A Review of Analytical Methods Used in Microplastics Quantification

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Abstract. Microplastics are pervasive contaminants that have attracted the attention of researchers over the last decades. Studies have detected and investigated different sizes of microplastics in the environment. However, the methods used for sample collection and quantification of microplastics for similar environmental matrices differ, based on the resources and equipment available to the researchers. This variation makes the comparison of results from different regions or researchers challenging. This paper presents a review of some of the methods that have been employed in microplastics quantification. We highlight the advantages and disadvantages of some of the methods and recommend that standardized methods need to be developed in order to simplify inter-study comparison.

1. Introduction
The Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection [1], the National Oceanic and Atmospheric Administration (NOAA) and the National Institute of Public Health and the Environment [2] define microplastics (MPs) as plastic particles that are less than 5 mm in size. The adoption of this size is based on field observations in various studies and also because primary microplastics are deliberately manufactured as spherical or cylindrical microbeads or pellets that are approximately 5 mm in size. Microplastics are divided into primary and secondary groups. Primary microplastics are created intentionally in sizes less than 5 mm either directly used as coarse grains for cleaning products or starting materials for some products. This class of microplastics are used in the cosmetics industry for personal care products such as facial scrubs, exfoliating hand scrubs, detergents, soaps, toothpaste, and other cleaning products varying in shapes, size and composition depending on the product.

Fibres from synthetic clothing are also regarded as primary microplastics, and they enter the aquatic environment through wastewater and disposed sewage. These types of microplastics are responsible for the direct input of microscopic plastic fragments into the marine environment [3]. Secondary microplastics are formed from the degradation and fragmentation of larger plastic components due to factors such as friction of tyre in traffic, exposure to UV radiation from sunlight and physical abrasion or mechanical action by sea waves. The rate of fragmentation of large plastics to form secondary microplastics vary depending on the intensity of the environmental factors and the polymer properties [4]. It was observed that studies across different parts of the world have employed diverse analytical methods of sample collection and quantification for microplastics. Furthermore, the sizes of microplastics investigated also vary widely; making inter-study comparisons difficult. This article presents a review of the sources of microplastics and some of the various analytical methods that have been used in the sampling and characterization of microplastics in different sample matrices.

2. Environmental sources of microplastics pollution
The sources of microplastics pollution was classified by [5] into primary and secondary sources. The main sources of microplastics in the environment are indicated as follows:

2.1 Primary Sources
The following are the primary sources of microplastics in the environment:

i. Consumer products like exfoliating beads in toothpaste, face and body wash. Microspheres in
shave foam, sunscreen creams, some lipsticks, glitter powder, mascara printer toner and other personal care products.

ii. Industrial products like abrasives used in sandblasting at shipyards, offshore maintenance, plastic blasting for garments and car parts.

iii. Microplastic dust from industrial production, dust from moulding, cutting, and polishing plastic items.

iv. Maintenance on plastic painted maritime equipment surfaces, buildings, bridges, offshore rigs and pipelines.

v. Dust and fibres from degradation of plastic products at home and offices, including paints, textiles, toys, furniture, and floor covering. Textile fibres from laundry machine and driers.

vi. Plastic particles in road dust from tyres and road paints. Abrasion of plastic ropes and surfaces.

vii. Accidental release of plastic dust during emissions and dust from fires and unrestrained burning.

2.2 Secondary sources
The secondary sources include:

i. Macroplastic debris from inappropriate and unregulated terrestrial waste handling, plastic particles in wastewater effluent.

ii. Macroplastic debris from poor maritime waste handling, waste thrown overboard, loss of fishing trawls, nets, floaters and ropes, abandoned equipment, plastics thrown directly into the sea during boating and recreation.

iii. Municipal effluent containing items like condoms and cigarette buds.

iv. Biological action of animals in shredding and boring of plastic materials.

All of these sources contribute to the mass of plastics debris found in both terrestrial and aquatic environments.

3. Analytical approaches in the investigation of the occurrence of microplastics
Microplastics have been identified and quantified in environmental samples ranging from seawater at different depths [6], sandy and muddy sediments [7], deep sea sediments [8], sewage effluent [9], washing machine effluents, plankton samples, and the guts of marine vertebrates and invertebrates [10][11]. The characterization and quantification of microplastics were achieved using various analytical techniques.

3.1 Sampling
Obtaining and preparing a representative sample of a target population is a crucial step in yielding accurate results for analysis. Samples must be obtained in a manner that deterrminate error is minimized. Some of the environmental matrices that have been sampled for microplastics quantification include:

3.1.1 Water
Microplastics suspended in water can be sampled directly or indirectly depending on the available equipment. Direct sampling involves the use of tools that pick up the microplastic particles without collecting the water into any container. In contrast, indirect sampling requires that a specified volume of water be collected into jars or flasks for MPs to be sieved, identified and quantified. MPs can be directly sampled from water using 333 – 335 µm mesh apertures with the following equipment:

i. Manta net, Neuston net and Niskin hydrophore [12] for surface water

ii. Bongo nets for mid-water depths

iii. Benthic trawls for sea bed

iv. Vessels for surface water and mid water depths [13][14].

A flowmeter is usually attached to the sampling net or trawl to measure the volume of seawater pumped over the time spent. In a study by [15], a motor water pump was used to collect water samples from the surface and bottom of the Antua river. The collected water samples were sieved using a mesh net for particles size range ≥ 0.055 mm and < 5 mm and stored in glass flasks at 4°C before analysis. The seawater may also be filtered through decreasing pore sizes (250, 125, and 62.5 µm) of sieves made from copper or stainless steel. The materials collected in the pores can then be rinsed with seawater and stored in glass vials [8].

Due to the cost of procuring and deploying the aforementioned equipment for water sampling, researchers have also used other cost-effective methods for water sampling in the determination of microplastics
occurrence. Surface water was collected using a one-litre glass jar at an unspecified depth and stored in the dark at 4°C until analysis [16]. In another study conducted by [17], about 5 litres of surface seawater was sampled at a depth of 0-12 cm by lowering a steel bucket from a boat. The water was transferred into sealable water containers and kept at 4°C until analysis. Bulk water sampling was carried out by [18], using a plastic bucket of 20 cm diameter to collect about 100 L of water at 20 cm depth. This was followed by filtration with net of mesh size of 50 µm aboard the vessel.

3.1.2 Sediments
Sediments for microplastics sampling can be collected from intertidal beach sand, seabed, or superficial depths of the seashore. According to a review by [19], most studies have focused on quantifying microplastic particles in sandy beaches other than in bottom sediments, core sediments, water surface or in biota due to cost and accessibility. The samples from beach sand is typically collected using stainless steel hand shovel, spoons or spatula at a specified depth ranging from 2 cm to 5 cm [20]. Box corer or core sampler is usually employed for superficial sediments and bottom trawl for collecting seafloor sediment samples [8].

3.1.3 Biological samples
The digestion of the biological material adsorbed to microplastics in organisms was observed to be carried out using various chemical reagents. A study reported the use of 35% H2O2 and 4% KOH for organic material digestion in fish samples [21]. An investigation on how best to digest the biological material adsorbed to microplastic particles in marine organisms and isolate them without any negative impact or degradation of the MPs was carried out by [22]. The researchers compared the efficiencies of acid, alkali and enzyme-mediated digestion procedures and the sequence of effective removal of biogenic substances from the MPs followed the order Proteinase-K (>97%) > 10 M NaOH (91.3±0.4%) > 1 M NaOH (90.0±2.9) > 2 M NaOH (85.0±5.0%) > 1 M HCl (82.6±3.7%). The results suggest that enzyme-mediated digestion is the most effective. However, for cost-effectiveness, low chemical hazard and simplicity of procedure, some studies have employed the use of 1 M NaOH for isolation of MPs from tissues of fish [23]. It was reported that fish samples were dissected from the top of the oesophagus to the anus using scissors, and the digestive tract was removed. The digestive tract was placed in the oven at 60°C for one hour after which 30 mL of 1 M NaOH solution was added to digest biological material. The mixture was shaken for 40 seconds and filtered using a 200 µm steel sieve. The residue was washed with distilled water and taken through the process of identification and quantification of MPs [23].

4. Separation of microplastics from samples and physical characterisation
The methods used in the separation of microplastics from environmental samples are influenced by physical properties such as density, size and shape of the microplastics [24]. Sieving (using different mesh sizes), density floatation and filtration have been commonly employed to separate microplastics from water, sand and sediments. The most common solution used for floatation is saturated sodium chloride, NaCl. It is recommended by the National Oceanic and Atmospheric Administration (NOAA) and the Marine Strategy Framework Directive (MSFD) Technical sub-group because of its low cost and environmental friendliness. The floatation technique requires that a saturated brine solution of intermediate density should be used to agitate the sample matrix such that particles with lower density than the brine solution would float and those with higher densities would sink to enable easy separation. Saturated NaCl solution has a density of 1.2 g cm⁻³ and plastics typically have a specific density range of 0.84 – 2.63 g cm⁻³ while sand and sediments have density of about 2.65 g cm⁻³.

Despite its low cost and eco-friendliness, saturated NaCl solution has the limitation of underestimating the quantity of microplastics in a sample in case there are higher density polymers or changes in polymer densities due to the input of additives. [24] demonstrated that higher density brine solutions resulted in better recovery of microplastics from samples; 25% ZnBr₂ (density 1.71 g cm⁻³) and NaI (density 1.56 g cm⁻³) gave better recoveries of microplastics than NaCl. However, achieving a density of 1.56 g cm⁻³ with NaI has proven to be very expensive. Therefore, many studies [25][26] have reported the extraction of microplastics using ZnCl₂ (density 2.91 g cm⁻³) which is relatively inexpensive and can float most kinds of microplastics. [25] used ZnCl₂ solution (density 1.6 – 1.7 kg/L) as the separation fluid which allowed the extraction of an extensive range of plastic particles from meso-sized fragments (5 mm) to smaller microplastic particles (<1 mm).

4.1 Quality control
Variations in the quality control measures were also observed across studies. In order to avoid ambient contamination of microplastics, precautions applied by researchers included the use of ultrapure water for
rinsing all apparatus, wrapping of samples in aluminium foil, avoiding contact of samples with other plastics, analysis of blanks using ultrapure water, floating artificial microplastics in sediment using the same density-floating solution (NaCl, ZnCl₂, ZnBr₂) employed for field microplastics [12].

5. Microplastics identification and quantification

5.1 Polymer characterisation

Microplastics were reported to be identified from sieved samples by visual inspection or optically using a binocular microscope [13] or stereomicroscope [28][29]. Other materials that were not microplastics were removed using a tweezer. Scanning electron microscopy (SEM), scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDS) and environmental scanning microscopy energy dispersive X-ray spectroscopy (ESEM-EDS) techniques have been reportedly used for surface morphology characterization. SEM-EDS and ESEM-EDS were used for characterizing the surface morphology of microplastics as well as the determination of the elemental composition of polymers [29]. However, the most common method of polymer identification reported was the use of Attenuated Total Reflectance-Fourier transform infrared spectrophotometry, ATR-FTIR [30][31][32]. The ATR accessory unit is simple, easy to use and makes it possible to study materials that are not transparent to IR radiation. Furthermore, no prior sample preparation in potassium bromide (KBr) is required as it is with the regular FTIR equipment.

5.2 Quantification of microplastics in sample matrices

After the separation of MPs from sample matrices, most researchers carried out the confirmation of polymer types, followed by counting and weighing of MPs. Microplastics from water samples were typically quantified as particles/m³; and particles/kg of dry weight or particles/m² of sediment. Measurements for biota were reported as particles/fish species or particles/organism’s weight [23].

6. Conclusion

Microplastics have become significant environmental contaminants across the globe. The current methods used in sampling, separation, polymer identification and quantification of microplastics show several dissimilarities. These variations in the analytical methods make inter-study comparison cumbersome. Hence, the standardization of the methods of analysis of microplastics is necessary for better scientific reporting and evaluation of data from different regions of the world.

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