TRAINING MODULE SERIES NO.2

# Training Module for Marine Microplastics Monitoring

Edited by
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National Marine Environmental Monitoring Centre
The Ministry of Ecology and Environment, PR China

Implementing the Strategic Action Programme for the Yellow Sea Large Marine Ecosystem: Restoring Ecosystem Goods and Services and Consolidation of a Long-term Regional Environmental Governance Framework (UNDP/GEF YSLME Phase II Project)

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**Training Module for Marine Microplastics Monitoring**

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# Preface

As early as in 2005, the United Nations Development Programme (UNDP) with financial support from the Global Environment Facility (the GEF) implemented a regional international waters project entitled Reducing Environmental Stress in the Yellow Sea Large Marine Ecosystem (YSLME). Five Regional Working Groups consisting of Chinese and Korean experts conducted transboundary diagnostic analysis (TDA) of state of pollution, biodiversity and ecosystems, fisheries, socioeconomics and governance of the YSLME. Based on the TDA, the two countries developed and adopted the YSLME Strategic Action Programme (SAP) in 2009 to restore the ecosystem carrying capacity of Yellow Sea. In response to eutrophication, contamination from heavy metals and POPs and excessive nutrients identified in the TDA, construction of artificial wetlands, ecological restoration, enhancing connectivity of marine protected areas, improving legal framework and law enforcement were proposed as the management measures in the SAP. At that time, microplastics had not come to the attention of both countries as a transboundary environmental issue.

In recent years, microplastics are found from river, seawater, beaches, sediments and biota. Scientists at the 3rd YSLME Science Conference held in July 2019 in Qingdao of PR China reported that a month's exposure of plastics can even produce nano- and micro-sized particles. Research also found that impact of macroplastics and microplastics can be economical, physical and chemical. Studies have proven that ingestion of micropastics by zooplankton is the fundamental link of microplastics and the food web. Lack of standardized monitoring protocols has hampered comparison of data across sites, risk assessment of ecological and human health impacts and the development of appropriate management and mitigation policies. Current governance strategies and approaches are still fragmented in approach and do not adequately address microplastics. Holistic approach is needed to address the multifaceted, widespread and complex nature of microplastics.

With support of the YSLME Phase II Project, Dr. Weiwei Zhang and Dr. Juying Wang of the National Marine Environmental Monitoring Center of the Ministry of Ecology and Environment (NMEMC/MEE) prepared the training modules for the monitoring of marine microplastics from seawater, sediment and biota. The draft module was also reviewed by Dr. Sang Hee Hong from Korea Institute of Ocean Science and Technology (KIOST). The module covers <5 mm of

plastic litter encountered in the marine environment, on shorelines, floating on the sea surface, deposited on the seabed and ingested by biota. The modules are intended for use by national, intergovernmental and international organizations, NGOs and sub-national institutions with responsibilities of managing the social, economic and ecological consequences of land and sea-based human activities on the marine environment. It is hoped that the modules can serve as a useful reference for developing programmes to monitor and assess the distribution and abundance of microplastics in the ocean.

**Juying Wang**
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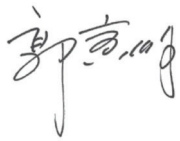

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CHAPTER 1

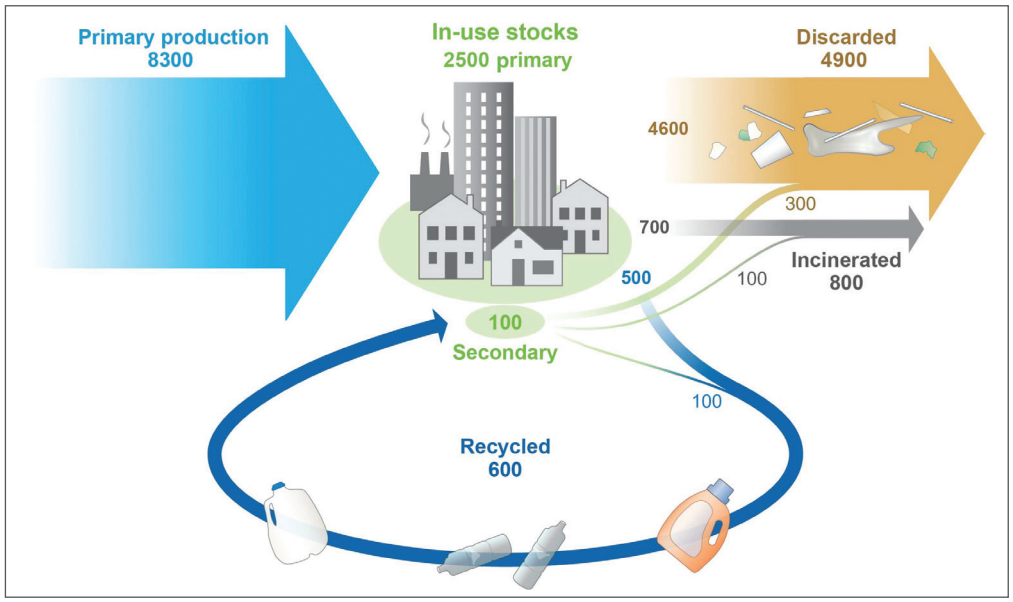
# Introduction

## 1.1 Plastic litter as a global ocean concern

The light weight, high durability, resistance to chemicals, plasticity, and high buoyancy of foamed and resin products and the cost-effectiveness of plastics (Thompson et al., 2009) make them so-called "essential" materials in our daily life.

A world without plastics, or synthetic organic polymers, seems unimaginable today, yet their large-scale production and use only dates back to the 1950s. Plastics have outgrown most man-made materials. World plastic production of $1.7\times10^6$ tons in 1950 increased 205 times to $3.5\times10^8$ tons in 2015 (Plastics Europe, 2017). Moreover, the global production of polyethylene (PE) and polypropylene (PP) (the most common marine microplastics) grew at a rate of 8.7% per year from 1950 to 2012 (Andrady, 2017). By identifying and synthesizing dispersed data on production, use, and end-of-life management of polymer resins, synthetic fibers, and additives, researchers present the first global analysis of all mass-produced plastics ever manufactured. It is estimated that 8,300 million metric tons (Mt) of virgin plastics have been produced to date. As of 2015, approximately 6,300 Mt of plastic waste have been generated, around 9% of which have been recycled, 12% have been incinerated, and 79% are accumulated in landfills or the natural environment. If current production and waste management trends continue, roughly 12,000 Mt of plastic waste will be in landfills or in the natural environment by 2050 (Geyer et al., 2017).

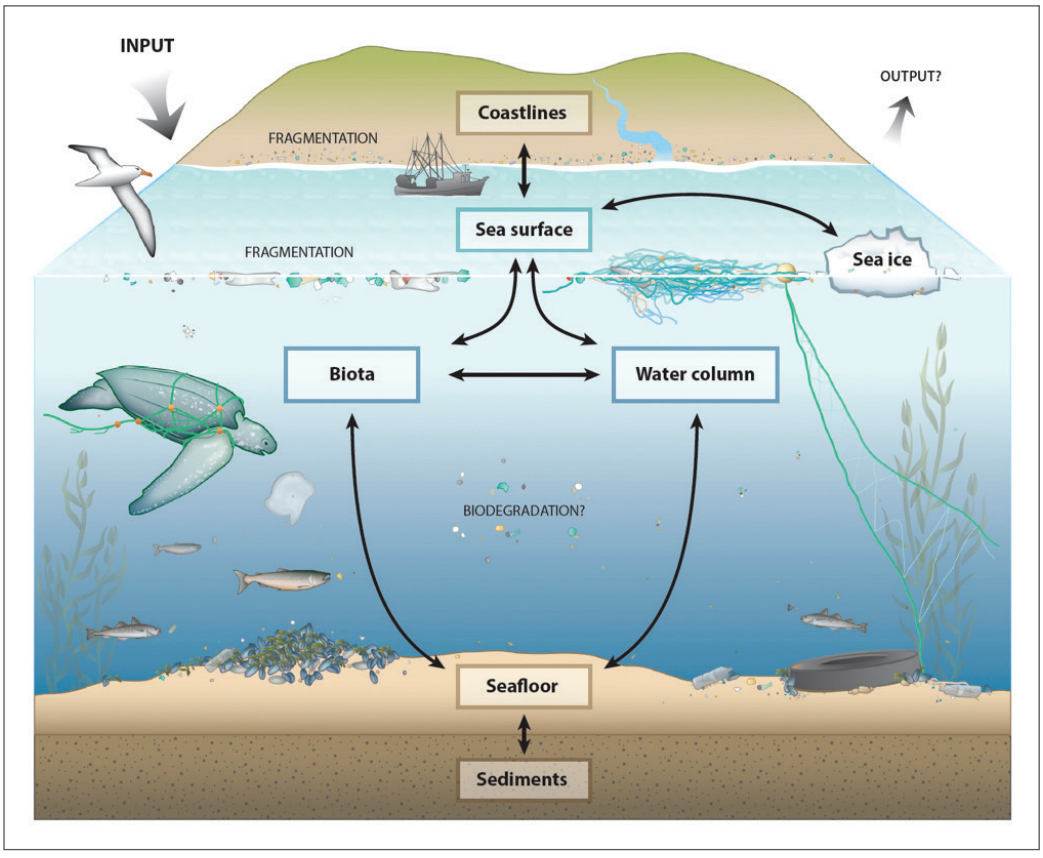
**FIG. 1-1** Global production, use, and fate of polymer resins, synthetic fibers, and additives (1950 to 2015; in million Mt). (Geyer et al., 2017)



The vast majority of monomers used to make plastics, such as ethylene and propylene, are derived from fossil hydrocarbons. None of the commonly used plastics are biodegradable. As a result, they accumulate, rather than decompose, in landfills or the natural environment (Barnes et al., 2009). The only way to permanently eliminate plastic waste is by destructive thermal treatment, such as combustion or pyrolysis. Thus, near-permanent contamination of the natural environment with plastic waste is a growing concern. This is a result of both land-based and sea-based human activities. Plastic debris have been found in all major ocean basins (Barnes et al., 2009). Contamination of freshwater systems and terrestrial habitats is also increasingly reported (Wagner et al., 2014; Rillig, 2012; Zubris & Richards, 2005), as is environmental contamination with synthetic fibers (Zubris & Richards, 2005, Dris et al., 2016). Plastic waste is now so ubiquitous in the environment that it has been suggested as a geological indicator of the proposed Anthropocene era (Zalasiewicz et al., 2016).

Since the 1950s, when large-scale production of plastics began, an increasing proportion of solid waste in the ocean has consisted of this material. It has been estimated that 4.8–12.7 million tons of plastic waste entered the oceans from land-based sources in 192 coastal countries in 2010 (Jambeck et al., 2015), accounting for 1.8%–4.7% of the global plastic production in 2010. Plastic litter is most obvious on shorelines, where litter accumulates as a result of current, wave and wind action, river outflows and by direct littering at the coast. However, plastic litter occurs on the ocean surface, on the seabed and in association with biota, due to entanglement or ingestion (Figure 1.2).

**FIG. 1-2** Microplastics do not reside permanently on the open water surface, but rather are subjected to a dynamic system of inputs and outputs between compartments (Law et al., 2017).



Microplastics have been reported and found globally in multiple seas, including the Arctic Ocean and the Antarctic Ocean. Once microplastics enter the sea, they persist and accumulate in water bodies and are transported around the world via winds and surface currents (Lusher et al., 2015). According to Co´zar et al. (2014), it has been estimated that approximately 7,000–35,000 tons of plastic, including microplastics, are floating and persistent in the open ocean. A similar study conducted by Eriksen et al. (2014) indicated that over 250,000 tons and more than 5 trillion pieces of plastic have accumulated in the ocean, including in the Atlantic, North Pacific, South Pacific, and Indian Ocean gyres, and the amount of plastic debris continuously increases. The occurrence of microplastics in the oceans differs, with a high abundance of microplastics found near the regions with high levels of industrial activities or high population densities and in remote areas far from human habitation. The image of a plastic bag found at 10,898 m in the famous Mariana Trench is a haunting one, suggesting that we have long passed the point where full or even partial recovery of extant plastic debris is feasible (despite some noble attempts in The Netherlands, the Adriatic Sea, and elsewhere) (Joanna & Peter, 2018).

The contamination of plastic debris including microplastics in the marine environment is regarded as a major risk for the health of marine organisms. Numerous studies have shown that many species suffer from plastic ingestion or entanglement (Gregory, 2009; Lusher, 2015; Auta et al., 2017). Marine organisms, including fish (Lusher et al., 2016), seabirds (Amelineau et al., 2016), sea turtles (Tourinho et al., 2010), invertebrates (Davidson & Dudas, 2016), and marine mammals (Besseling et al., 2015), are directly and indirectly vulnerable to microplastic ingestion. More importantly, microplastics can adsorb hydrophobic contaminants or heavy metals from the surrounding seawater and potentially act as a vector for these contaminants to enter the food web (Reisser et al., 2014). Therefore, it is essential to understand the distribution and the potential hotspots of plastic debris including microplastics. Marine litter, including microplastics, is now a global challenge. In particular, pollution of the marine environment by microplastics has been recognized as a serious international issue over the past decade as microplastics are likely to affect marine ecosystems and are extremely difficult to recover. To promote policy planning based on a more concrete scientific knowledge while making a head start with preventive measures for plastic litter in the ocean, and determining the current status of distribution and quantity of microplastics in the ocean is an urgent task.

## 1.2 Purpose and Objectives

In response to the growing interest worldwide in microplastics in the ocean, monitoring of microplastics (sampling and indoor analysis) has been carried out by many institutions around the world using various methods, and accordingly, findings are gradually accumulating. Since 2004, when Thompson et al. (2009) pointed up marine microplastics as a new problem of high concern for our global ecosystems, over 2,200 researchers published approximately 700 scientific articles on this topic. In this context, microplastics identification within a broad variety of environmental compartments, e.g., aquatic systems, sediments or organisms is an important aspect (Käppler et al., 2015). However, researchers criticize the lack of standardized analysis techniques and protocols which lead to insufficient results comparability, or even worse, uncertain conclusions (Gerrit et al., 2018).

It is expected that more monitoring will be conducted in the future, but as different sampling and analytical methods are used depending on the purpose of the survey of each country and research institution, there is now a global concern about lack of comparability of the accumulated data. There is also speculation that research conducted under limited resource availability, technical capacity and institutional arrangements, or monitoring by the latest instrument that are not yet globally common will be carried out. Inability to compare data obtained by different monitoring methods may pose an obstacle to research determining the global distribution and fate of microplastics in the ocean. Hence, it is recognized that standardization and harmonization of monitoring methods for marine litter, including microplastics, are important undertakings.

The principal purpose of this Module is to provide advice and practical guidance, for establishing programmes to monitor and assess the distribution and abundance of microplastics in the ocean. This training module is a product of the YSLME Phase II Regional Working Group on Pollution. The main audience is intended to be national, intergovernmental and international organizations, NGOs with responsibilities for managing the social, economic and ecological consequences of land- and sea-based human-activities on the marine environment. The decision to produce the Module reflects the lack of an internationally-agreed methodology to report on a key aspect of ocean (and freshwater) microplastics contamination, which is attracting increasing concern. Although the focus is on the marine environment, it is recognized that many of the sampling methods and material characterizations will apply equally to freshwater systems.

The intention is to promote a more harmonized approach to the design of sampling programs, the selection of appropriate indicators (i.e., type of sample), the collection of samples or observations, the characterization of sampled materials, dealing with uncertainties, data analysis and reporting the results. The Module covers < 5 mm of plastic litter encountered in the marine environment, on shorelines, floating on the sea surface, suspended in the water column, and deposited on the seabed.

## 1.3 How to Use the Module — Structure

The Module is divided into seven chapters:

Chapter 1 – Introduction, addressing background, purpose, objectives and structure of the Module
Chapter 2 – Definitions and Terminology: providing definitions of common terminology used in existing marine microplastics monitoring
Chapter 3 – Introduce the protocol of microplastic collection in seawaters and sample preparation procedure
Chapter 4 – Provide microplastic collection methods in sediments, beaches and subtidal sediments
Chapter 5 – Present a range of laboratory-based techniques of microplastics detection in biota
Chapter 6 – Introduce microplastics identification technologies
Chapter 7 – Present the necessary quality control and quality assurance

CHAPTER 2

# Definition and types of marine litter and microplastics

## 2.1 Definition of marine litter and microplastics

In 1995, UN Environment defined marine litter as any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment (UNEP, 1995). Marine litter consists of items that have been made or used by people and deliberately discarded into the sea or rivers or on beaches; brought indirectly to the sea with rivers, sewage, storm water or winds; accidentally lost, including material lost at sea in bad weather (fishing gear, cargo); or deliberately left by people on beaches and shores. Marine debris comprise of various material types, and can be classified into several distinct categories, including plastics, metal, glass, processed timber, paper and cardboard, rubber, clothing and textiles.

Once plastic enters the marine environment, it is difficult to degrade completely due to its biodegradation-resistant properties. Therefore, large plastic debris degrade into smaller fragments via different mechanisms such as weathering, photodegradation, and biodegradation and thus become small plastic fragments (Andrady, 2011).

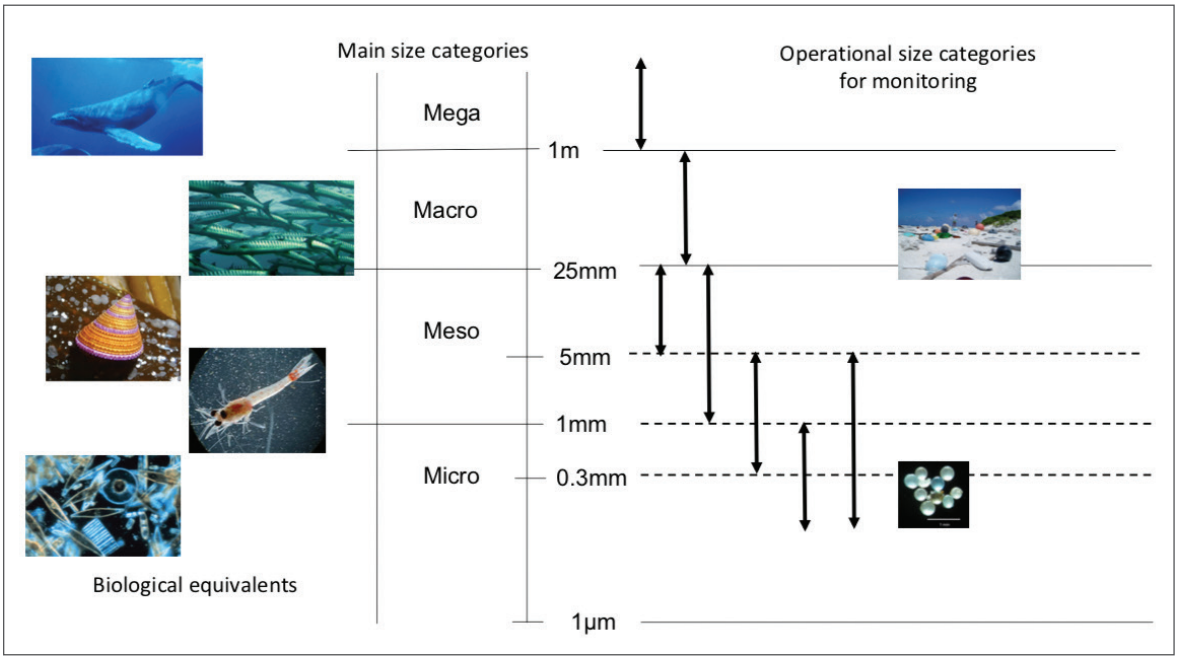
**Definitions of microplastics**

When reported in 2004, the term microplastics was used to describe fragments of plastic around 20 µm in diameter. However, while these early reports referred to truly microscopic particles, they did not give a specific definition of microplastics. In 2008, the National Oceanic and Atmospheric Administration (NOAA) of the USA hosted the first International Microplastics Workshop in Washington, and as part of this meeting, formulated a broader working definition to include all particles less than 5 mm in diameter (Arthur et al. 2009). Particles of this size (i.e., < 5 mm) have been very widely reported, including publications that considerably pre-dated the use of the term "microplastics".

However, some scientists have argued that microplastics should be defined as being < 1,000 µm (<1 mm) (Harmann et al., 2019). The global assessment reports published by GESAMP (2015, 2016) mentioned the lack of an internationally-agreed size definition of microplastics. GESAMP recommends < 5 mm diameter as the 'common definition' of the upper size boundary for microplastics for monitoring purposes.

Microplastics can be subdivided by usage and source as primary or secondary (Wright et al., 2013). Primary microplastics are commonly defined as manufactured raw plastic material (Browne et al., 2007; Arthur et al., 2009), such as microbeads in personal care products that enter waterways through wastewater, or loss of virgin plastic pellets during production and transport; secondary microplastics result from the fragmentation of larger plastic materials.

**FIG. 2-1** Schematic showing field descriptors, typical aquatic organisms in that size category, examples of marine litter and common size divisions (GESAMP, 2019).




## 2.2 Types and size of plastics

There are many hundreds of different types of polymer and mixtures of polymers in commercial production, but the market is dominated by: polyethylene (as both high-density, HDPE, and low-density, LDPE); polypropylene (PP); polyvinyl chloride (PVC); polyurethane (PUR); polystyrene (PS); and polyethylene terephthalate (PET). These six polymers make up about 80% of plastics production and are likely to form a large proportion of most marine litter (GESAMP, 2019). Table 2.1 provides examples of common products and their associated polymer resin, as well as their density as a virgin material and percentage of the global market.

**Table 2-1** Common polymers and abbreviation codes.

| Polymer | 聚合物 | Abbreviation |
|---|---|---|
| Acrylonitrile-butadiene-styrene | 丙烯腈 - 丁二烯 - 苯乙烯 | ABS |
| Acrylate-styrene-acrylonitrile | 丙烯酸酯 - 苯乙烯 - 丙烯腈 | ASA |
| Butadiene rubber | 丁二烯橡胶 | BR |
| Cellulose acetate | 醋酸纤维素 | CA |
| Cellulose acetate-butyrate | 醋酸纤维素 - 丁酸酯 | CAB |
| Cellulose acetate propionate | 醋酸纤维素丙酸酯 | CAP |
| Cellulose | 纤维素 | CE |
| Carboxymethyl cellulose | 羧甲基纤维素 | CMC |
| Cellulose nitrate | 硝酸纤维素 | CN |
| Cellulose propionate | 丙酸纤维素 | CP |
| Polychloroprene (neoprene) | 聚氯丁二烯（氯丁橡胶） | CR |
| Chlorosulfonated polyethylene | 氯磺化聚乙烯 | CSM |
| Ethylene chlorotrifluoroethylene | 乙烯三氟氯乙烯 | ECTFE |
| Ethylene-propylene rubber | 乙丙橡胶 | EPR |
| Expanded polystyrene | 膨胀聚苯乙烯 | EPS |
| Ethylene vinyl acetate | 乙烯醋酸乙烯酯 | EVA |
| Ethylene vinyl alcohol | 乙烯乙烯醇 | EVOH |
| Fluorinated ethylene propylene | 氟化乙烯丙烯 | FEP |
| High-density polyethylene | 高密度聚乙烯 | HDPE |
| Hydroxyethyl methacrylate | 甲基丙烯酸羟乙酯 | HEMA |
| High-impact polystyrene | 高抗冲聚苯乙烯 | HIPS |
| Low-density polyethylene | 低密度聚乙烯 | LDPE |
| Linear low-density polyethylene | 线性低密度聚乙烯 | LLDPE |
| Methacrylate butadiene styrene | 甲基丙烯酸酯丁二烯苯乙烯 | MBS |
| Medium-density polyethylene | 中密度聚乙烯 | MDPE |
| Melamine formaldehyde | 三聚氰胺甲醛 | MF |
| Acrylonitrile butadiene rubber | 丙烯腈丁二烯橡胶 | NBR |
| Natural rubber | 天然橡胶 | NR |
| Polyamide (nylon) | 聚酰胺（尼龙） | PA |
| Nylon 4,6 | 尼龙4,6 | PA 46 |
| Nylon 6 | 尼龙6 | PA 6 |
| Nylon 6,10 | 尼龙6,10 | PA 610 |
| Nylon 6,6 | 尼龙6,6 | PA 66 |

**Table 2-1** Common polymers and abbreviation codes. (cont.)

| Polymer | 聚合物 | Abbreviation |
|---|---|---|
| Nylon 6,6/6,10 copolymer | 尼龙6,6 / 6,10共聚物 | PA 66/610 |
| Nylon 11 | 尼龙11 | PA 11 |
| Nylon 12 | 尼龙12 | PA 12 |
| Polyarylamide | 聚芳香酰胺 | PAA |
| Polyamide imide | 聚酰胺酰亚胺 | PAI |
| Polyacrylonitrile | 聚丙烯腈 | PAN |
| Polybutylene | 聚丁烯 | PB |
| Polybutylene terephthalate | 聚对苯二甲酸丁二醇酯 | PBT |
| Polycarbonate | 聚碳酸酯 | PC |
| Polycaprolatone | Polycaprolatone | PCL |
| Polyethylene | 聚乙烯 | PE |
| Polyether block amide | 聚醚嵌段酰胺 | PEBA |
| Polyetheretherketone | 聚醚醚酮 | PEEK |
| Polyester elastomer | 聚酯弹性体 | PEEL |
| Polyester imide | 聚酯酰亚胺 | PEI |
| Polyetherketone | 聚醚 | PEK |
| Polyether sulfone | 聚醚砜 | PES |
| Polyethylene terephthalate | 聚对苯二甲酸 | PET |
| Polyethylene terephthalate glycol-modified | 聚对苯二甲酸乙二醇酯改性 | PETG |
| Phenol formaldehyde | 苯酚甲醛 | PF |
| Perfluoroalkoxy alkane | 全氟烷氧基烷烃 | PFA |
| Polyhydroxybutyrate | 聚羟基丁酸酯 | PHB |
| Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) | 聚（3-羟基丁酸酯 - 共-3-羟基戊酸酯） | PHBV |
| Polyhydroxyvalerate | 聚羟基戊酸 | PHV |
| Polyimide | 聚酰亚胺 | PI |
| Polyisocyanurate | 聚异氰脲 | PIR |
| Polylactic acid | 聚乳酸 | PLA |
| Poly (methyl methacrylate) | 聚（甲基丙烯酸甲酯） | PMA |
| Polymethylpentene | 聚甲基戊烯 | PMP |
| Polyoxymethylene | 聚甲醛 | POM |
| Polypropylene | 聚丙烯 | PP |
| Poly (p-phenylene ether) | 聚（对亚苯基醚） | PPE |

**Table 2-1** Common polymers and abbreviation codes. (cont.)

| Polymer | 聚合物 | Abbreviation |
|---|---|---|
| Poly (p-phenylene oxide) | 聚（对苯醚） | PPO |
| Polyphenylenesulphide | 聚苯硫醚 | PPS |
| Polyphenylenesulphide sulfone | 聚亚苯基硫醚砜 | PPSS |
| Polyphenylenesulfone | 聚亚苯基砜 | PPSU |
| Polypropylene terephthalate | 聚对苯二甲酸丙二醇酯 | PPT |
| Polystyrene | 聚苯乙烯 | PS |
| Polysulfone | 聚砜 | PSU |
| Polytetrafluoroethylene | 聚四氟乙烯 | PTFE |
| Polytrimethylene terephthalate | 聚对苯二甲酸丙二醇酯 | PTT |
| Polyurethane | 聚氨酯 | PUR |
| Polyvinyl acetate | 聚乙酸乙烯酯 | PVA |
| Polyvinyl butytral | 聚乙烯醇缩丁醛 | PVB |
| Polyvinyl chloride | 聚氯乙烯 | PVC |
| Chlorinated polyvinyl chloride | 氯化聚氯乙烯 | PVCC |
| Polyvinylidene chloride | 聚偏二氯乙烯 | PVDC |

## 2.3 Shape of microplastics

Microplastics samples are usually sorted into different shapes according to observed morphology. GESAMP recommends five general categories, including fragment, foam, film, line and pellet (GESAMP, 2019). It is recommended that the original data in these finer subdivisions with the recognition that subdivisions can be combined for ease of harmonizing and comparing data. For example, separate the "Line" category into "Filaments" (from fishing) and "Fibers" (from textiles). The standard of GESAMP is recommended to be cited in this report.

**Table 2-2** Morphological descriptors for marine plastic particles and some larger plastic objects (GESAMP, 2019).

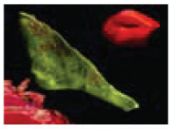
| Field Description | Alternative descriptor | Characteristics | Example |
|---|---|---|---|
| **Fragment** | Granule, flake | Irregular shaped hard particles having appearance of being broken down from a larger piece of litter |  |
| **Foam** | EPS, PUR | Near-spherical or granular particle, which deforms readily under pressure weathering state |  |

**Table 2-2** Morphological descriptors for marine plastic particles and some larger plastic objects (GESAMP, 2019). (cont.)

| Field Description | Alternative descriptor | Characteristics | Example |
|---|---|---|---|
| **Film** | Sheet | Flat, flexible particle with smooth or angular edges |  |
| **Line** | Fibre, filament, strand | Long fibrous material that has a length substantially longer than its width |  |
| **Pellet** | Resin bead, Mermaids' tears | Hard particle with spherical with spherical, smooth or granulat shape |  |

CHAPTER 3

# Sampling and Analysis of Microplastics in seawaters

## 3.1 Sampling of microplastics in seawater

### 3.1.1 Sampling of microplastics on sea surface

Because of their relatively low concentrations in the environment, sampling of microplastic particles generally requires large sample volumes. Thus, samples from open water are usually taken with plankton nets of different mesh sizes. This approach allows for the sampling of large volumes and surface areas of water relatively quickly in a volume-reduced method resulting in a relatively small, concentrated final sample. The size of the particles retained and also the filterable volume is a direct consequence of the mesh size used. The mesh size used for sampling in previous studies ranged between 0.05 mm and 3 mm (Hidalgo-Ruz et al., 2012); a mesh size of approximately 330 µm is used most commonly, with a tendency to become a standardized method of collection (Lusher et al., 2015).

There is no defined definition of what constitutes the surface water layer in microplastic sampling. Nevertheless, it has been described as the water surface layer less than 15 cm deep, which is where 95% of small plastic debris is concentrated (Carson et al., 2011). However, in most studies, the depth of the surface layer is not specified as well as where it has been.

There are numerous standard operating procedures (SOPs) available from different monitoring agencies, such as NOAA (Lippiatt et al., 2013, Masura et al., 2015). This module shows a general sampling procedure for surface water microplastics.

**FIG. 3-1** Flow diagram for the analysis of microplastics in water samples suggested by NOAA (2015).

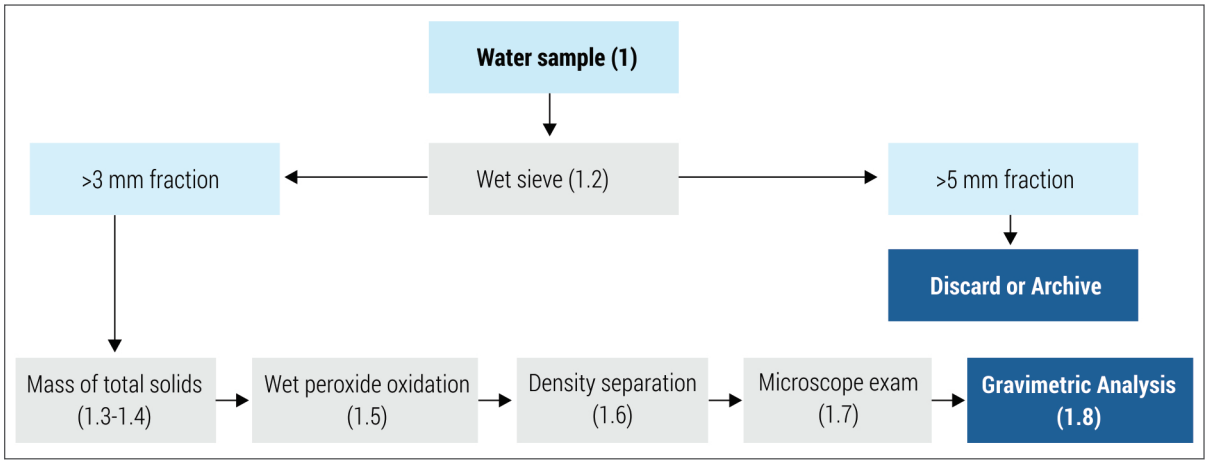



**FIG. 3-2** Neuston catamaran.

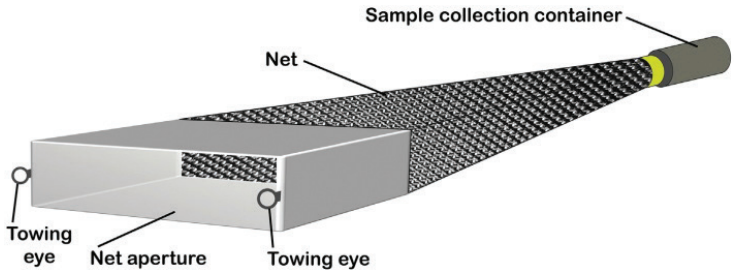



**FIG. 3-3** Manta trawl.

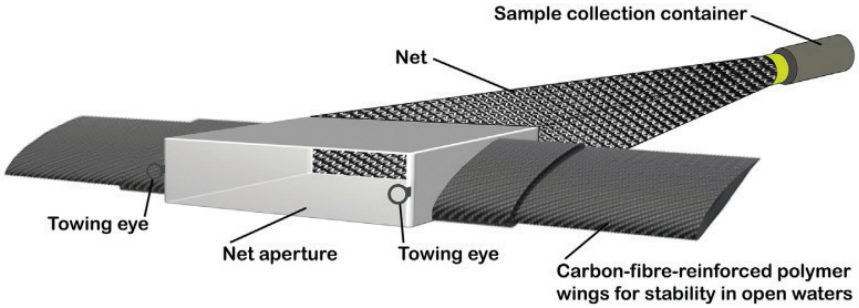
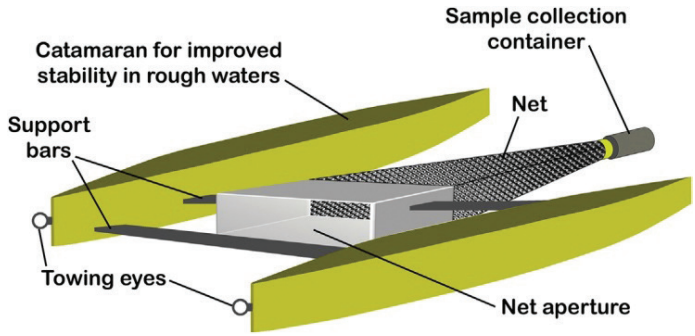



**FIG. 3-4** Neuston trawl.



- A catamaran, manta or nueston trawl may be used to sample microplastics, with a body of 330 µm mesh size and of approximately 3-4 m in length, attach the flowmeter midway between the center and the net rim.
- Clean the net and check for any contamination before use. Steps to avoid microplastic contamination should be undertaken, such as avoiding wearing synthetic garments, especially manmade fabrics.
- Record the net aperture length and the mesh size. Check if the cod end of the net is closed. Record the date, time, location, weather conditions, vessel course and speed. Write down the initial GPS coordinates and initial time in the data sheet.
- Lower the net into the water to the correct depth. Wherever possible, a boom should be used to collect samples from the side of the boat (approx. 3 - 4 m distance from the boat) in order to prevent collecting water affected by turbulence inside the wake zone. The angle between the net and the ship's route is about 20°.
- Tow the net at a constant speed (2-3 knots) horizontally at the surface for a set period of time, such as 20 minutes per sample.
- Care should be taken to ensure that the net has not become clogged. If this has occurred, reduce the trawl time/speed.
- Once the tow is complete, retrieve the net and allow the water to drain away. Wash the debris collected by the net to the cod end of the net using a hose.
- Transfer the collected sample to an appropriate container for storage and preserve it where necessary, such as with 4% formaldehyde, 70% ethanol or freezing.
- The net is then cleaned and checked for any contamination, after which it is ready for reuse.
- Using the data from the flowmeters, the measurement of the net aperture, and the abundance of microplastics in the sample, calculate the results.
- The results can then be reported as the number of microplastics per $m^3$ of water.

When collecting samples in the environment it is important to take into consideration and to record the prevailing weather conditions, not only on the day of sampling but in the period leading up to sampling. On the day of sampling, it is necessary to note the wind direction as this may influence any potential contamination from the person carrying out the sampling, as well as from others nearby. Poor weather and sea conditions often cause the trawl to be difficult to balance on the surface water. It is recommended that researchers collect a sample of vessel paint if possible to compare to anthropogenic debris that may not look like plastic, or sinks in seawater. In addition, also get a small sample of the materials used for sampling, such as the net and rope, as these are often composed of plastic materials and can be used to exclude sampling induced contamination. Also, field blanks should be collected, whereby a sample is collected from the equipment without it actually having touched the water.

### 3.1.2 Water column

Besides common net sampling, other techniques are occasionally used for assessing microplastic concentrations in the water column: bulk sampling with subsequent filtration (Ng & Obbard 2006; Dubaish & Liebezeit 2013), screening Continuous Plankton Recorder (CPR) samples (Thompson et al. 2004) or using direct in situ filtration (Norén & Naustvoll 2010). There are some advantages for pump filtration compared to plankton nets such as: flexible to use smaller filter papers (or glass fiber filters), exactly measured sample volume, applicable stationary for point measures. However, limitations are: often too little sample volume for 300 µm in practice to reach statistically reliable particle counts, and do not sample sea surface layer so effectively floating particles will be missed (Karlsson et al., 2018).

## 3.2 Sample preparation

Since environmental samples usually contain organic matter, algae, wood products and other interfering impurities, it is generally impossible to directly analyze the microplastics in samples. It is necessary to separate and extract the microplastics, e.g., density separation and biochemical separation (digestion). The pretreatment methods used for microplastics in different environmental media are different. It is recommended to use the NOAA steps in the "Laboratory Methods for the Analysis of Microplastics" (Masura et al., 2015) and EU pre-treat steps in the "Guidance on monitoring of marine litter in European seas" (European Commission, 2013).

In the laboratory, samples were poured through stacked 5.0-mm and 0.3-mm stainless steel mesh sieves. The plastics that were retained on the 5.0-mm stainless steel mesh sieve were separated using stainless steel tweezers. Solids collected in the 0.3-mm sieve were transferred to a clean beaker using minimal rinsing with a low density polyethylene squirt bottle containing distilled water. To remove the organic material mixed in the sample, wet peroxide oxidation process was conducted. A solution of 20 mL of aqueous 0.05 M Fe (II) (prepared by adding 7.5 g of $FeSO_4 \bullet 7H_2O$ to 500 mL of distilled water and 3 mL of concentrated sulfuric acid) and 30% $H_2O_2$ solution were added to the beaker containing the 0.3-mm size fraction of collected solids (Yonkos et al., 2014; Masura et al., 2015). After the reaction stopped, the beaker was heated on a 75°C hotplate for 30 min. If natural organic material was still visible, another 20 mL of 30% $H_2O_2$ was added. Repeat until no natural organic material is visible. If interference materials are remained, add ~6 g of salt (NaCl) per 20 mL of sample to increase the density of the aqueous solution (~5 M NaCl) for further density separation. Solids in the mixed solution were filtered using a vacuum system. Subsequently, the filter placed in clean petri dishes which was covered with tinfoil paper and allowed to dry for further analysis.

CHAPTER 4

# Sampling and Analysis of Microplastics in Sediment

## 4.1 Sampling Microplastics in Sediment

### 4.1.1 Sediments

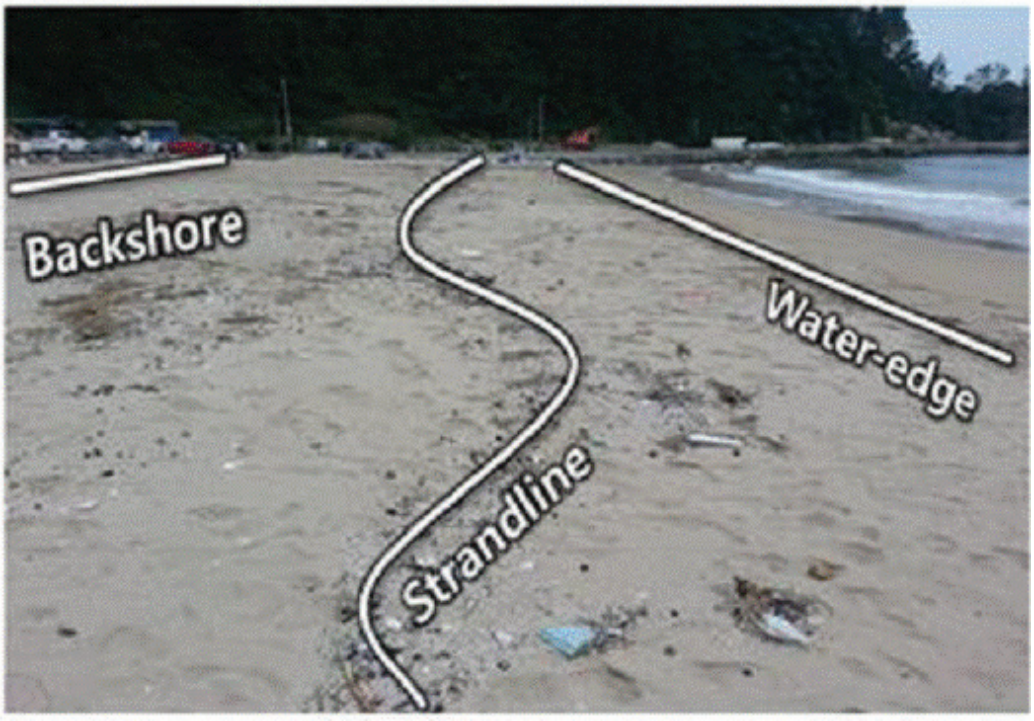
Analyzing sediment samples for the presence of microplastics began to appear in scientific literature about 20 years ago and with greater frequency in the last 10 years. Sediment types investigated include deep sea (core) sand, beach sand, river sand, intertidal mangrove mud and municipal soil, as a result, sampling methods vary greatly. Maximum depth collected varied from 2 cm to 5 cm to sediment cores of unknown depths (Miller et al., 2017). Microplastics in sediments or beaches are currently more frequently analyzed than microplastics in the water column. Sampling approaches depend on the sampling location, i.e., sampling sediments directly on beaches or sampling subtidal sediments from a ship.

### 4.1.2 Beach sediments

Sampling beaches for microplastics is relatively easy and requires nothing more than a non-plastic sampling tool (tablespoon, trowel or small shovel), a frame or a corer to specify the sampling area, and a container (if possible non-plastic) to store the sample. The quantity of samples reported in the literature varies between less than 500 g to up to 10 kg. While sampling on a beach poses no problem *per se*, the positioning of the sampling location on the beach is still a matter of scientific debate as the distribution of microplastics is as dynamic as the beach itself (Hidalgo-Ruz et al., 2012). The high-tide line where flotsam accumulates is sampled mostly (Browne et al., 2010). Commonly applied sampling strategies include random sampling at several locations on the beach, on transects perpendicular or parallel to the water or in single squares. Often, several samples are pooled for an integrated estimate of the microplastic contamination of a beach. Every single sampling location for the pooled sample is then defined as described above. Another point of concern is the sampling depth. Sampling the top 5 cm is a common approach (as also suggested by the MSFD GES Technical Subgroup on Marine Litter (TSG ML)), but sampling to a depth of 0.3 m is also reported in the literature. If corers are used for sampling, different depth layers can be sampled so that microplastic concentrations can be related to sediment depth and eventually to the age of the corresponding sediment layer. The units of microplastic abundance reported depend on

the sampling approach. Thus, abundance is normalized to sampling area, sediment weight or volume. Sampling sediments for microplastics at beaches might appear trivial. However, currently, no standard protocol exists for sampling microplastics with respect to location, sampling technique and sample quantity, and thus the comparability of the data produced is limited. Accordingly, there is an urgent need for the development of standardized sampling approaches (procedures). Because the patchy distribution of microplastics at beaches are standardized, spatially integrating sampling design appears reasonable and would facilitate the generation of comparable data. A first step towards the standardization of sampling microplastics at beaches in the EU has been made by the TSG-ML (Hanke et al., 2013). Three 100 m stretches parallel to the shoreline were selected to collect representative samples at each beach (Figure 1b). The upper shoreline is the point farthest from the sea, but in front of vegetation or artificial structures, where quadrats can be placed; the water edge line is the point closest to the sea where dry sand can be sampled at low tide; and the middle-line is the point midway between the upper shore and water edge lines (Eo, et al., 2018). It recommends to monitor microplastics at sandy beaches at the strandline with a minimum of five replicate samples separated by at least 5 m and to distinguish two size categories: large microplastics (1–5 mm) and small microplastics (20 µm–1 mm). Small microplastics should be sampled from the top 5 cm with a metal spoon by combining several scoops at arm length in an arc-shaped area at the strand line to collect ca. 250 g of sediment; large microplastics should be sampled from the top 5 cm and several kilograms of sediment sample can be reduced by sieving over a 1-mm sieve directly at the beach (Löder & Gerdts, 2015).

**FIG. 4-1** The referent lines for beach sediment sampling (IOC-WESPAC, 2018).



### 4.1.3 Subtidal Sediments

Subtidal sediments can be sampled from vessels with grabs, e.g., Van Veen or Ekman grab or corers of different design, e.g., a multiple corer. Grabs tend to disturb the sediment and are suited for surface (e.g., top 5 cm) or bulk sampling, whereas undisturbed core samples enable the simultaneous sampling of surface and depth layers but yield smaller sample volumes. The size of the instrument (equipment) applied, as well as the time needed for its retrieval depends strongly on the water depth at the sampling location. The use of corers enables sampling to a water depth of more than 5,000 m (Van Cauwenberghe et al., 2013). Sediment samples are usually stored frozen or dried, and kept in the dark until further analysis (Löder & Gerdts, 2015).

## 4.2 Separation of microplastics in sediment

Density flotation methods using either sodium chloride (NaCl, 1.2 g $cm^{-3}$) or sodium iodide (NaI, 1.6 g $cm^{-3}$), and Zinc Chloride ($ZnCl_2$, 1.6 g $cm^{-3}$) were widely used, regardless of the sediment type or depth of sampling. NaCl is commonly used for sediment samples due to low cost and low toxicity.

Most studies suspended the sediments in hypersaline NaCl solution after which they were allowed to settle (10 minutes to overnight), while others (Claessens et al., 2011; Miller & Motti, 2017) conducted multiple (exhaustive) settlements to ensure all microplastics were recovered. However, some polymers have a higher density and will not be taken into account from this point.

Horton et al. (2017) implemented a 3-step procedure involving visual inspection of whole sample, density flotation in $ZnCl_2$, followed by further visual inspection of unfloated sample. This procedure revealed the inefficiency of visual sorting through sediment samples (37% recovery of total microplastics), yet the effectiveness of a $ZnCl_2$ density separation (75% recovery). While these recovery rates were not established from spiked samples, the difference demonstrates the importance of density flotation separation when processing sediment samples. Maes et al. (2017) similarly suggests a $ZnCl_2$ density separation, saying a solution with density of 1.37 g $mL^{-1}$ will allow for the flotation of PA, PS, PVC, PET, PE, and PP. In addition, Maes et al. (2017) proposed an alternative method allowing for the identification of microplastics from sediments by staining samples with a Nile Red (NR) acetone solution. While this method proved effective at allowing for slightly faster visual inspection and promises (with further validation) general microplastic categorization, it is unknown whether this additional step (~60 min) would speed up analysis of samples. In addition, any subsequent FTIR (Fourier-transform infrared) analyses of NR-stained microplastics is reliant on the use of "very small amounts", i.e., final concentration of 1, 10 or 100 µg $mL^{-1}$ suspension, and requires adaptation of the FTIR imaging optics (Maes et al., 2017). Masura et al. (2015) suggested using a commercial separator lithium metatungstate solution as an alternative due to its greater density (1.62 g $cm^{-3}$) compared to NaCl. This allows for denser particles (i.e., PVC, PET) to be recovered more readily (Quinn et al., 2017).

**Table 4-1** Commonly employed solutions for density separation of microplastics.

| Salt | Density (g cm-3) | Reference |
|---|---|---|
| Sodium Chloride (NaCl) | 1.2 | Hidalgo-Ruz et al. 2012 |
| Sodium Polytungstate (PST) | 1.4 | Hidalgo-Ruz et al. 2012 |
| Sodium Iodide (NaI) | 1.6 | Claessens et al. 2013 |
| Zinc Chloride ($ZnCl_2$) | 1.7 | Imhof et al. 2012 |
| | 1.6 | Zobkov and Esiukova 2017 |

Claessens et al. (2013) used elutriation, whereby an air stream lifts lower density particles to the surface, followed by decanting and sieving. They suggested the implementation of thorough cleaning, as well as procedural blanks when using an elutriation method for field samples, since there is the potential for contamination during extraction. Wessel et al. (2016) used a custom-made automated density flotation separator with > 35 PSU filtered water, which achieved an average recovery rate of 97.25% (± 2.5) in only 26 min. Crichton et al. (2017) proposed an innovative and cost-effective flotation methodology exploiting the oleophilic properties of microplastics by using retail-grade canola oil, yielding average recovery rates of 96.1%, and proving a more time-efficient method than NaI or $CaCl_2$ methods, although this method will impact on any subsequent chemical analysis, particularly Fourier-transform infrared (FTIR). More recently, Fuller & Gautam (2016) investigated pressurized fluid extraction using methanol and dichloromethane as a means of chemically extracting the microplastics. This extraction procedure dissolved the plastics, producing plastic residues, thereby destroying the morphology of microplastics (not all the microplastics are particles) making physical characterization impossible. Only three studies reported using an alkaline, acid or oxidative digestion on sediments (Fuller & Gautam, 2016; Masura et al., 2015; Quinn et al., 2017; Claessens et al., 2013).

Similar to the seawater samples, only a small number of sediment studies conducted recovery checks to establish robustness of their methods. Claessens et al. (2013) spiked uncontaminated sediment samples with known amount of microplastics and achieved a recovery efficiency range of 68.8%–97.5% dependent on sediment and polymer type. In another experiment using elutriation, clean sediments were spiked with known amount of PVC or PE, and fibers collected from environmental samples, with a 100% and 98% separation efficiency, respectively. Claessens et al. (2013) achieved similar recovery rates to Claessens et al., (2011) study, reporting a 69–98% recovery with control beach samples (unknown plastic polymer types). Quinn et al. (2017) observed higher recovery rates with increasing solution density, from a 55%–90% range in saturated NaCl (1.17 g $cm^{-3}$), to 91% in saturated NaI (1.57 g $cm^{-3}$) and 99% in saturated 25% $ZnBr^2$ (zinc bromide, 1.71 g $cm^{-3}$). Nor & Obbard (2014) obtained recovery rates for spherical PE

beads from spiked mangrove sediment samples of 55%–72% after grinding samples with a mortar and pestle, followed by two density flotation separations using NaCl. Implementing a grinding step is not recommended for environmental samples as it can physically damage and break apart microplastics, especially if already weathered (Pers. Observation). Fuller & Gautam (2016) spiked composted municipal waste sediments with known plastic polymers and, after grinding, separated the microplastics using a pressurized fluid (dichloromethane) extraction protocol, producing a microplastic residue and average recoveries of >80%. FTIR analysis of the microplastics was also performed before (beads) and after (residue) spiking. Although the appearance of the microplastic beads was altered due to the solvent extraction process, the FTIR spectra revealed no significant chemical changes to the microplastic residue. However, the application of this technique is limited by the fact that the residue may contain mixtures of microplastics requiring sophisticated spectral deconvolution.

Based on the review of the recovery rates from density flotation techniques applied to sediments, the use of $ZnBr_2$ is recommended (Quinn et al., 2017), however, this method has not been validated for all polymer types. To ensure all microplastics (fragments and fibers) are recovered from sediment samples, an elutriation method, similar to that reported by Claessens et al. (2013), is also recommended. As for seawater samples, there is a need to establish a reliable, standardized and efficient approach for the separation and characterization of microplastics from sediments, with an emphasis on determining recovery rates.

# Sampling and Analysis of Microplastics in Biota Detection methods of microplastics in biota

CHAPTER 5

While visual detection and separation is a mandatory step for the removal of debris and naturally occurring organic fragments, in many cases it may be impractical to completely rely on visual detection and separation to remove microplastics from biological material. For this reason, techniques have been developed that allow faster separation of microplastics from organic material by digesting away the organic material to leave behind the microplastics for quantification. This is typically accomplished with the use of acids, bases and enzymes. Ultimately, this approach is particularly appropriate for smaller organisms where the entire body or visceral mass may be digested, as well as with fish stomachs, and ensures that all of the microplastics present are collected. Nonetheless, care has to be exercised when utilizing this approach since although the reagent may successfully digest away the biological tissue, it may also have a chemical impact upon the microplastics themselves, especially with small items such as fibers (Rocha-Santos T. et al., 2015).

## 5.1 Sampling Microplastics in Biota

Shellfish and mussels can be sampled on-site or purchased in-market. Samples can be collected directly by hand or with the help of clamps and net tools according to different stations. The latitude and longitude of the sampling points are recorded in detail, and the environmental conditions of the sampling sites are described on site. If purchased from fishing harbors or markets, the purchase place, sea area where farmed should be recorded in detail. Samples can be stored in a metal or glass container in a freezer. If the sample is processed 24 hours after sampling, the sample can be wrapped in aluminum foil, put into a sealed bag and stored in a cryogenic refrigerator. For long distance transportation, samples should be frozen.

## 5.2 Separation of microplastics in biological tissues

### 5.2.1 Acid digestion

One of the most extensively studied methods is based upon the principle of wet digestion of biological tissues using acids. Indeed, the most successful is the acid destruction method and is recommended by the International Council for the Exploration of the Sea (ICES, 2015) as part of a preliminary protocol which was introduced for the monitoring of microplastics in fish stomachs and shellfish. The acid destruction method (also known as the acid mix method) uses a mixture of 65% nitric acid ($HNO_3$) and 68% perchloric acid ($HClO_4$) in a 4:1 ratio ($HNO_3$:$HClO_4$ 4:1 v:v) and completely digests the tissues and removes other organic material,

leaving behind only silica and microplastics. This method has the added advantage that it also removes rayon fibers, which are common fibers composed of regenerated cellulose and are not considered to be microplastics, and which have been known to skew results. However, the technique is still under development and variations on the concentrations of the acids may be required since there have been some reports of detrimental effects on nylon fibers, which are known to be sensitive to acids and alkalis.

Other methods which have been developed involved the use of nitric acid, hydrogen peroxide and have demonstrated effective rates of tissue digestion (particularly in mussels), with high recovery yields of polystyrene microbeads at 94–98%, but highly variable results for nylon fibers at 0–98% recovery (Claessens et al., 2013). Furthermore, considerable variation was found based on the size and type of the microplastics extracted. A review of the presence of microplastics in organisms from natural habitats reported that crustaceans, fish, molluscs (mostly *Mytilus edulis* mussels) and polychaetes (lugworms) were assessed using visual detection and separation, as well as with tissue dissociation methods utilizing potassium hydroxide (KOH), hydrogen peroxide and various acid destruction methods. Treatment with hydrogen peroxide was undertaken in various studies (Foekema et al., 2103; Mathalon, 2014; Wesch et al., 2014) but was demonstrated in other studies to result in incomplete tissue dissociation and a significant loss of microplastics of specific sizes from the sample.

### 5.2.2 Alkaline digestion

Relative to any acid digestion, studies have found an alkaline hydrolysis utilizing a strong base (which denature proteins and hydrolyze chemical bonds) more efficient and generally less damaging to inherent microplastics, especially with regard to fish and invertebrates (Claessens et al. 2013, Cole et al. 2014, Lusher et al. 2017). The impact of this method on microplastics depends on the type of plastic, with some conflicting reports for certain polymers. PE, PP, and PAs are all reported to be resistant, while PC and polyesters seem to be degraded (Lusher et al., 2017), which limits the applicability of these reagents. The optimized alkaline digestion protocol recommends 40 mL of 10 M KOH per 0.2 g dry weight of sample maintained at 60°C for 24 h.

### 5.2.3 Enzymatic digestion

To avoid the prospect of dissociation of the microplastics themselves, the use of enzymes, such as proteinase, lipase, cellulase and chitinase, have been recommended for use in tissue dissociation as an alternative to acids and alkalis. Indeed, a study which investigated the ingestion of microplastics in zooplankton, the enzyme proteinase-K was used in a sample clean-up step to remove large amounts of biogenic material (97% by weight) which was successfully filtered from water samples without destroying any microplastics present. Cole

et al. (2014) optimized the original enzymatic protocol. A homogenizing solution (400 mM Tris-HCl buffer, 60 mM EDTA 105 mM NaCl, 1% SDS) is added to a dried sample. The mixture is incubated for 15 min at 50°C prior to the addition of 500 g/mL of Proteinese-K per 0.2 g dry weight of the sample. This mixture is then further incubated at 50°C for 2 hr. Sodium perchlorate (5 M) is added and samples shaken at room temperature for at least 20 min. The solution is then physically homogenized a second time using a finer (21G) needle prior to further incubation at 60°C for 20 min. After ultrasonication on ice (to prevent excess heat) using a sonication probe, samples are vacuum filtered, with filters being rinsed with Milli-Q water, removed, covered and dried at 60°C. Filters are then visually examined with the use of an optical microscope, for microplastics.

### 5.2.4 Oxidative digestion

The U.S. National Oceanic and Atmospheric Administration (NOAA) marine debris program recommends the use of a wet peroxide oxidation (WPO), utilizing Fenton's reagent, for the removal of sestonic material (Masura et al., 2015), which has been utilized and supported in a number of other studies (e.g., Zobkov & Esiukova 2017; Tagg et al., 2017; Hurley et al., 2018). In brief, 20 mL each of an iron (II) catalyst solution (7.5 g of $FeSO_4°7H_20$ in 500 mL of DI water with 3 mL of concentrated sulfuric acid) and 30% hydrogen peroxide is added to a sample and allowed to react in a covered beaker. Subsequent additions of hydrogen peroxide can be utilized until little to no labile organic material remains. The sample is then filtered or sieved prior to visual analysis (Masura et al. 2015). While the original protocol utilizes elevated temperatures (i.e., 70°C) to accelerate the reaction, more recent studies (Munno et al., 2018; Hurley et al., 2018) found that such temperatures may lead to the loss of some types of microplastic particles. Thus, while there will be some lag time as this exothermic reaction initiates, it is recommended to perform this digestion at room temperature or lower (through the use of an ice bath).

CHAPTER 6

# Microplastic identification

## 6.1 Visual Identification

Following sample collection and separation, the final stage in the assessment of microplastics in the environment is the positive identification of those items suspected to be composed of plastic. Noren (2007) suggested the following strict criteria to identify microplastics, which work best for microplastics in the size range of 0.5-5 mm.

- The particle or fiber in question has no observable organic or cellular structures.
- In the case of fibers, the diameter should be consistent along the length with no evidence of tapering or bending in three-dimensional space. If the fiber is not straight, biological origin is suspected.
- In the case of red colored fibers, additional scrutinization with high-magnification microscopic examination, fluorescence microscopy and staining of chloroplasts is required to preclude algal sprouts.
- Microparticles should be clear and unvaryingly colored.
- In the case of transparent, opaque or white particles, further high-magnification microscopic examination, as well as fluorescence microscopy, should be undertaken to preclude the possibility of biological origin.

It is strongly recommended to subsequently analyze sorted microplastics by techniques that facilitate a proper identification of plastics (Hidalgo-Ruz et al., 2012; Dekiff et al., 2014) because the quality of the data produced by visual sorting depends strongly on: (1) the counting person; (2) the quality and magnification of the microscope; and (3) the sample matrix (e.g., plankton, sediment, gut content). Another fundamental drawback of visual sorting is the size limitation, i.e., particles below a certain size cannot be discriminated visually from other material or be sorted because they are unmanageable due to their minuteness. Furthermore, visual sorting is extremely time-consuming. In summary, even an experienced person cannot discriminate all potential microplastics unambiguously from sand grains, chitin fragments, diatom frustule fragments, etc. Thus, the error rate of visual sorting reported in the literature ranges from 20% (Eriksen et al., 2013) to 70% (Hidalgo-Ruz et al. 2012) and increases with decreasing particle size.

## 6.2 Fourier-transform infrared (FTIR) spectroscopy

FTIR spectroscopy is the most popular and widely used technique for the positive identification of the type of plastic that microplastics in environmental samples are composed of. The reason for the popularity of the technique is due to its straightforwardness and reliability, the predominant reason is that FTIR is highly accurate in identifying the type of plastic present by producing highly specific infrared (IR) spectra which contain distinct band patterns, thereby allowing differentiation between plastic materials and natural materials. The technique relies on the actuality that most molecules absorb light in the IR region of the electromagnetic spectrum.

Large particles can be easily analyzed by an FTIR surface technique—"attenuated total reflectance" (ATR) FTIR spectroscopy— at high accuracy in less than one minute. A step forward with respect to the characterization of small-sized particles is the application of FTIR microscopy. Although micro-FTIR mapping has been successfully applied for microplastics identification, however this technique is still extremely time-consuming when targeting the whole sample filter surface at a high spatial resolution because it uses only a single detector element (Harrison et al., 2012). Focal plane array (FPA)-based FTIR imaging, allows for detailed and unbiased high throughput analysis of total microplastics on a sample filter (Levin & Bhargava, 2005). This technique enables the simultaneous recording of several thousand spectra within an area with a single measurement and thus the generation of chemical images. By combining FPA fields, whole sample filters can be analyzed via FTIR imaging. It's important to note that samples must be dried prior to measurement via IR spectroscopy as water strongly absorbs IR radiation.

## 6.3 Raman spectroscopy

Raman spectroscopy can also be coupled with microscopy (Raman microspectroscopy) to identify microplastics as small as 1 µm (Cole et al., 2014). It is a straightforward, efficient and reliable technique which requires minimal sample preparation and has been successfully used for the identification of microplastics that have been separated from environmental samples.
Like FTIR spectroscopy, Raman spectroscopy is a non-destructive technique that does not affect the sample. Thus, further analysis can be undertaken following identification of the microplastic, such as the extraction of any adsorbed persistent organic pollutants (POPs) for identification and quantification via gas chromatography–mass spectrometry (GC–MS). However, unlike the transmission and reflectance methods utilized in IR spectroscopy, Raman spectroscopy is a scattering technique. This is advantageous over IR spectroscopy in that thicker and strongly absorbing microplastics can be analyzed. Furthermore, in comparison to FTIR, microplastics of a very small size can be analyzed by Raman spectroscopy and a wider range of IR wavelengths can be utilized for analysis of the sample. Nevertheless, Raman spectroscopy tends to be the second choice in polymer identification after FTIR spectroscopy. This is mainly a result of issues with sample fluorescence (Lenz et al., 2015). Most plastics are rarely the pure polymer and are typically of impure composition as a result of the incorporation of a wide variety of additives and coloring pigments during manufacture.

## 6.4 Pyrolysis–gas chromatography–mass spectrometry (Pyr-GC-MS)

Pyr-GC–MS is a technique which thermally decomposes the large high-molecular weight molecules of a sample via heat mediated cleavage in the presence of an inert atmosphere. The composition of these moieties is subsequently determined by mass spectrometry (MS) and provides characteristic information as to the structural composition of the samples' large high-molecular weight molecules, thereby allowing the sample composition to be identified (Nuelle et al., 2104). As a destructive technique which thermally decomposes the sample, further analysis of the microplastics is precluded. Consequently, this may be a limiting factor in some cases. Nevertheless, the great advantage of Pyr-GC–MS is that the technique utilizes direct introduction of the sample with minimal pre-treatment. The technique can be utilized for the identification of microplastics in environmental samples, as well as simultaneously identifying any plastic additives present. However, since samples must be manually placed in the instrument and analyzed individually, the analysis of large quantities of microplastics is limited, as well as the range of sizes of items which can be effectively handled (Löder et al., 2015). This method requires a well-trained and experienced operator as well as considerably more time and effort for instrument runs and data processing compared with FTIR spectroscopy (Käppleret al., 2018).

CHAPTER 7

# Contamination mitigation

Due to their ubiquitous nature, the contamination of a solid or liquid environmental sample with microplastics that were not originally part of that sample is one of the major issues involving the examination of samples for microplastics and consequently it has been raised by several researchers. Indeed, the processes involved in the collection, separation and identification of samples for microplastics often result in the inadvertent introduction of microplastics that would not otherwise be found in the sample. Mini-microplastics (particularly microfibers) can be introduced from the ambient air, but also via the use of sampling or laboratory equipment, improper storage of samples or even from the clothing of the researchers themselves. In many cases this contamination can compromise the analysis.

The following contents list the microplastic analysis quality control steps (Quinn, 2017)

### Step 1: Preparation

- A clean white cotton laboratory coat and nitrile gloves should be worn at all times.
- Only clothing composed of natural fibers should be worn, synthetic garments should be avoided, even if worn underneath a laboratory coat.
- Air movement in the laboratory should be minimized by closing all windows and doors.

### Step 2: Cleaning

Ensure that the laboratory is kept clean and free from dust. Avoid working below overhead fixtures which may have accumulated settling dust.

- Clean all equipment with 70% ethanol and then rinse three times with distilled water.
- After cleaning, cover all equipment with aluminum foil.
- Wipe all work surfaces with 70% ethanol three times prior to the commencement of work.
- Examine all petri dishes, filter papers and forceps with a dissection microscope before use.

### Step 3: Solid particulate surface monitoring

- This process is carried out before and after all analyses of microplastics in samples.
- While ensuring that gloves are worn at all times, the adhesive side of fresh 5 $cm^2$ sections of transparent high tack adhesive tape is pressed three times onto the work surface and then lifted. Any solid particulates present should adhere to the adhesive on the tape.

- Each 5 cm2 section of adhesive tape is then adhered to a clean piece of cellulose acetate film.
- The date and time that each 5 $cm^2$ section of adhesive tape was used should be written next to it on the cellulose acetate film with a permanent marker pen.
- The 5 $cm^2$ sections of adhesive tape on the cellulose acetate film are then examined under a microscope for the presence of mini-microplastics, such as microfibers and microfragments.
- Further analysis, and positive identification, of any mini-microplastics found adhered to the 5 $cm^2$ sections of adhesive tape can be undertaken by IR spectroscopy and subsequently excluded from the sample of interest.

### Step 4: Solid airborne particulate monitoring

- Before work commences, clean pieces of dampened filter paper are placed in 9 cm standard glass petri dishes, ensuring that the filter paper covers the entire internal area of the petri dish.
- The petri dishes are then placed around the work surface, where they remain for the duration of the laboratory work.
- Upon completion of the work, the filter paper is then examined for the presence of mini-microplastics using a microscope, or a glass lid is placed on the petri dish and labelled with the date and time for subsequent microscopic analysis at a later date.
- Further analysis, and positive identification, of any mini-microplastics found on the filter paper can be undertaken by IR spectroscopy and subsequently excluded from the sample of interest.

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