Mekong River Commission
For Sustainable Development

PROTOCOL FOR MONITORING MICROPLASTICS IN FISH

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

Protocol for Monitoring Microplastics in Fish:

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January 2024

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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, which 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 strangling 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 aimed 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 in five different areas: (i) hydrology and hydraulics; (ii) sediment and discharge; (iii) water quality; (iv) aquatic ecology; and (v) fisheries. The MRC has long experience with environment and fisheries monitoring in these key areas; the MRC WQM dates back to 1993 and to date is carried out in 22 WQM sites spread throughout the mainstream and major tributaries in the LMB. The MRC fisheries monitoring began in 1994 and consists of three types of monitoring: (i) fish abundance and diversity monitoring (FADM); (ii) fish larvae and juvenile drift monitoring (FLDM); and (iii) dai (bag net) fishery monitoring. Implementing agencies in the four Member Countries has been implementing the FADM programme 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 each in Cambodia (Mekong and Tonle Sap Rivers), Viet Nam (Mekong and Bassac Rivers) and Lao PDR (Mekong and Sekong Rivers). Dai fishery monitoring has been implemented only at Tonle Sap River in Cambodia since 1995, which is located in the lower section of the River, spanning more

than 30 km across the municipality of Phnom Penh and Kandal Province. Due to the transboundary nature of monitoring, 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 the United Nations Environment Programme (UNEP) have signed a Memorandum of Understanding (MoU) to, inter alia, work on water quality monitoring including plastic waste leakage into the Mekong river system. Under this partnership arrangement, in 2019, the MRC supported 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, which includes regional workshops, capacity mapping for plastic pollution in the Mekong Basin and support to the pilot projects in the four MRC Member Countries, i.e. 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 have agreed on several areas of cooperation including in 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 final 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 processes.

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 LMB countries and the 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 protocols for monitoring riverine macroplastics, riverine microplastics 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).

1.1 Rationale

Today, marine plastic debris is a worldwide issue for which every country must take urgent action. 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, account for 88–95% of transportation of the global load.

Riverine and marine plastic debris have diverse sources of leakage from land. Large plastic debris, such as macroplastics (larger than 25 mm in diameter), is considered to leak mainly 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 the disintegration of larger debris. However, the actual

behaviour of plastic debris is yet to be clarified including its leakage sources and transportation in the water.

To solve these issues, several organizations have established a working plan on monitoring riverine and/or marine plastic debris, such as the Association of Southeast Asian Nations (ASEAN) Regional Action Plan. Still, the LMB does not have 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 (whose definitions are provided in the following chapter), and to gather information and knowledge to inform decision-making for the 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** should cover riverine macroplastics, riverine microplastics and microplastics in fish, and will be developed based on the following approaches:

- **Pillar 1**: The 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**: The protocol for riverine microplastics monitoring should be conducted every five years at a **fewer selected monitoring stations** along the Mekong mainstream and its major tributaries in the four Member Countries by relevant national research institutes or line ministries of the four Member Countries for sample collection in the field and a qualified national laboratory in one of the Member Countries for laboratory analysis.
- **Pillar 3**: The protocol for microplastics monitoring in fish should be conducted every five years at **fewer selected monitoring stations** along the Mekong mainstream and its major tributaries in the four Member Countries by relevant national research institutes or line ministries of the four Member Countries for sample collection in the field and a qualified national laboratory in one of the Member Countries for laboratory analysis.

This protocol will provide information on how the number of microplastics present in fish species is an indicator of overall environmental pollution. It will not explain how microplastics negatively impact fish species and human health, such as from exposure to chemicals associated with the plastics. It will clarify the correlation between the surface microplastics monitoring results and the microplastics found in the digestive tracts of fishes. Since it is difficult to link the microplastics ingested by aquatic birds and mammals with the survey sites' environment, such analyses are outside the scope of this protocol. However, the analysis methods showcased in this report could be used as a reference towards learning about the microplastics ingested by aquatic birds, mammals, and other shellfish and benthic organisms.

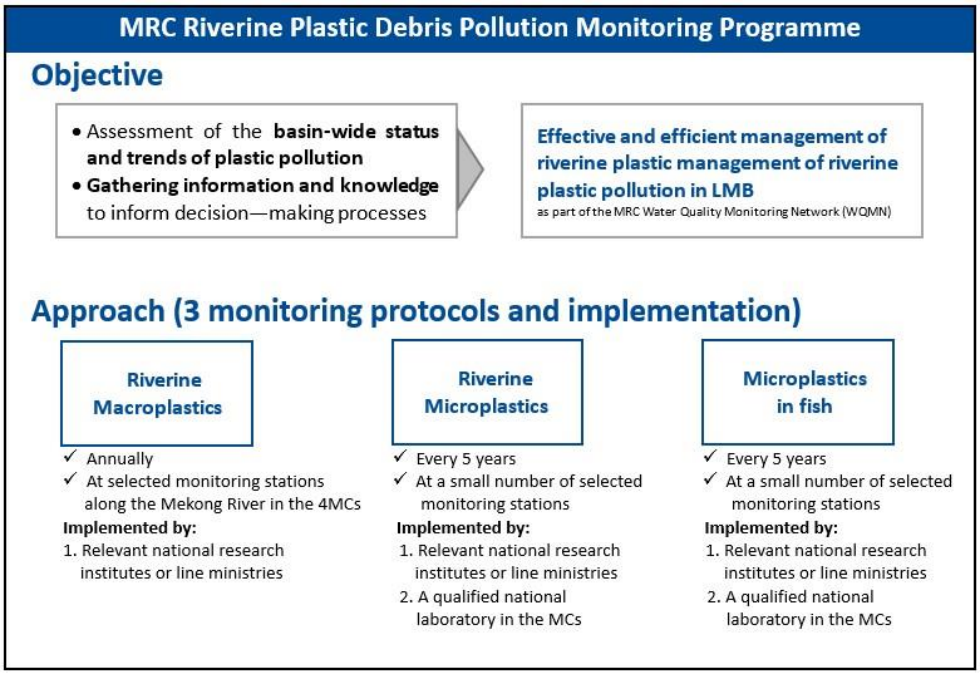


Figure 2.1. The scope of the monitoring programme and monitoring protocols

2.2 Objectives and scope of monitoring microplastics in fish

(1) Objectives and scope of microplastics in fish

The purpose and scope of monitoring microplastics in the digestive tracts in fishes are to understand the level of plastics and microplastics contamination in rivers, contamination taken into fishes, the trends as well as their distribution throughout the Lower Mekong Basin (LMB).

(2) Definition

a) Microplastics

In the field of marine debris monitoring, the Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) has introduced the following size range categories of plastic marine debris:

- Megaplastics (>1 m)
- Macroplastics (25 mm – 1 m)
- Mesoplastics (5–25 mm)
- Microplastic (<5 mm).

Regarding riverine debris monitoring, United Nations Environment Programme (UNEP) defines the same debris size range categories as the GESAMP ones 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.

b) Plastics

Plastics are individual substances artificially configured into useful shapes using high-molecular substances (mostly synthetic resin) as the main ingredients. Regarding riverine debris monitoring, rubber, paint and adhesives are excluded because it is difficult to analyse them since their particles are too small for the conventional analysis method; most of these that are found in the environment would pass through a sampling net and are often made of various materials, and some mixed with natural ingredients.

2.3 Steps in the procedures for monitoring microplastics in fish

Monitoring follows the steps of planning, sampling, analysis, and reporting, as shown in Figure 2.2, and their respective methods are shown in chapter 4. Survey plan, chapter 6. Sampling method, chapter 7. Analysis method, and chapter 9. Data interpretation and reporting. Also, the methods for the blank test, which checks for any contamination that may have occurred between the sampling and analysis, and the spiked recovery test, which checks whether adequate values have been obtained in the analysis, are shown in chapter 8. Quality assurance and quality control.

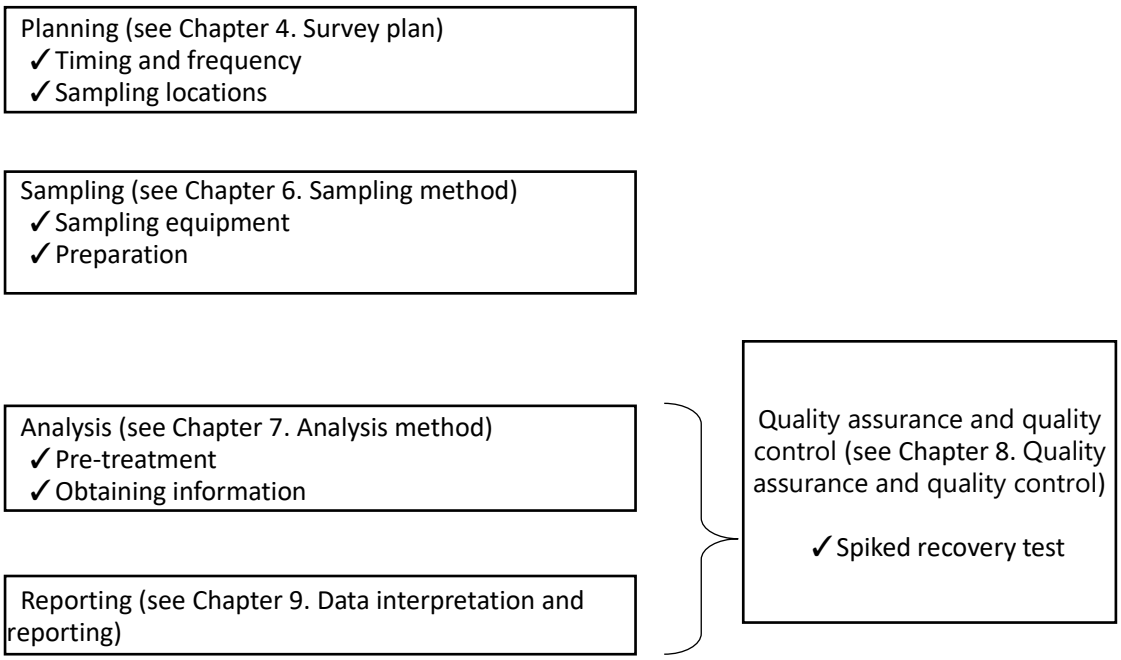


Figure 2.2. Steps in riverine microplastics monitoring

3. Summary of the survey method

The protocol for monitoring microplastics in fish consists of two components:

1. **Sampling:** collection of fish together with local fishers.
2. **Analysis:** analysis of microplastics that are contained in the digestive tracts of collected fish through chemical processing, and identification of the characteristic such as colour, shape and material in laboratories.

The following section summarizes the two monitoring components and the necessary equipment, and each is discussed in the following chapters: chapter 4. Survey plan, chapter 5. Selection of fishers, chapter 6. Sampling method, chapter 7. Analysis method, chapter 8. Quality assurance and quality control, and chapter 9. Data interpretation and reporting.

Microplastics in fish

Planning (see chapter 4. Survey plan)

Timing and frequency

✓ Twice per year (once in the wet and once in the dry season) to obtain the average river condition for each season.[1]

Survey locations

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

Selection of fishers (see chapter 5. Selection of fishers)

✓ It is recommended that at least three experienced fishers be chosen for cooperating in the monitoring according to capabilities and availability.

Sampling (see chapter 6. Sampling method)

Collecting samples

✓ Any fishing method can be applied in collecting fish samples. However, if there is concern about the method for implementing the procedures, refer to the Standard Sampling Procedures for Fish Abundance and Diversity Monitoring in the Lower Mekong Basin (Ngor et al., 2016).

Preparation for the analysis

✓ Wash and keep the samples of the target species in the container with labels (gear, mesh size).
✓ Keep the packed samples in an icebox filled with ice, and transfer the samples to a laboratory within an hour; should it take longer, keep the samples cool by adding ice.

[1] The monitoring frequency may be adjusted to be aligned with the frequency of the MRC's Multi-Media Monitoring and Assessment Study, where concentrations of selected heavy metals, pesticides, and fish are monitored once every five years to support the preparation of the MRC's State of the Basin Report.

- ✓ In the laboratory, weigh the samples and measure their body length.
- ✓ Record the species of the sample, together with the weight and body length.

Analysis (see chapter 7 Analysis method)

Anatomy

- ✓ Remove the digestive tracts using an anatomy scissors and tweezers.
- ✓ Measure the wet weight.

Decomposition of tissues of digestive tracts

- ✓ Add the 10% potassium hydrate solution to the digestive tract. Leave it in an incubator set at 50℃ for 2–3 days.
- ✓ Clean the sample in a hand-net with pure water or tap water.

Oxidation

- ✓ Add the cleaned sample and 100 ml of the hydrogen peroxide. Leave it at the room temperature for 2–3 days.
- ✓ Clean the sample in a hand-net with pure water or tap water.
- ✓ Move the sample to a glass petri dish.

Obtaining information

- ✓ Follow the same procedures as those stated in the Riverine Microplastic Monitoring Protocol.

Also, follow the quality assurance and quality control steps in chapter 8 to ensure the data quality.

Reporting (see chapter 9. Data interpretation and reporting)

- ✓ Captured fish: Record the location and fishing gear in which the fish was caught. Then, record its species, body length, and wet weight, as well as wet weight of its digestive tract.
- ✓ Microplastics in digestive tracts: Follow the same analytical steps as detailed in Chapter 6 of the Protocol for Riverine Microplastics Monitoring.

Necessary equipment

- ✓ Items in the following table will allow for an efficient implementation of the survey.

Category	Group	Item
Sampling (microplastics in fish)	**Ship equipment**	Any fishing gear including but not limited to dai (bag net), gillnet, lift net, push net, seine net
		Stakes, floats, weights for gillnets if necessary
	Measuring	Calibration weights
		GPS – used for recording positions where nets were set; check settings.
	Storing	Buckets for washing/holding fish
		Ice-chests and ice. Generally, three ice-chests with 20 L of ice and another empty ice-chest are required as a minimum.
		Small cloth towels
	Safety	Personal safety equipment (one unit include: safety hat, google, life vest, water boot)
		Head torches, mosquito repellent, hats, sunscreen
	Sanitation	First Aid Kit
		Insect spray (knockdown spray for flying insects)
	Stationary	Forms/logbook (chapter 7. *Data Interpretation and reporting*)
		Waterproof field data sheets
		Waterproof sample labels
		Waterproof markers
		Waterproof labels
		Digital camera – used for recording each catch and details of net setting locations. Record time and date, and set the parameters for maximum resolution.
Recording	**Anatomy**	Buffered formalin, (10% formaldehyde solution)
		Dissecting kit with scissors, scalpel, forceps
		Screwdrivers and cutters
	Measuring	Fish measuring boards, 1 m in mm increments.
		Calibrated electronic balance(s), 5 kg x 0.1 g; 10 kg x 10 g
		Calipers
		Plastic trays for fish photography.
	Stationary	Fish identification books and guides suitable for the sampling location

4. Survey plan

4.1 Field sampling variables to be recorded

(1) Season and moon phase
Fish catches are strongly affected by large-scale migrations and by moon phases. Fish are easiest to catch during the new moon and for the following four days; however, this must be checked by interviews at each site during the quarterly site visit of the responsible agency staff. Migrations are seasonal; many fish move upstream early in the wet season and return to their refuge areas late in the wet season, although fish from Tonle Sap Lake migrate upstream at the end of the wet season or early dry season. Recording catches every day allows for later analyses to determine the effect of these factors.

(2) Time and duration
Many river fish are more active and catchable at certain times during the year. Recording the day time or night time, i.e. the light conditions under which fishing gear are used, allows for further analysis of any effect of differences in the time that gears are set.

(3) River levels and discharge
Fish often migrate upstream at times of rising discharge, and downstream during falling discharge after the flood. These factors are generally controlled by sampling at the same time each year, since the Mekong's discharge pattern is highly regular by world standards, so fish migration timing is relatively predictable. Discharge data for analyses are obtained from the Technical Support Division of the MRCS.

(4) Water quality
High turbidity usually increases fish catch efficiency, because fish cannot see the net. General observations should be recorded at the time of sampling. Water quality data (excluding water turbidity) for analyses are obtained from the Environmental Management Division of the MRCS.

(5) Weather
Wind and rain, as well as sun shine and cloud cover, may affect fish behaviour and thereby catches. Details are entered on the field sheet (chapter 7. Data interpretation and reporting) at the time of sampling.

(6) Habitat
Fish tend to be found at certain ranges of depth and current speeds, and within snags and brush, depending on species and size. Information on these topics should be included in fishers' logbooks and noted on the maps of the sampling locations. These parameters must also be noted for the multi-panel gillnetting surveys and collected on each occasion.

(7) Fish migration
In addition to responding to external cues such as discharge and water quality changes, migration is driven partly by each fish's internal chemistry and physiology. Migrating fish become more catchable at certain times and places, so a good understanding of migration

patterns and timing should be gleaned for each location.

4.2 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 as follows:

- Microplastic in fish monitoring should be conducted both during the rainy and dry seasons, representative river conditions for each season, more than once in each season (same as the river survey methods).
- It is advisable to conduct the surveys in the seasons and times of day when it is easier to capture the target fish species.
- When conducting a survey in an estuarine basin, it is advisable to choose the same points in the tidal cycle each time, i.e. at the rising tide or at the ebb tide.
- In order to understand the level and the distribution of contamination, it is advisable to conduct a survey more than once every five years.
- In order to understand the trend of contamination, it is advisable to conduct a survey every year. (same as in the riverine microplastics surveys).

4.3 Sampling locations

It is recommended that survey locations be selected among ongoing MRC monitoring programmes including the Water Quality Monitoring Network (WQMN), FADM, FLDM, or hydrological monitoring. 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).

When selecting locations for monitoring microplastics in fish, the following should be considered:

- There should be a balanced number of monitoring locations among Member Countries, with monitoring locations for each country located upstream, midstream and downstream of the Mekong River.
- Monitoring locations should represent a major fishing location.
- Monitoring locations should be located downstream of major cities or industrial areas;
- Monitoring locations should be located in the tributaries if data and information on plastic leakage sources are desired.
- Monitoring locations should be located 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);
- A monthly survey should preferably be conducted at least twice, in the dry and rainy seasons if the budget allows.
- A survey conducted should be at three locations including the centre, the right bank and the left bank of the river in order to generally cover the fish species inhabiting

various environments at each survey point.

- Many of the fish species in the Mekong River migrate seasonally. In the rainy season (the period with much water), they move to tributaries and return to the mainstream in the dry season (the period with little water). When selecting a survey site, it is advisable to consider these behaviours of the target species.
- In the estuary area, the sample should be taken at ebb or low tide. Estuaries are subject to complex flow dynamics because they are influenced by both the tide and the freshwater discharge, which in turn influence plastic transport and export into the ocean. It is recommended to take into account the complex dynamics in the estuaries. It is also recommended to select a location upstream of the deltaic section of the river.

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

5. Selection of fishers

As currently practised, it is proposed that a minimum of three experienced fishers be selected at each sampling location. This decision represents a compromise between the need for precision and affordable survey costs. If possible, it is recommended that the number of fishers per location be increased or the frequency of sampling catches be reduced to 2–3 days per week (randomly selected), and the number of fishers is doubled.

The selected fishers must meet the following criteria:

- They are 'typical' medium-scale, full-time fishers at the survey location. They fish most days of most weeks (<200 days per year), and fishing is their primary occupation for income generation.
- They fish from motorized boats, depending on the season, and use medium-scale gears, such as the gillnet, trammel net or trawl. It is recommended that all fishers use gillnets as a standardized gear because it enables enhanced comparisons between locations. The mesh size of the gillnets used should be recorded on each sampling occasion to account for selectivity.
- They are able to read and write.
- They are willing to participate in the survey and must be honest in recording fish catches.
- Priority is given to fishers associated with organized fisher groups, such as community fisheries or a fish refuge pond community, if these types of organizations are present in the survey location.
- They have adequate experience and capacity, as well as good eyesight for identifying fish and recording data.

The fishers must be trained and tested to ensure that they are able to:

- set up and maintain a clean and tidy work area properly;
- identify fish accurately;
- check and calibrate balances;
- measure fish accurately and record the data; and
- take clear photos of fish laid out in the correct way to facilitate data checking.

Where possible, fishers participating in data collection activities should be retained as long as possible and continuously trained and tested in measuring samples and recording the relative information

Fishers use a variety of fishing gears, varying in accordance with seasonality of fish occurrence. Therefore, all fishers must record their daily catches according to each gear type and the respective habitat on a given day. They must record the fishing effort accurately, including the dimensions of the fishing gear, especially the mesh size, and duration of tow/soak.

6. Sampling method

6.1 Target species

For the MRC's Microplastic in Fish Monitoring Protocol, fish sampling will not be based on specific species, but rather on a combination of feeding habits (carnivorous/herbivorous), habitat (bottom mud/middle layer/surface layer), and feeding method (filter feeding or not).

6.2 Sampling method and equipment

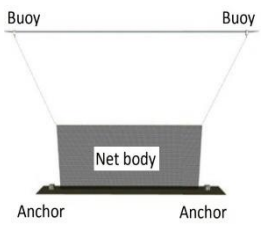
Any fishing gear used for sampling must be suitable for catching the target species to ensure the achievement of the objective of the monitoring. For this protocol, it is more important to obtain fish species for further laboratory analysis. The type of fishing gear used is not as important. However, if there is a concern about the method of implementing procedures, refer to the Standard Sampling Procedures for Fish Abundance and Diversity Monitoring in the Lower Mekong Basin (2019).

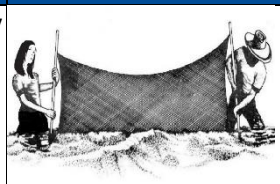
In order to accurately identify the locations of the catches and to observe the correlation with the quality of the water, on-site sample collection is recommended in line with the MRC's FADM programme rather than purchasing the samples from markets. However, in the case that the sufficient amount and the target fish species cannot be obtained using the sampling method of the FADM and an exact location for sampling cannot be identified, then purchasing samples at markets is also a possible choice.

The captured fish should be preserved by either freezing or by using the 10% formalin concentration of 10%. Since formalin is toxic for human beings, the use of a freezer is recommended where possible.

Examples of survey methods for the fish species are shown in section 6.1 Target species.

Table 6.1. Examples of survey methods for the fish species

Method	Description	Image
Dai (bag net)	• The dai net consists of a nylon bag net suspended between scaffolding formed from tree trunks. The Dai net is positioned near the outflow of the lake and in the river to catch fish as they migrate down river. • Typically, 25–45 m wide and 100 m long.	
Gillnet	• The gillnet is a common throughout the LMB and composed of vertical panels of netting that hangs from a line with regularly spaced floaters that hold the line on the surface of the water. Most nets are 50 m or longer.	

Method	Description	Image
Seine net	• The seine net is a fishing gear that captures fish by seining the water column.	
Lift net	• The lift net is submerged to a certain depth and then lifted out of the water vertically. The lift net comes in various sizes.	
Cast net	• The cast net is a circular net with small weights distributed around its edge. Contemporary cast nets have a radius of 1.2 m to 3.6 m) (4 to 12 feet).	
Push net	• The push net is a small triangular fishing net with a rigid frame that is pushed along the bottom in shallow waters.	

Note: * In Cambodia, the Fisheries Law prohibits the use of any gillnets for personal use that are longer than 10 m. In addition, *say yoeun*, i.e. nylon mosquito netting fences with traps, are illegal gear.
** In Thailand, fishing with seine nets and bag nets is illegal.

Source: Inland Fisheries Research and Development Institute (2020), MRC (2004a), MRC (2007), MRC (2013a)

6.3 Sample collection

For the sampled fish, record the necessary information, such as the monitoring location, name of the sampler, and the survey duration (Table 6.2). Also, in the record form, record the fishing gear in which the fish was caught as well as its characteristics such as species, body length, and wet weight, as well as the wet weight of its digestive tract (Table 6.3).

Table 6.2. Record form for the survey profile

Items	Record
Name of observer	
Affiliation	
Survey duration	
Survey location (name)	
Latitude	
Longitude	

Table 6.3. Record form of each fish caught

Date	Fishing Gear	Species name (Scientific name)	Species name (local name)	Habitat (demersal, pelagic, etc)	Body length (cm)	Standard length (cm)	Wet weight (g)	Wet weight of the digestive tracts (g)	Analysis of microplastic (name of excel file)

The statements below are taken from the Standard Sampling Procedures for Fish Abundance and Diversity Monitoring in the Lower Mekong Basin (2019), which must to be followed in recording the characteristics of the captured fish.

(1) Preparation

In the field, the fish are removed from each gear, washed and stored in plastic containers or bags labelled with unique names (including type of gear and its mesh size). Other aquatic organisms such as shrimps, crabs and snakes should be released to the environment.

Samples should be stored in an icebox in layers of crushed ice and transported to a field laboratory for processing within one hour of removal from the fishing gears. Should the processing be delayed, the fish should be stored in refrigerators, or if on ice, the ice must be periodically re-filled.

Do not combine any samples from different gears. Store and process each sample individually.

For each sample, proceed as follows:

1. Prepare a clean work area and flat work table that is free of any unnecessary items.
2. Ensure that the working bench area is well-lit by natural or artificial light.
3. Keep onlookers and domestic animals away to prevent them from distracting people from their work.
4. Use a spirit level to make sure that the balance and measuring board are horizontal.
5. Shield the balance from any wind or breezes.
6. Wash all fish in clean fresh water and remove all detritus and sediment.
7. Drain off the water and arrange all fish in trays, with the fish from each gear in separate groups, and arranged by location, gear, replicate, species and size (in descending order), using waterproof labels and a metric ruler. Labels should include the location, gear, date and time of gear retrieval, as well as the fisher's name. The fish on the trays should be covered with wet cloth towels to ensure that they neither absorb excess moisture nor dry out.
8. Identify fish correctly; it is fundamental for obtaining useful monitoring data.

(2) Length measuring

The fish should be measured and data recorded in a logical order by location, gear, species and size (in descending order). All fish should be individually measured and their details entered the field record sheet by responsible staff of the implementing national line agencies (chapter

7. Data interpretation and reporting).

For measuring each fish sample, the following steps must be carried out:

1. Each fish is measured on a measuring board, with its snout pressed gently against the vertical piece and its mouth closed.
2. The fish should not be stretched, bent or manipulated in any way, and should be in a natural position.
3. Measure each fish's fork length (FL) as shown in Figure 5.2. For fish with non-forked caudal fins (rounded, truncate or pointed), the total length is measured. When a fish is in position on the measuring board, a pencil or probe is placed on the ruler at the measurement point (behind the caudal fin), and the fish is then removed so that the ruler is clearly displayed.
4. One fished read aloud the number and units form the measurement (e.g. four-one-six mm for 416 mm) while another record the reading in the field data sheet. Fishers must be tested for their comprehension of the units of cm and mm, and what to record.
5. The fisher who measures the fish must ensure that the fisher who records the data correctly hears and records the measurements.

Figure 6.1. Body length measurement

(3) Weighing fish

For weighing the fish, the following steps must be carried out:

1. Use the same type of digital electronic balances wherever possible. Use other balances only for very large fish or large catches, or in the event of malfunction of electronic balances.
2. Calibrate each balance prior to use using a weight set. Calibration weights should be handled using forceps and cleaned with tissue paper.
3. Fish must be weighed in a plastic pan. Never put fish directly on the balance because water and slime may run inside the balance and damage it.
4. Tare the balance (i.e. to 0.0) with the pan on it before weighing each fish.
5. Check that the airflow is not moving the pan, and use shields to prevent drafts.
6. Blot excess water from each fish using a damp cloth towel; fish should be weighed damp.
7. Place each fish in the pan and read the units aloud (e.g. four-six-point-three) for 46.3.
8. The fisher who weighs the fish must ensure that the fish who records the data correctly hears and records the measurements.
9. Clean any excess water from the weighing pan and re-tare the balance before each measurement.

(4) Photographing fish

Photos are used in order to check data.

For photographing the fish, the following steps must be carried out:

1. Check camera settings – correct date, time, and highest image quality and size.
2. Check the camera lens and clean it as necessary.
3. On sunny days, take photos in the shade to reduce contrast, or take photos under a white sheet, which acts as a diffuser.
4. On shady days, take photos in the open.
5. Take photos about 1 m from the tray to reduce distortion or blurring.
6. Position the camera lens directly above the centre of the tray.
7. Check photos for clarity before processing the sample; if necessary, adjust the light conditions to take a better photo.

(5) Data storage

As soon as possible, data from field sheets are entered into standardized databases. Ideally, data are entered in the field, or immediately upon return to the office. The staff that carried out field sampling should enter the data or supervise the data entry. Field sheets should be photocopied or scanned for a backup and then stored systematically in separate files.

7. Analysis method

Figure 7.1 shows the steps of the microplastics analysis.

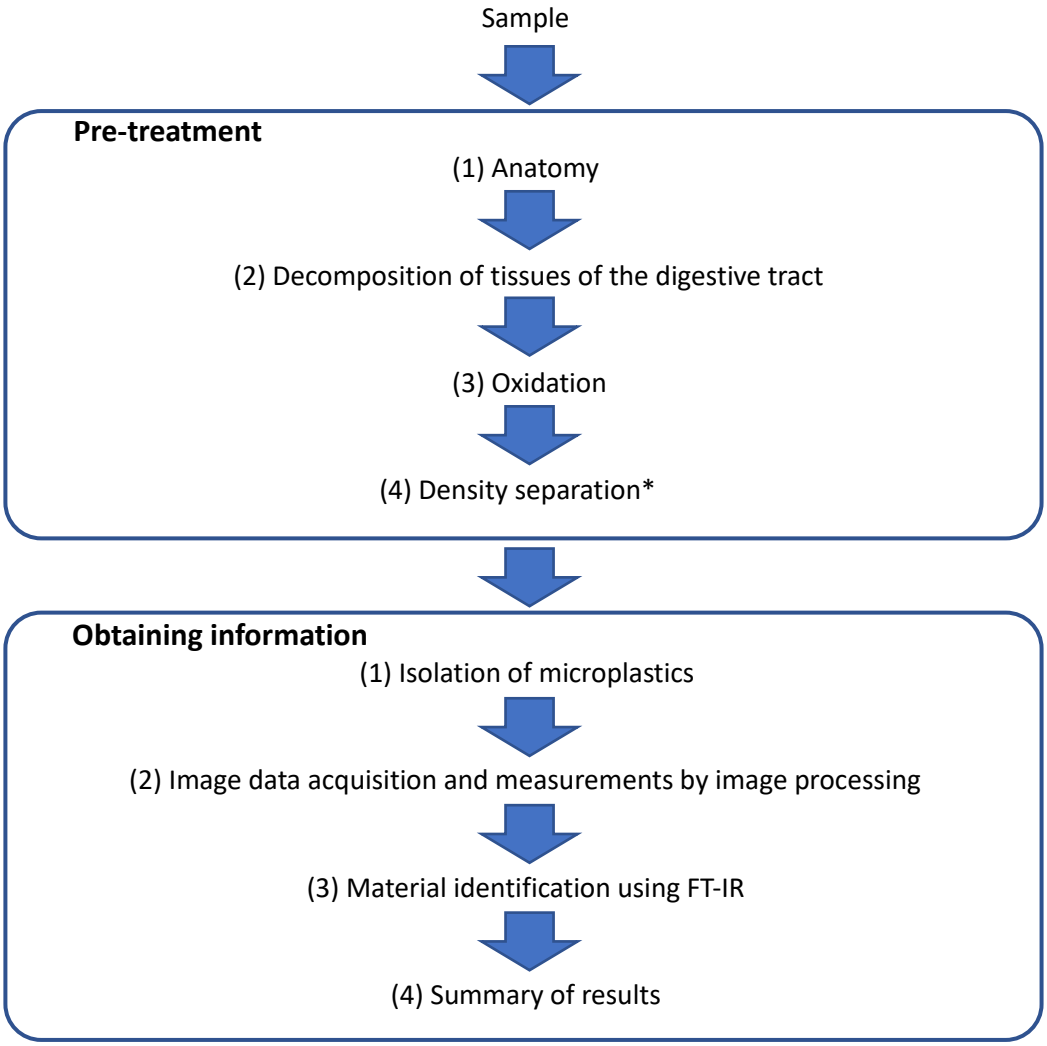


Figure 7.1. Steps for measure and analysing the number density of the samples

Note: *This process is applied when it is difficult to directly isolate the microplastics due to a large amount of suspended matter.

7.1 Pre-treatment

(1) Anatomy

Wash the samples thoroughly prior to the anatomy. Remove the digestive tract from each sample for the wet weight measurement.

Equipment

- Stainless steel trays
- Stainless steel anatomy scissors
- Stainless steel tweezers
- Electronic scale.

Procedures

1. Place a sample on a trey and remove the digestive tract (from the top of the esophagus to the end of the rectum) using an anatomy scissors and tweezers. Pay attention not to damage the digestive tract during the process since this can prevent the accurate count of the microplastics. If damaged, carefully collect the contents while gently cleansing with water.
2. Measure the wet weight of the removed digestive tract using a scale.
3. (The number of fish contained in each sample will be decided on according to the results of pilot projects.)
4. For small/medium samples, continue to (3) Oxidation section below.
5. For large samples, the content of the digestive tracts will be removed because it is difficult to chemically treat the digestive tract as a whole by the following additional steps.

Additional steps for the contents of large samples

1. When the digestive tract is taken out, open it with a knife and wash the content of the digestive tract with water.
2. Sift the washed samples using a hand-net with a mesh size of 0.1 mm.
3. If there is a small amount of content in the digestive tract**,** follow the same process as **(3) Oxidation section below**.
4. If there is a large amount of content in the digestive tract or if contains large material that are not easily degradable such as a whole fish, follow the same process as sections **(2) Decomposition of tissues of digestive tract** and **(3) Oxidation**.

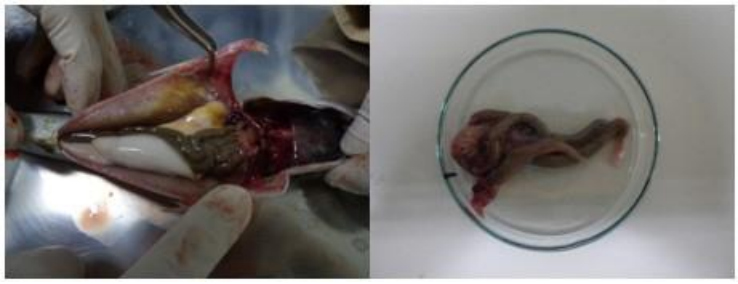


Figure 7.2. Anatomy (left) and the digestive tract removed (right)

(2) Decomposition of tissues of the digestive tract

A chemical treatment is applied in order to separate the digestive tract, digested matters and microplastics.

Equipment

- 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).

Procedures

1. Add the 10% potassium hydrate solution to the digestive tract (the amount can vary depending on the size of the digestive tract), and leave it in an incubator set at 50 °C for two to three days; the number of days can vary depending on the status of decomposition.
2. Transfer the treated digestive tract sample to a hand-net and clean it thoroughly with pure water or tap water; any trace of the potassium hydrate solution will react to hydrogen peroxide, which will be used afterwards. The potassium hydrate solution now drained will be properly treated for disposal.

(3) Oxidation

Equipment

- Hydrogen peroxide
- Container (pre-washed with pure water or tap water
- Hand-net (mesh size 0.1 mm) (pre-washed with pure water or tap water)
- Washing bottle (for water).

Procedures

1. Add the cleaned sample and 100 ml of the hydrogen peroxide (the amount can vary depending on the sample amount), and leave at room temperature for two to three days. (The number of days can vary depending on the status of decomposition.)
2. Transfer the treated digestive tract to the hand-net and clean it thoroughly with pure water or tap water.
3. Transfer the sample on the hand-net to a glass petri dish (Figure 7.3).

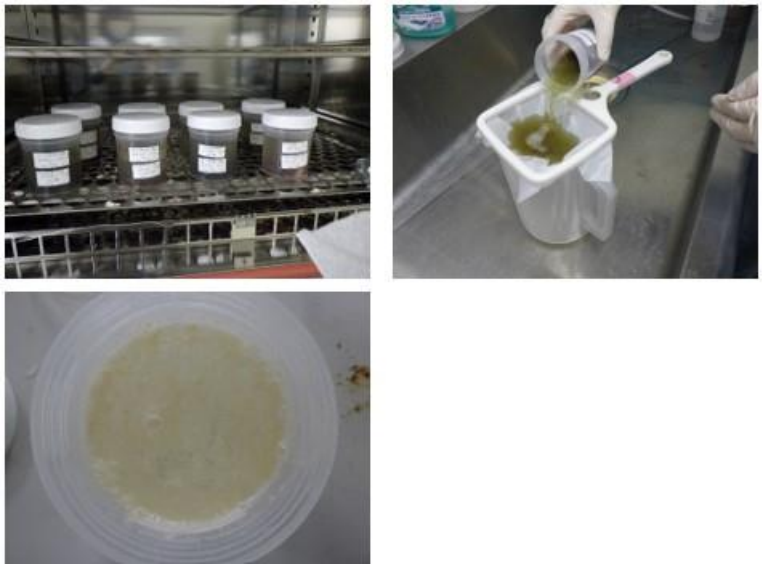


Figure 7.3. Oxidation procedure

Note: Add 10% potassium hydrate solution, and leave the sample at 50℃ (upper left). After the treatment with 10% potassium hydrate solution, clean it by filtering it through a 0.1-mm mesh (upper right) and add hydrogen peroxide (bottom left)

(4) Density separation (follow the same procedure as that for the Protocol for Monitoring Riverine Microplastics)

Additional note

In order to save labour and streamline the process, it is recommended to pre-process many samples together and analyse them as one sample.

7.2 Obtaining information

Refer to section 6.2 Obtaining information in Protocols for Riverine Microplastic Monitoring.

8. Quality assurance and quality control

8.1 Sampling

Changing the sample procedure or sampling under varying conditions or with different staff or field crews may cause large variations in results, so it is important to follow the Standard Operating Procedures and fill in the field data sheets accurately. Should it be necessary to modify the procedure at a location, full details should be recorded for future reference and for possible adjustment at other locations.

(1) Fish identification
An expert taxonomist compares a summary of the species at each location from databases against the set of photos from the field sampling. The taxonomist then signs off on the field data sheet and in the database with his/her confirming signature.

(2) Data check
The final computerized data are cross-checked against the contents of the field sheet, and against the photographs of each sample, in terms of:

- species recorded:
- total counts or approximate counts; and
- approximate size and weight ranges.

The data are also checked against the historical data for the location, and if any major changes are evident (i.e. lack of previously abundant taxa or dominance of previously rare taxa), the laboratory data recording book and photographs are re-checked.

8.2 The spiked recovery test

This section describes the quality assurance and quality control procedures for field sampling and laboratory analysis (including for the blank test or the recovery test).

(To prevent contamination, avoid using equipment with plastic component, and thoroughly wash equipment before each use)

This test confirms whether the analyses are being conducted adequately by the laboratory technicians. It is advisable that any technician undertaking analytical work for this protocol also conduct the spike recovery test, as part of their routine work.

Steps for undertaking spiked recovery test:

- A 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. Use roughly the same amount as the

samples collected and measure them according to section 5.2 Obtaining information.

- Put the microplastics and about 10–20 g of minced fish, which were previously prepared and measured, into a sample container. Fill the container with water filtered through a 0.1µ filter to the same level as the other container of the sample collected. This constitutes 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 with the predetermined results prepared by the examiner, and confirm the discrepancy between the results. The discrepancy is confirmed as small if the difference between the test result and predetermined result is with a 10% margin of error. If there is a significantly large discrepancy gap (greater than 10% margin of error), then the causes for the discrepancy must be investigated, and training must be provided to the examinees as necessary.

9. Data interpretation and reporting

The results of analysing the microplastics in digestive tracts of fish are recorded on the datasheet similar following the same procedures as those of the Riverine Microplastic Monitoring Protocol. The data recorder must ensure that all relevant information recorded in Tables 6.2 and 6.3 are transferred into the datasheet for each sample.

Table 9.1. An example form for entering the relevant data

Items			Results Input	Unit
Laboratory analysis				
Density separation	**Whether or not density separation was conducted**			-
	Type of solution used for density separation			-
	Concentration of solution used for density separation			%
	Processing Time			min
Biological digestion and chemical treatment	**Whether or not biological digestion or chemical treatment was conducted**			-
	Methods used for digesting organic matter			-
	Temperature during processing			℃
	Reaction time			min
Sample splitting	**Whether or not sample splitting was conducted**			-
	Method or equipment of splitting			-
	Estimated relative error range caused by your splitting process			%
Picking of microplastic particles	**Whether or not pretreatment before picking out particles conducted**			-
	Type of pretreatment			-
	Whether or not picking was conducted under stereo microscope			-
Counting and measuring sizes of particles	**Method of size fractionation**			-
Identification of microplactics	**Whether or not composition analysis was conducted**			-
	Method of composition analysis			-
	Percentage of the particles subjected to composition analysis			%
Weight measurement	**Temperature of sample drying**			℃
	Humidity of sample drying			%
	Processing time of sample drying			min
	Methods of weight measurements			-
QA/QC	**Blank tests**	• **Whether or not blank tests were conducted**		-
		• **Results**		Particles/ sample
	Spiked recovery	• **Whether or not blank tests were conducted**		

	tests	• Results							particles/ sample
Results									
Weight and number of plastic particles	**Maximum Feret's diameter 1.0 ≤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
	Maximum Feret's diameter d<1.0	• **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
	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
Properties of the plastic particles	**1.0 ≤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%
	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%

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