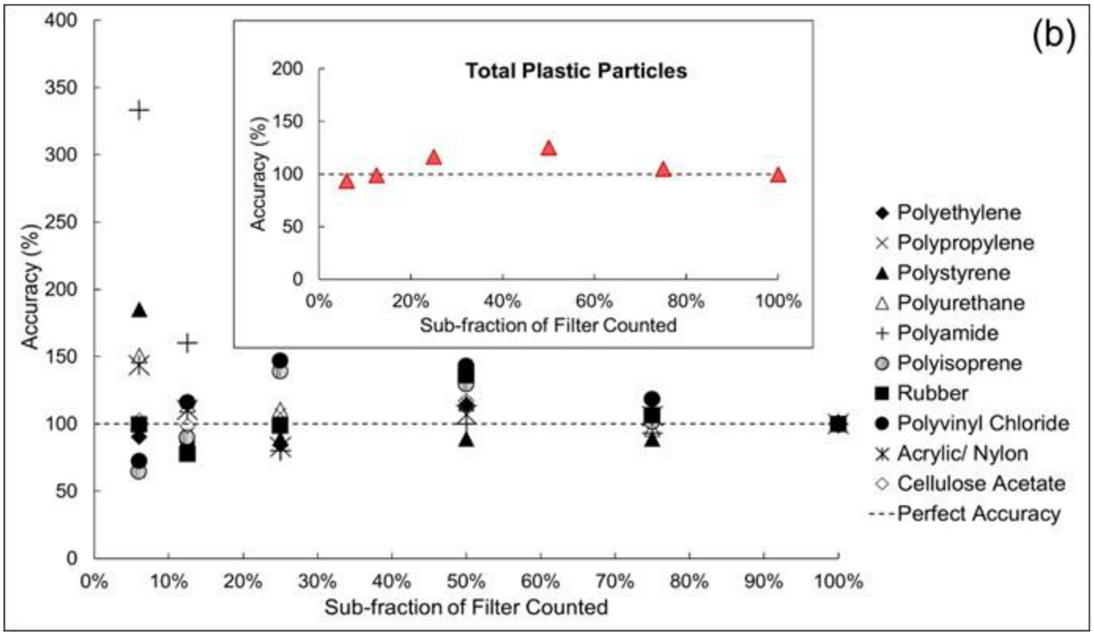


Figure (2).

U.S. EPA regional sediment sampling sites (a) Narraganset Bay, RI, USA (Region 1), (b) Coney Island, NY, USA (Region 2), (c) Delaware River, MD, PA and NJ, USA (Region 3), and (d) Long and Seal beaches, CA, USA (Region 9).


(a)
Total Plastic Particles
Accuracy (%)
200
150
100
50
0
0%
20%
40%
60%
80%
100%
Sub-fraction of Filter Counted
300
250
200
150
100
50
0
Accuracy (%)
Sub-fraction of Filter Counted
● Polyethylene (Microbeads)
■ Polyvinyl Chloride (Fragments)
◆ Polyethylene Terephtalate (Fibers)
- - Perfect Accuracy


(b)
Total Plastic Particles
Accuracy (%)
200
150
100
50
0
0%
20%
40%
60%
80%
100%
Sub-fraction of Filter Counted
400
350
300
250
200
150
100
50
0
Accuracy (%)
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Sub-fraction of Filter Counted
◆ Polyethylene
× Polypropylene
▲ Polystyrene
△ Polyurethane
+ Polyamide
● Polyisoprene
■ Rubber
● Polyvinyl Chloride
✳ Acrylic/ Nylon
◇ Cellulose Acetate
---Perfect Accuracy

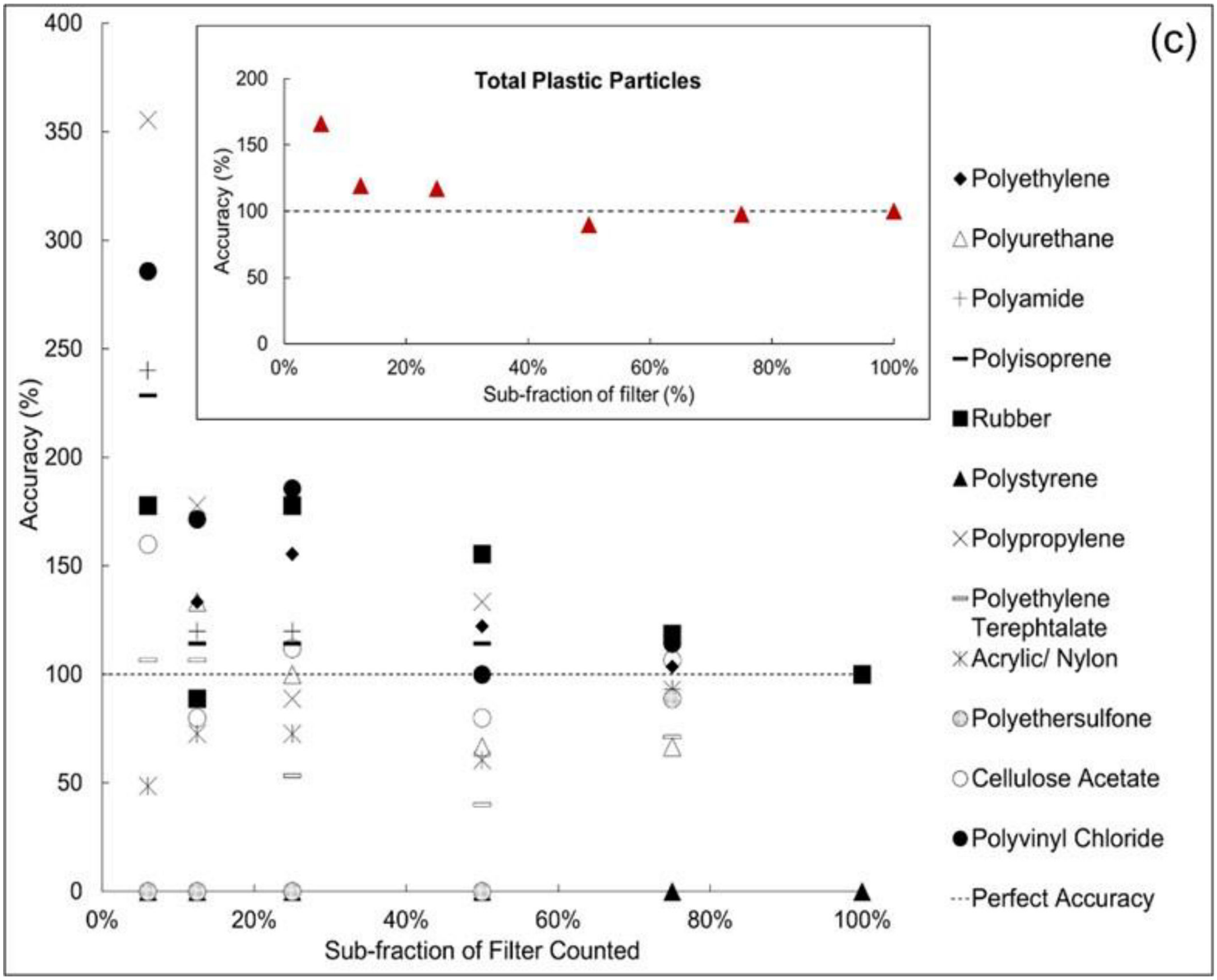


Figure (3).

Extrapolated accuracy values for several polymers for the (a) DI water MP solutions, (b) Region 1 station 4, and (c) Region 9 station 5. The insets illustrate ‘total plastic’ extrapolation accuracy. Accuracy is defined as the ratio of the number of polymer particles counted in a filter fraction and the polymer particles counted in the entire filter multiplied by 100. In each plot, perfect accuracy is represented by the horizontal dashed line.

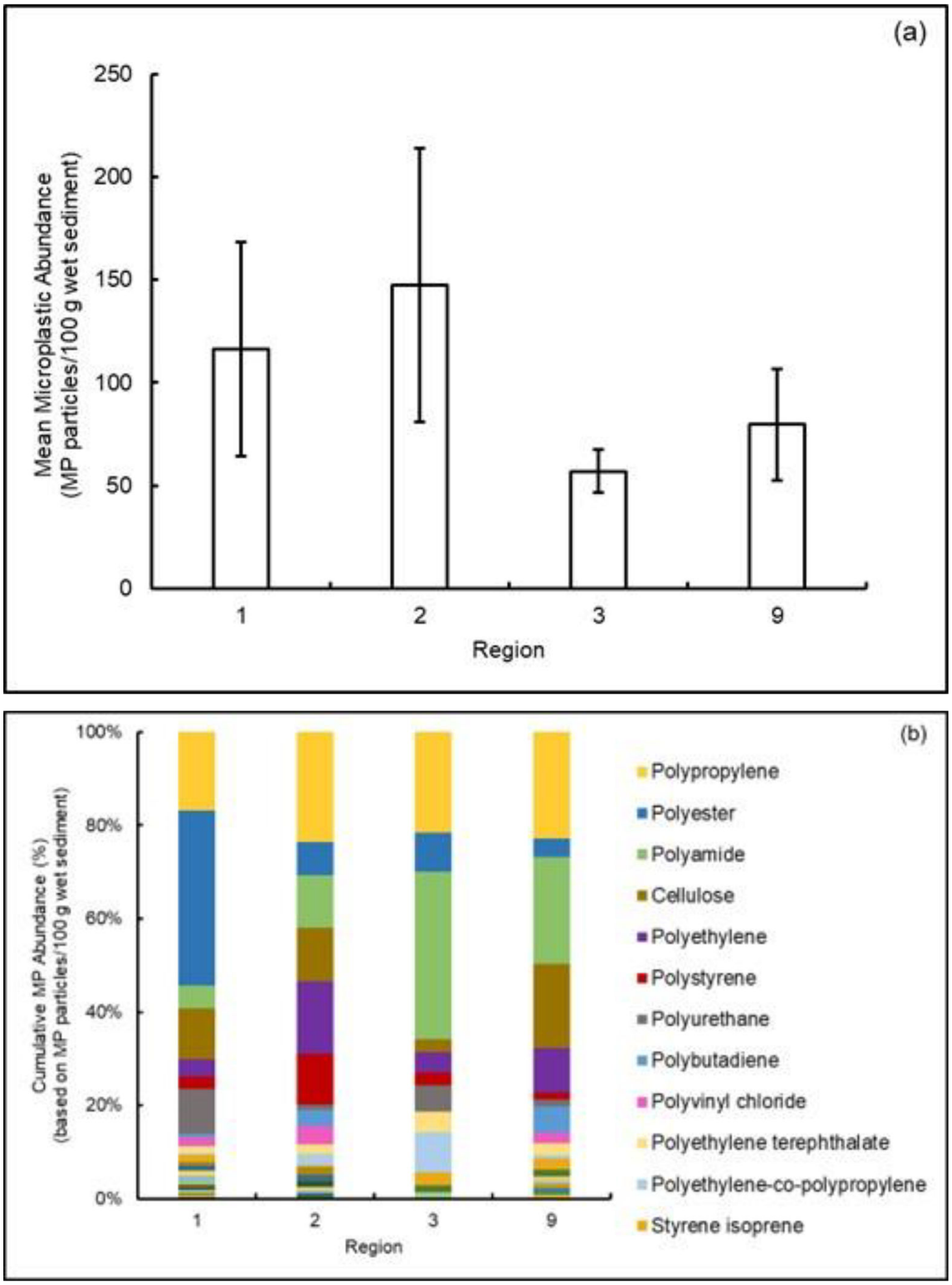
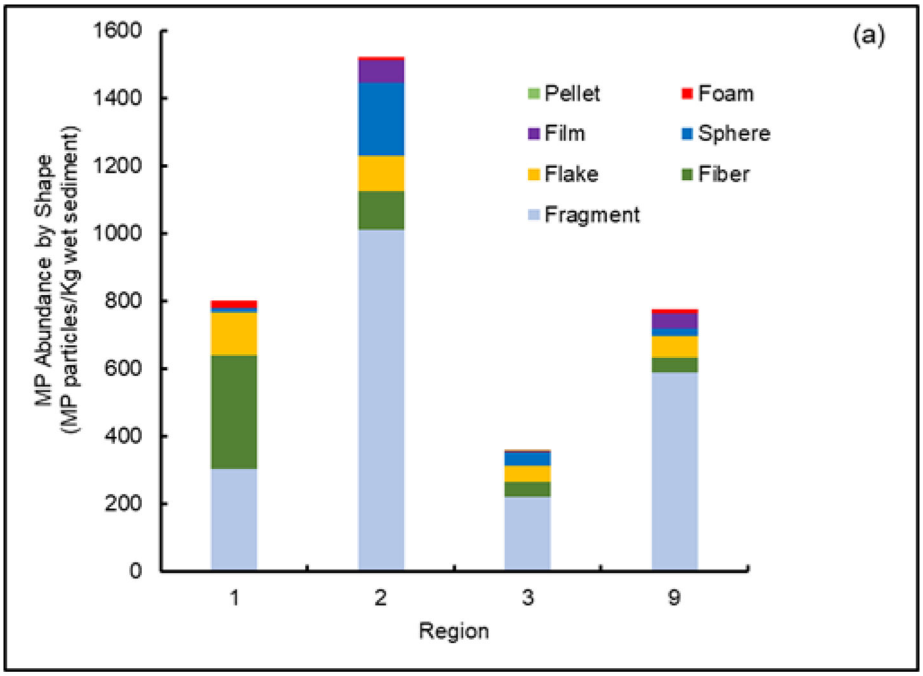
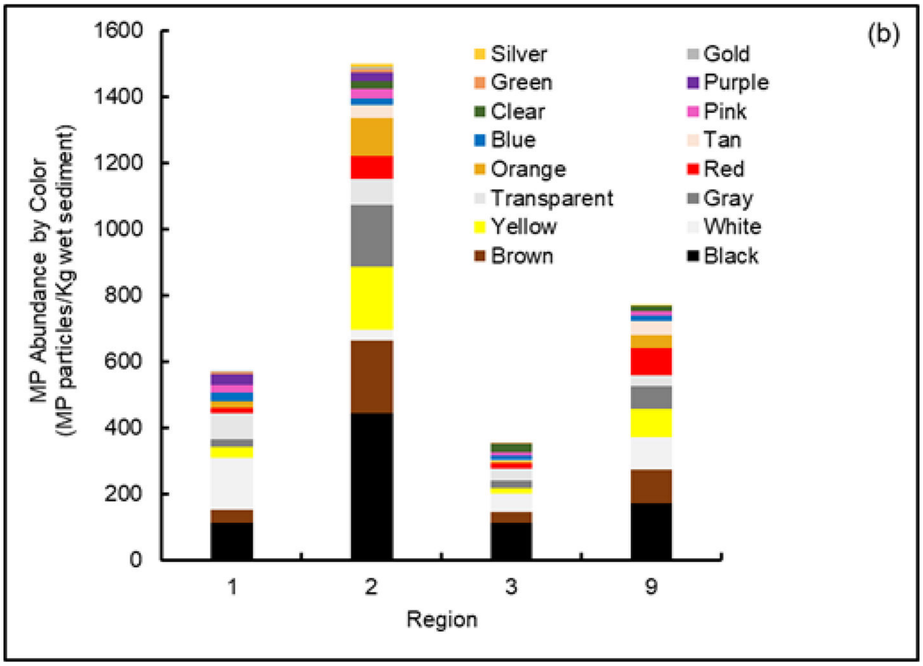


Figure (4).

Summary of results of analyses on regional sediments including (a) mean ± standard deviation abundance of microplastic particles (in 100 g wet sediment) and (b) cumulative abundance (%) of the detected polymers. Note: the 12 most abundant polymers are listed here, SM Figure S3 lists all polymers detected. Regions 1, 2, 3 and 9 consisted of 7, 11, 5 and 5 stations, respectively. Data for Region 1 from Cashman et al.[52].


(a)
MP Abundance by Shape
(MP particles/Kg wet sediment)
0
200
400
600
800
1000
1200
1400
1600
Pellet
Foam
Film
Sphere
Flake
Fiber
Fragment
1
2
3
9
Region


(b)
MP Abundance by Color
(MP particles/Kg wet sediment)
0
200
400
600
800
1000
1200
1400
1600
Silver
Gold
Green
Purple
Clear
Pink
Blue
Tan
Orange
Red
Transparent
Gray
Yellow
White
Brown
Black
1
2
3
9
Region

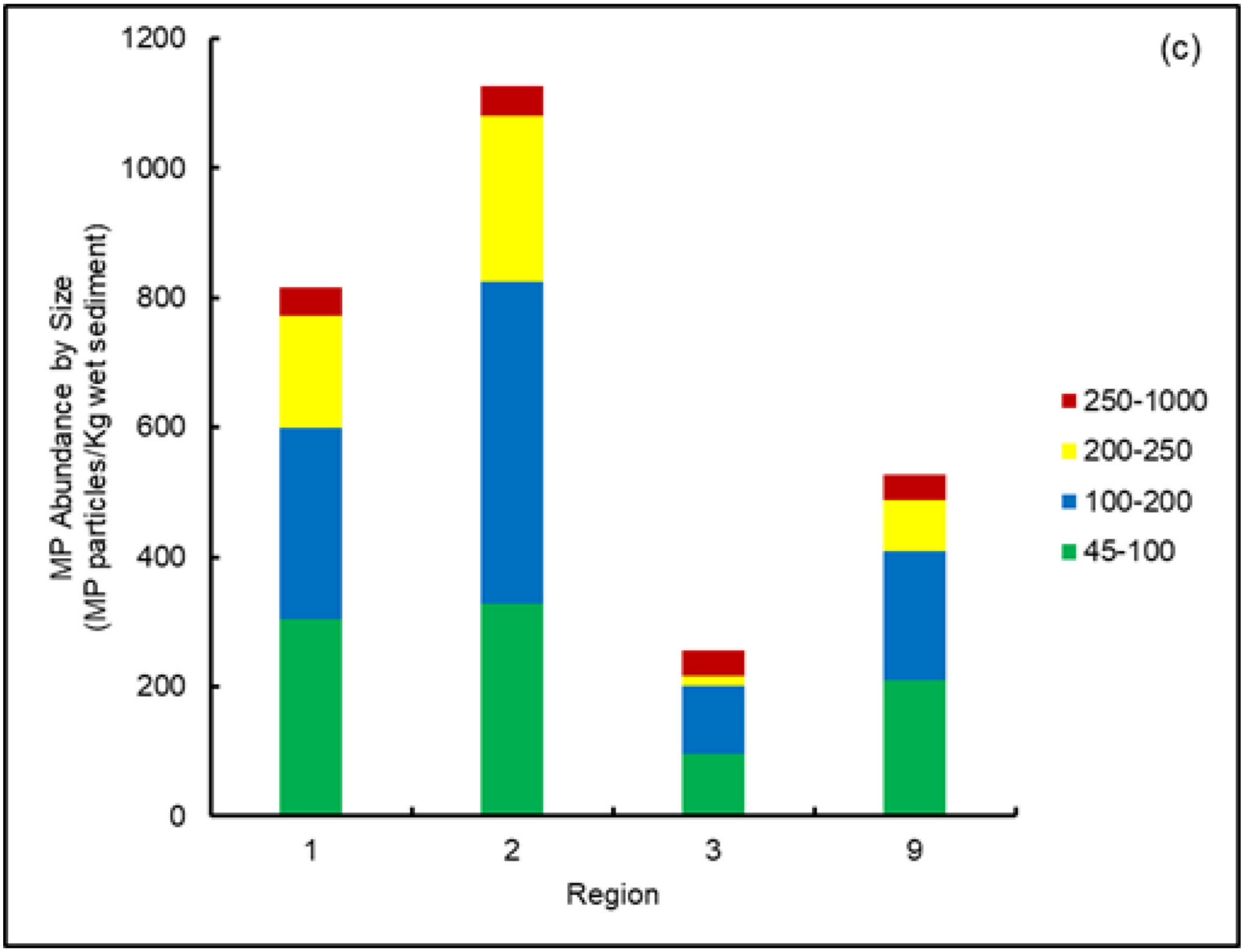


Figure (5).

Summary of the abundance of microplastic particles across regions by (a) shape, (b) colors, and (c) size ranges (based on 100 g wet sediment). Data for Region 1 from Cashman et al.[52].

Table 1)

Polymer type, color, morphology, sizes, amounts and sources of MP amended to deionized water solutions for the sub-sampling component of this study. For the 'All Polymers' treatment, the specific information below for each individual polymer was combined into a single treatment.

Polymer type	Color and morphology	Polymer size range (μm)	Amount (mg)	Source
Polyethylene (PE)	Blue Microbeads	180–212	100	Cospheric, Goleta, CA, USA
Polyvinyl chloride (PVC)	Orange Fragments	500–1000	100	PVC pipe, Home Depot, North Kingstown, RI, USA
Polyethylene terephtalate (PET)	White Fibers	50 wide x 3000 long	100	Embroidery floss, Mini Fibers, Inc., Johnson City, TN, USA

Table 2)

Summary of statistical analyses performed on DI water and two environmental sediments as part of the subsampling component of this study. For these analyses, the dependent variable was the sum of all polymers detected.

Sample Type	Sample ID	Statistical Tests	Statistical Endpoint	Difference Among All Filter Fractions	Individual Differences between Filter Fractions
DI water	1	Multi-factor ANOVA followed by Dunnett's Test (100% used as control)	Significant difference between means	No: F-test: 2.79; p = 0.057	No significant differences detected, not performed.
	2			No: F-test: 0.61; p = 0.691	No significant differences detected, not performed.
	3			Yes: F-test: 3.76; p = 0.021	Extrapolation of 6% filter wedge was greater than 100% counted
	4			Yes: F-test: 4.98; p = 0.007	Extrapolation of 6% filter wedge was greater than 100% counted
Sediment 1	Region 1; 4A-B1	Kruskal-Wallis followed by Dwass, Steel, Critchlow-Fligner pairwise comparisons	Relative accuracy	No: $X^2 = 5.02$; p = 0.286	No significant differences detected
			Absolute bias	Yes: $X^2 = 12.3$; p = 0.015	Extrapolation of 6% filter wedge was greater than the 75% filter wedge extrapolation
Sediment 2	Region 9; 5B-B1	Kruskal-Wallis followed by Dwass, Steel, Critchlow-Fligner pairwise comparisons	Relative accuracy	Yes: $X^2=10.0$, p = 0.040	No significant differences detected.
			Absolute bias	Yes: $X^2=23.6$, p < 0.001	Extrapolation of 6% filter wedge was greater than 12, 50 and 75% filter wedge extrapolations

Table 3)

Summary of time consumed during Raman analysis scanning of four replicate filter samples in the subsampling component of the DI water study. Preparation includes placing the filter on a 5 mm × 5 mm grid sheet using Skin Tac liquid adhesive and holding it in-place on the Raman microscope stage using magnets.

Raman Time Consumption (Minutes)							
		6.25%	**12.5%**	**25%**	**50%**	**75%**	**100%**
Sample 1	Filter Preparation	20	20	20	20	20	20
	Generating Maps	150	200	240[b]	170	180	150
	Scanning Time[a]	44	71	148	307	493	609
	Total Time	214	291	408	497	693	779
Sample 2	Filter Preparation	25	25	25	25	25	25
	Generating Maps	40	90	180	190	160	170
	Scanning Time	44	59	133	361	429	466
	Total Time	109	174	338	576	614	661
Sample 3	Filter Preparation	15	15	15	15	15	15
	Generating Maps	40	100	190	180	170	160
	Scanning Time	43	66	129	289	410	529
	Total Time	98	181	334	484	595	704
Sample 4	Filter Preparation	20	20	20	20	20	20
	Generating Maps	30	90	140	150	170	170
	Scanning Time	51	83	170	294	360	420
	Total Time	101	193	330	464	550	610
Average Time Consumption (min)		131	210	353	505	613	689
Standard Deviation		55.9	54.7	37.1	49.1	59.7	71.5
Standard Error		27.9	27.4	18.6	24.5	29.9	35.8

[a] Scanning time depends on the number of particles. Normally, it requires 1.2 minutes to scan one particle.

[b] Technical difficulties – the computer and instrument needed to be restarted several times.