Mekong River Commission
For Sustainable Development

PROTOCOL FOR RIVERINE MICROPLASTICS MONITORING

A detailed methodology for long-term and cost-effective monitoring of riverine plastic debris pollution in the Lower Mekong River

Protocol for Riverine Microplastics Monitoring:
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1. Background and rationale

1.1 Background

The Mekong River Commission (MRC) was established by the 1995 Agreement on Cooperation for the Sustainable Development of the Mekong River Basin, between the Governments of Cambodia, Lao PDR, Thailand and Viet Nam. The role of the MRC is to coordinate and promote cooperation in all fields of sustainable development, utilization, management and conservation of the water and related resources of the Mekong River Basin.

The MRC Secretariat (MRCS) is the operational arm of the MRC. It provides technical and administrative services to the Joint Committee and the Council to achieve the MRC's mission.

The Environmental Management Division (ED) is responsible for environmental monitoring, assessment, planning, and management to support basin planning management and development for sustainable development of the Mekong River.

The Mekong River Basin is one of the largest and most biodiverse river basins in the world, spreading over more than 795,000 km2 and extending over 5,000 km through six different countries, and providing a home to more than 70 million people alone in its lower reaches (Lower Mekong Basin). However, the Mekong River is also one of the 10 major contributors to marine plastic pollution. Collectively, these major contributors discharge about 95% of the plastic overwhelming the world's oceans.

In 2019, with the commitment of 180 countries including the MRC Member Countries, the United Nations Environmental Assembly agreed on measures aiming at curtailing global plastic pollution and leakage into the world's oceans. The main goal is to reduce the use of single-use plastic products. However, it is known that this will not be enough to effectively address the magnitude of plastic waste that pollutes our freshwater ways and our oceans.

The MRC has six river basin management core functions including assessments and analysis, monitoring of environmental status and trends, and the implementation of MRC procedures. Among the five MRC procedures are the Procedures for Water Quality (PWQ) and the Procedures for Data and Information Exchange and Sharing (PDIES). One of the key objectives of the MRC core function for monitoring is the continuous assessment and identification of basin changes of five different areas (i) hydrology and hydraulics; (ii) sediment and discharge;(iii) water quality, iv) aquatic ecology; and (v) fisheries. The MRC has long-lasting experience with environment and fisheries monitoring of key disciplines. The MRC's water quality monitoring (WQM) activity dates back to 1993, and to date, 22 WQM sites have been established throughout the mainstream and major tributaries in the Lower Mekong Basin (LMB). The MRC Fisheries monitoring began in 1994 and consists of three types of the monitoring: (i) fish abundance and diversity monitoring (FADM); (ii) fish larvae and juvenile drift monitoring (FLDM); and (iii) Dai (bagnet) fishery monitoring. FADM has been implemented in the four Member Countries for about 10 years with 38 monitoring stations in the LMB. FLDM was implemented by Cambodia in 2000, Lao PDR in 2019, and Viet Nam in 1999. The monitoring stations are located in two sites in Cambodia (Mekong and Tonle Sap Rivers), Viet Nam (Mekong and Bassac Rivers), and Lao PDR (Mekong and Sekong Rivers). Since

1995, Dai fishery monitoring has been implemented only at Tonle Sap River in Cambodia. It is located in the lower section of the Tonle Sap River spanning more than 30 km across the municipality of Phnom Penh and Kandal Province. Due to their transboundary nature, only FADM and FLDM were included in the Joint Environmental Monitoring (JEM) Programme for the Mekong mainstream hydropower projects. These procedures and monitoring activities lay the groundwork for this assignment.

The MRC and United Nations Environment Programme (UNEP) signed a Memorandum of Understanding (MoU) to, inter alia, conduct water quality monitoring, including of plastic waste leakage into the Mekong River system. Under this partnership arrangement in 2019 the MRC has been supporting the first phase of the UNEP Project on Promotion of Countermeasures Against Marine Plastic Litter in Southeast Asia (CounterMEASURE) funded by the Government of Japan including regional workshops, capacity mapping for plastic pollution in the Mekong basin and support to the pilot projects in the four MRC Member Countries – Cambodia, Lao PDR, Thailand, and Viet Nam.

To build on the initial efforts under the first phase of the CounterMEASURE project, the MRC and UNEP agreed on several areas of cooperation, including the identification of sources of plastic waste leakage and the development of a standardized methodology for plastic waste assessment and monitoring in the Mekong River. The ultimate goal is to provide timely data and information on transboundary plastic waste pollution status and trends, and to report on these status and trends to inform policy decision-making.

To achieve this, the MRC carried out two key activities in 2020, including a review of the status and trends of plastic waste management in the Lower Mekong Basin countries and a development of a concept note for a long-term and cost-effective assessment and monitoring methodology of riverine plastic debris pollution in the Mekong River. Following the completion of these activities, and upon the availability of funds, the MRC further developed and finalized a detailed methodology for the long-term and cost-effective assessment and monitoring of plastic waste in the LMB, followed by national and regional capacity building to implement this methodology in collaboration with UNEP through the CounterMEASURE project. The methodology consists of the following three monitoring protocols for riverine macro plastic, riverine microplastic, and microplastics in fish. Following its finalization, the detailed methodology will be utilized for systematic riverine plastic debris pollution monitoring in the LMB, as part of the MRC Water Quality Monitoring Network (WQMN).

1.2 Rationale

Today, marine plastic debris is a worldwide issue, and all countries must take urgent action accordingly. Rivers are known as the main contributors in transporting most of the plastic debris into the sea. Schmidt et al. (2017) estimated that the world's 10 largest contributing rivers, including the Mekong River, accounts for 88–95% of transportation of the global load.

Riverine/marine plastic debris has various sources of leakage from land. Large plastic debris, such as macroplastics (larger than 25 mm in diameter), is considered to mainly leak from illegal dumping sites, uncontrolled open dumpsites, and citizens' littering activities. Small plastic debris, such as microplastics (smaller than 5 mm in diameter), is considered to mainly leak from consumer products such as toothpaste and skin care products, industrial sources using

plastic resin pellets, and from the disintegration of larger debris. However, the actual behaviour of plastic debris is yet to be clarified including its leakage sources and transportation in water.

To solve these issues, several organizations have established a working plan on monitoring riverine/marine plastic debris, such as the Association of Southeast Asian Nations (ASEAN) Regional Action Plan. Still, the LMB has neither regular monitoring programmes nor a standardized method for monitoring riverine plastic debris that would enable a precise analysis and comparison of data over areas and time.

Therefore, this protocol shall provide the region with the appropriate and harmonized method for monitoring riverine plastic debris to support efficient policymaking for the reduction of plastic debris

2. Objectives

2.1 Objectives of the MRC Riverine Plastic Debris Pollution Monitoring Programme

The objectives of the MRC Riverine Plastic Debris Pollution Monitoring Programme" are to assess the basin-wide status and trends of plastic pollution, including both macroplastics and microplastics (definitions are provided in the following chapter), and gather information and knowledge to inform decision-making for effective and efficient management of riverine plastic pollution in the LMB as part of the MRC Water Quality Monitoring Network (WQMN).

The **MRC Riverine Plastic Debris Pollution Monitoring Programme** covers riverine macroplastics, riverine microplastics, and microplastics in fish, and will be developed based on the following approaches:

- **Pillar 1**: Protocol for Riverine Macroplastics Monitoring should be conducted annually at **selected monitoring stations** along the Mekong mainstream and its major tributaries in the four Member Countries by the relevant national research institutes or line ministries.
- **Pillar 2**: Protocol for Riverine Microplastics Monitoring should be conducted every five years at **selected smaller number of monitoring stations** along the Mekong mainstream and its major tributaries in the four Member Countries by (i) relevant national research institutes or line ministries of the four Member Countries for sample collection in the field; and (ii) a qualified national laboratory in one of the Member Countries for laboratory analysis.
- **Pillar 3**: Protocol for Monitoring Microplastics in Fish should be conducted every five years at **selected smaller number of monitoring stations** along the Mekong mainstream and its major tributaries in the four Member Countries by: (i) relevant national research institutes or line ministries of the four Member Countries for sample collection in the field; and (ii) a qualified national laboratory in one of the Member Countries for laboratory analysis.

Figure 2.1 provides an overview of the scope of the monitoring programme and monitoring protocols.

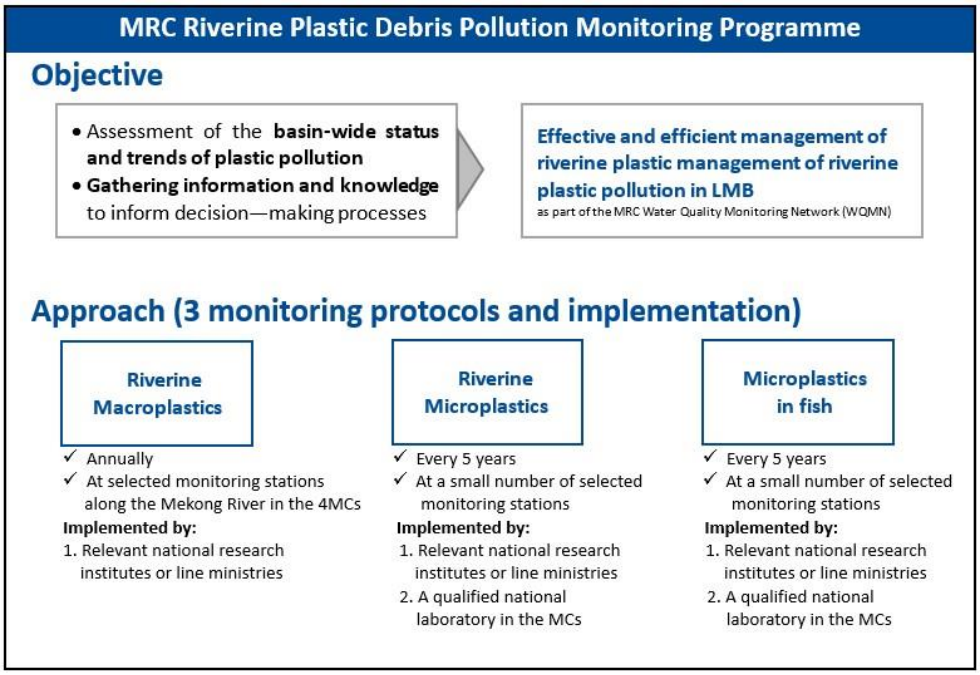


Figure 2.1. The scope of the monitoring programme and monitoring protocols

2.2 Objectives and scope of riverine microplastics monitoring

(1) Objectives and scope of riverine microplastics monitoring

The objectives and scope of riverine microplastics monitoring are to understand riverine plastic pollution levels, trends and their distribution throughout the LMB, with a view to preventing impacts on human health and ecosystems.

(2) Definition

a) Microplastics

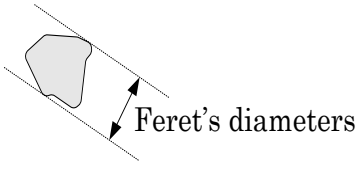
In the field of marine debris monitoring, GESAMP introduces the following size range categories of plastic marine debris:

- Megaplastics (>1 m)
- Macroplastics (25 mm – 1 m)
- Mesoplastics (5–25 mm)
- Microplastics (0.3 mm – 5 mm).

Regarding riverine debris monitoring, UNEP defines debris size range categories exactly the same as mentioned above.

Plastic particles with diameters less than 5 mm are defined as microplastics in this Protocol. The diameter refers to the long diameter measured as Feret's diameter unless otherwise indicated.

The **Feret diameter,** or **Feret's diameter**, is a measure of an object size along a specified direction. In general, it can be defined as the distance between the two parallel planes restricting the object perpendicular to that direction.



b) Plastics

"Individual substances artificially configured into useful shapes using high-molecular substances (mostly synthetic resin) as the main ingredients, however, excluding rubber/paint/adhesives." They are excluded due to the difficulties of analysing them because their particles are too small for the conventional analysis method since most of them in the environment would pass through a sampling net and they are often made of various materials, some of which are mixed with natural ingredients.

Flow of riverine microplastics monitoring

Monitoring is conducted according to the steps of planning, sampling, analysis, reporting, as shown in Figure 2.2, and their methods are shown in Chapter 3. Survey plan, Chapter 4. Sampling method, Chapter 5. Analysis method, and Chapter 7. Data interpretation and reporting. Also, the blank test, which aims to check any contamination that may have occurred between sampling and analysis, and the spiked recovery test, which aims to check whether adequate values have been obtained during the analysis, are illustrated in Chapter 6. *Analysis method*.

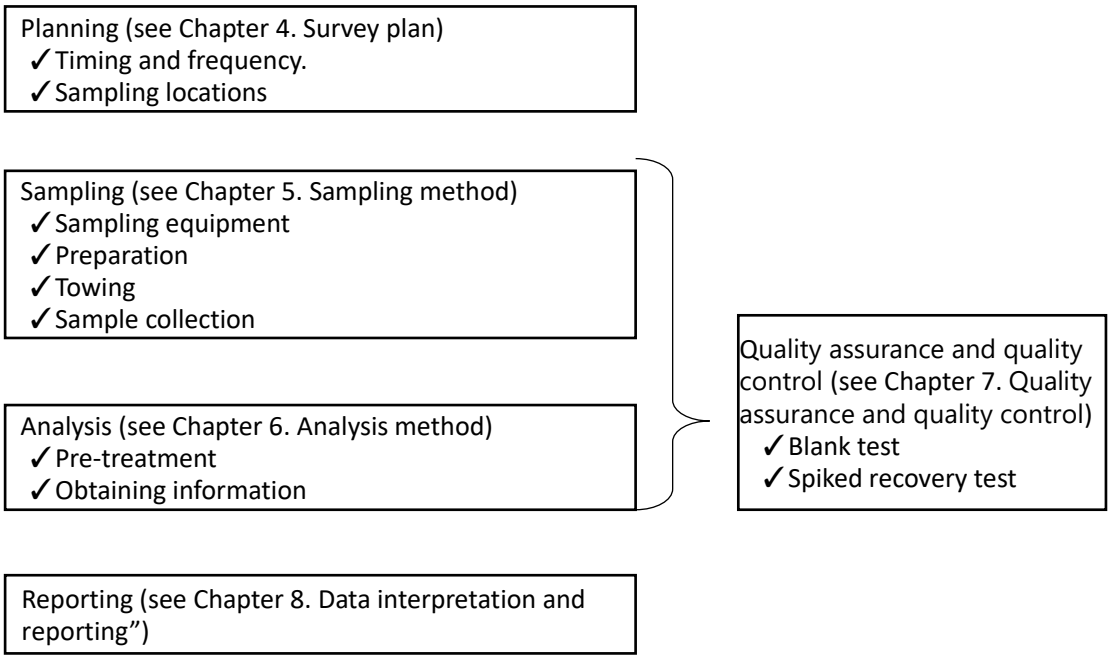


Figure 2.2. Steps in riverine microplastics monitoring

3. Summary of the survey method

Protocol for monitoring riverine microplastics consists of two components:

1. Sampling: collection of riverine microplastics by towing a net on a vessel in the river;
2. Analysis: analysis of collected samples through chemical processing and identification of the characteristics such as colour, shape, and material in laboratories.

Below is a summary of the two components and the respective equipment; each is detailed in the corresponding chapters: Chapter 4. Survey plan, Chapter 5. Sampling method, Chapter 6. Analysis method, Chapter 7. Quality assurance and quality control, and Chapter 8. Data interpretation and reporting.

Riverine microplastics

(1) Sampling

Planning (see Chapter 4. Survey plan)

Timing and frequency

✓ Twice a year, with one sampling to be carried out during the dry season when the average dry season flow is observed, and the other to be carried out during the wet season when the average wet season flow is observed.

Survey locations

✓ Select the locations near the stations of ongoing monitoring programmes by the MRC.
✓ Select the locations according to the purpose of the monitoring.

Sampling (see Chapter 5. Sampling method)

Preparation (the day before sampling)

✓ Confirm that all the necessary equipment functions appropriately. Most importantly, the mesh size of the net should be about 0.3 mm, which is a common size for conducting research.
✓ Make a final decision on the implementation day based on weather, and the availability of manpower and equipment.
✓ Conduct the safety check.

Blank sampling

✓ Collect the blank sample from the net, which is not yet towed in the water, using distilled water by following the exact same process as in the regular sampling (see **"Sample collection"** below).

Equipping the net and the vessel

✓ Fasten a flowmeter to the lower net frame. Set the reading at 0.
✓ Attach the floaters to the side of the net.
✓ Fix the net to the pipe.

Towing

✓ Record the necessary data before, during, and after the towing.
✓ Start towing as close to the centre of the river as possible considering safety as the priority.
✓ Make sure that the flow velocity is 0.5 m/s to 1.5 m/s; if any faster, stop the boat with anchors then start towing again.

- ✓ For Neuston nets, less than 90% of the net should be immersed in the water (e.g. 50%, 75%).
- ✓ Keep the net submerged for the planned tow duration (time estimated to be required to collect 100 pieces of debris, or 5 minutes if there is no precedent survey).

Sample collection

- ✓ Haul the net slowly and lift it up. During and at the end of this process, wash the net from the outside to wash down the particles inside to the cod end.
- ✓ Transfer the particles collected in the cod end to the sample container by opening the outlet of the cod end.
- ✓ Seal and store the container.
- ✓ Transfer to laboratories.

Riverine microplastics

(2) Analysis

Pre-treatment (see Chapter 6. Analysis method)

Sieving of samples

- ✓ Sieve the sample using two sieves (upper: mesh size 5.0 mm, lower: mesh size 0.1 mm) and reduce the amount of unnecessary substances.

Oxidation

- ✓ Remove the natural organic matter by using a 30% hydrogen peroxide solution and iron sulfuric solution (Fenton's reagent). This treatment will make the isolation of particles more efficient and improve the accuracy of quality assessment by Fourier-transform infrared (FT-IR).

Density separation

- ✓ Add saturated sodium chloride solution for an efficient isolation of the microplastics. This process is applied when direct isolation of microplastics is difficult due to a large amount of suspended matter such as sand.

Obtaining information (see Chapter 6. Analysis method)

Isolation of microplastics

- ✓ Isolate possible microplastics in the samples on the petri dishes.
- ✓ Lay out the isolated particles on a separate petri dish.
- ✓ Classify the particles according to their shape/form and colour.

Image data acquisition

- ✓ Take a photo of the particles on the glass petri dishes using a digital camera attached to the stereo microscope to obtain image data. The photo will be imported to the image processing software with each of the particles numbered, before measuring the longest and shortest diameters as well as the dimensions.

Material identification using FT-IR

- ✓ The spectrum of sample will be displayed by FT-IR.
- ✓ Confirm the spectrum and identify the material of the particle for recording.
- ✓ Following the measurement of each set of samples, weigh the microplastics.
- ✓ Ensure the quality of the data by following the quality assurance and quality control steps in Chapter 7. Quality assurance and quality control.

Reporting (see Chapter 8. Data interpretation and reporting)

Summarize the results in the form

- ✓ Indicate the shape/form and colour recorded in the "Isolation of microplastic" section above.
- ✓ Indicate the longest/shortest lengths and dimensions of particles measured by image data acquisition/image processing in the "Image data acquisition" section above.
- ✓ Indicate the materials of the particles obtained in the "Material identification using FT-IR" section above.
- ✓ From the data for each particle, integrate the number, materials, shape/form and colour of the plastic particles excluding non-plastic ones.
- ✓ Classify the size of microplastics into three diameter categories: $d < 1$ mm, $1 \leq d < 5$ mm, and $5 \leq d$.
- ✓ Calculate the densities of the particles (particles/m^3, mg/m^3, particles/m^2 and mg/m^2) from the volume and area of the filtered water.

Necessary equipment

Items in Table 4.2 will ensure the efficient implementation of the survey.

Table 4.2 Equipment and materials for laboratory analysis of microplastics

Category	Group	Item
Microplastics analysis (necessary for both riverine debris and in fish)	Sieving	Wash bottle
		Glass beaker (500 ml)
		Watch glass
		0.5 mm stainless steel sieve
		0.1 mm stainless steel sieve
		Stainless washtub
		0.1 mm hand net
	Oxidation	30% hydrogen peroxide
		Steel tray for the water-bath
		Dispensing spoon
		Wash bottle
		Glass beaker (500 ml)
		Watch glass
		0.1 mm stainless steel sieve
		Stainless washtub
		Iron sulphate solution
		0.1 mm hand net
		Drafts, masks, etc. to prevent inhalation of harmful gases
		Wastewater tank, funnel
	Density Separation	Saturated sodium chloride solution
		0.1 mm stainless steel sieve
		Stainless washtub
		0.1 mm hand net
		Waste tank, funnel
		Glass beaker (500 ml)
		Watch glass
		0.1 mm stainless steel sieve
		Stainless washtub
		0.1mm hand net
	Isolation of microplastics	Stereo microscope (Olympus SZX16, comprehensive magnification 7X to 11.5X)
		Light source device (MicroNet LED light source, 4 seasons)
		Two precision tweezers
		Wash bottle
		Glass petri dish (10 cm)
		Nitrocellulose filter
	Image acquisition	Stereo microscope (Olympus SZX16, magnification × 7 to 115)
		Digital camera (WRAYMER WRAYCAMNF500, approximately 5 million pixels)
		Digital Camera Control Software (WRAYSPECT)
		Personal computer
		Reference scale
		Monitor(s) (preferably 2)
		Image processing software (ImageJ)
		Trace table
	FT-IR	Glass petri dish with sample

		FT-IR: Detector (Shimadzu IRAffinity-1S) + PC
		A separate PC from above
		Precision tweezers
		Paper wiper (Japan Paper Cresia Kim Wipe)
		Mister filled with ethanol (or methanol)
	Measurement	Electronic balance
		Glass bottle (5 ml)
Additional requirement for analysing fish	For anatomy of fish	Stainless steel trays
		Stainless steel anatomy scissors
		Stainless steel tweezers
		Electronic scale
	Decomposition of tissues of digestive tract	10% potassium hydrate solution
		Container (pre-washed with pure water or tap water
		Incubator
		Hand-net (mesh size 0.1 mm) (pre-washed with pure water or tap water)
		Washing bottle (for water)

Necessary equipment

Items in Table 4.1 will allow for an efficient implementation of the survey.

Table 4.1: Equipment and materials for riverine microplastics sampling

Category	Group	Item
Sampling (riverine microplastic)	Ship equipment	0.3 mm microplastic tow net built to the required specification as described in the Toolbox 5 (x2)
		Floater (multiples)
		Sinker (multiples)
		Water pump (1)
		Large bucket or tube for collecting water to clean the tow net at the end of each tow
	Measuring	Flowmeter (x2)
		GPS receiver (1)
	Storing	Non-plastic sample containers for storing the sample to be transported and analysed at the laboratory
		Formalin (analytical grade) as a preservative
		Cold box
		Ice block
	Safety	Personal safety equipment (1 per staff member participating in the pilot)
	Sanitation	Gloves and personal hygienic supply
	Stationary	Time chart sheet for relevant field observation data
		Sample labels
		Office supplies (logbook, printing paper, pen, etc.) and printing

4. Survey plan

4.1 Timing and frequency

Riverine plastic abundances can be highly variable over time due to river flow, inhomogeneous/random distribution or human activities. Thus, it is recommended to focus on relatively frequent and long-term monitoring. The recommended timing is as follows:

- The survey should be conducted both in the rainy and dry season, with the average river condition representing these seasons, and more than once in each season;
- It is best to conduct a survey more than once every five years if the aim is to fully understand the pollution level and its distribution
- In order to fully understand the pollution trend, it is recommended to conduct a survey every year.
- It is recommended to conduct a survey more than once a month at least in order to measure the amount of microplastics that drift downstream throughout a year.

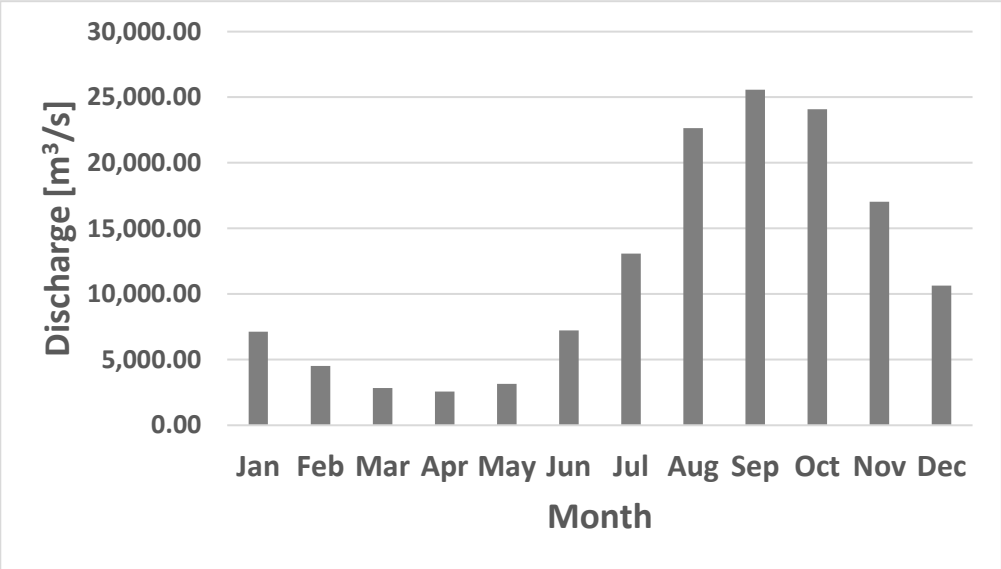


Figure 4.1. Mean monthly river discharge at Tan Chau (Mekong) and Chau Doc (Bassac) in Viet Nam

4.2 Sampling locations

The survey location should be selected according to the purpose of the monitoring. Survey locations are recommended to be selected among those covered by ongoing MRC monitoring programmes, including Water Quality Monitoring Network (WQMN), FADM, FLDM and hydrological monitoring programmes. Also, it is recommended to choose the same locations as those for other riverine plastic debris pollution monitoring programmes. This allows to efficiently use resources and available metadata (e.g. water quality, river discharge, fish populations). *A monthly survey would be preferable. At least twice in the dry and rainy season if the budget is limited.*

Potential selection criteria for the survey locations

- *Upstream, midstream and downstream in each country*
- *Near a water intake for municipal water supply or industrial supply*

- *A major fishing area*
- *Upstream of confluence points*
- *Downstream of confluence points (the location where mainstream water and tributary water are well mixed)*
- *Downstream of major cities or industrial areas.*
- *At some tributaries, which are preferable for the identification or monitoring of leakage sources*
- *Upstream of the diversion points*
- *At the centre of the river stream: the river current near a riverbank tends to slow down and thus microplastics tend to accumulate and settle).*
- *At ebb or low tide in the estuary area.*

Based on the above criteria, survey locations should also be chosen according to the survey objectives such as the following:

- To fully understand the pollution source: Downstream of major cities and industrial areas should be chosen.
- To evaluate the impact on water quality and fish species: Upstream of water intake and at fishing location should be chosen.
- To estimate plastic flux with transboundary impact: Upstream, midstream, and downstream in each country, and nearby of confluence and diversion points should be chosen.

In riverine microplastics monitoring, survey sites should be selected from the sites established in the MRC monitoring programmes, for example, upstream of water intake, with a view to preventing impacts on human health and ecosystems. To be specific, based on the results of water quality surveys, sites where pollution is of particular concern and sites where analysis of microplastics in fish is also conducted should be chosen.

Where in the location should sampling take place?
In selected survey locations, the tow starting point should be chosen as near the centre of the river as possible to collect the data that represent its regional characteristics.

Figure 4.2. Current monitoring stations and locations in the Mekong River

5. Sampling method

5.1 Sampling equipment

(1) Sampling vessels

A sampling vessel should have a device to fix a net at the bow in order to prevent any disturbances from undertows from the boat. When using a boat other than a research vessel, such as a fishing boat, some outfitting is required to fix a net at the bow (Figure 5.1).

The sampling vessel must be able to ensure a constant stable towing at the speed of 1–2 knots in the intended direction despite any load potentially caused by net clogging.

Furthermore, when several sample collections are conducted continuously with one net, or when it is deemed inadequate to continue the collection due to net clogging during towing, the hired boat should have a work space for washing the net and collecting samples.

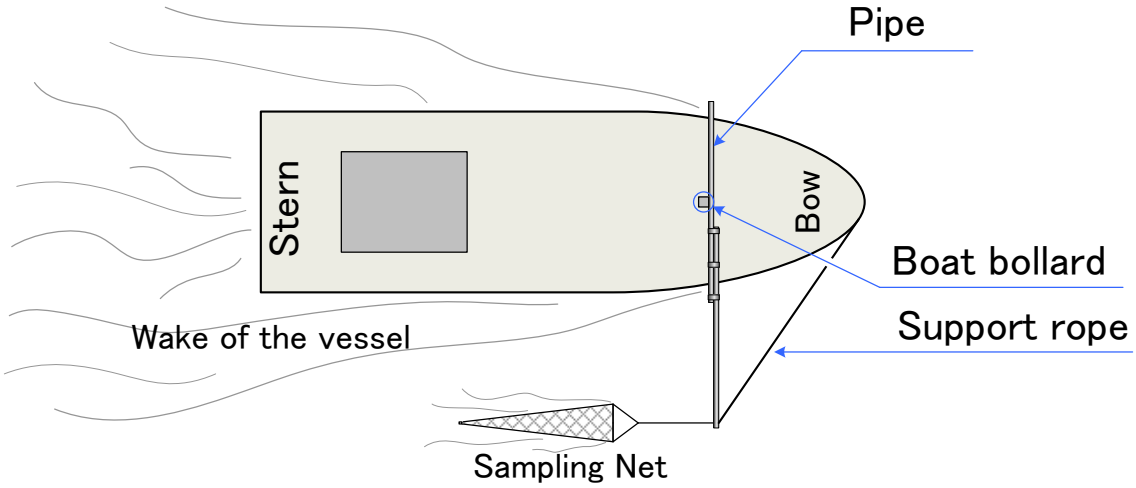


Figure 5.1. Towing performed by simple outfitting on fishing boat

For a small fishing boat requiring outfitting, the following points should be noted to avoid damaging the boom when towing a net from the side:

a) Set an adequate length of the outfitting pipe to keep the net close to the body of the vessel. Also, in order to avoid impact from undertows, the pipe equipped on the side should be as close as possible to the bow. This is also effective for preventing contamination.
b) The pipe should be fixed at a safe and stable location where the vessel strength is high such as boat bollards.
c) In order to prevent damage to the mounting hardware, stabilize the edges of the pipe by tension using support ropes.

For further prevention of damage, it is important to pay the utmost attention to avoid damage to outfitted pipes and contamination from coating and buffer materials onboard.

(2) Sampling nets

a) Net types

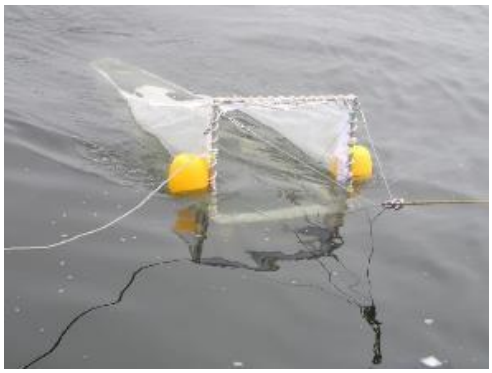
Neuston nets and manta nets are recommended (Figure 5.2).

Neuston nets are easy to handle onboard a vessel and on land due to their lightweight net frame. Also, they can easily capture the water surface layer. And yet, it is difficult to estimate the volume of water filtered accurately due to the fluctuations of the immersion depth of the net mouth during towing, which requires stabilizing operations and equipping the vessel with floats.

Manta nets are equipped with floats to maintain a constant immersion depth of the net mouths during towing. Some are configured to have the upper end of the net mouths to capture the water surface at all times, which makes it easier to tow with the immersion depth maintained at a certain level. However, due to their large size and weight, more manpower is required for handling them onboard a vessel or on land.

The particle density obtained with Neuston and manta nets is thought to be comparable when the nets have similar immersion depth. However, when a typical Neuston net and a manta net are compared, the immersion depth of the manta net is shallower relative to the width of its mouth. The density of microplastics tend to increase nearer the water surface and more so in calmer conditions. As such, it is necessary to report weather and river conditions at the time of sampling together with net immersion depth.

For marine surveys, nets with a mouth width of about 1 m are often used. Small nets may simplify the work on board a ship but will require more trawling time in order to filter the necessary amount of water. Also, longer trawling time means more clogging, which would lead to more net washing during trawling. Furthermore, nets with a lower mouth height would be closer to the water surface than those with a higher mouth height and filtrating river water with a higher concentration of plastic particles. Hence, it is necessary to choose a net of adequate size according to the conditions of the sampling sites as well as the size of the survey boats used.



Neuston net

Manta net

Figure 5.2. A Neuston net (left) and a manta net (right)

b) Mesh openings

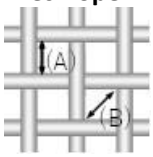


Figure 5.3. Mesh openings

As shown in Figure 5.3, the mesh openings, as indicated in this protocol, are expressed as the side length of a quadrangle separated by mesh thread and through which sea water passes, (A), but in some cases, the length of the diagonal line (B) is used as the mesh opening. The researcher should confirm which mesh opening is referred to and record the mesh opening used for the survey.

Mesh openings of about 0.3 mm for sampling zooplanktons are commonly used. In order to compare pollution from floating microplastics in various areas, or from a broader, global perspective, the use of the most common mesh opening of 0.3 mm is considered desirable.

However, microplastics whose shortest length is smaller than the mesh opening, even with the longest length larger than the mesh opening, may still pass through a net. The density of the microplastics obtained with the mesh openings of around 0.3 mm are under-sampled, particularly when the longest length is < 1 mm. Therefore, it is advisable to measure and report particles < 1 mm separately from particles 1 mm – 5 mm.

Monitoring using a net with finer mesh openings would be useful because data on smaller particles are essential for understanding the behaviour of microplastics as well as the effect of uptake by organisms. However, it should be noted that a net with 0.1 mm mesh or less would tend to get clogged. Also, given a report that the accuracy of microplastics analysis is reduced for particles with less than 1-mm diameter, it is advisable to use a net with the mesh openings that are capable of collecting particles with enough varying diameters, which would secure the accuracy of the analysis.

(3) Flowmeter

Microplastic abundance is reported as particle number or weight per unit water volume or unit surface area. Filtered water volume or area is obtained by multiplying the tow distance by the filtered area (net width x net immersion depth or the net width), respectively. It is advisable to use a flowmeter for calculating tow distances.

When using a flowmeter, it is necessary to select a model that can accurately measure the filtered water distances at different towing speeds. In addition, it is important to ensure that the measurement can be conducted accurately at the towing speeds, which were planned before the towing.

If a flowmeter is not available, it is possible to substitute with a tow distance relative to river water calculated by continuously measuring vessel speed or current speed during towing. However, since net clogging will cause a gap between filtered and tow distances, it is necessary to wash the net regularly during towing in areas with large amount of natural floating substances.

(4) Cleaning equipment

After towing, the samples in the net are washed down to the cod end using water from outside. A pump would make this process easier (Figure 5.4).

If a pump is not available, it would be effective to use a bucket or tub that is large enough so as to put the net from its cod end to the net mouth. Soak the net from the cod end side to the net mouth and push the collected samples down to the cod end using water from outside as the net is raised slowly.

Figure 5.4. Net washing (left) and pump (right)

(5) Sample collection containers

In order to minimize the risk of contamination, it is advisable to use sample collection containers made of non-plastic materials (Figure 5.5). If plastic containers must be used, it is recommended to use new ones because old ones may be damaged by deterioration and could contaminate the samples. The sampling location, sampling date, and the serial number of the sample should be written on the container with a waterproof method before conducting sampling. When the mouth of the container is smaller than the outlet of the cod end, a funnel with the inlet larger than the outlet of the cod end should be used in order to avoid dissipation of samples.

Figure 5.5. Sample container

(6) GPS receiver

The start and the end point during towing are the most primary information obtained through a survey. Whether or not the amount of filtered water is measured as the distance recorded by a flowmeter or as the total tow distance obtained from the vessel speed relative to the ground speed, a GPS receiver will be necessary to record these two points during towing.

Figure 5.6. GPS receiver

5.2 Preparation

(1) Preparation of equipment

The day before conducting sampling, all the equipment for sampling should be in place and their functions checked.

For the nets, particular attention should be paid to detect any tear in the texture and damage to the net mouth and the cod end.

Any equipment such as nets and sample containers that may come in contact with the samples collected should be washed thoroughly and stored, avoiding any contamination.

Every item of the equipment used shall be assigned a serial number, which should be displayed on the equipment.

(2) Final decision on the choice of sampling day

Consideration must be made on whether the survey may go ahead based on the forecast of weather and river conditions on the sampling day. In addition, the full availability of manpower and equipment should be confirmed, before deciding to proceed with the sampling.

(3) Safety check

Before boarding, the conditions of safety equipment such as life jackets and helmets should be checked, and the schedule for the day should be communicated to all onboard.

Safety guidelines in Standard Sampling Procedures for Fish Abundance and Diversity Monitoring in the Lower Mekong Basin (Ngor et al., 2016)

(1) Boats

The boat safety checklist should be completed prior to departure for sampling. In particular and as for all small-boat use:

- ✓ No smoking is allowed on the boat.
- ✓ All personnel must wear full lifejackets at all times.
- ✓ Boat drivers must be briefed on safety issues and requested not to smoke and to be considerate of other people on the water and to watch out for submerged nets and obstructions.
- ✓ All crew must watch for overhanging branches and inform others when there is any chance of injury.
- ✓ The driver must avoid rapid acceleration or deceleration while setting nets.
- ✓ No more than one extra person (e.g. observer) may be on board while sampling, and this person must be briefed by the supervisor, must wear the safety equipment, and must stay near the rear of the boat.

(2) Protective clothing

- ✓ All personnel must wear safety boots or shoes to protect their feet from any dropped nets, containers, or other items.
- ✓ All personnel must wear hard-hats to protect against tree branches
- ✓ All personnel must wear sunglasses to reduce the effect of surface glare on the water and also to protect against branches and net-handles.
- ✓ Personnel may wear gloves if required.

(3) First Aid

- ✓ At least one person in the sampling team must have completed First Aid and CPR training.
- ✓ There must be a full First Aid Kit on the boat.

5.3 Towing

(1) Towing steps

Blank sampling

Before towing the net, conduct blank sampling, which consists of collecting a sample from the net that is not yet towed in the water using distilled water by following the exact same regular sampling process to collect blank sample. This aims to check whether or not there is contamination in the net.

Upon reaching the tow starting point, the serial numbers of the net used for towing and the flowmeter and other equipment should be recorded in the field book.

Towing should begin by dropping the net gently on the water surface. Gradually increase the vessel speed while making sure that the towing is smooth by checking that the alignment is orderly from the net's mouth to the cod end and the net immersion depth is stable at the predetermined depth. Upon reaching a pre-determined vessel speed, continue to tow at a certain speed and monitor the floating substances in the net and on the water surface. When net clogging or another malfunction is detected during towing, determine the cause of the problem by lifting up the net. If the net is clogged, either collect the samples or change the net and resume the towing.

(2) Towing speed/duration

Towing should be conducted at the speed of 0.5–1.5 m/s; if any faster, the water is not properly filtered and may flow backwards through the net mouth.

Keep the net submerged for the planned tow duration (the estimated time required to collect 100 pieces of debris, or 5 minutes if there is no precedent survey).

Calculation of tow duration

Tow duration is an important factor in obtaining representative results. If the particle density is 5 particles/m^3, the volume of water to be filtered will be more than 20 m^3. If a net with a width of about 0.5 m and the immersion depth of 0.25 m is towed at 1 m/s, the towing duration will be more than 160 seconds.

What If the net is clogged?

In river areas that are abundant with floating debris, net clogging can occur before reaching a desirable duration, undermining normal sample collection. In these areas, set a shorter towing duration and conduct multiple towing in order to secure the desirable duration.

What if the net rotates?

Install floats of appropriate size on both sides of the net opening and weights of appropriate size at the bottom of the net opening.

Also, it can be assumed that the towing speed is high. Tow the net at a speed of about 1.5 m/s relative to the river water.

(3) Towing positions

Samples should be collected by a net fixed on the side of the boat to avoid disturbances from the wake.

The density of microplastics near the surface layer generally increases towards the river surface. Disturbances from the wake will mix the high density water shallower than the immersion depth with low density water deeper than the immersion depth, which reduces the density of water passing through the net mouth.

(4) Net immersion depth

The immersion depth of a manta net is usually designed to match the height of the net mouth so that the upper end of the net mouth can catch the water surface.

A Neuston net is commonly used for towing at 1/2 to 3/4 of the height of the net mouth. However, in river areas abundant with floating substances, the immersion depth gradually increases due to net clogging.

In order to reach the pre-determined immersion depth, place sinkers and buoys of adequate weight and make adjustments to the length of the towing net. During towing, regularly observe the condition of the net mouth to record the immersion depth (Figure 5.7).

If the net is clogged and immersion depth cannot be kept at a certain level, either haul the net to collect the samples, or change the net before continuing the towing.

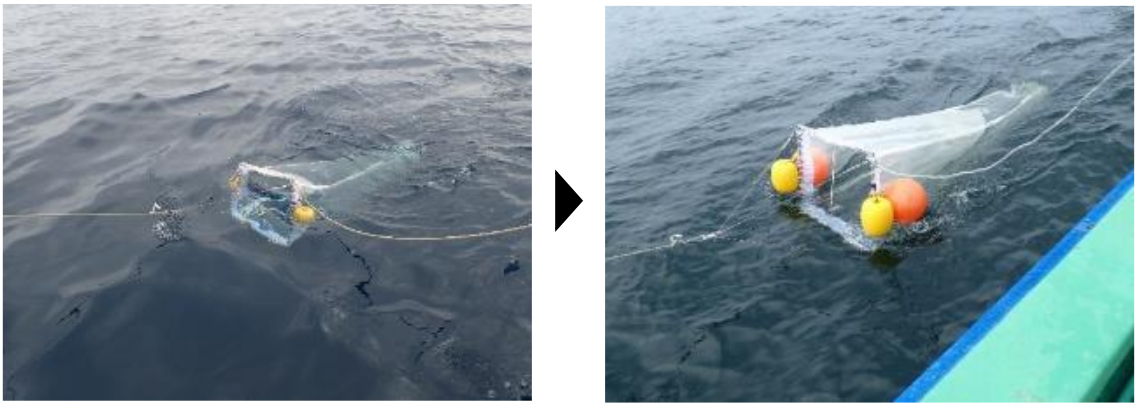


Figure 5.7. Adjustment of immersion depth of the Neuston net by attaching appropriate floats

(5) Collection of relevant data

Conduct various observations to obtain and record relevant data before, during, and after towing.

The time chart for relevant data observations is shown in Table 5.1.

If a flowmeter is not available and the filtered volume is calculated from the current speed or GPS log, record the results from consecutive measurements taken at a certain interval during towing.

Table 5.1. Time chart for relevant data observations

	Before towing	Towing	Towing (interruption)	After towing
Time	◉	◉	◉	◉
GPS Log	◉	◉	◉	◉
Flowmeter indication	◉			◉
River flow velocity	○			○
Other types of water quality data	○			○
State of the net		◄►	◄►	
State of floating debris on the river surface		◄►	◄►	

◉ Items for which observation is compulsory

○ Items for which observation is desirable in order to understand the microplastics contamination

◉ Items to be observed when towing is interrupted, or the post-towing condition changed greatly from the pre-towing condition.

◄►

5.4 Sample collection

(1) Net cleaning/sample collection

When towing is finished, haul the net slowly with the mouth side up at all times (Figure 5.8). During and at the end of this process, the net is washed with water from the outside to wash down the particles inside to the cod end while particles on the outside are washed away. The water can be drawn from the river by a pump or a bucket.

Figure 5.8. Net hanging

When it is difficult for all the particles to be collected in the sample container due to large pieces of rubbish inside the net, larger items that are clearly not plastic particles should be removed first. Before the removal, wash them carefully inside the net to separate the small particles attached to them. Water that is clear of plastic contamination, prepared before boarding, should be used. Separated particles will be funnelled into the cod end. The sampling positions and serial numbers shown on the containers should be checked for accuracy.

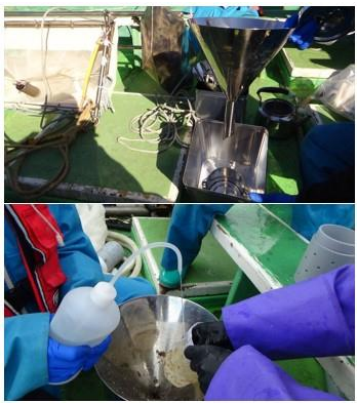


Figure 5.9. Sample collection

Particles piled up in the cod end will be transferred to the sample container by opening the outlet of the cod end over it when the outlet of the cod end if smaller; however, should it be larger, then the container, a funnel larger than the outlet of the cod end, should be used over the container. The cod end will be washed with distilled water free of plastic contamination to remove any residual particles to transfer all the particles into the container (Figure 5.9).

Figure 5.10. Collected samples

(2) Transfer to laboratories

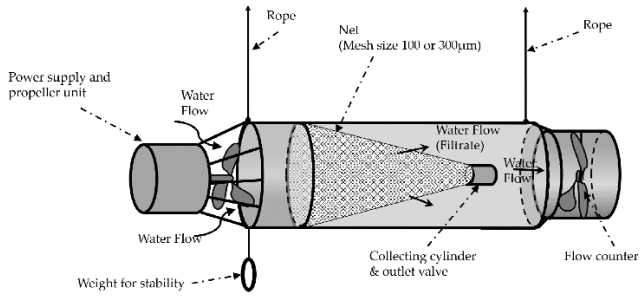
Samples should be transferred to a laboratory swiftly in an ice cooler box and buffer materials to avoid damage to the container (Figure 5.11).

Figure 5.11. Sample containers in an ice cooler box

Alternative sampling method (without using boat)

The concentration of riverine microplastics collected by a net with a mesh size of about 0.3 mm ranges from around 1 to 99 pieces/m^3. In order to obtain stable survey data for macro and microplastic, it is necessary to filter a large amount of riverine water through the net. For this reason, towing nets by boat is the most recommended method for large rivers such as the Mekong River. However, at a location with a flowing current, water can be filtered in the natural current by either hanging the net from a bridge (if the water is deep), or by fixing the net by researcher (if the water is shallow).

At locations where chartered boats cannot enter and the river flow is slow, neither tow net nor hanging net methods can be applied. To address this issue, Abeynayaka et al. (2020) developed a sampling device called Albatross, which combines a net and a flowing water pump, to conduct surveys. However, the Albatross method is only suitable for riverine microplastics and not for river macroplastics.



The structure of the Albatross device

Sampling by the Albatross device

6. Analysis method

Figure 6.1 shows the steps of the microplastics analysis.

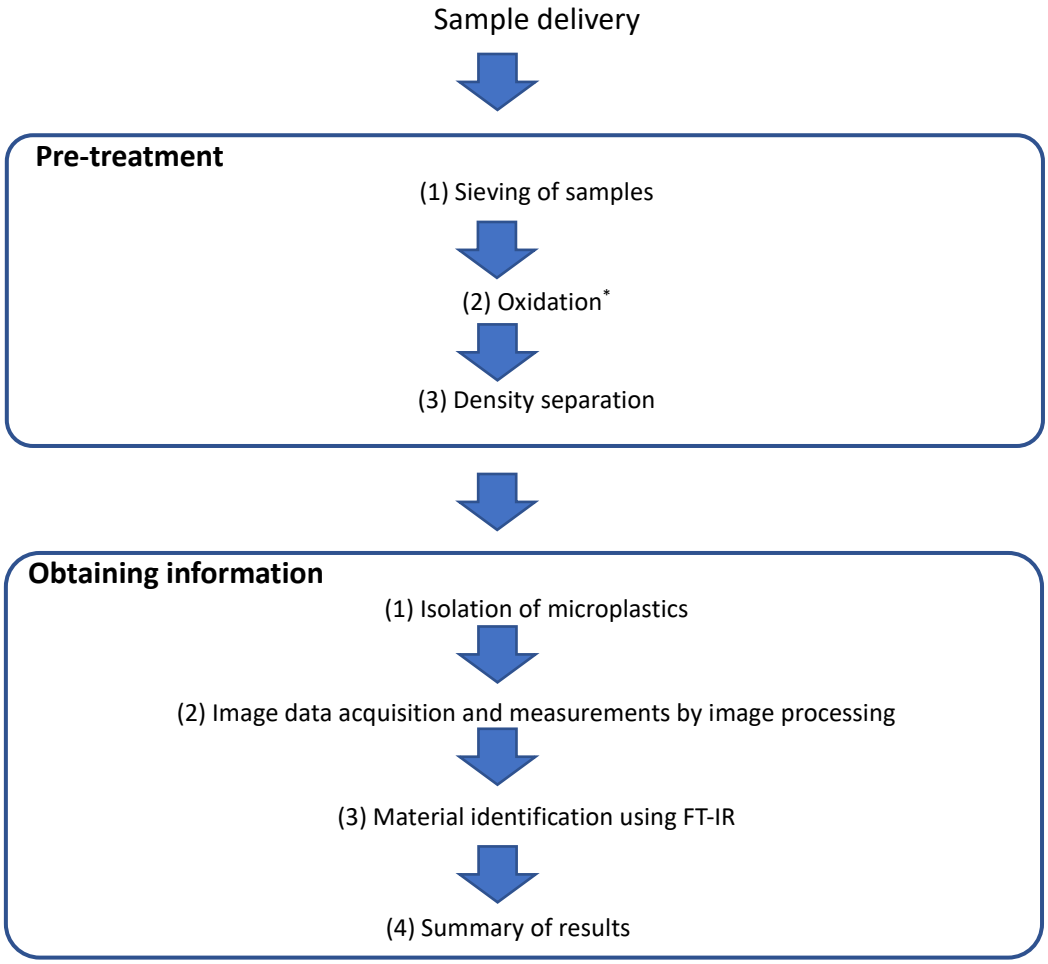


Figure 6.1. Steps in microplastics analysis

Note: *This process is applied when direct isolation of microplastics is difficult due to large amount of suspended matter.

6.1 Pre-treatment

(1) Sieving of samples

Samples are sieved using sieves with mesh sizes of 0.1 mm (Figure 6.1).

Equipment

- Stainless steel basin
- Glass beaker (500 ml volume)
- Hand net (mesh size 0.1 mm)
- Stainless steel sieve (mesh size 0.1 mm)
- Water – tap water, purified or distilled water, or water filtered through a 0.1-mm mesh net
- Washing bottle (for water).

Procedures

1. Place sieves (upper mesh 0.1 mm) over a stainless steel basin and sieve the samples through. Return the filtered water to the sample container (as sample backup).
2. Wash thoroughly the large piece in the sieve, such as wood chips, leaves and jellyfish. Return the washed pieces back to the container (as a sample backup). If any clear large pieces of plastic such as those from a plastic bag or a Pet bottle were mixed in at this time, wash them thoroughly with water and keep them separately before adding them as microplastics.
3. Wash the remaining samples in the container with water and sieve through the 0.1 mm sieve. The filtered water can be discarded.
4. Wash the samples on the 0.1 mms sieve with water and transfer to a hand net. Repeat the process until the transfer of the entire samples to the hand net is visually confirmed.
5. Wash the samples on the hand net with a washing bottle during the transfer to the glass beaker. Repeat the process until it is visually confirmed that no particles are left on the hand net.

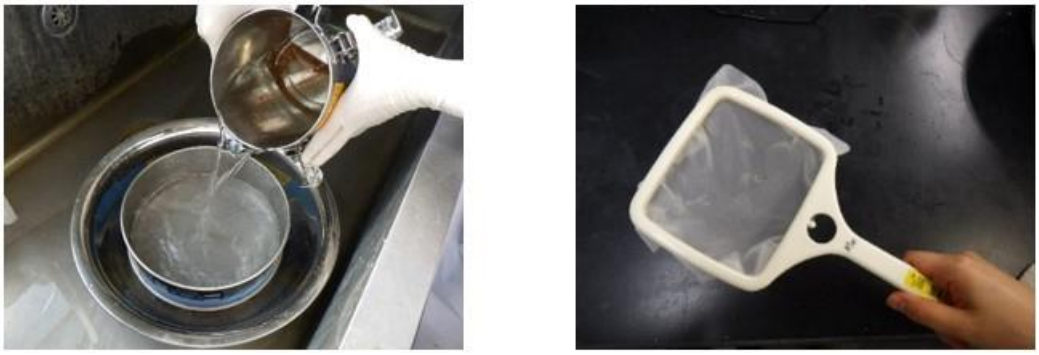


Figure 6.2. Sieves (left, mesh size 0.1 mm) and hand net (right, mesh size 0.1 mm)

(2) Oxidation

In order to remove and dissolve the organic matter that is attached to the surfaces of microplastics, oxidation treatment is conducted using a 30% hydrogen peroxide solution and iron sulfuric solution (Fenton reagent). Dissolving organic matter other than plastics will make the isolation of particles more efficient, and removing organic matter on the plastic surfaces will improve the accuracy of quality assessment of the materials when using FT-IR (Figure 6.3).

Oxidation using only a 30% hydrogen peroxide solution is often conducted when there are fewer non-plastic substances. In these cases, allow approximately one week for oxidation.

Equipment

- drafts and masks to prevent inhaling harmful gases;
- an iron sulphate solution (iron (II) sulphate heptahydrate: 7.5 g, concentrated sulfuric acid: 3 ml, diluted in a 500-ml flask with water);
- a 30% hydrogen peroxide solutions;
- watch glass;
- water;
- a hand net (mesh size 0.1 mm);
- a stainless vat;
- a washing bottle (distilled water);
- a stainless steel basin.

Procedures

1. Fill the vat with water up to several centimetres.
2. Place the beaker in the vat. (Purpose: to keep the temperature inside the beaker at a certain level to reduce deformation/degeneration of microplastics.)
3. Add 50 mL each of a 30% hydrogen peroxide solution and an iron sulfuric solution to the beaker.
4. Place the watch glass above the beaker containing the samples and leave for three days, carefully preventing any plastic particles in the air from mixing into the samples.
5. If not enough oxidation has occurred in the three days, continue monitoring the progress of the treatment one day at a time. On the fourth day, apply 50 mL of the 30% hydrogen peroxide solution and apply the iron sulfuric solution, if necessary. Leave the samples for a week if only the 30% hydrogen peroxide solution is used.
   Note: If the samples are not properly soaked in the hydrogen peroxide solution due to bubbling, gently mix the solution several times a day.
6. The oxidation is completed when there are no more non-plastic materials (yellow arrow in Figure 6.3).
7. After the oxidation is completed, transfer the samples to the hand net. The drainage from the hand net should be given to a waste liquid treatment specialist or discarded after proper treatment such as pH adjustments.
8. Follow one of the two processes below depending on the amount of non-plastic materials:
   - **a large amount** of non-plastic materials → **(Step 3: Density separation under Section 6.1 *Pre-treatment*)**
   - **a small amount** of non-plastic materials → **(Step 1: Isolation of microplastics under Section 6.2 *Obtaining information*)**

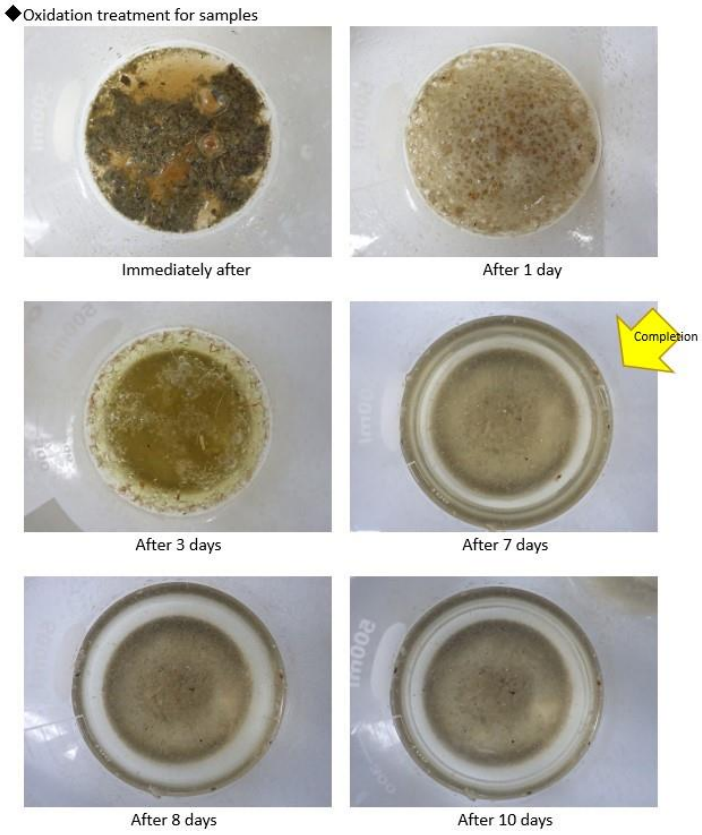


Figure 6.3. Oxidation daily changes

Note: With this sample, oxidation was completed on day 7.

(3) Density separation

Most of the plastic particles collected at the water surface have a low density. For density separation, heavy fluid (saturated sodium chloride) is added for efficient isolation of microplastics (Figure 6.4). This process is applied when it is difficult to directly isolated the microplastics due to a large amount of suspended matter such as sand.

Note: Saturated sodium chloride solution should be prepared at least one week in advance of the density separation process to allow time for undissolved sodium chloride solid to settle.

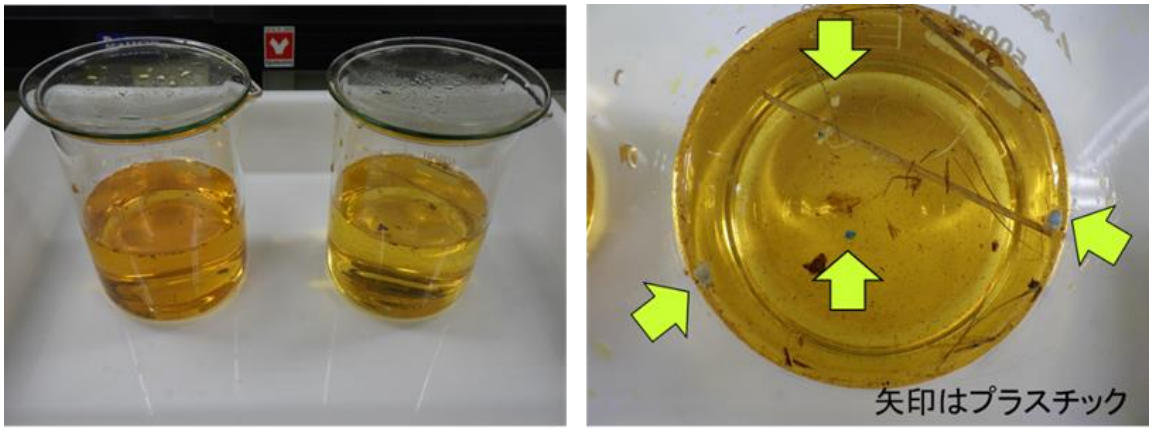


Figure 6.4. Density separation

Note: Particles including microplastics float in the upper layer. However, plastics that have high specific gravity may be found in the bottom layer (sunk particles), which need to be confirmed (arrows indicate plastics).

Equipment

- saturated sodium chloride solution (dissolved 350 g of solid sodium chloride or table salt in 1L of distilled water);
- glass beaker (500 ml volume) (a funnel for density separation can be used instead);
- glass beaker (500 ml volume) (for upper layer transfer);
- hand-net (mesh size 0.1 mm);
- washing bottle (filled with saturated sodium chloride solution);
- stainless steel basin;
- glass petri dishes (two: for the upper and lower layers).

Procedures

1. Transfer the post-oxidation samples to the hand net.
2. Wash the samples in the hand net using a washing bottle filled with a saturated sodium chloride solution, and then transfer them to the beaker. Add approximately 300 ml of saturated sodium chloride solution.
3. Cover with a watch glass and leave it overnight.
4. Separate and transfer the upper layer into the beaker. Leave the lower layer as it is.
5. Transfer the samples in the upper layer to the hand net and wash with the washing bottle (water).
6. Transfer the samples in the hand net to the glass petri dish.
7. Follow steps **5)** and **6)** for the samples in the bottom layer as well.

Method for analysing a proportion of the entire sample

When the collected sample from the field is expected to contain a high concentration of microplastics, it may be necessary for the laboratory to only analyse a known proportion of it. Should the decision be made to only analyse only a certain proportion of the sample, the following procedures will need to be carried out:

- Record the total volume of the sample (in mL) in the datasheet.
- Shake the water sample in the collected bottle rigorously to ensure that the sample is well mixed.
- Pour a small amount of sample into a cleaned borosilicate glass measuring cylinder and record the volume in the measuring cylinder.
- Analyse the samples collected in the glass measuring cylinder as per Section 6.1 and Section 6.2 to obtain microplastics information, including the total weight of the microplastics from the measuring cylinder.
- The total weight of the sample can then be estimated using the following formula:

  $W_T = W_p \times V_T / V_p$

  Where W_T and V_T represent the total weight and total sample volume, respectively, and W_p and V_p represent the weight and volume of the proportion of sample analysed, respectively.

6.2 Obtaining information

Step (1) Isolation of microplastics

Isolate possible microplastics in the samples on the petri dishes using a stereo microscope as well as by visual inspection (Figure 6.5). Isolated particles are laid out on a separate petri dish to obtain image data and for image processing.

Fractionation of plastics by morphological traits such as fragments, fibres and beads should be conducted at this time.

Equipment

- Stereo microscope
- Lighting system (LED light source)
- Glass petri dish
- Precision tweezers (2 pairs)
- Washing bottle (distilled water).

Procedures

1. Isolate possible microplastics from the samples on a petri dish with precision tweezers, using a stereo microscope or by visual inspection (Figure 6.5). The petri dish should be soaked in water to prevent the particles from sticking to the tweezers due to static electricity when dry.
2. The particles are fractionated by morphological traits (fragments, fibres, beads) and also by the colour (opaque, translucent, transparent). Further classification by colour is recommended for opaque particles. UNEP (2020) identifies microplastics morphologies as 1) fragments, 2) fibres/filaments, 3) beads/ spheres, 4) films/sheets, and 5) pellets. The report also states that categories of the reported colour, if any, should be based on simple classification schemes such as the 13 categories provided by the ISCC-NBS (Inter-Society Color Council and National Bureau of Standards) system to avoid subjective bias.
3. Fractionated particles are laid out on a separate petri dish for ease of photographing.

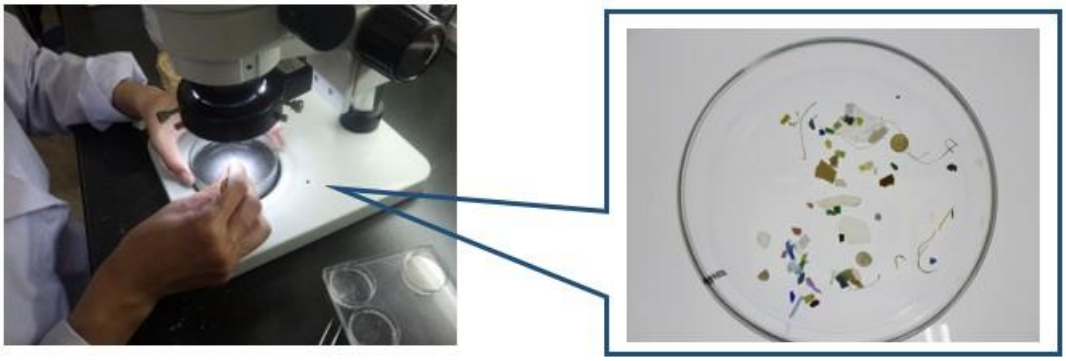


Figure 6.5. Isolation of microplastics

Table 6.1. ISCC-NBS colour classification system

Colour	Abbreviation	Example	Colour	Abbreviation	Example
Pink	Pk		Green	G	
Red	R		Blue	B	
Orange	O		Purple	P	
Brown	Br		White	Wh	
Yellow	Y		Gray	Gy	
Olive	Ol		Black	Bk	
Yellow green	YG				

Step (2) Image data acquisition and measurements by image processing

The particles on the glass petri dishes are photographed with a digital camera attached to the stereo microscope to obtain image data. The data obtained are imported to the image processing software (Image J) with each of the particles numbered, before measuring the longest and shortest diameters as well as their dimensions (Figure 6.1).

Equipment

- Stereo microscope
- Lighting system (LED light source)
- Reference scale
- Digital camera (for connecting to the microscope)
- Digital camera control software (WraySpect)
- Personal computer
- Image processing software (ImageJ).

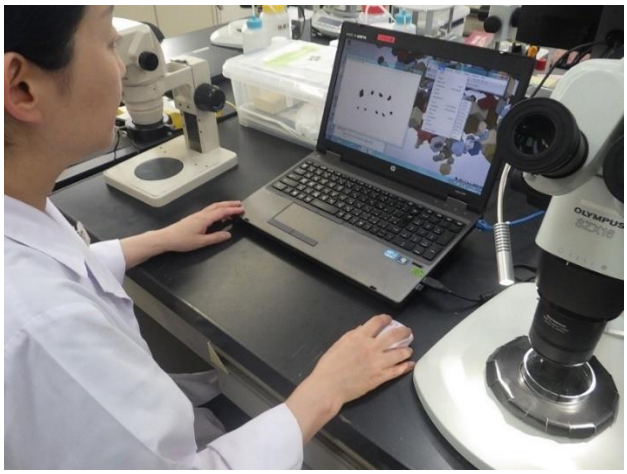


Figure 6.6. Photographing and image processing

Procedures

1. Focus the microscope on the particles on the petri dish. Once set, do not change the focus.
2. Photograph the reference scale with the digital camera attached to the stereo microscope and set the size for image processing software to 1 pixel per mm of microplastic.
3. Photograph the particles.
4. Change the image to grayscale, adjust the brightness of the particles to emphasize the contrast with non-particle parts.
5. Convert to a binary image (Figure 6.1).
6. Click on each target particle, and record the particle number and measure the longest/shortest lengths and the dimension.
7. Export the longest/shortest lengths and the dimensions of all particles in the sample in Excel format.

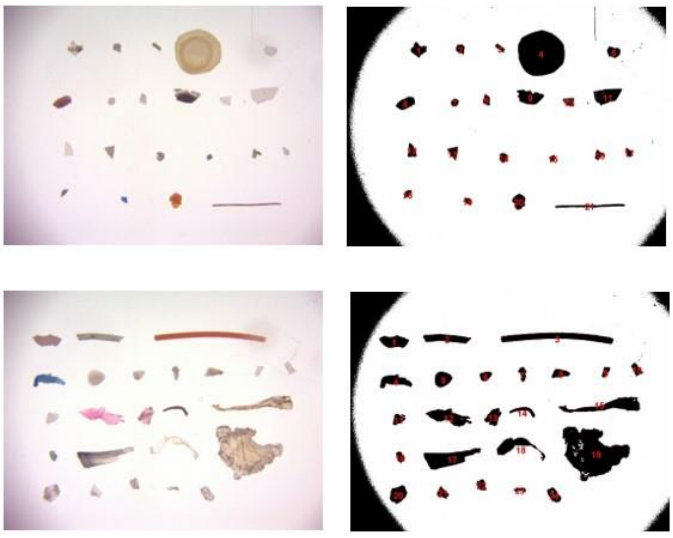


Figure 6.7. Digitally photographed image of particles on petri dish (left) and binary image of numbered particles by image processing software (right)

Step (3) Material identification using FT-IR

FT-IR is used for material identification of particles.

Each equipment comes with different instructions for use, which need to be followed, and

support from manufacturers on instrument operational and maintenance may be necessary. Extra attention should paid not to damage the sample when using FT-IR.

Equipment

- FT-IDetector (Shimazu IRAffinity-1S)＋personal computer
- Personal computer (for inputting identification results)
- Precision tweezers
- Pre-weighed screw-top glass bottle (6 ml volume, 3 bottles) (Items: for microplastics, macro plastics and non-plastics 1 bottle for each)
- Paper cloth
- Ethanol (methanol allowed).

Procedures

This process should be carried out using **Shimadzu Corporation's IRAffinity-1S**.

Launch the LabSolutions IR program

1. Turn on the power of the detector and the PC.
2. Launch the Windows' start menu or the desktop program.
3. Press the <Equipment> button on the screen followed by the <Connect> button to connect the detector to the personal computer.
4. Once initialized, attach the part to be detected.

Background measurement

1. Wipe the sensor with a paper cloth soaked with ethanol.
2. Click on the green <BKG> button on the screen. After confirming that there are no samples inside, click <OK>.

Sample measurement

1. From the image of the particles for which the numbers were assigned during the Image data acquisition and measurements by image processing steps (page 27), use tweezers to pick up the particles that correspond to the number for the measurement, and press it to the sensor.
2. Enter the sample name and click on the <Sample measurement> button.
3. The measurement process begins.
4. When the measurement process is finished, the spectrum will be displayed (Figure 6.8)
5. Confirm the spectrum and identify the material of the particle for recording. Also, enter the colour information for the particle.

Identification of materials of the particles

1. Click on the <Search> button in the analysis menu.

2. The material of the particle and Hit Quality (HQI) will be displayed.

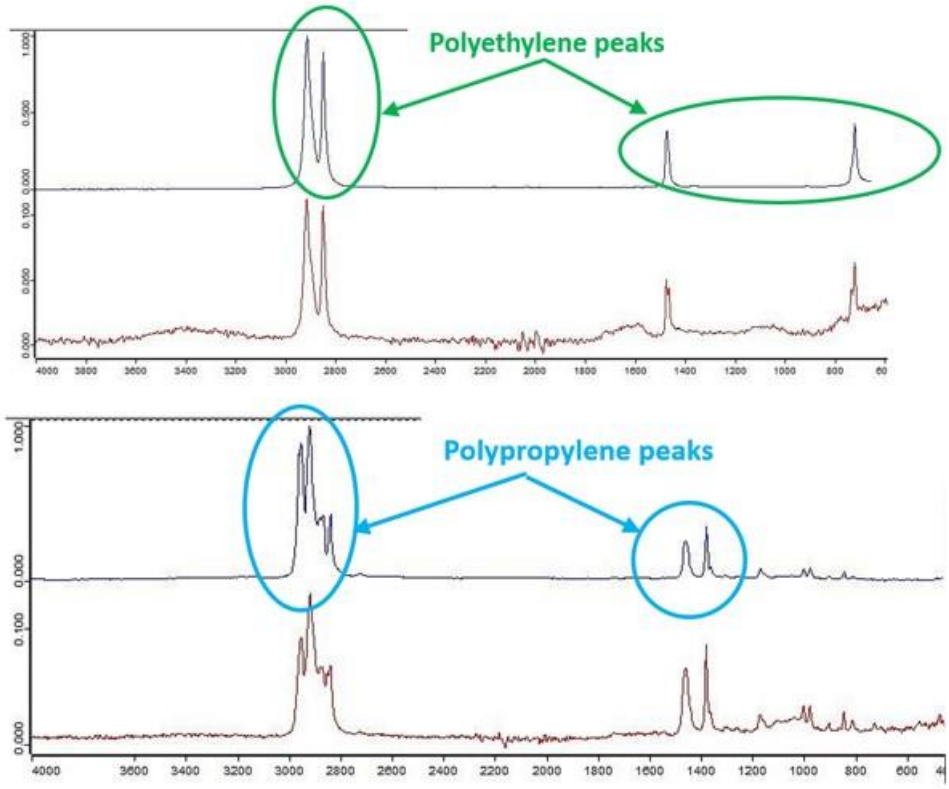


Figure 6.8. Example of spectrums from FT-IR measurement
(above: polyethylene, below: polypropylene)

Note: The blue line (above) indicates the curves of particles from the database,
while the red line (below) indicates those from the samples.

3. Record the measurements of the particles related to the information obtained from the process in (2) above.

4. The particles that are identified as plastics will be collected in a screw-top glass bottle for microplastic particles. The particles with their longest length of 5.0 mm or longer (macroplastics) will be collected in a screw-top glass bottle prepared especially for those particles. These particles identified as non-plastics will be put into a screw-top glass bottle prepared for non-plastic particles.

5. Repeat the background/sample measurements and the spectrum search as necessary.

Weight measurement of microplastics and macroplastics

1. When the measurement is completed for one set of samples, the screw-top glass bottle for the microplastics and the glass bottle for the plastics will be weighed; these weights of the bottles will be subtracted to yield the total weight of the microplastics and macroplastics.

Notes

Prisms and sample clamps should be cleaned with ethanol each time the sample is replaced.
HQI is an indicator that shows the degree of consistency between the curves in the FT-IR

database and those of the measured particles that were identified as plastics. While HQI is an indicator to determine the materials of the particles, it should be noted that methods for calculating the consistency level differ depending on the manufacturers and types of equipment.
Attention is required for the condition of the crimp on the prism and covered areas as they can alter the HQI.

Step (4) Summary of the results
For each of the particles, the following data will be collated for tabulation (Table 6.2):

- the shape/form and colour recorded in (step 1 – Isolation of microplastics);
- the longest/shortest lengths and the dimensions of the particles measured by image data acquisition/image processing in (step 2 – Image data acquisition and measurements by image processing) above;
- the materials of the particles obtained in (step 3 – Material identification using FT-IR) above.

Table 6.2. Example of data collation for individual microplastics

Sample name	Sample serial no.	Image no.	Serial no. within image	Longest length (mm)	Shortest length (mm)	Dimen-sions (mm^2)	Materials	Shape/ F form	Colour
S.T.1_01	1	s01a	1	1.977	1.330	1.388	non-pla.	Fragment	White
S.T.1_01	2	s01a	2	1.541	0.814	0.520	PE	Fragment	White
S.T.1_01	3	s01a	3	1.038	0.682	0.365	PE	Fragment	White
S.T.1_01	4	s01a	4	1.919	1.072	0.902	PS	Fragment	White
S.T.1_01	5	s01a	5	1.959	1.185	1.102	non-pla.	Fragment	White
S.T.1_01	6	s01a	6	2.410	1.799	2.540	PP	Fragment	White
S.T.1_01	7	s01b	1	2.273	1.458	1.669	PP	Fragment	Green
S.T.1_01	8	s01b	2	1.940	1.021	0.819	PP	Fragment	Green
S.T.1_01	9	s01b	3	1.967	1.093	0.938	PP	Fragment	Green
S.T.1_01	10	s01b	4	1.772	0.791	0.491	PE	Fragment	Green
S.T.1_01	11	s01b	5	1.741	0.999	0.783	PP	Fragment	Green
S.T.1_01	12	s01b	6	1.263	0.740	0.430	PP	Fragment	Green
			(2) Measurement data of the longest/shortest lengths, dimensions of particles by image data acquisition/image processing.				(3) Data obtained through material identify-cation using FT-IR.	(1) Categorized by the same shape and colour per each petri dish upon particle identification. Identical within the same image number.	

Note: non-pla= non-plastic; PE= polyethylene; PS= polystyrene; PP = polypropylene

From the tabulated data for each particle, aggregate the number, materials, shape/form, and colour of the plastic particles excluding non-plastic into the three categories: longest diameter below 1 mm, 1 mm or above and below 5 mm; and 5 mm or more.

Table 6.3. Example of results collation per sample

Diameter (mm)	Number (particles/sample)			Shape/form		Colour		Weight (mg/ sample)
	Number	Materials						
		PE	PP	Fragments	Fibres	Transparent	White	
d < 1	237	108	63	87	72	56	87	46.8
1 ≤ d < 5	185	75	42	122	65	45	93	
5 ≤ d	23	9	6	18	2	13	7	10.2

Also, calculate the volume and area of the filtered water from the data measured at the time of sampling in order to obtain the number and weight of the particles within the filtered volume (particles/m^3 and mg/m^3), and the number and weight of the particles within the filtered area (particles/m^2 and mg/m^2) for tabulation just as in Table 6.3.

7. Quality assurance and quality control

7.1 Blank test

This test aims to detect any contamination in microplastics during the process of sampling and a series of analyses. It is advisable to carry out the test on the day of each sampling run.

- The test is conducted based on the following steps: Just before collecting samples, obtain a blank test sample by applying the procedures described in Chapter *5.4 Sample collection* to the net not being used for trawling.
- Analyse the blank test sample following the procedures described in Chapter *6. Analysis method* in the same manner as for the actual samples obtained by trawling.
- If the number of the microplastics confirmed in the blank test sample is more negligible than the number of microplastics obtained through trawling (approximately 5% or more), the causes of the contamination will be determined, and the necessary measures will be put in place before deciding whether or not to conduct another survey.

7.2 The spiked recovery test

This test confirms whether the analyses are being conducted adequately. It is advisable to test all those who undertake the analytical work:

- The spiked recovery test sample will be prepared by an individual other than the examinee. The examinee will not be notified of the prepared content.
- Samples from past analyses and other microplastics obtained by crushing plastic products will be made available for the test. Take roughly the same amount as the samples actually collected and measure them according to *Chapter 6.2 Obtaining information*.
- Put the microplastics and other natural particles that were prepared and measured into a sample container. Fill the container with water filtrated through a 0.1µ filter to the same level as the other container of the sample actually collected. This will serve the spiked recovery test sample.
- The examinee will analyse the spiked recovery test sample according to the steps described in Chapter *5. Analysis method*.
- Compare the results reported by the examinee and the results measured by the individual at the time of preparing the spiked recovery test sample and confirm whether or not gap between the two results is small within 10% margin of error. If there is a wide discrepancy, the causes will be determined, and training will be provided to the examinees as necessary.

8. Data interpretation and reporting

Sampling methods and conditions at the time of sampling are summarized together with the analysis methods and results in the prescribed form.

Table 8.1 provides of an example of a form for entering relevant data.

Table 8.1. An example of a form for entering the relevant data

Items				Results Input				Unit	Explanation/ Input Examples
Sampling date and location	Sample name/ ID							-	
	Enter Time difference from GMT							-	
	Sampling date							-	Date/ month/ year
	Sampling time (Initial)							-	Hour/ minute/ second
	Sampling time (Final)							-	
	Season								
	Sampling Location (Name)							-	e.g., Tokyo Bay (Tama Riv. estuary)
	GPS Log	•Input style						-	Select sexagesimal (base 60) notation or decimal notation to input coordinates.
		• GPS Log (Initial Position)	-Latitude					N	Enter the coordinates in sexagesimal (base 60) or decimal notation.
			-Longitude					E	
		• GPS Log (Final position)	-Latitude					N	
			-Longitude					E	
Sampling equipment	Classification of net frame	• Type of net frame						-	Manta, Neuston or other nets
		• Model number and manufacturer						-	Eg., JMA Neuston net, RIGO Co., Ltd., No.5552
	Net aperture	• Shape of net aperture						-	Rectangular, square, circular, others
		• Size of net aperture	-Width					m	
			-Height					m	
			-Area					M^2	
	Length of net							m	
	Mesh	•Openings						mm	
								-	Select one side length or diagonal length
		•Model number and manufacturer						-	
	Tow distance	•Distance						m	Distance relative to water

Tow Parameter		• **Calculation method**					-	Describe the method used to calculate the tow distance such as: 1: Flow meter, 2: GPS (Recorded only initial and final points), 3: Vessel speed and duration time
		• **Calculation formulas**	Distance=		Rotation*factor		-	
	Trawl sweep area	• **Sweep area**					m^2	Report sweep area and the equations used to calculate it.
		• **Calculation formulas**	Area=				-	
	Filtered water volume	• **Water volume**					m^3	Report filtered water volume and the equation used to calculate it
		• **Calculation formulas**	Volume =					
	Tow duration						min	
	Vessel speed						m/s	Speed relative to water e.g., 1,5 m/s
	Tow position							The side of a vessel or the stem of a vessel
	Distance from vessel						m	
	Net immersion	• **Net immersion depth**					m	
		• **Percentage of net immersion depth to size of net**					%	
		• **Whether or not there was any change in the immersion depth during tow**					-	
	Tow direction		Current → Wind→				-	e.g., direction relative to land, wind, ocean current, sources (reverse, etc)
	Blank test	• **Whether or not blank tests were conducted**					-	Evaluate the effect of contamination on sea-surface plastic concentrations during
		• **Results**					Particles/ sample	

					onboard sampling
Laboratory analysis					
Density separation	**Whether or not density separation was conducted**			-	Record “Conducted” or “Not conducted”.
	Type of solution used for density separation			-	e.g., NaCl, $ZnCl_2$
	Concentration of solution used for density separation			%	
	Processing Time			min	Optional
Biological digestion and chemical treatment	**Whether or not biological digestion or chemical treatment was conducted**			-	Record “Conducted” or “Not conducted”.
	Methods used for digesting organic matter			-	Acid treatment, alkali treatment, enzyme treatment, oxidation treatment, etc
	Temperature during processing			°C	
	Reaction time			min	
Sample splitting	**Whether or not sample splitting was conducted**			-	Record “Conducted” or “Not conducted”.
	Method or equipment of splitting			-	e.g., Folsom
	Estimated relative error range caused by your splitting process			%	
Picking of microplastic particles	**Whether or not pretreatment before picking out particles conducted**			-	Record “Conducted” or “Not conducted”.
	Type of pretreatment			-	e.g., removing non-plastic particles, size classification of plastics using sleves
	Whether or not picking was conducted under stereo microscope			-	Record “Used” or “Not used”
Counting and measuring sizes of particles	**Method of size fractionation**			-	Whether maximum diameter was measured or sleves were used
Identification of	**Whether or not composition analysis was conducted**			-	“Conducted” or “Not conducted”.
	Method of composition analysis			-	e.g., FTIR, Raman

microplactics					spectroscopy, etc. When using methods other than spectroscopy to check the material (pricking with a heated needle, grinding with a forceps, etc), describe them.
	Percentage of the particles subjected to composition analysis			%	
Weight measurement	**Temperature of sample drying**			˚C	
	Humidity of sample drying			%	
	Processing time of sample drying			min	
	Methods of weight measurements			-	e.g., weighing the particles directly on a scale, weighing the mass of the vial and microplastic together and subtracting the mass of the tared vial to provide the mass of the microplastics.
QA/QC	**Blank tests**	• **Whether or not blank tests were conducted**		-	“Conducted” or “Not conducted”.
		• **Results**		Particles/ sample	Outline procedure and results of blank tests in the laboratory analysis
	Spiked recovery tests	• **Whether or not blank tests were conducted**			“Conducted” or “Not conducted”.
		• **Results**		particles/ sample	Outline procedure and results of blank tests in the laboratory analysis
Results					
	Maximum Feret’s diameter 1.0 ≤d ≤5.0	• **Number of particles**		particles/ sample	Record data in at least one of the three units given on the left, and provide information for converting

Weight and number of plastic particles										data, if possible
		• **Particle density (per filtered water volume)**							particles/m^3	
		• **Particle density (per trawl swept area)**							particles/m^2	
		• **Total weight**							g	
	Maximum Feret's diameter d<1.0	• **Number of particles**							particles/ sample	Please note that for particles less than 1 mm, final results could be regarded as underestimated (See pp. 15*18, pp 47*48 in the Guidelines).
		• **Particle density (per filtered water volume)**							particles/m^3	
		• **Particle density (per trawl swept area)**							particles/m^2	
		• **Total weight**							g	
	Maximum Feret's diameter d≥5.0	• **Number of particles**							particles/ sample	
		• **Particle density (per filtered water volume)**							Particles/m^3	
		• **Particle density (per trawl swept area)**							Particles/m^2	
		• **Total weight**							g	
	Total	• **Number of particles**							particles/ sample	
		• **Particle density (per filtered water volume)**							particles/m^3	
		• **Particle density (per trawl swept area)**							particles/m^2	
		• **Total weight**							g	
	1.0 ≤d ≤5.0	• **Shapes of microplastic particles**	- Category	Fragment	Beads	Pallets	Fibers	Others	Total	
			-Percentage						0.0%	

Properties of the plastic particles		• **Material of microplastic particles**	- Category	LDPE	PP	Others	HDPE	PU	Total	Please input the top five categories in descending order of the observed characteristics of the collected plastic particles in each sample. When entering, please also enter the percentage (%) data. <Shape> Fragments, beads, foam, pellets and fibers are classification categories by shape commonly seen in many studies that currently perform classification by shape. <Material> e.g., PP, HDPE, LDPE, PU <Color> Black, blue, white, transparent, red, green, multicolors and others are introduced as the most common classification categories.
			-Percentage						0.0%	
		• **Colors of microplastic particles**	- Category	Transparent	White	Red	Orange	Yellow	Total	
			-Percentage						0.0%	
	d<1.0	• **Shapes of microplastic particles**	- Category	Fragment	Beads	Pallets	Fibers	Others	Total	
			-Percentage						0.0%	
		• **Material of microplastic particles**	- Category	Transparent	White	Red	Orange	Yellow	Total	
			-Percentage						0.0%	
		• **Colors of microplastic particles**	- Category	Transparent	White	Red	Orange	Yellow	Total	
			-Percentage						0.0%	
	d≥5.0	• **Shapes of microplastic particles**	- Category	Fragment	Beads	Pallets	Fibers	Others	Total	
			-Percentage						0.0%	
		• **Material of microplastic particles**	- Category	LDPE	PP	Others	HDPE	PU	Total	
			-Percentage						0.0%	
		• **Colors of microplastic particles**	- Category	Transparent	White	Red	Orange	Yellow	Total	
			-Percentage						0.0%	
	Total	• **Shapes of microplastic particles**	- Category	Fragment	Beads	Pallets	Fibers	Others	Total	
			-Percentage						0.0%	
		• **Material of microplastic particles**	- Category	LDPE	PP	Others	HDPE	PU	Total	
			-Percentage						0.0%	
		• **Colors of microplastic particles**	- Category	Transparent	White	Red	Orange	Yellow	Total	
			-Percentage						0.0%	

Note: In “Category” column, “Basic” indicate basic information for producing horizontal distribution maps of microplastic densities. “Essential” indicate information essential for allow comparisons with other data. Data without “Basic” or “Essential” in the column are those to be obtained optionally depending on the specific purpose if individual surveys or instrument availability. Reporting all data obtained, including meta data sets (refer Chapter4, pp62*65), is recommended.

9. References

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