Guidelines for River and Lake Microplastic Monitoring Methods

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Ministry of the Environment, JAPAN

Contents

1 Introduction

Plastics smaller than 5 mm in diameter (hereinafter referred to as "microplastics") are found in the ocean around the world. Their impacts on marine environments are concerned. Microplastics have also been found in the seas around Japan and considered measures against their sources. For this consideration, it is necessary to understand the distribution of microplastics in the seas from land-based sources.

The guidelines set survey methods for the distribution of microplastics in surface water of rivers and lakes, the main route of the outflow from land. Based on the results of the surveys, it is expected that the measures against the sources of microplastics are advanced by cooperations between local governments and relevant organizations and citizens.

The guidelines will be modified as necessary according to new knowledge.

1.1 Target readers

Target readers of the guidelines are the staff of local public agencies and other organizations who conduct the survey on the distribution of microplastics in the surface water of river and lake, and cooperative researchers, research institutions, and business operators.

1.2 Target microplastics

The guidelines are applied to the survey on plastic and fiber pieces smaller than 5 mm in surface water of rivers and lakes.

It is assumed that nets with approximately 0.3 mm mesh openings are used to collect microplastics. However, plastics smaller than 1 mm are not completely collected with the 0.3 mm mesh nets, even if they are 0.3 mm or larger[I]. Therefore, these smaller plastics are measured as supplemental references. Additionally, plastics ranging from 5 to 25 mm (known as mesoplastics) are also measured as supplemental references, as these measurements can help us understand the distribution of plastic in surface water of rivers and lakes.

[I] Isobe et al., 2019. An interlaboratory comparison exercise for the determination of microplastics in standard sample bottles. Marine Pollution Bulletin. 146, 831–837.

2 Purpose

2.1 Purpose of survey

Purpose of survey is to understand the distribution of microplastics in surface water of rivers and lakes which are one part of microplastics that flow out from land to sea.

2.2 Outline of survey and measurement procedure

Figure 2-1 shows the survey and measurement procedure on river and lake microplastics.

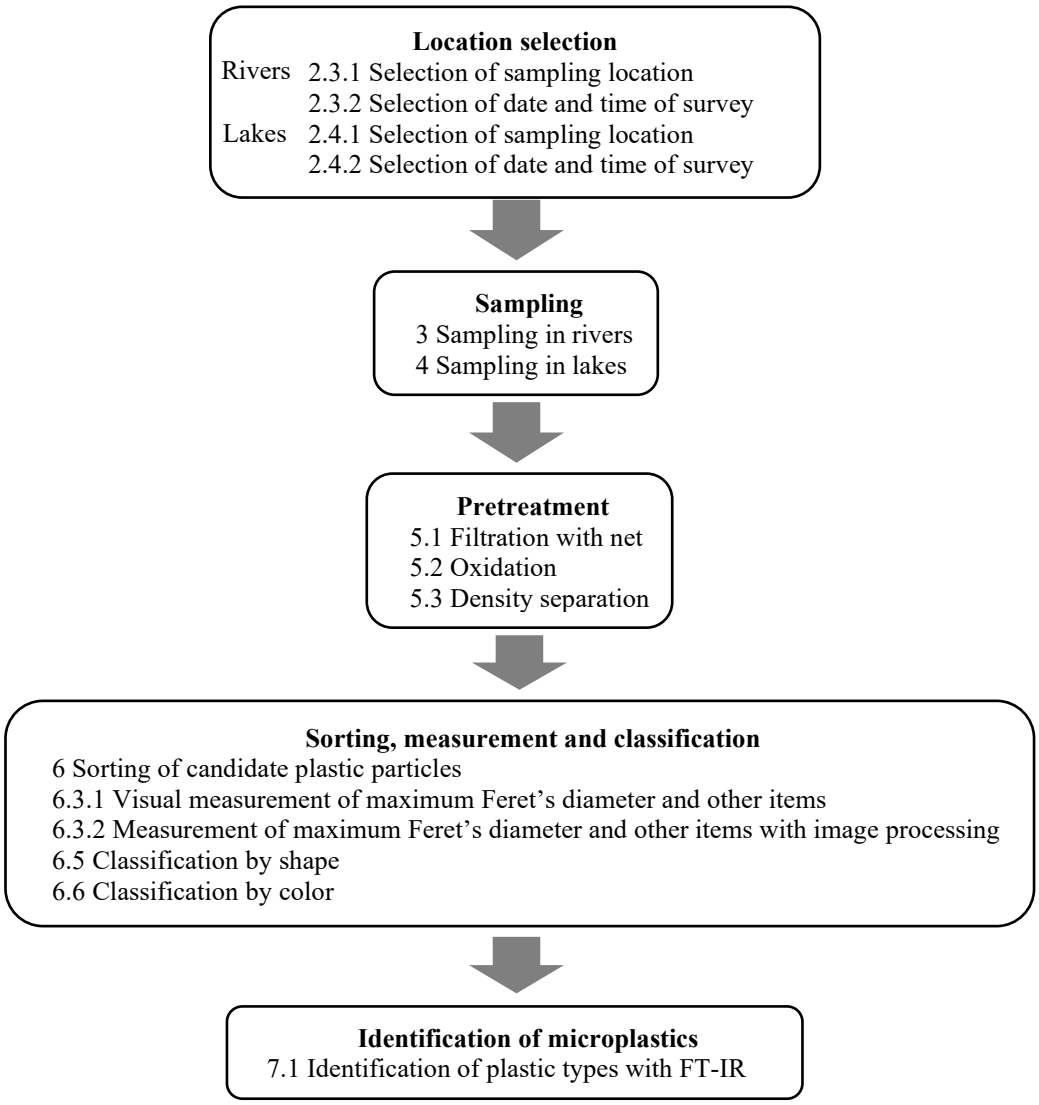


Figure 2-1. Survey procedure on river and lake microplastics.

2.3 Survey scheme on river microplastics

2.3.1 Selection of sampling location

1. For on-site filtration sampling, it is important to choose locations where the water flow velocity is equal to or greater than 0.3 m/s and the water depth is equal to or greater than 50 cm. If these conditions are not met, sampling can be done by net towing of boats (see “4.2 Net tow sampling”), using motorized survey devices (see "4.3 Sampling using motorized survey devices “) or with pumps (see “4.4 Sampling with pumps”) from the riverside. It is important to note that the density and composition of microplastics can vary widely in areas with low water flow velocity, and it may be challenging to ensure that the samples collected are representative of the overall area.
2. The locations designated as reference points for water quality or water level can be the sampling location. To survey the distributions of microplastics throughout the entire length of a river, multiple locations in upper, middle, and lower reaches of the river should be chosen.
3. In addition to those mentioned in 2, locations where plastic waste or microplastics flow out into rivers are suitable.

Examples of suitable survey location:

- Locations close to densely inhabited district (DID)
- Locations where a large amount of plastic waste or microplastics is on river flood plains.
- Locations designated as reference points for environmental monitoring where biochemical oxygen demand (BOD) and suspended solids (SS) are high.
- Locations where tributaries or irrigation canals meet the mainstream.[II]

2.3.2 Selection of date and time of survey

1. As specified in the water quality survey procedure (MoE water quality control No. 30, September 30, 1971), the day following a sunny streak should be chosen as the survey date to ensure stable water quality. The weathers at the sampling location must be recorded for seven days before the survey.
2. When the sampling location is in a tidal area, the sampling time is set in consideration of temporal variation in tide level and other factors. The period in which seawater does not run up and river flows from upstream to downstream (e.g., low tide period) is suitable.

[Reference] Research during or after flood event

Since the amount of microplastics outflow will increase during or after flood event, there may be a case in which field sampling will be conducted during the flood event. In such a case, the precipitation in catchment areas and water level must be recorded.

Under such conditions, it is necessary to pay attention to safety measures and avoid stepping into rivers to collect samples. In addition, it is necessary to pay sufficient attention to increased drags of nets and large drifting objects due to the increase in flow rate.

[II] Hale, R.C., Seeley, M.E., La Guardia, M.J., Mai, L.& Zeng, E.Y. (2020). A Global Perspective on Microplastics. *Journal of Geophysical Research: Ocean,* 125(1)

2.4 Survey scheme on lake microplastics

2.4.1 Selection of sampling location

1. The guideline considers sampling by net towing on boats as a primary method (hereinafter referred to as “net tow sampling”). Therefore, these locations must be sufficiently deep for safe cruising and towing. Locations with a risk of stranding and obstacles must be avoided (Photo 2-1). Because distances between the bottoms of boats and lakebeds differ depending on the boats, sufficiently deep locations should be checked in advance.

Photo 2-1. An example of a location with many obstacles (aquatic plants)

2. The locations designated as reference points for water quality or water level can be sampling locations. To survey the distributions of microplastics throughout the lake, approximately three locations in the center of the lake are chosen, and samples are obtained via net tow sampling.
3. Besides those mentioned in 2, locations where plastic waste or microplastics flow into lakes are suitable. However, locations where rivers join the lake and are affected by river water and groundwater must be avoided.
4. When it is difficult to prepare boats for surveys, sampling should be conducted from the lakeside using motorized survey devices or pumps. Considering the fact that the density and composition of microplastics vary depending on the surrounding environment, their representativeness of the whole lake may not be ensured.
5. When the outflow regions of the lakes had (have) sufficient streams, on-site filtration sampling (see “3 Sampling in rivers”) is available.

Examples of suitable sampling location:
- Locations designated as reference points for environmental monitoring where chemical oxygen demand (COD) and suspended solids (SS) are high.
- Locations close to densely inhabited district (DID)
- Locations where large amounts of plastic waste or microplastics are present on lakeshores.
- Locations where rivers flowing via density inhabited district (DID) inflow.

2.4.2 Selection of date and time of survey

1. As specified in the water quality survey procedure (MoE water quality control No. 30, September 30, 1971), the day following a sunny streak should be chosen as the survey date to ensure stable water quality. The weather at the sampling location must be recorded for seven days before the survey.
2. The sampling time should be set considering the flow phenomena of lake water masses caused by changes in water temperature and wind direction and velocity. For example, the time when the wind blows strongly in one direction is not suitable. The sampling time must be recorded.
3. In deep lakes, underwater environments differ greatly between seasonally circulated and uncirculated periods.

[Reference] Flow phenomena in lakes[III]

In lakes, various flows are caused by a combination of extrinsic factors (e.g., winds, hearts, outflows and inflows of river water and seawater, gravities, and air pressures) and intrinsic factors (e.g., the topography of the lake bottom, lake areas, and depths). These flows continue for various periods (e.g., daily and seasonal). Seasonal changes in the water temperature are related to large-scale flow phenomena. Generally, in deep lakes, the surface water becomes warm and light, and water masses are stratified (i.e., thermal stratification) in spring and autumn (i.e., uncirculated period). However, the surface water becomes cold and heavy in winter and sinks to the bottom. Subsequently, vertical large-scale circulations occur (i.e., the circulation period).

3 Sampling in rivers

3.1 Outline

The samples containing microplastics are collected from surface water of rivers.

3.2 Equipment

3.2.1 Net

Plankton nets are used for sampling.

A flow meter and a bottom pipe (or cod end) made of rubber or metal are installed to the mouth and the end of a commercial short-cone-shaped plankton net (mouth size diameter: 30 cm, mesh opening size: 0.3 mm, length: 75–100 cm) that is used for collecting underwater plankton in rivers (Photo 3-1). A float is attached near the bottom pipe (or cod end) for even spreads of the net (Photo 3-2).

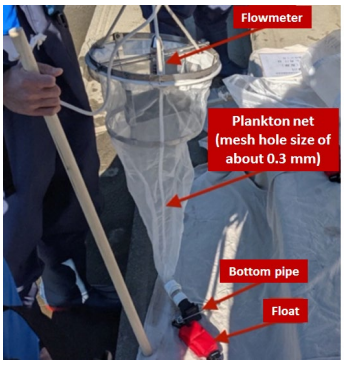


Photo 3-1. An example of plankton net

Photo 3-2. An example of sampling with a plankton net

[III] MLIT Ministry of Land, Infrastructure, Transport and Tourism. "Chapter 1. Hydraulic and Water Quality Phenomena in Lakes". MLIT Ministry of Land, Infrastructure, Transport and Tourism. https://www.mlit.go.jp › kosyo › tec › pdf › 1.pdf, (accessed on 2023-03-08) (in Japanese)

[Reference] Square net

- A square net used to collect benthic organisms is modified for the sampling (Photo 3-3).
- A net with approximately 0.3 mm mesh openings is laid on a square frame, and a metal bottom pipe (or cod end) is installed to the bottom of the net.
- It is necessary to place a customer order in order to procure a square net with specifications indicated below. The price of a custom-made square net is higher than a commercial plankton net.

A typical square net (frame size: 30 × 30 cm) that is available on the market has large mesh openings (0.475 mm) and a small open area ratio. Therefore, it is not suitable for collecting microplastics. To collect microplastics, it is necessary to use a net with approximately 0.3 mm mesh openings and an open area ratio calculated using the expressions below:

*Open area ratio: filtration area of a net to the net mouth

It is calculated using the following equation:

(For a net with 0.3 mm mesh openings,
the open area ratio must be approximately 3.6.)

$$R = a\beta / A$$

R: open area ratio, a: surface area of net gauze,

β: porosity of net gauze, A: net mouth area

The porosity of net gauze is calculated using the following equation:

$$\beta = m^2 / (d + m)^2$$

m: mesh width, d: meshwork strand diameter

(Source: Guideline of Ocean Observations, 4th Edition, vol.6)

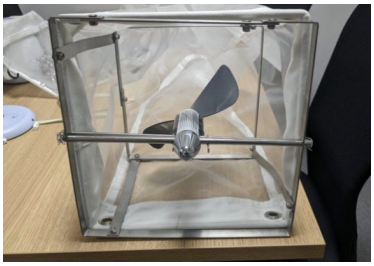


Photo 3-3. An example of square net

3.2.2 Flow meter

1. A flow meter is installed at the mouth of a net and water volume that has passed through the net (hereinafter referred to as “filtered water volume”) is calculated from the count of revolutions of the rotor.
2. As rotors in flow meters sometimes do not work accurately due to breakdowns and aging degradations, it is necessary to verify that the rotor functions smoothly with the water flow in every sampling.

3.2.3 Specifications of flow meter

- In the sampling under a low-flow condition in rivers, a digital flow meter with a rotor for low flow velocity is connected (Photo 3-4).
- Measuring range
  Low flow velocity rotor: 0.02–1.00 m/s
  High flow velocity rotor: 0.10–7.90 m/s

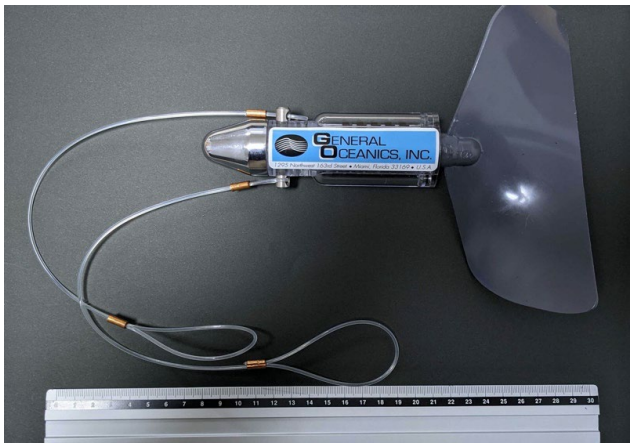


Photo 3-4. Flow meter and low flow velocity rotor

- Other type of flow meter
  In addition to the digital flow meters mentioned above, there is another flow meter for plankton nets (small-sized integrating flow meter) used for oceanic plankton surveys.

3.2.4 Installation and fixation of flow meter

- A flow meter is installed at the center of the net frame and fixed with a non-plastic strings or metal frames.
- The tip of the flow meter is adjusted at a few centimeters outside of the net to prevent the rotor from contacting the net while it is running.
- Plastic strings must not be used because there is a high risk of contaminating samples with plastic pieces (hereinafter referred to as “contamination”).

Case 1. Fixation of the flow meter with strings (Photo 3-5)
- The flow meter is fixed to the net frame at three points with strings.
- It is not necessary to fasten strings at three points as long as the flow meter is secured.

Case 2. Fixation of the flow meter with a metal frame (Photo 3-6, Figure 3-1)
- A metal frame allows the flow meter to be fixed more stably at the center of the net.
- The fixation with a metal frame allows more accurate measurements than Case 1, because of the stability and the low possibility of contact with the rotor and net.

- However, frames for fixing flow meters are not commercially available; thus, it is necessary to custom-make it according to the net being used.

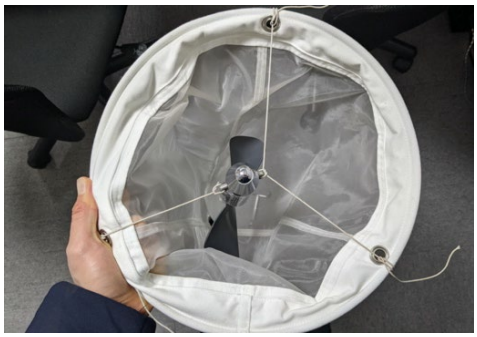


Photo 3-5 Case 1. An example of fixation of the flow meter with strings

Photo 3-6 Case 2. An example of fixation of the flow meter with a metal frame

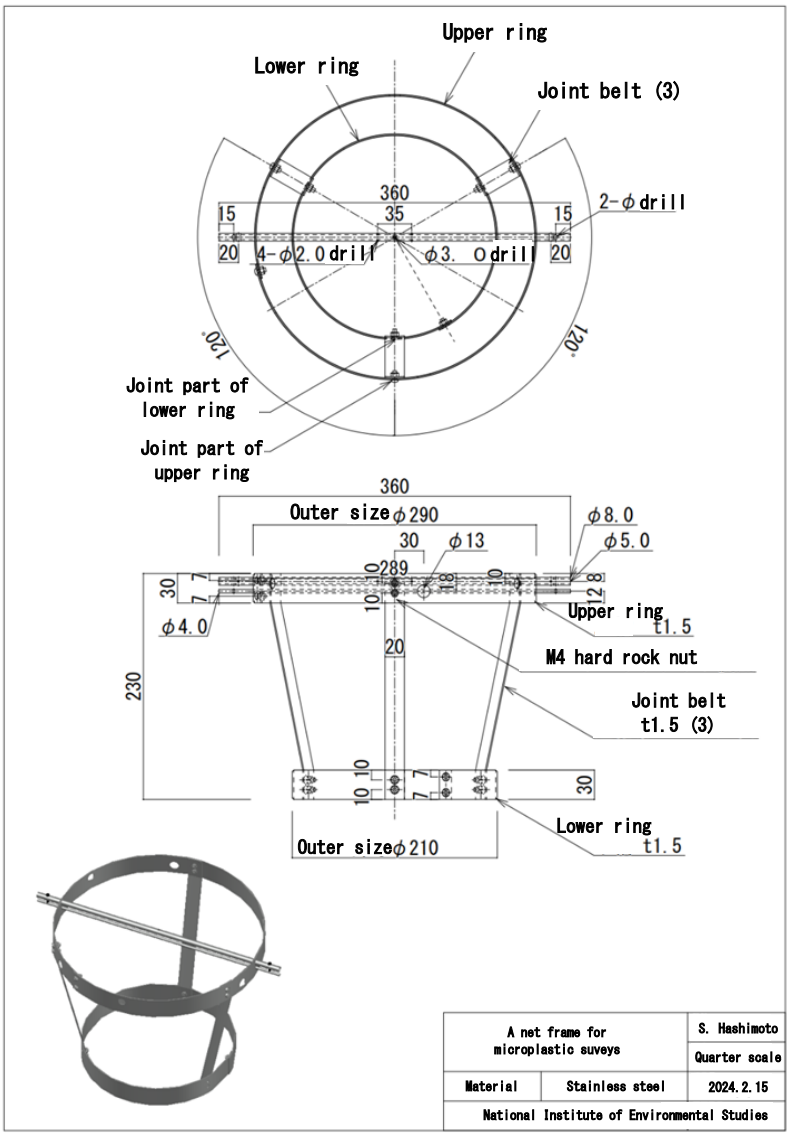


Figure 3-1. An example of metal frame

(provided by Seichi Hashimoto from National Institute of Environmental Studies)

3.3 Sampling position

In principle, the center of streams (the location of the fastest flow) in the cross section of rivers are chosen as positions for sampling. In the case of wide river, it is recommended to set three or more sampling points

(e.g., the center of a stream, a point distant 10 m from right bank, and a point distant 10 m from left bank).

At the sampling locations, the net is hung from a bridge, or installed in a stream by stepping in a position where the water level is low.

When the net is hung from bridges, the net should be hung toward downstream to observe the net condition easily and adjusted its position to avoid turbulences caused by piers and sandbars. If it is difficult to collect samples at the center of the stream, another location where sample collection is possible should be chosen.

In the case that the right or left bank is selected as the sampling location, it is recommended to record the distance from banks and maintain approximately 2–3 m distance from them to avoid contamination by impurities falling from river flood plains.

Stepping in rivers to collect samples is possible at locations where the water level is 1 m or less and the streambed does not subside. It will become easy to install the net in the stream and check the rotation of the flow meter visually.

3.3.1 Setting of net in stream

The mouth of the net is immersed fully in the stream under the river surface (so that the upper part of the mouth of the net lies just below the surface) (Photo 3-7).

The guidelines are intended to collect microplastics in surface water of river water; thus, to avoid contaminations by particles deposited on the riverbed, the upper part of the mouth of the net should not be set deep under the surface of the river.

Photo 3-7. Setting of net in stream

3.3.2 Fixation of the net in stream at locations for sampling

The mouth of the net is fixed at three points with ropes (Photo 3-8).

In the case of hanging from bridges, the net is lowered to rivers with a rope (Photo 3-9).

In the case of stepping into the river, the rope is connected to a rod and set the net in rivers. When the flow velocity is slow and it is difficult to collect samples by using a rod, it is permitted to set the net directly in the stream by hand (Photo 3-10).

Photo 3-8. An example of fixation of plankton net

Photo 3-9. Hanging a net from a bridge

* When samples are collected from bridges, and when the flow velocity is slow and it is difficult to set the net into the water, it is recommended to shorten one of the ropes fixed to the frame of the net to set into the water easily.

Photo 3-10. Stepping into the river and collecting samples

* Researchers should be careful not to affect the inflow of the river water into the net by their feet and body when they hold the bar or ropes and stand toward upstream.

3.4 Sampling methods

3.4.1 Preparation

To arrange survey environments, sheets are spread on the sampling locations to avoid contamination of the net by coating debris falling from bridges, plastic debris, and other impurities. When samples are collected from bridges, the bridge's handrails are wrapped in sheets to prevent contamination of the net by coating debris falling from the bridge handrails and other impurities. In this case, natural fiber cloth sheets should be used, and those made of plastic-based cloth must not be used.

Tools are carefully checked to make sure that rotors in flow meters rotate smoothly, rotors do not separate easily, and nets are not torn or frayed and have no attached impurities.

3.4.2 Measurement of flow velocity

Flow velocity is measured before collecting samples. The measurement time during a flow meter has ≥ 10 m^3 filtered water volume is checked. To measure flow velocity, the rotation number of flow meter removed from the net is checked by setting it at the location underwater for one minute (60 seconds) (Photo 3-11). When it is conducted from bridges with ropes, wind effects for flow meters should be concerned.

Photo 3-11. Measurement of flow velocity with flow meter

3.4.3 Sampling

After checking that the bottom pipe (or cod end) is closed tightly, the net is set in streams until the filtered water volume reaches 10–20 m^3. The flow velocity measured in Section 3.4.2 is not constant due to the resistance and clogging of the net; thus, it is recommended to keep the net underwater for a specific period determined by calculation until the amount of filtered water volume reaches approximately 13–14 m^3. However, a sufficient number of plastic (approximately 30 particles) was collected in less than 10 m^3 of water. Sampling does not need to be continued. Confidence intervals for the number of collected microplastics are illustrated in Figure 3-2. Error of approximately 50%, 33%, 25%, and 20% were recorded for 10, 30, 50, and 100 particles, respectively.

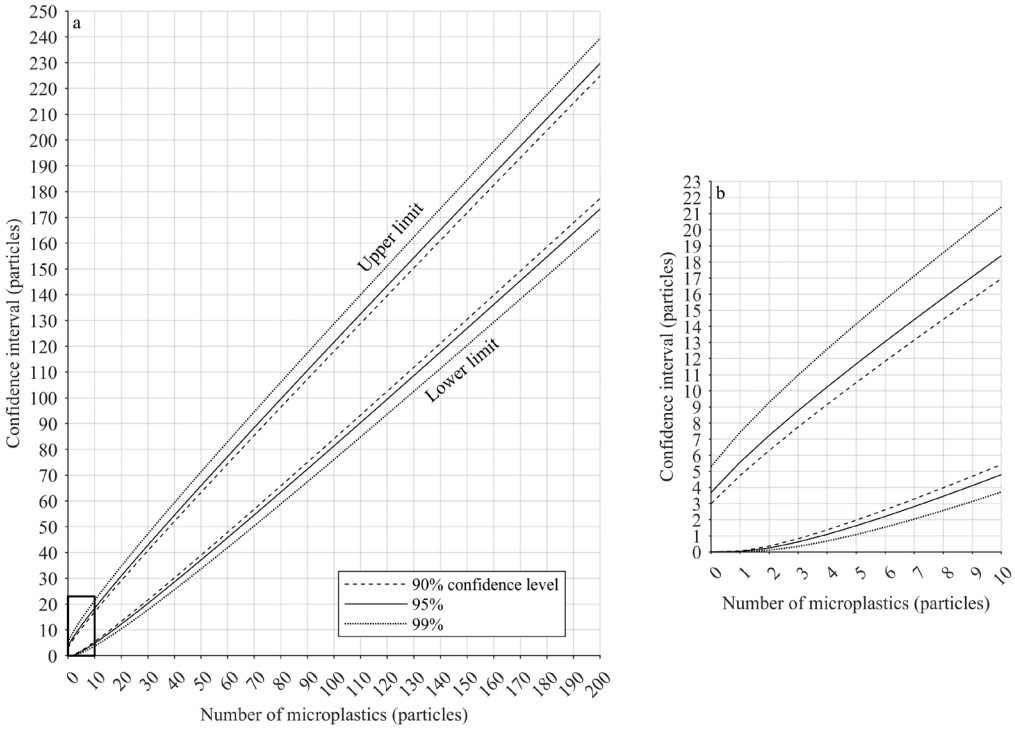


Figure 3-2. Confidence intervals for the number of collected microplastics[IV]

Additionally, sampling does not need to continue with clogged nets when a sufficient amount of microplastics is visually confirmed in samples (Photo 3-12). If the amount is insufficient, sampling should be resumed after the clogged nets are washed from the outside. Be sure not to rotate the rotors of flow meters place in nets while washing.

After pulling up the net, the rotation number of the flow meter is recorded immediately. It must be careful not to rotate the rotor of the flow meter by wind or other factors before reading the rotation number of the flow meter.

Photo 3-12. Normal and clogged nets

(provided by Tomoya Kataoka, associate professor at Ehime University Graduate School)

[IV] Tanaka, M., Kataoka, T., Nihei, Y., 2023. An analytical approach to confidence interval estimation of river microplastic sampling. Environmental Pollution. 335, 122310.

3.4.4 Removal of impurities

When the pulled-up net contains aquatic plants, dead leaves, and other impurities, surfaces of these impurities are washed with water which is gotten in the field and filtered through the net with 0.1 mm mesh openings (hereafter referred to as “washing”) before they are removed.

3.4.5 Transfer of samples from net to storage containers

In principle, to avoid contamination in the field, it is recommended to transfer samples from net to storage containers in the laboratory. In this case, samples in the net are collected to the bottom pipe (or cod end) by pouring tap water to the net from the outside, and then transferred to storage containers. Samples that adhere to the bottom pipe (or cod end) are transferred to storage containers with a small amount of tap water (photo 3-13, Photo 3-14). When the samples are transferred to the storage containers, be careful to prevent contaminants in the container and not to leave any samples in the net. When the nets are taken back to laboratories, their mouths are covered with cloths, and they are put in cloth bags and carried to the laboratories (photo 3-15, Photo 3-16).

When multiple nets are not available, samples are transferred on site. In this case, washing is used instead of tap water (photo 3-17).

Photo 3-13. Collection of samples in a bottom pipe (or cod end)

Photo 3-14. Transfer of samples from a bottom pipe (or cod end) to a container

Photo 3-15. An example of cloth cover

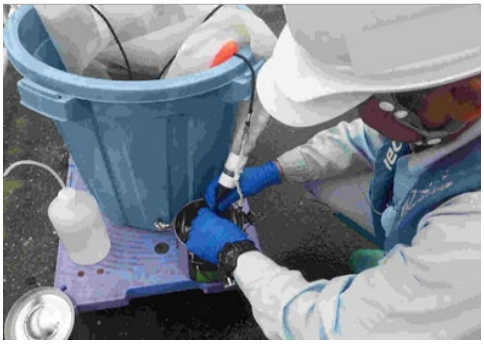
Photo 3-16. An example of cloth bag

Filtration of river water with a net with 0.1 mm mesh openings

(With a watering can)

(With a spray)

Pouring of washing to a net from the outside



Transfer of samples from a bottom pipe (or cod end) to a storage container

Photo 3-17. Sampling scenes

3.4.6 Numbering of containers

Storage containers are numbered.

Before the nets are brought back to the laboratory, the bags containing the nets are numbered in advance.

3.4.7 Storage of containers

When no more sampling is to be conducted on the day, storage containers are stored in refrigerator, and the subsequent described below are conducted within a few days.

3.5 Measuring and recording in sampling

3.5.1 Required items and recommended items

The items listed below are measured and recorded during sampling. The recommended items are measured and recorded as far as possible.

Required items

1. Location name
   Names of sampling locations including sampling positions must be recorded.
   (Example: Tama River, Maruko Bridge, Downstream, center of flow)
   It is recommended to take photographs of the location and make records regarding it to ensure that the location where samples are collected is identifiable.
2. GNSS data for sampling positions
   Data on the latitude and longitude of sampling location from Global Positioning System (GPS) are obtained using mobile terminals or GNSS receivers and are recorded.
3. Water depth
   Water depth at the sampling locations must be measured and recorded to confirm safety at this location and at the center of a stream.
4. Flow velocity (measured with flow meter)
   Flow velocity must be measured and recorded to estimate the reference time needed for the required filtered water volume.
5. Measurement time (starting and ending time)
   Measurement time must be recorded to calculate the filtered water velocity.
6. Rotation number and filtered water volume
   In sampling with flow meters which include rotors, the rotation number of flow meter rotor is read before and after sampling. The amount of water flowing through flow meter is calculated and recorded.
7. Sampling methods
   Hanging the net from bridges or stepping into riverbeds must be recorded, and sampling locations must be photographed.

[Reference] Examples of formulae for filtered water volume and velocity

In the case of using a sampling net with 30 cm in diameter, filtered water volume v is calculated as below,

$$v = 0.15^2 \pi a f$$

f is the distance per one revolution of the flow meter, a is the number of revolutions of the flow meter, and π is the ratio of the circumference of a circle to its diameter.

Filtered water velocity m is calculated as below,

$$m = v/t$$

t is the measurement time.

Recommended items

1. Weather
   Weather on a survey day and the number of preceding sunny days in a week around sampling locations are recorded to assess the influence of rainfall.
2. Turbidity
   Water turbidity should be measured and recorded to estimate the degree of net clogging and examine implementations of density separation.
3. Tides
   In sampling locations in tidal areas, time and conditions from high and low tide should be recorded to examine effects of backflows from downstream on rotations of flow meter and possibilities of contamination by drifting objects.
4. Condition of riverbed at sampling locations
   The condition of riverbed should be recorded to confirm safety during sampling in rivers for sampling

and assess the potential for sample contamination by riverbed sediment.

5. Local circumstances
   Local circumstances around sampling locations that may affect distributions of surface water microplastics, such as upstream works, and drifting debris, should be checked and recorded.
6. Flow rate
   In the case that official data is not available, actual flow rates should be measured and recorded on site.
7. Water level
   To understand conditions of streams (e.g., during typical weather and after rain), water level data for seven or more days should be gathered from water level observatories near sampling locations.

3.5.2 Precautions for sampling

1. At sampling locations, it should be checked that surveys will not be subject to influence from construction works or other factors nearby in advance.
2. To ensure safety, sampling should be conducted with multiple people. Researchers must put on life jackets and safety belts. Helmets must be also put on at places with risks of falling.
3. In the case of stepping in rivers for sampling, researchers must approach sampling locations from downstream and be careful not to kick up gravel on riverbeds. After conditions of streams become stable, sampling should be started. As mentioned above, they must check that the mouth of the net is immersed under the river's surface and the flow meter rotor rotates normally while paying attention not to disturb streams.
4. In the case that something that disturbs water flow through the net or damages the net and flow meter (e.g., driftwood and large floating objects) comes into the net while sampling, it is permitted to temporarily stop sampling and remove it. The temporary stop and circumstances must be recorded.
5. Plastic items such as synthetic-rubber gloves and synthetic fiber cloth must not be put on.
6. The contamination by dust or fiber waste must be avoided while sampling.
7. In the field, researchers must consider people around them (e.g., workers engaged in river work, ships, fishermen, and walkers on riverbanks) and ensure their safety.

4 Sampling in lakes

4.1 Outline

Samples containing microplastics are collected from surface water of lake water. This guideline considers net tow sampling, conducted at the center of lakes from boats, as the primary method. Sampling from lakesides with motorized survey devices or pumps is considered as substitute methods. Considering the fact that the density and composition of microplastics vary depending on the surrounding environments, their representativeness of the whole lake may not be ensured.

4.2 Net tow sampling

4.2.1 Equipment

4.2.1.1 Net

Refer to Section 3.2.1 for the sampling net used in lake survey (Photo 4-1).

Photo 4-1. An example of sampling with a plankton net

[Reference] Neuston net

A net with 0.3 mm mesh openings and a metal bottom pipe (or cod end) are attached to a square frame.

While the Neuston net (Photo 4-2) (frame size: 75 cm × 75 cm, net length: 300 cm) is generally used for ocean surface microplastic surveys, it is necessary to pay attention to handling it on small boats in lake surveys because it is large compared to small boats.

A square net (frame size: 30 × 30 cm) (see Reference in "3.2.1 Net") is more manageable than Neuston net on small boats.

Photo 4-2. An example of neuston net

4.2.1.2 Flow meter

Refer to Section 3.2.2 for the flow meter used in lake survey.

4.2.1.3 Specifications of flow meter

Refer to Section 3.2.3 for the specification of flow meter used in lake survey.

4.2.1.4 Installation and fixation of flow meter

Refer to Section 3.2.4 for the method of installation and fixation of flow meter used in lake survey.

4.2.1.5 Boat

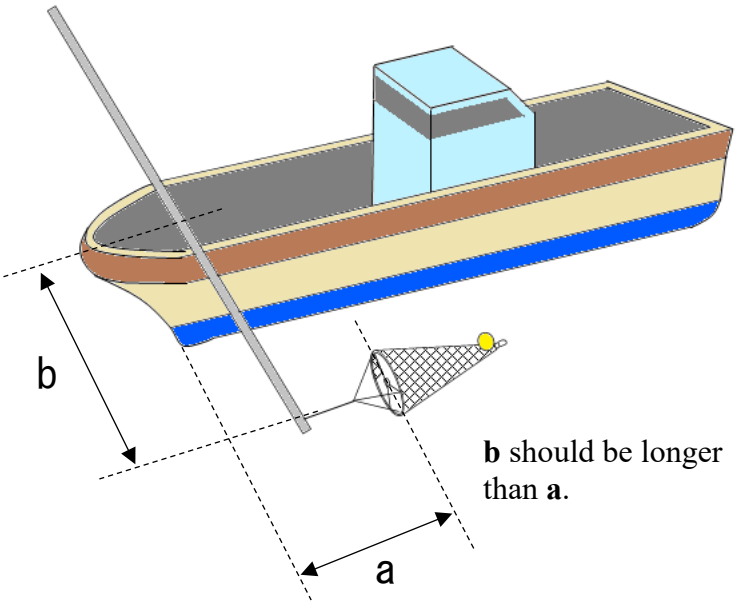
Boats that support safe sampling should also be used. Boats that meet the following conditions do not require an operational license:

- The registered boat length, which is 90 % of the overall length in most cases, is < 3 m.
- Engine size is < 1.5 kw (approximately 2 hp).
- The boat has mechanisms that stop the propeller as soon as it gets orders or guard people against it (e.g., emergency stop switch and propeller guard).

Boats that do not satisfy these conditions require operational licenses. Rubber rafts should not be used because they are unsuitable for safe sampling.

4.2.1.6 A place where sampling nets are to be installed on boats

Boats are run tentatively in advance, and the extent of wake wave is checked. The nets are installed far from the side of the boat with bars to avoid the effects of wake waves. The nets should be installed close to the stem and turned forward as much as possible (Figure 4-1).



a: the distance between the stem and the net mouth

b: the distance between the center line of boat and the net mouth

Figure 4-1. An example of installation of plankton net

- It is recommended to use metallic bars to bear the drag load during towing.
- The bars should be stably fixed with metal fixtures and ropes.

4.2.2 Sampling position

In principle, the center of a sufficiently deep lake for cruising should be selected as the sampling position. Considering the lakebed configuration, the towing direction should be set to avoid stranding and uneven expansion of the sampling nets.

4.2.2.1 The setting of the net in water

The mouth of the net is fully immersed in the water under the lake surface (so that the upper part of the net mouth lies just below the surface). Floats and weights are installed at its mouth to control the net's depth.

Because this guideline is set for microplastics in the surface water column, to avoid contamination with plastics and other particles accumulated at the bottom of lakes, the upper part of the net mouth must not be immersed deeper than the surface (Photo 4-3).

Photo 4-3. An example of mud accumulated at the bottom of a lake

4.2.3 Sampling methods

4.2.3.1 Preparation

- Check the equipment carefully to ensure smooth rotation of the flow meter against easy separation of the rotor, torn or frayed conditions of the net, and no attached impurities.
- To prevent contamination of the nets, sheets are spread on the boats. In addition, to prevent contamination by boat-bottom paint, the sides of the boats on which the sampling nets are brought must be protected with sheets. Natural fiber cloth sheets should be used, and those made of plastic-based cloth should not be used.

4.2.3.2 Sampling

- Immerse nets in water and tow at 1−2 knots until the filtered water volume reaches 10−20 m^3. When a net with 30 cm in diameter is towed 200 m, the filtered water volume is approximately 14.1 m^3. However, a sufficient number of microplastics (approximately 30 particles) can be collected in less than 10 m^3 of water, and sampling using clogged nets does not need to be continued.
- In the case that sufficient amount of filtered water is not get at once, turn boats back, and conduct additional towing. Then, for accurate measurement of filtered water volume, the net is brought on the boat while the boat is turned back. After the boat is turned straight, the net is immersed again.
- After towing, pull the net up and immediately record the rotation number of the flow meter. It must be careful not to rotate the rotor of the flow meter by wind or other factors before reading the rotation number of the flow meter.
- Record the towing distance and boat wake using GNSS (GPS). The duration of towing should also be recorded.

[Reference] Net clogging

- Capture rates decrease with clogging nets[V].
- Turbidity is measured to estimate the degree of net clogging.
- In addition to turbidity, SS and chlorophyll data will help to explain the causes of net clogging.
- It is possible to understand the degree of net clogging from the relationship between the towing distance and filtered water volume in the towing test (Figure 4-2).
- In addition to net clogging, the type and size of impurities should be checked during towing test.

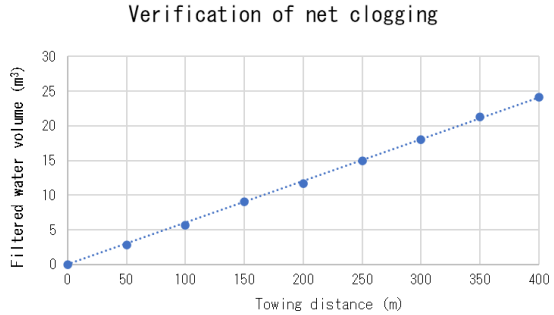


Figure 4-2. An example of verification of net clogging

4.2.3.3 Removal of impurities

Refer to Section 3.4.4 for the details of removal of impurities.

4.2.3.4 Transfer of samples from net to storage containers

Refer to Section 3.4.5 for the method of transfer of samples from net to storage containers in lake survey. Be sure to be careful against falling overboard and overturning when they work on unstable boats.

4.2.3.5 Numbering of containers

Refer to Section 3.4.6 for numbering of containers in lake survey.

4.2.3.6 Storage of containers

Refer to Section 3.4.7 for the method of storage of containers.

4.3 Sampling using motorized survey devices

4.3.1 Equipment

1. Motorized survey device
   The motorized survey device consists of submersible waterproof power supply unit with a propeller, net and flow meter (Photo 4-4). The propeller causes water to flow, and the water is filtered through the net. For more information on the net and flow meters that compose the device, please refer to Sections 3.2.1 and 3.2.2, respectively.

[V] Kataoka, T., Tanaka, M., Mukotaka, A., Nihei, Y., 2023. Experimental uncertainty assessment of meso- and microplastic concentrations in rivers based on net sampling. Science of Total Environment. 870, 161942.

Photo 4-4. An example of motorized survey device

4.3.2 Sampling position

The devices are lowered parallel to lakesides and then sunk under the water surface of the lakes. The positions of the devices are controlled using a rope. Before sampling, the front parts of the devices are turned toward the center of the lakes at an angle of approximately 45 degree (Photo 4-5). Sampling locations should be selected where sufficient water depth is available to prevent resuspension of bottom sediment, and the immersion depth should be adjusted by the length of the rope suspending the device. Since this guideline aims to collect microplastics from the surface water of lakes, excessive submergence of the device's opening should be avoided to prevent contamination by microplastic particles deposited on the lake bottom.

Photo 4-5. An example of sampling using a motorized survey device

4.3.3 Sampling methods

4.3.3.1 Preparation

- Check the instruction manual of the device and measure the flow velocity.
- Check the device to ensure sufficient battery life, smooth rotation of the flow meter, torn or frayed condition of the net, and for no attached impurities.
- To prevent device contamination, sheets are spread on the sampling sites. Natural fiber cloth sheets should be used, and those made of plastic-based cloth must not be used.

4.3.3.2 Sampling

- Sink the device under the water surface in the lake and keep it until the filtered water volume reaches 10–20 m^3. However, a sufficient number of microplastics (approximately 30 particles) is collected in less than 10 m^3 water, further sampling does not need to be continued with clogged nets.
- After sampling, pull the device up and record the rotation number of the flow meter immediately.
- Thereafter, remove the net from the device and collect the sample.

4.4 Sampling with pumps

4.4.1 Equipment

1. Pump
   Pumps with sufficient power to attain the required flow rate should be used even if connected to hoses and strainers. When selecting a pump, the vertical distance from the suction water surface to the discharge water surface, i.e., actual head, should be determined at the location where the pump is intended for use. Furthermore, the pipe loss, pressure, and velocity heads of the designated pump and hose should be confirmed using an instruction manual or similar documentation, and the total head should be calculated to confirm whether the pump can be used. If the pump has plastic components, the color and material must be checked and recorded prior to use. The pump should be cleaned before use to prevent contamination by solids accumulated inside the pump.

2. Strainer
   To avoid clogging the hoses and pump failures, metal strainers (e.g., stainless-steel strainers) with more than 5 mm mesh openings are connected to the inlet ports of the hoses. Those with no mesh in the bottom are better at avoiding plastic contamination in the bottom sediments (Photo 4-6).

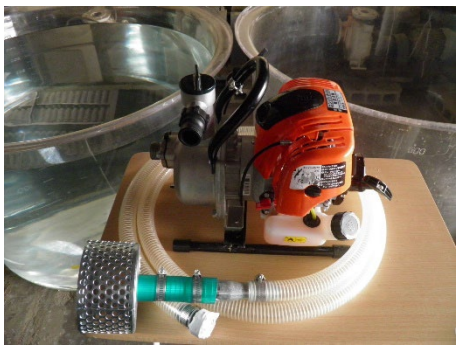


Photo 4-6. An example of a pump and a strainer

3. Hose
   Hoses with sufficient pressure resistance should be used. Additionally, they must be sufficiently long to set a distance of more than 5 m between the inlet and outlet ports. When the hoses are made of plastic, the color and materials must be checked before use.

4. Net
   Nets with approximately 0.3 mm mesh openings as indicated in Section 3.2.1 are used to filter the pumped water and obtain samples.

5. Flow meter
   The use of clamp-on flow meters is recommended to accurately measure the filtered water volume (Photo 4-7).

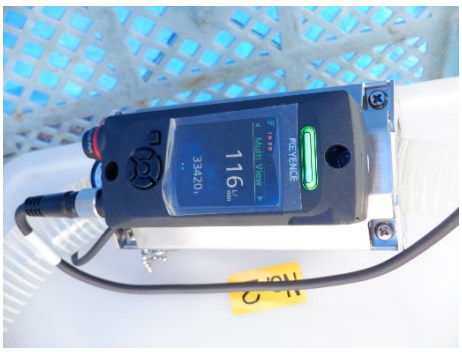


Photo 4-7. An example of a clamp-on flow meter

4.4.2 Sampling position

The pump and related equipments are installed at the lakeside. Sampling locations are selected where sufficient water depth is available to prevent suction of bottom sediments. The strainer connected to the inlet port is submerged under the surface water of the lake during sampling. Because this guideline aims to collect microplastics from the surface water of lakes, and to avoid plastic contamination from lake bottoms, sampling locations must have sufficient depth, and the strainer must not be sunk deep (Photo 4-8).

Photo 4-8. An example of the depth of a strainer

4.4.3 Sampling methods

4.4.3.1 Preparation

- Connect a hose to the inlet and outlet ports of the pump. Fix the tip of the hose connected to the outlet port inside the net to filter the pumped water. Additionally, fix the net to ropes.
- Check the equipment carefully, and focus on the following aspects:
  - ・Damages to strainers and hoses
  - ・Amount of fuel and oil of pumps
  - ・Tears and frays of nets
  - ・Contamination of equipment
- Set the inlet and outlet ports at a distance of more than 5 m from each other.
- Test the operation of the pumps before sampling.
- Attach clamp-on flow meters tightly to hoses.
- Instead of flow meters, water buckets are used to measure the time it takes to fill them. The known volumes of the buckets are used to calculate the time to fill 10–20 m^3 of water, and to estimate the volume flow rates of the pumps. To account for any decrease in the pumps' power due to continuous use, the flow rates at the beginning and end of sampling are checked, and the average flow rates are used to estimate the filtered volume.

[Reference] Points to note when checking flow rates with water buckets

Table 4-1 shows the differences in the volume flow rates measured with a water bucket and clamp-on flow meter. Although accurate flow rates and accumulated water volumes can be measured using clamp-on flow meters, they are usually expensive. Therefore, another method using water buckets instead of clamp-on flow meters was examined. The volume flow rates measured using a clamp-on flow meter are shown in Figure 4-3 and Table 4-2. The mean value calculated from the flow rates measured at the start and end of the sampling was used to calculate the relatively accurate filtered volume (Table 4-3). To consider the decline in pump power caused by continuous use, a coefficient was multiplied by the equation for sampling time. An example shown in below used "1.1" as a coefficient.

$$10000[\mathrm{L}] / 127[\mathrm{L/min.}] \times 1.1 = 86.6[\mathrm{min.}]$$

Before the pump is turned off, the flow rate should be measured at the estimated time, and sampling should be extended if necessary.

Table 4-1. The differences in volume flow rates measured with a water bucket and clamp-on flow meter

	Water bucket	Clamp-on flow meter
	Water volume [L/50sec.]	Water volume [L/50sec.]
1	73	71
2	71	71
3	72	70

Table 4-2. The volume flow rates measured using a clamp-on flow meter

Time	Flow rate	Accumulated water volume
[min.]	[L/min.]	[m^3]
1	93	—
6	127	—
86	112	10.06

Table 4-3. The mean value calculated from the flow rates measured at the start and end of the sampling

Based on the flow rate:	Estimated water volume [m^3]	Matching rate with actual water volume
at warming up (1 min. passed)	8.00	80%
at the start (6 min. passed)	10.92	109%
at the end (86 min. passed)	9.63	96%
of the mean of the start and end	10.28	102%

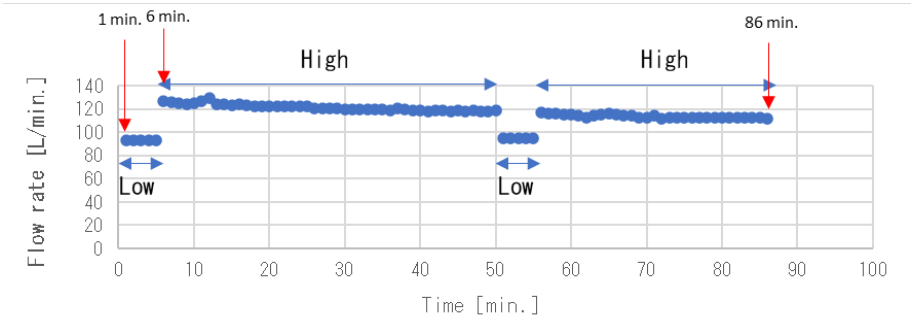


Figure 4-3. The volume flow rates measured using a clamp-on flow meter

4.4.3.2 Sampling

- Connect the strainer to the inlet port, and then sink the strainers below the lake`s surface with the strainer bottom facing downward.
- Arrange the positions of the strainers as close to the lake surface as possible. Subsequently, the strainers are not exposed to the air.
- Fill hoses and pumps with lake water using water buckets and start the engines.
- After the pumps operate stably, the tips of the hoses are connected to outlet ports inside the nets until 10–20 m^3 of water is filtered. However, a sufficient number of microplastics (approximately 30 particles) were collected in less than 10 m^3 water, sampling does not need to be continued with clogged nets.
- In the case where refueling is needed in the middle of sampling, suspend the sampling to refuel and resume where it is stopped. The suspension time should be recorded.
- After obtaining a sufficient filtered volume, turn off the pumps and collect samples from the nets.

4.5 Measuring and recording in sampling

4.5.1 Required items and recommended items

The items listed below are measured and recorded during sampling. The recommended items are measured and recorded as much as possible.

Required items

1. Location name
   To specify the sampling position, photographs should be taken and information regarding the location should be recorded.
2. Water depth
   The water depth at the sampling locations must be measured and recorded to confirm safety at this location and at the center of the lake. The measurements are conducted using the following items:
   - Rope with weight at the tip
   - A long rod
   - Pressure sensor (e.g., conductivity temperature and depth profiling system (CTD))
3. Rotation number and filtered water volume/accumulated water volume
   In sampling with flow meters which include rotors, the rotation number of the flow meter rotor is read before and after sampling. The amount of water flowing through the flow meter is calculated and recorded.
   The accumulated water volume is recorded by sampling using pumps. When water buckets are used, the mean value calculated from the flow rates measured at the start and end of sampling is used to calculate the filtered volume.
4. Measurement time (starting and ending time) (net tow sampling)
   The measurement time must be recorded to calculate the filtered water velocity.
5. GNSS data for the sampling locations (boat wake)
   Data on the latitude and longitude of the sampling location and boat wake in net tow sampling from the Global Positioning System (GPS) are obtained using GNSS receivers on boats and are recorded.
6. Towing distance (Net tow sampling)
   The towing distance must be recorded from the data in (5) to determine the sampling conditions.
7. Boat velocity (Net tow sampling)
   The boat velocity must be recorded to grasp the sampling conditions.
8. Sampling position on boats and distances between boats and nets (net tow sampling)
   To understand the effects of boat wake wave and contamination by boats, the sampling position (starboard or port) and the distance between the boat and net must be recorded.
9. Wind direction and velocity and wave direction
   To understand the distribution of microplastics and determine the towing direction in net tow sampling, wind direction, velocity, and wave direction must be recorded in the field.

Recommended items

1. Weather
   Weather on the survey day, and the number of preceding sunny days in a week around the sampling location are recorded to assess the influence of rainfall.
2. Turbidity
   Water turbidity should be measured and recorded to estimate the degree of net clogging and examine the implementation of density separation.
3. Local circumstances
   Local circumstances around the sampling location that may affect the distribution of surface water microplastics, such as rivers, upstream work, and drifting debris, should be checked and recorded.
4. Water level
   To understand the conditions of the stream (e.g., during typical weather and after rain), water level data for more than seven days should be gathered from the water level observatory near the sampling location.
5. Information about rivers that flow into/out of lakes
   Rivers that flow into/out of lakes and possibly affect survey results should be checked.

4.5.2 Precautions for sampling

Refer to Section 3.5.2 for the precaution for lake sampling.

5 Pretreatment of samples

In pretreatment, to remove impurities from samples and to efficiently capture particles that appear to be plastic (hereinafter referred to as *candidate plastic particles*), oxidation and/or density separation are used together with filtration in accord with the amount of samples and impurities and conditions of samples. Oxidation is effective when there are many organic impurities such as plant particles in samples, and density separation is effective when there are many inorganic impurities such as soil particles in samples.

It needs to regard that there are possibilities that oxidation causes denaturation or decomposition of microplastics, and density separation allows microplastics with high specific density to be included in precipitate.

5.1 Separation of solids from samples with nets

5.1.1 Outline

Solids from samples are separated with nets with 0.1 mm mesh openings. If a high volume of solids is present and filtration becomes time-consuming, suction filtration may be employed exclusively at the beginning of the process (Figure 5-1). However, care must be taken when crushing plastic particles; if fragile particles are included, they should be separated before suction.

Figure 5-1 Suction filtration
(Courtesy of Hisano Watanabe, National Institute for Environmental Studies)

5.1.2 Reagents and equipment

1) Net with 0.1 mm mesh openings
2) Two glass containers or beakers
3) Purified water
4) Tweezers
5) Pipette (made of glass)

5.1.3 Procedure

1. Put a net on glass container A and carefully pour samples through the net to filter out impurities (Photo 5-1).
2. Remove impurities that are ≥ 5 mm in size with tweezers (Photo 5-2). Wash matters that stick to the impurities with purified water and collect them on the net.
3. Remove the net from container A and put it on container B (Photo 5-3).
4. Carefully pour filtered water in container A into container B through the net (Photo 5-4).
5. Remove the net from container B and put container A on the net to cover the samples with its mouth so that the samples face to the inside of container A. (Photo 5-5).
6. Pour a small amount of purified water (or reagent used for oxidation when the samples are oxidized without drying after filtration) on the net on container A with a pipette to transfer the samples from the net to container A (Photo 5-6).

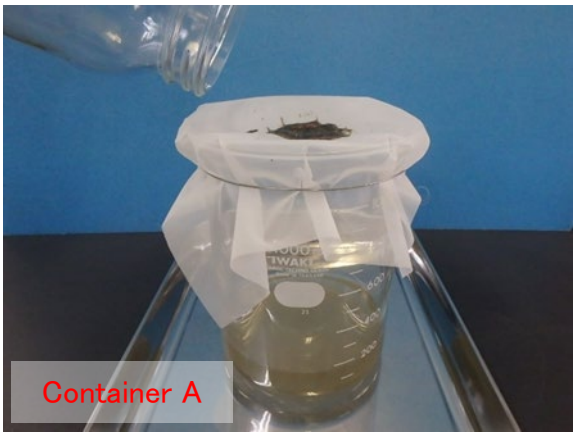


Photo 5-1. Filtration of impurities through a net

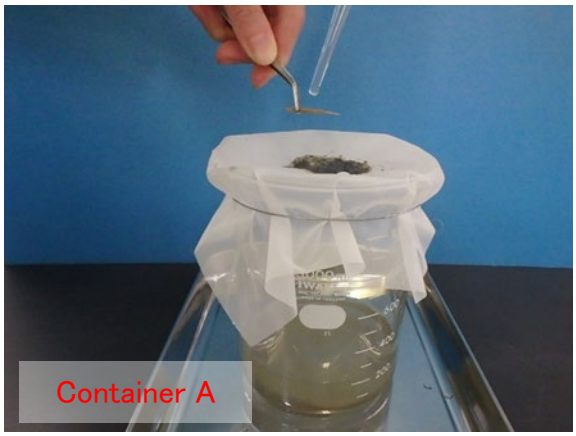


Photo 5-2. Removal of large impurities

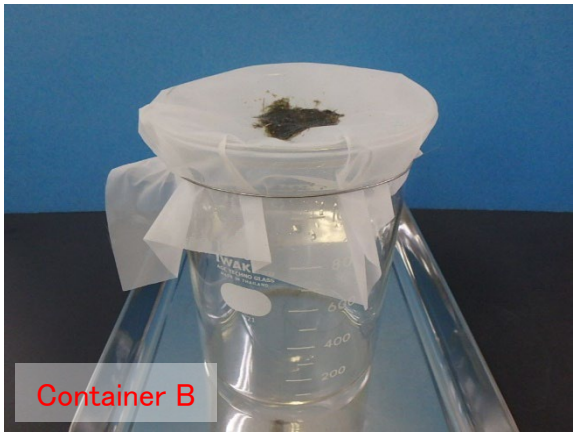


Photo 5-3 . Putting of a net on container B

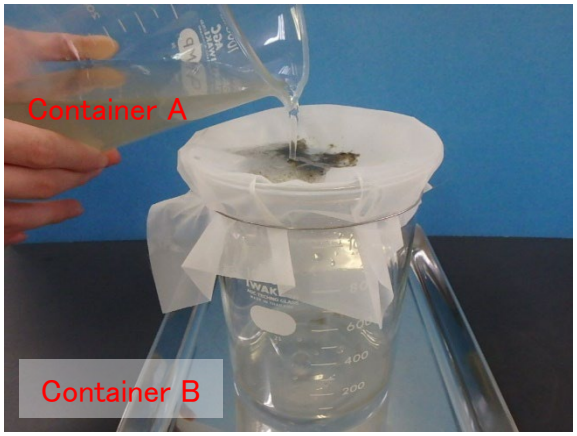


Photo 5-4. Filtration of filtered water

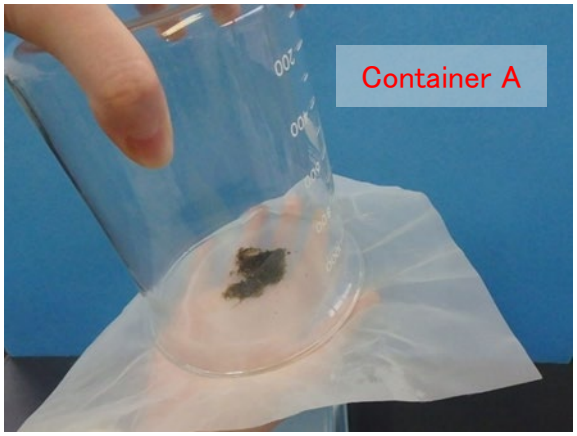


Photo 5-5. Putting container A on the net with samples side facing inward

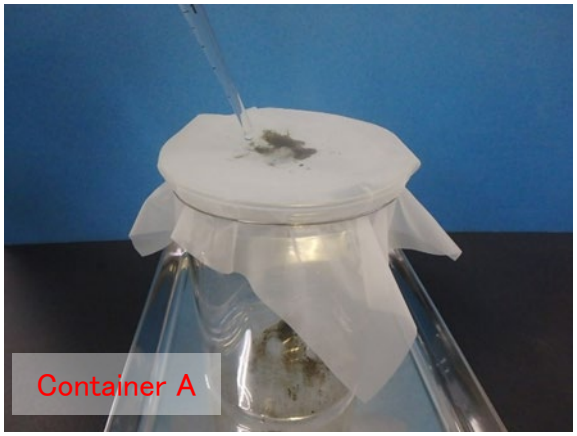


Photo 5-6. Drop of the samples sticking on the net into container A

5.2 Oxidation

5.2.1 Outline

Matters that stick to microplastics and organic impurities in samples are removed by oxidation. As a result, visual identification and sorting of candidate plastic particles become easy. The samples are oxidized with 30 % hydrogen peroxide solutions while warming.

5.2.2 Reagents and equipment

1) Oven
2) Water bath (constant temperature water bath) or incubator
3) Watch glass
4) Net with 0.1 mm mesh openings
5) Glass container or beaker
6) Glass container or glass Petri dish
7) 30% hydrogen peroxide solution

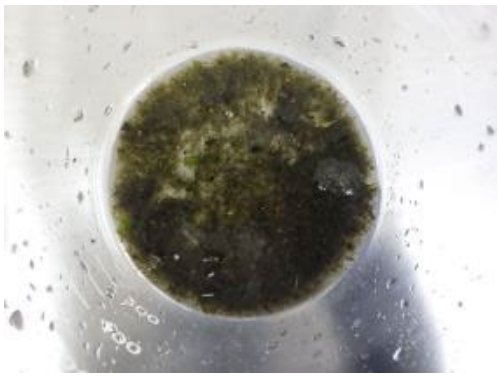
5.2.3 Procedure

1. Put watch glasses on samples in containers and warm at 60 °C or less in ovens to remove water content from the samples. However, do not dry them up completely (the amount of sample in a 1-L container shall end up being approximately 50 mL). A large amount of sample should be divided into additional containers (Photo 5-7, Photo 5-8, Photo 5-9).

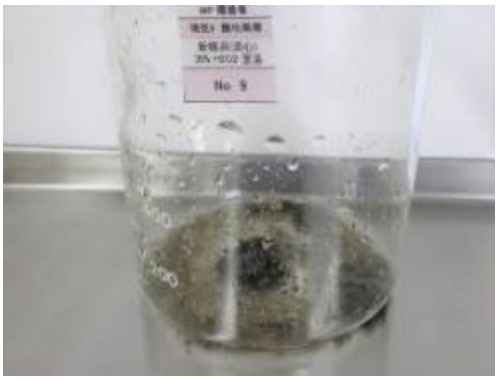
*Notes: - Dry samples at 60 °C or lower to prevent deterioration of the plastics.
- "Completely dried condition" refers to a condition that samples do not move when containers are tilted.
- Be careful if water content in the dried samples is too high, because oxidizing agent is diluted and reduces the effect of organic impurity decomposition.

Photo 5-7. Warming of containers in an oven

Photo 5-8. A dried sample



Before drying (top view)



Before drying (side view)

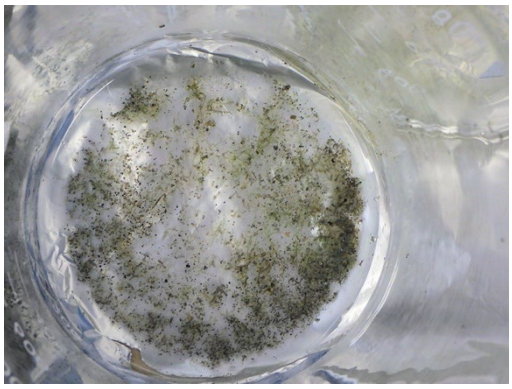
After drying for 2.5 hours (top view); the end of drying process

After drying for 2.5 hours (side view); the end of drying process

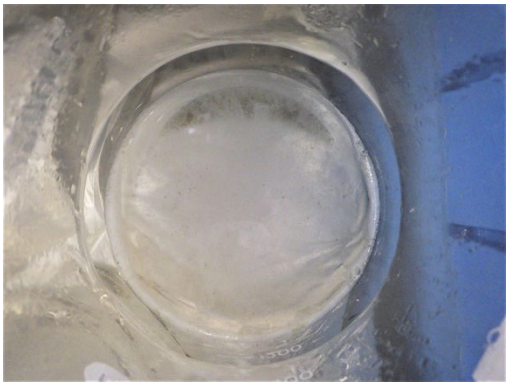
Photo 5-9. Examples of samples dried properly without solidification

2. Add 100 mL of 30 % hydrogen peroxide solutions to the containers and put watch glasses on the top. Warm them at 55 °C in water baths or incubator and leave for three days.
   * It is recommended to gently shake the containers occasionally to assist their reactions. However, do not shake them too much or stir the samples with stirring rods to avoid damages to plastic pieces.
   * Add 30 % hydrogen peroxide solutions in fume hoods with goggles and gloves to protect your eyes and skin (Photo 5-10, Photo 5-11, Photo 5-12).
   *To avoid a violent reaction between 30 % hydrogen peroxide solution and samples which contain large amounts of organic matter, 30 % hydrogen peroxide solution should be added little by little.
3. When white gel-like suspensions in the solutions do not disappear after leaving for three days, add another 100 mL of 30% hydrogen peroxide solutions and keep them warm for one day.
4. Repeat Step 3 until the suspensions disappear.

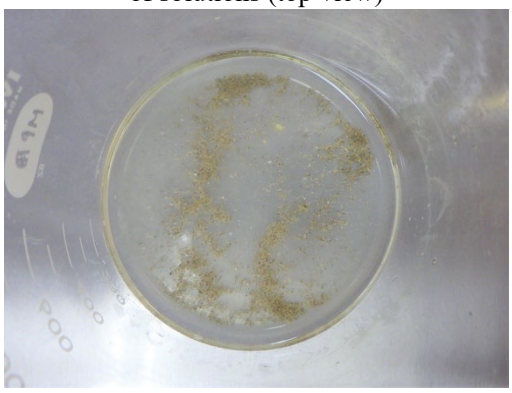
Photo 5-10. Warming of containers in a water bath



A condition immediately after the addition of solutions (top view)



A condition after the addition of solutions (24 hours later) (top view)



An end condition of oxidization (top view)

Photo 5-11. Examples of the progress of oxidation (with 100 mL 30% hydrogen peroxide solutions at 50°C)

5. Cover the containers with nets and carefully spill the solutions.
6. Rinse the containers used for oxidation with a small amount of purified water and carefully pour the water into the other containers through the nets (more than once).
7. Pour a small amount of purified water on the samples remaining on the nets to transfer them into glass containers or glass Petri dishes.

Photo 5-12. Samples on a glass Petri dish

[Reference] Methods to check activities of solutions

- In oxidation process, hydrogen peroxide sometimes reacts completely and becomes inactive. The activities of solutions can be checked by taking the following steps:

1) Collect a small amount of solutions used for oxidation, drop it on pieces of liver (catalase), fermented soybeans or manganese dioxide and check their foaming.
2) If they contain active hydrogen peroxide, they foam. If hydrogen peroxide is deactivated, they do not foam.

5.3 Density separation

5.3.1 Outline

A large number of inorganic impurities such as soil particles are sometimes included in samples. In such a case, to efficiently capture plastics, they are separated from the samples by utilizing differences in specific densities of inorganic impurities and plastics. In this guideline, a funnel and a silicon tube are utilized; however, alternative instruments may be employed if the sediment and suspended matter can be reliably separated.

5.3.2 Reagents and equipment

1) Funnel (made of glass)
2) Funnel stand
3) Silicon tube
4) Pinch cock (clip)
5) 5.3 M sodium iodide solution (ex. Approximately 800 g of sodium iodide diluted to 1 L with water)
6) Glass container
7) Net with 0.1 mm mesh openings

5.3.3 Procedure

1. Connect a silicon tube to a funnel. Bend the tip of the tube and secure it with a pinch cock (clip) (Photo 5-13).

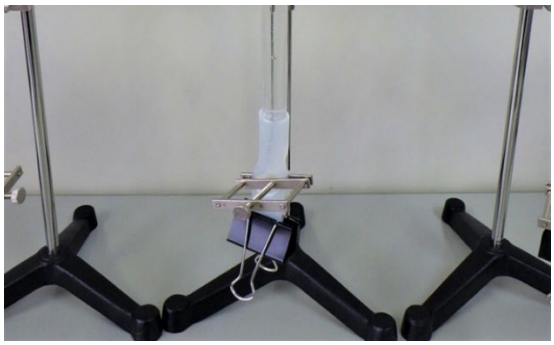


Photo 5-13. Pinching the tube tip

2. Add an appropriate amount of 5.3M sodium iodide solutions to samples in a container. Stir it slowly with a glass rod (Photo 5-14).

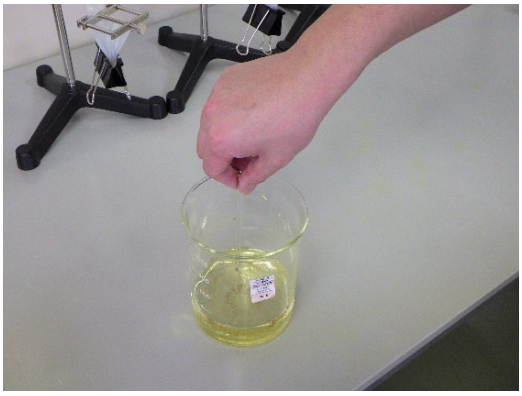


Photo 5-14. Stirring of a sample with a glass rod

3. Pour solutions into the funnel (Photo 5-15).

Photo 5-15. Pouring solutions into funnels

4. Cover the top of the funnel with a watch glass to prevent contamination by falling objects. Leave it to stand for approximately three hours (Photo 5-16).

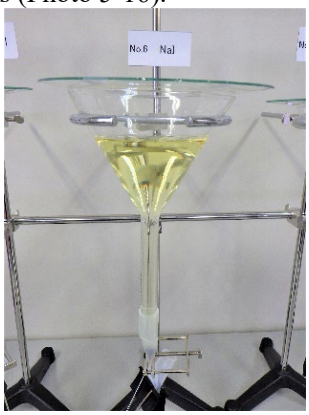
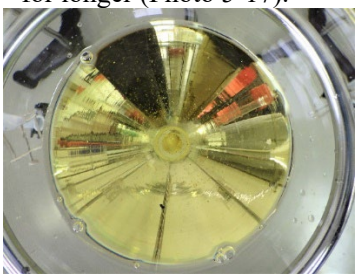
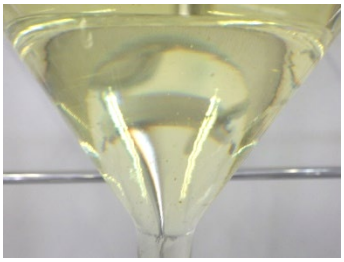


Photo 5-16. Leaving of solutions to stands

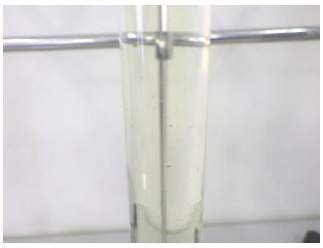
5. Check the condition of the solutions in the funnel. When the separation is insufficient, leave it to stand for longer (Photo 5-17).



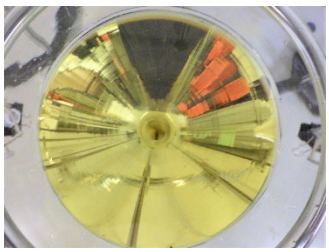
Top view



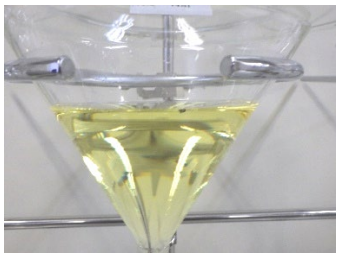
Upper and middle part
At the start of density separation



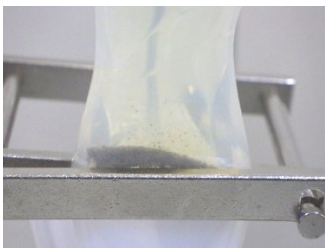
Lower part



Top view



Upper and middle part
At the end of density separation



Lower part

Photo 5-17. Solutions at the start and end of density separation

6. Set a container under the tube. Remove the clip and drain the lower part solutions while retaining the upper part solutions (Photo 5-18).

Photo 5-18. Density separation

7. Rinse out the samples remaining in the upper part with purified water (Photo 5-19).

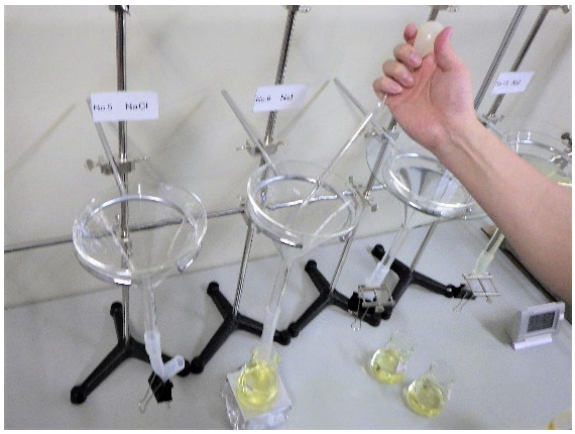


Photo 5-19. Rinsing out the remaining samples

8. Filter the water to collect the samples with a net with 0.1 mm mesh openings and wash the samples with purified water (Photo 5-20).

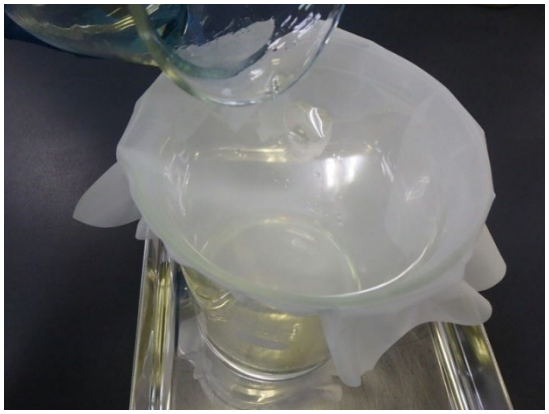


Photo 5-20. Filtration of water to collect samples

9. Filter the precipitates in the lower part of the tube with a net in the same way and check for candidate plastic particles (Photo 5-21).

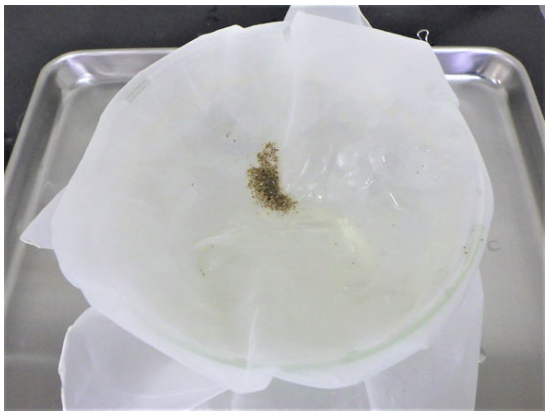


Photo 5-21. Precipitate in a lower part on a net after filtration

10. Transfer the filtered samples to glass Petri dishes (Photo 5-22).

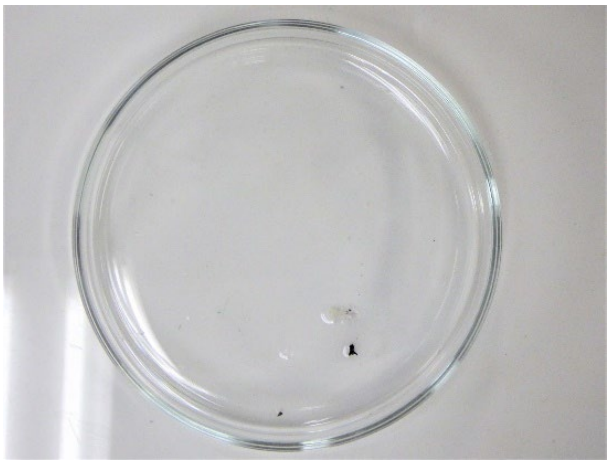


Photo 5-22. Filtered samples on a glass Petri dish

[Reference] Other method of density separation

Since sodium iodide is expensive, saturated aqueous solutions of sodium chloride are used for density separation as an alternative.

The specific gravity of saturated aqueous sodium chloride solutions is 1.2 g/cm^3, which is lower than the specific gravity of aqueous 5.3M sodium iodide solutions (1.5 g/cm^3). Thus, polyethylene terephthalate (PET) and other heavy microplastics are sometimes included in precipitates. It needs to check for microplastics in separated precipitates carefully.

While much time is required to sort out samples, it is acceptable to skip density separation and conduct sorting of candidate plastic particles mentioned in chapter 5 depending on conditions of samples (e.g., low turbidity) to avoid losses of microplastics and costs of density separation. However, for research conducted for the same purpose, it is recommended to unify the pretreatment process and contents as much as possible for comparison between each sample.

[Reference] Contraction

In certain rivers, sampling 10–20 m3 of water can yield more than 1,000 microplastic candidate particles. Because the principle involves a total analysis, the volume of water collected should be reduced and the analytical workload adjusted at sites where microplastic concentrations are known to be high based on previous surveys. However, if a large number of microplastic candidate particles is obtained, it can be splitting after transferring to a glass petri dish in 5.3.3 (10). Nevertheless, at least 100 candidate particles should be secured. Finally, the number of microplastics identified as plastics is multiplied by the splitting factor to determine the number of microplastics at that particular point.

Example: Conic quadrant

First, the candidate particles are stacked in a cone shape, and radially spread from the center with a spatula to form a flat disk. Next, a straight line is drawn passing through the center of the disk and dividing the circumference into quarters; subsequently a diagonal pair of opposite quadrants is collected and used as the analytical sample (Figure 5-24). If the number of candidate particles is large, the above process is repeated. It is desirable to confirm that splitting is conducted properly with weight control and marker particles.

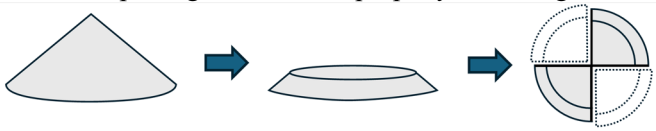


Figure 5-2　4 Conic quadrant

5.3.4 Check of contamination in a test room

Before taking pretreatments written in Sections 4.1 to 4.3, Petri dishes filled with purified water are placed at hand, and blank tests are conducted to check plastic contamination. After each pretreatment, it is checked whether contaminant plastics and other impurities are in it.

When they contaminate the water in the Petri dishes, their components are identified and recorded through the following methods written in chapters 6 to 7. When identified contaminant particles are plastic and plastics that have the same components and characteristics as they (e.g., shape and color) are found in the samples, the number of microplastics subtracted with the number of contaminant plastics are recorded as references for Item 14 and 15 on Subsection 2 in Section 9.2.

It is recommended to confirm the degree of contamination in the test room in advance. In addition, by checking whether contamination occurs every time or during specific operations, it is possible to estimate the causes of contamination and examine countermeasures.

6 Sorting out of candidate plastic particles

6.1 Outline

From the treated samples, candidate plastic particles are sorted out. Then, maximum Feret's diameters of them are measured, and they are sorted by color and shape.

It is easy to sort them out by adding approximately 10 mL water to samples. The excessive water is dried in ovens at 60 °C or less until it becomes suitable. However, the samples must not be dried completely, or the candidate plastic particles will stick onto the residues of impurities, and it becomes difficult to sort them out.

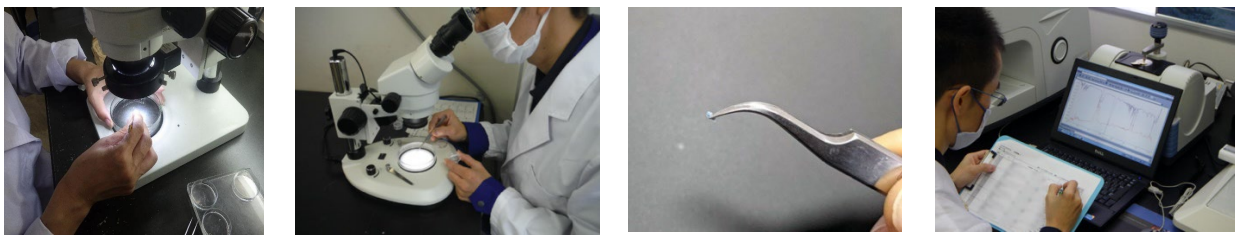
6.2 Equipment

1) Stereomicroscope
2) Precision tweezers
3) Micrometer (for visual measurements of maximum Feret's diameter and other items)
4) Digital camera (for measurements of maximum Feret's diameter and other items with image processing)
5) Personal computer (for measurements of maximum Feret's diameter and other items with image processing)
6) Image editor application (for measurements of maximum Feret's diameter and other items with image processing

6.3 Procedure

6.3.1 Visual measurement of maximum Feret's diameter and other items

1. Observe samples on Petri dishes with stereomicroscopes and sort out plastic candidate particles with precision tweezers (for details, refer to Section 6.4.) Assign identification numbers to them and measure their maximum Feret's diameter with micrometers.
2. Transfer the candidate plastic particles to another Petri dish.
3. Classify the candidate plastic particles by shape and color (for details, refer to Sections 6.5 and 6.6).
4. For the respective candidate plastic particles, make records about the maximum Feret's diameter, shape, and color.



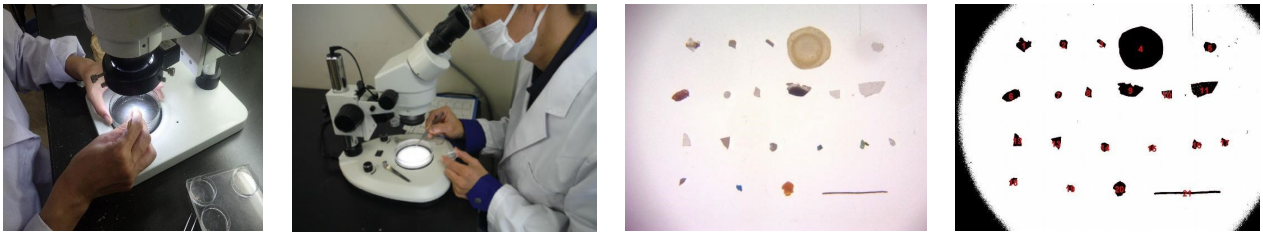
1 2 3 4

Photo 6-1. Work sceneries

6.3.2 Measurement of maximum Feret's diameter and other items with image processing

1. Observe samples on Petri dishes with stereomicroscopes and sort out plastic candidate particles with precision tweezers.
2. Transfer the candidate plastic particles to other Petri dishes.
3. Take images of them on the Petri dishes with digital cameras.
4. Assign identification numbers to them in the images with image editor applications.
5. For each of them, measure the major axis, minor axis, and projected area. The maximum and minimum Feret's diameters[VI] of each candidate particle are measured and used as the major and minor axes, respectively (Figure 6-3). However, because the fibrous plastic candidate particles may be overestimated or underestimated when measured using the Feret`s diameter, the length measured along the fiber and width are, if possible, recorded as the major and minor axes, respectively (Figure 6-4). Furthermore, for instances in which the fibers are entangled and become a lump (hereinafter referred to as "fiber mass"), the maximum and minimum Feret's diameters and the projected surface area of the fiber mass should be measured as a single candidate particle without forcibly unwinding it.
6. For each of them, make records about the maximum and minimum Feret's diameters, the projected surface area, shape, and color (for details of classification by shape and color, refer to Sections 6.5 and 6.6).

* The following treatments are not needed for candidate plastic particles which maximum Feret's diameter is over 5 mm.
* Depending on the shapes of plastic pieces, errors in the areas become big by calculating from maximum and minimum Feret's diameters. Thus, image processing software is required to accurately evaluate the projected surface area.
* Projected surface area was defined as the maximum visible area observed from above when each plastic particle was placed with its most stable plane facing downward under a stereoscopic microscope.



1 2 3 4

Photo 6-2. Work sceneries

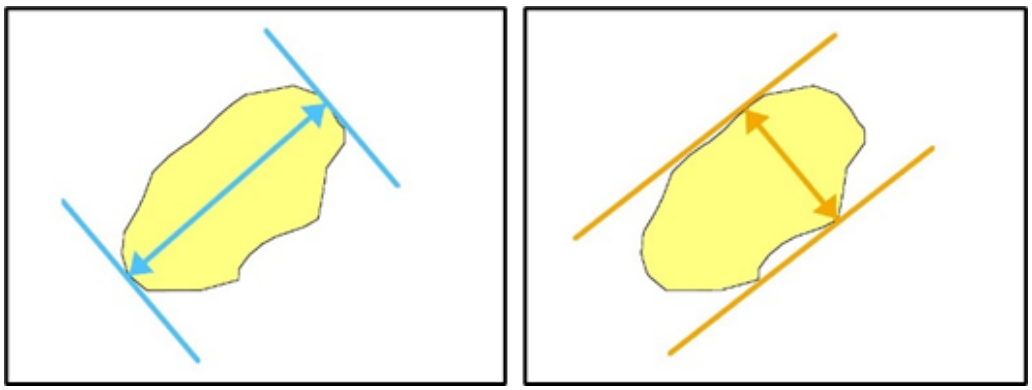


Figure 6-3 Left: maximum Feret's diameter, right: minimum ferley's diameter

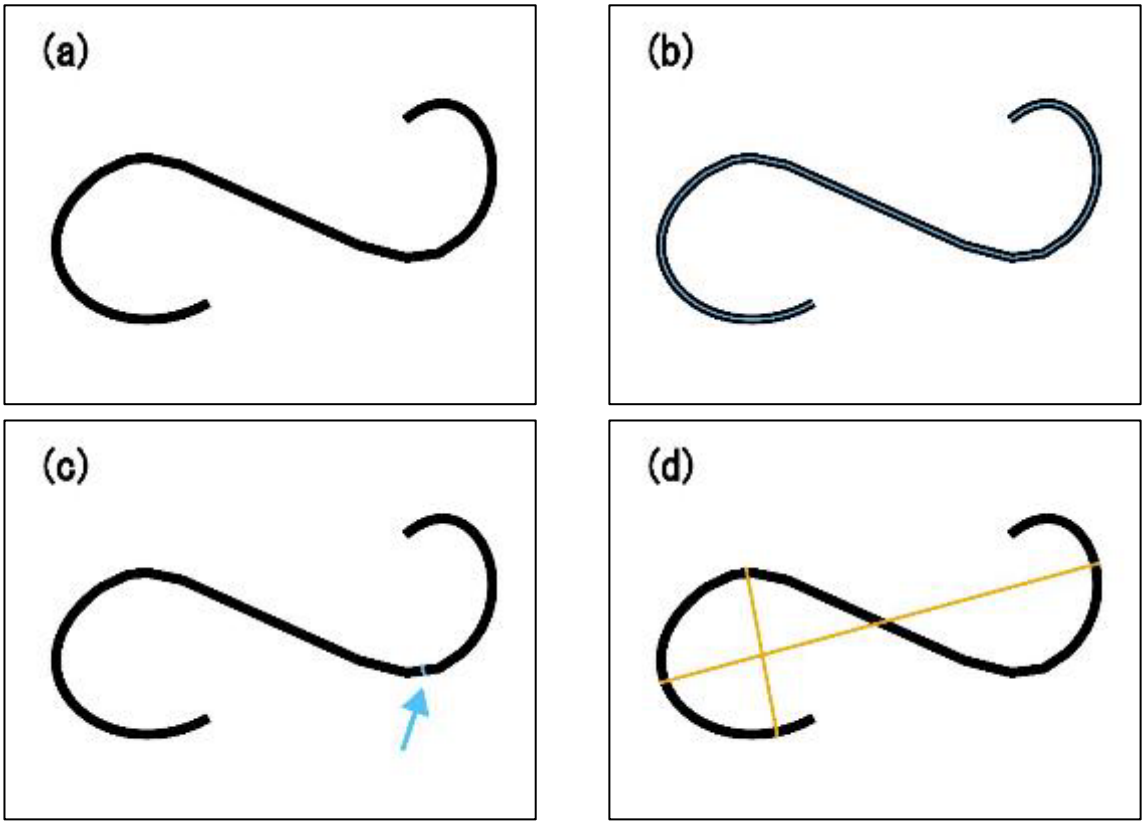


Figure 6-4 Measurement part of fibrous plastic (schematic)
((a) original drawing, (b) major axis, (c) minor diameter,
and (d) maximum and minimum Feret's diameters)

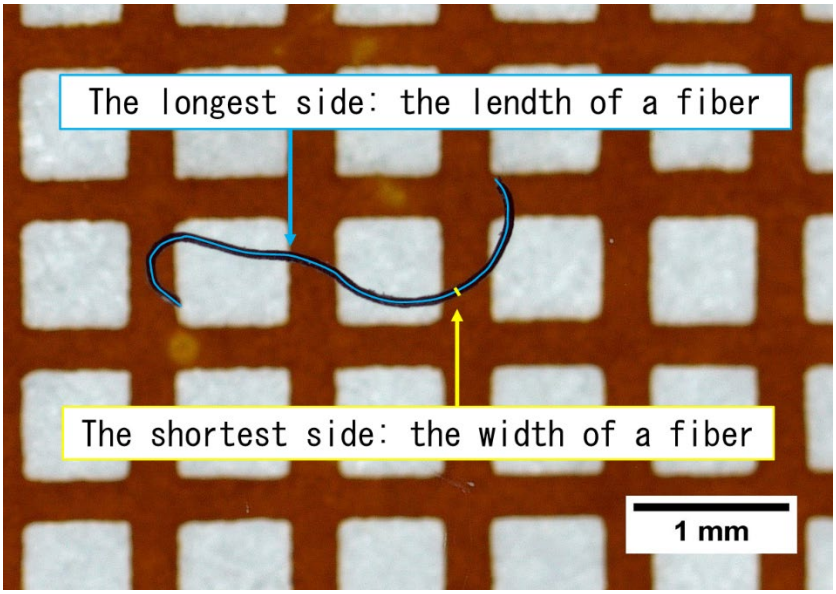
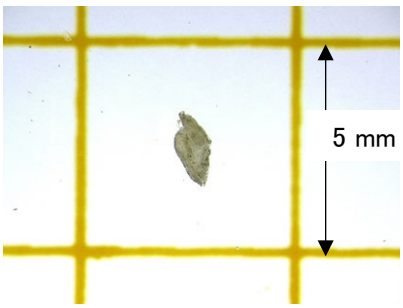


Photo 6-3. An example of measured sides of a fibrous plastic

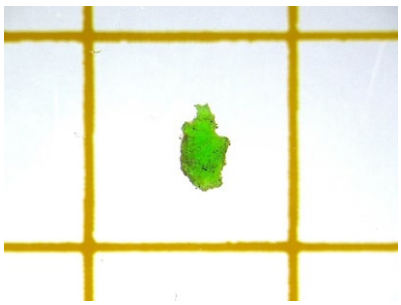
6.4 Identification of candidate plastic particles

Photo 6-4 shows typical candidate plastic particles by type.

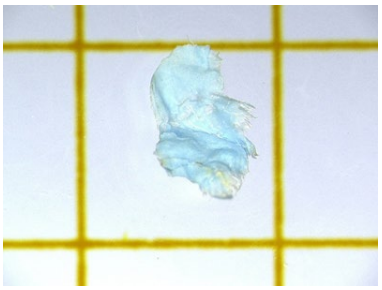
* Some candidate plastic particles are fragile and damaged with tweezers easily, so that it needs to treat them carefully.



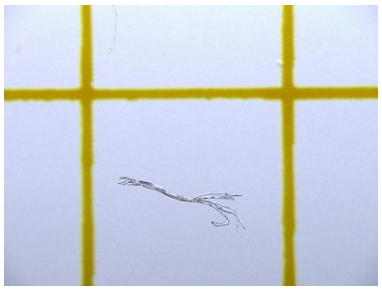
Polystyrene (PS)



Polyethylene (PE)



Acrylic resin (PMMA)



Nylon

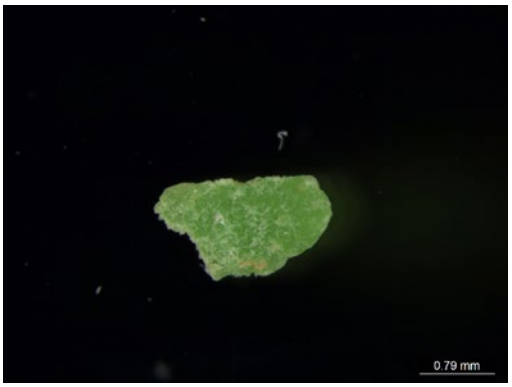
Photo 6-4. Examples of typical candidate plastic particles
Yellow frames are ruled into 5 mm squares.
(Photos above are examples. The same types of materials have different shapes and colors.)

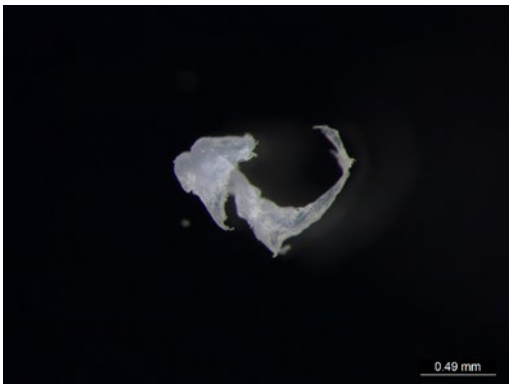
6.5 Classification by shape

Candidate plastic particles are classified into the following shape categories:

1. Fragment
2. Film and sheet
3. Bead
4. Foam (expanded)
5. Cylinder and sphere (pellet)
6. Fiber
7. Fiber cluster
8. Plastic-coated fertilizer
9. Artificial turf, artificial grass
10. Others

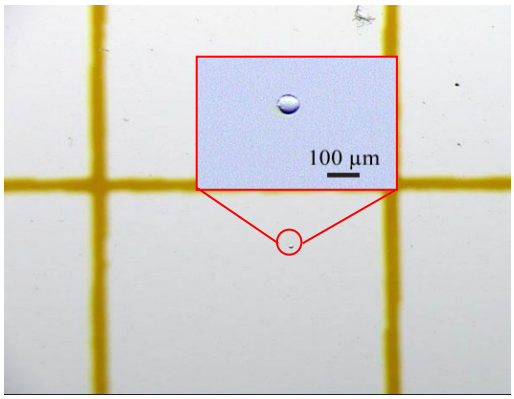
Photo 6-5 shows examples of plastic pieces by shape.





1. Fragment: fragment-like pieces that do not have specific shapes

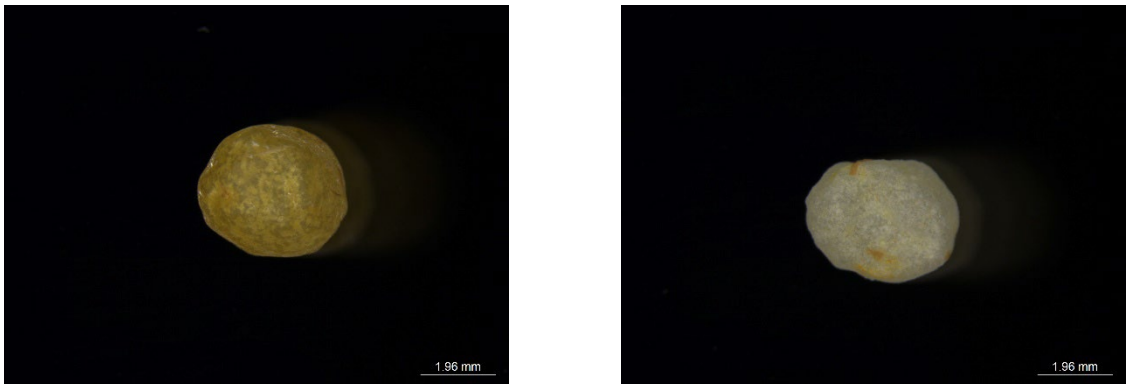
2. Film and sheet: thin sheet-like pieces



Yellow frames are ruled into 5 mm squares.

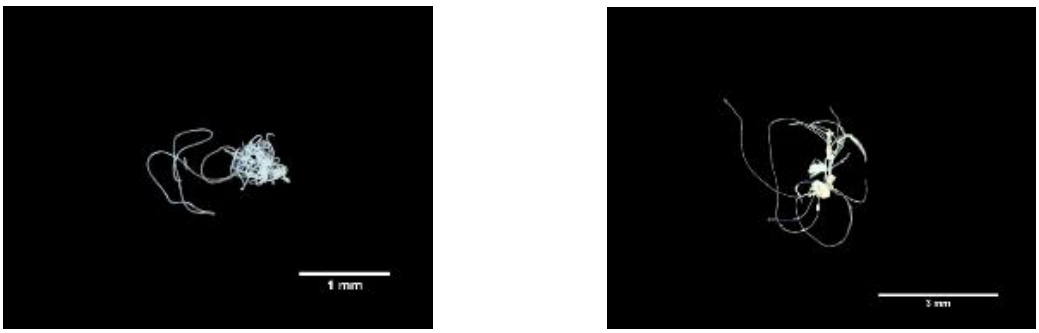
3. Bead: almost spherical pieces

4. Foam (expanded): expanded pieces



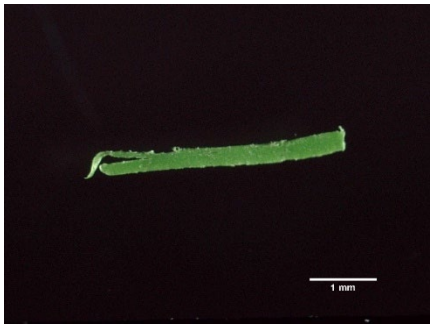
5. Cylinder and sphere (pellet)

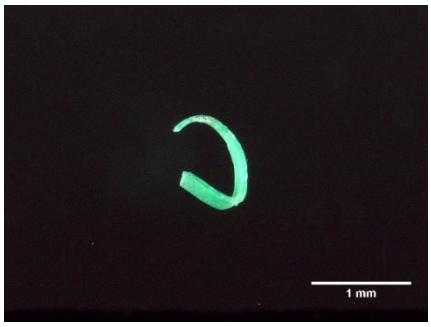
6. Fiber: elongated string-like and rod-like pieces



7. Fiber cluster: tangled fibers

8. Plastic-coated fertilizer





9. Artificial turf, Artificial grass

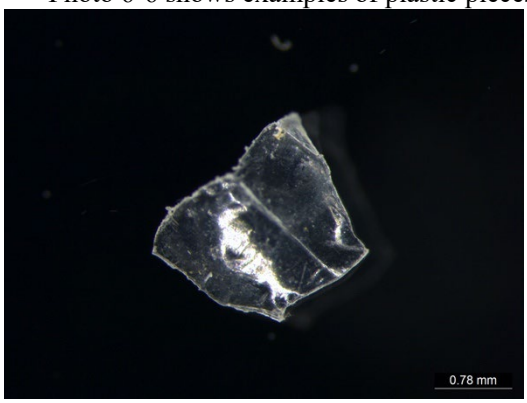
Photo 6-5. Examples of plastic pieces by shape

6.6 Classification by color

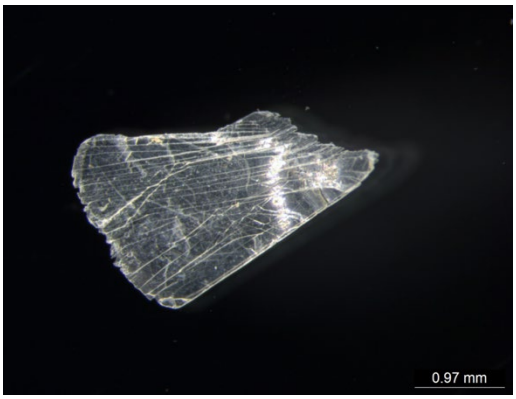
Candidate plastic particles are classified into the following color categories:

1. Transparent
2. White
3. Red
4. Orange
5. Yellow
6. Green
7. Blue
8. Purple
9. Black
10. Compound (mixed color)
11. The others

Photo 6-6 shows examples of plastic pieces by color.

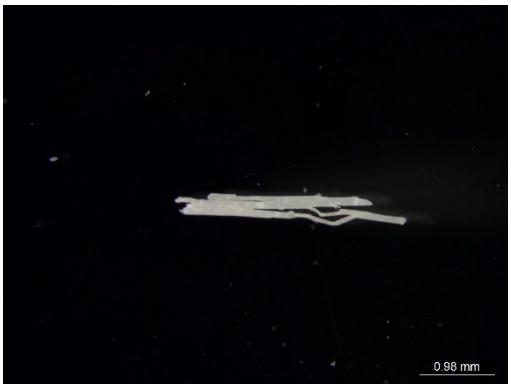


1. Transparent

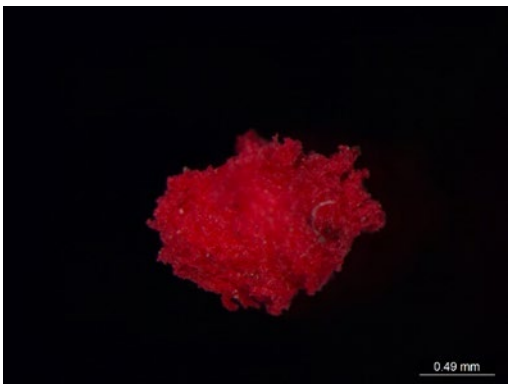


1. Transparent

2. White

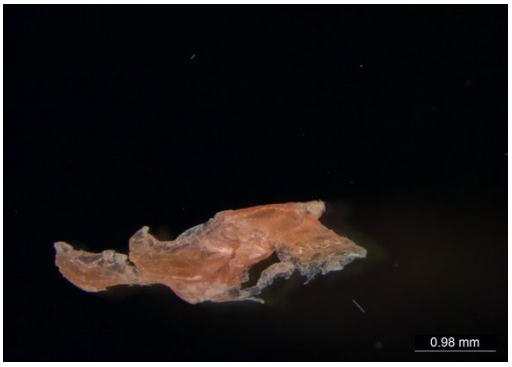


2. White

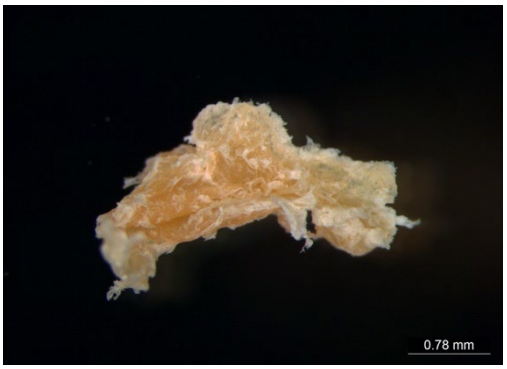


3. Red

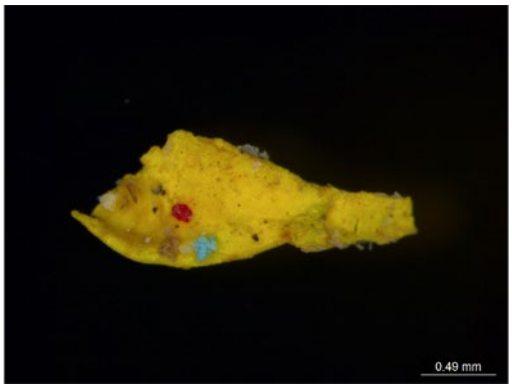
3. Red



4. Orange

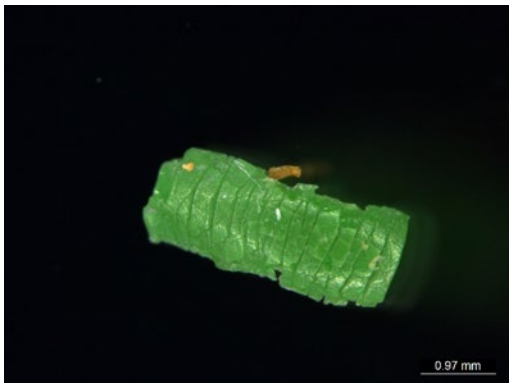


4. Orange



5. Yellow

5. Yellow

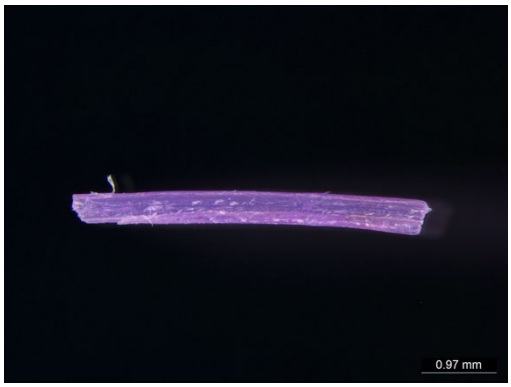


6. Green

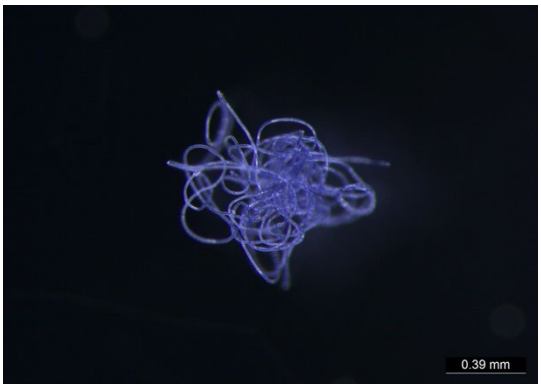
6. Green

7. Blue

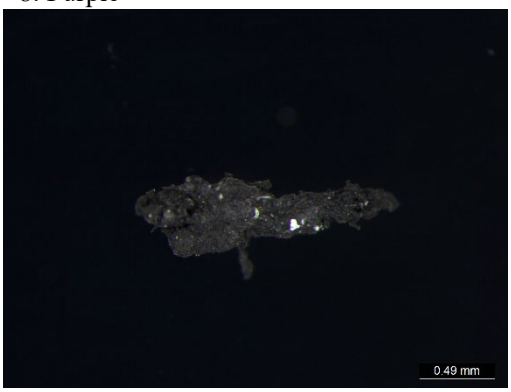
7. Blue



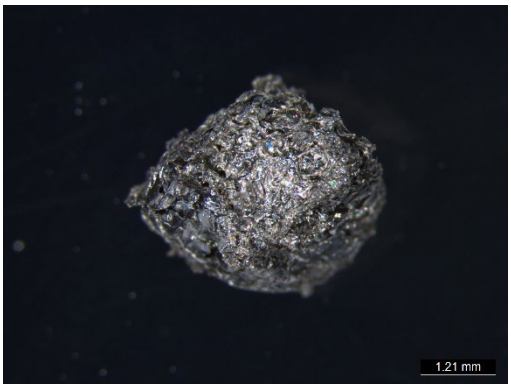
8. Purple



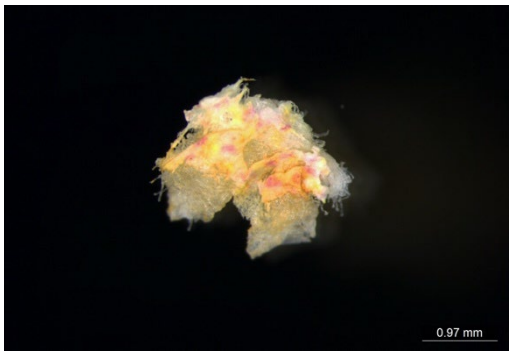
8. Purple



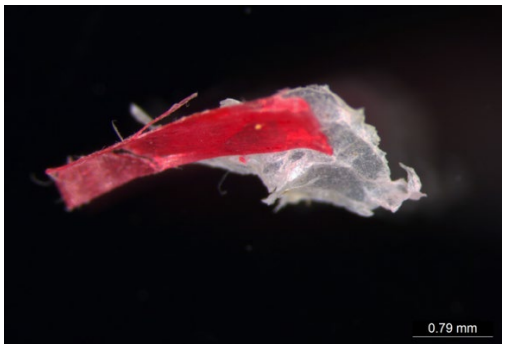
9. Black



9. Black



10. Compound (mixed color)



10. Compound (mixed color)

Photo 6-6. Examples of plastic pieces by color

[Reference] Spike-and-Recovery test with standard plastic particles (example)

The accuracy of sorting of candidate plastic particles with microscopes is sometimes varied depending on the operator. When multiple operators are involved in sorting, it is recommended to conduct spike-and-recovery tests to manage separation accuracy.

Procedure

1. Add standard plastic particles to collected samples before sorting them out.
2. Sort out the standard plastic particles from the collected samples.
3. Check how accurately the standard plastic particles are sorted and collected.

* Multiple sizes of standard plastic particles should be selected. The sizes are selected from 0.3, 0.5, 1, and 2 mm in consideration of sizes of candidate plastic particles.
* The number of standard plastic particles should be approximately 50, and recovery rates should be calculated.
* Colors of standard plastic particles should be unusual colors in nature to distinguish them from collected samples.

7 Identification of plastic types

7.1 Identification of plastic types with Fourier transform infrared spectrophotometer

7.1.1 Outline

This section describes how to identify plastic types with Fourier transform infrared spectrophotometer with attenuated total reflection spectroscopy (FT-IR ATR), which is generally used.

FT-IR is not suitable for identifying some shapes and sizes of candidate plastic particles such as fine fibers. In these cases, it should be recorded specifically that FT-IR ATR spectroscopy has not been used.

To identify plastics with finer particle sizes, a micro-Fourier transform infrared spectrophotometers or Raman spectroscopes are used.

7.1.2 Equipment

1) Fourier transform infrared spectrophotometer (FT-IR)
2) Precision tweezers
3) Personal computer for spectrum analysis
4) Static eliminator (ionizer) (recommended)

* When handling dry plastic pieces, it prevents them from becoming charged and jumping up.

7.1.3 Procedure

1. Wipe sensors with paper wipes.
2. Configure measurement conditions.

* Measurements of microplastics are performed with the number of sample scans set to five or more and the conserved wave area set to 4000 to 650 cm^{-1} or much-extended range.

3. Perform background measurements.
4. Clamp candidate plastic particles sorted out in Chapter 6 to the sensors one by one with precision tweezers.
5. Perform measurements.

* Candidate plastic particles are easily lost before and after measurements and sometimes crushed after measurements. In these cases, record them in remarks.
* When particles are crushed, their fragments often stick onto the FT-IR sensors. To remove such fragments, wipe the sensors with paper wipes moistened with ethanol. After that, perform a background measurement and then restart the measurements.

6. For displayed infrared spectrum, identify plastic types by doing spectral retrieval in the library. Save the obtained spectral data in csv and image formats.
7. When hit qualities are low and plastic types cannot be identified through spectral retrieval, visually identify the plastic types from characteristic peaks on infrared spectra. After recording the hit qualities, save the spectral data. If it is difficult to visually identify them, they are not counted as plastics.
8. Record the identified plastic types, hit qualities, implementation of visual identification based on the spectra.

7.1.4 Notes

1. Hit quality

- Hit quality refers to matching rates of infrared spectra between libraries created by FT-IR manufacturers and measurement samples.
- Since hit quality calculations are specific to the manufacturers, and degrees of clamping to the sensor are specific to devices, hit quality values vary depending on FT-IR manufacturers even with the same samples.
- The libraries vary depending on the manufacturers. In addition, manufacturers have multiple types of libraries, and for deteriorated plastics, hit quality is sometimes improved by using libraries with different condition (e.g., heat deterioration and UV deterioration) even for the same material.
- In academic study, the lower limit of hit quality is set, and some samples less than this limit are excluded from collected data. However, this survey aims to understand actual distributions of microplastics

carried out by local governments and other organizations. Therefore, even if hit qualities are low, plastic types are visually identified from shapes of similar spectra based on characteristic peaks of infrared spectra.

2. Characteristic peak shapes by plastic types

- Photo 7-1 show examples of infrared spectra based on plastic types. For reference, the spectra in cases that impurities are included are also shown to show differences in appearance of peaks. When peaks unique to the components shown below are observed, samples should be identified even if they contain impurities. In some figures, the spectra of non-plastics are shown.

(a) Polyethylene (PE)

High C-H stretching vibration is observed at 2845 and 2915 cm^{-1}, and CH_2 rolling vibration peaks are observed near 717 and 730 cm^{-1}. For high-density polyethylene (HDPE), peaks are observed near 1462 and 1472 cm^{-1}, and for low-density polyethylene (LOPE), CH_2 variable angle vibration is observed near 1462 and 1467 cm^{-1}. As for C-H stretching vibration around 2845 and 2915 cm^{-1}, many natural objects display corresponding peaks, so be sure to check for CH_2 rolling vibrations and angular vibration peaks as well.

PE is easily oxidized by acid and heat. For oxidized PE, absorption peaks derived from functional groups and bonds indicating oxidation such as C=O, C-O, etc. are observed around 1700 cm^{-1} and 1200 cm^{-1}.

(b) Polypropylene (PP)

Four characteristic C-H stretching vibration peaks are observed around 3000 cm^{-1}. In addition, relatively high CH_2 angular vibration peaks are observed near 1455 and 1377 cm^{-1}.

(c) Polystyrene (PS)

Out-of-plane angular vibration on the high-intensity aromatic ring C-H is observed near 694 cm^{-1}, and 6 to 7 characteristic peaks derived from benzene rings are observed around 3000 cm^{-1}. In addition, aromatic ring stretching vibration is observed near 1492 and 1601 cm^{-1}, and aromatic ring C-H variable angular vibrations are observed near 1027 cm^{-1}.

(d) Polyethylene terephthalate (PET)

Out-of-plane angular vibration on the aromatic ring C-H is observed near 720 cm^{-1}, C-O stretching vibration is observed near 1094 and 1241 cm^{-1}, and characteristic peaks for C=O stretching vibration are observed near 1713 cm^{-1}.

(e) Nylon

NH variable angle vibration and CN stretching vibration are observed near 1538 cm^{-1}, C=O stretching vibration is observed near 1634 cm^{-1}, CH stretching vibration is observed near 2858 and 2932 cm^{-1}, and characteristic sharp peaks on NH stretching vibration are observed near 3298 cm^{-1}.

(f) Polyurethane (PU)

It exhibits characteristic peaks for the N-H stretching vibration near 3291 cm^{-1}, CH_2 stretching vibration near 2858 and 2938 cm^{-1}, C=O stretching vibration near 1734 cm^{-1}, C-N-H bending vibration near 1538 cm^{-1}, and C-O stretching vibration near 1109 cm^{-1}.

(g) Polyvinyl chloride (PVC)

It exhibits characteristic peaks for the CH_2 stretching vibrations near 2862 and 2911 cm^{-1}, CH_2 bending vibration near 1426 cm^{-1}, CH_2-Cl bending vibration near 1254 cm^{-1}, and C-Cl bending vibration near 637 and 694 cm^{-1}.

(h) Mixed PE and PP (PE/PP)

Similar to PE, it exhibits peaks for the C-H stretching vibration at approximately 2845 and 2915 cm^{-1}, CH_2 bending vibration at approximately 1462 cm^{-1}, and CH_2 rocking vibration at 717 cm^{-1}. In addition, Similar to PP, it has a relatively strong CH_2 bending vibration peak around 1455 and 1377 cm^{-1}.

(i) Protein

It needs to pay attention because the spectrum for protein is similar in shape to that for nylon. The protein

spectrum tends to have broad peaks, and the peak wavenumber is often different from that of nylon. In addition, protein products look different from plastics, as shown in the image. Protein products are visually identifiable so that cell tissues are often observed on microscopes.

(j) Cellulose (natural fiber)

Broad peaks are observed near 1000 to 1100, 2800 to 3000, and 3300 to 3400 cm^{-1}.

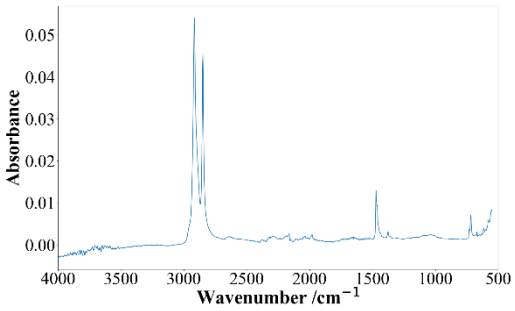
(k) Inorganic substances

Glass and other inorganic substances have a broad and monotonous peak. For glass, a broad and high-intensity peak is observed around 1050 cm^{-1}.

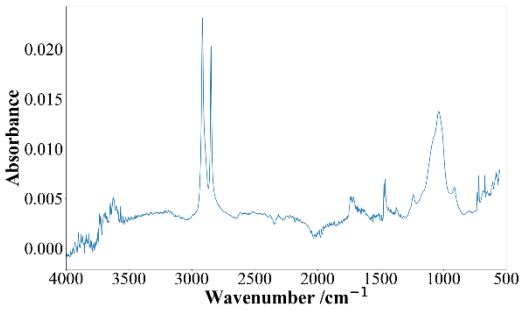
(l) Black substance

Black substances (rubber or the like) whose clear peaks are not detected in the IR spectrum are separated as candidate plastic particles.

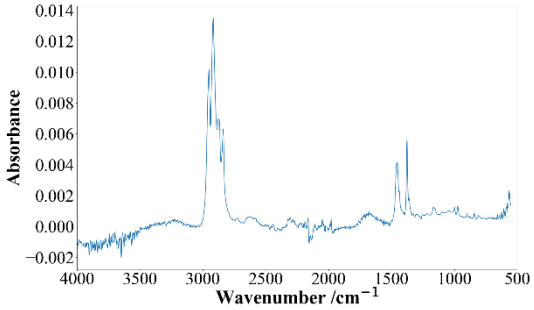
* In such black substances, their spectra are sometimes observed through ATR spectroscopy with germanium (Ge) prisms which have a higher refractive index.



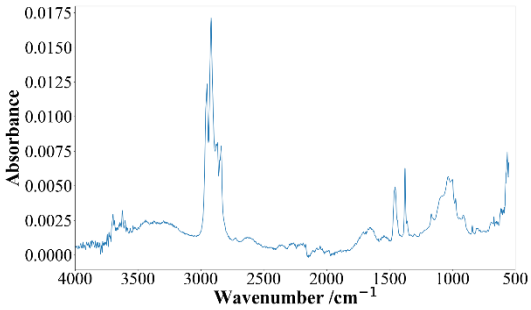
(a) Polyethylene (PE)



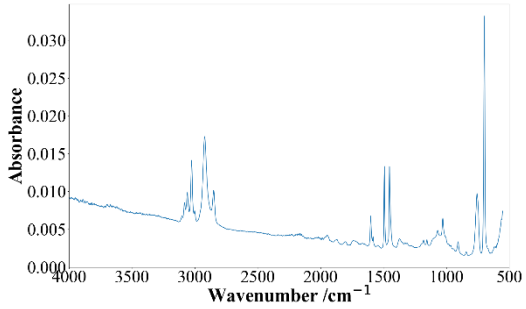
PE + impurities



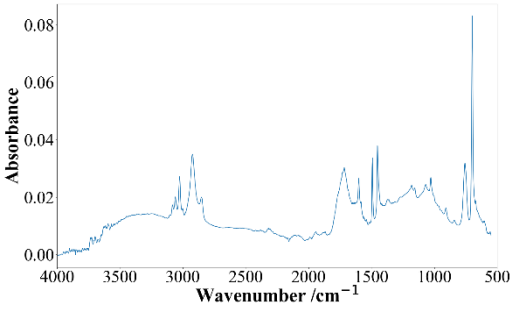
(b) Polypropylene (PP)



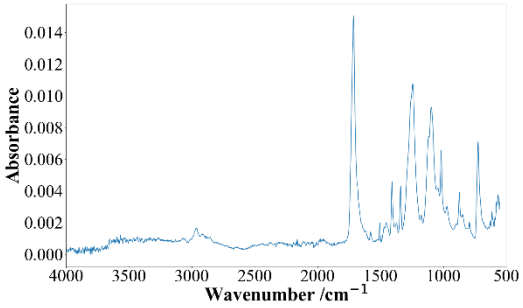
PP + impurities



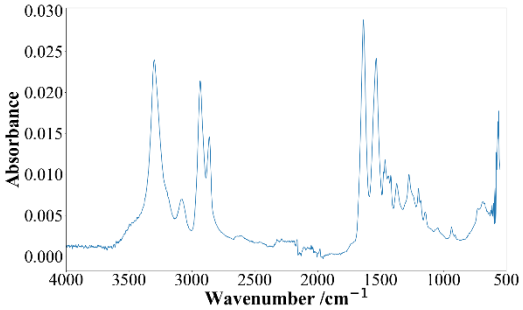
(c) Polystyrene (PS)



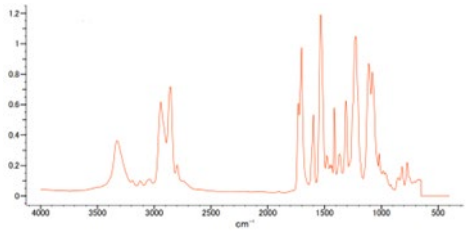
PS + impurities



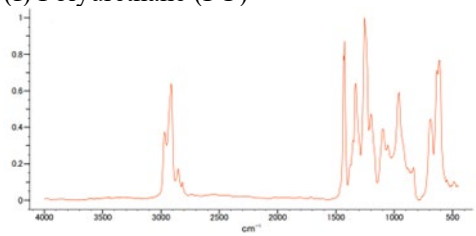
(d) Polyethylene terephthalate (PET)



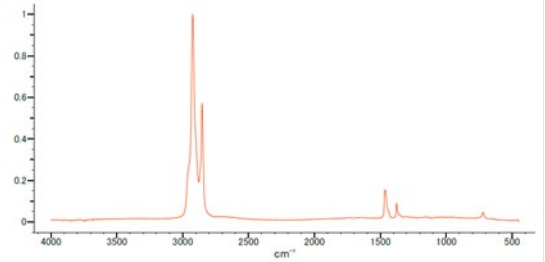
(e) Nylon



(f) Polyurethane (PU)

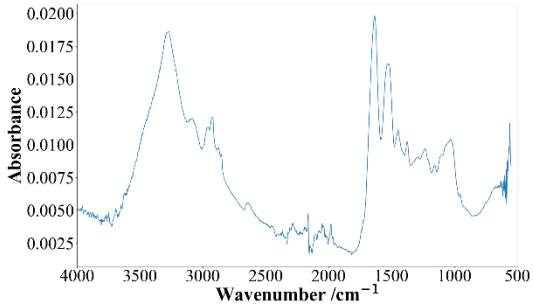


(g) Polyvinyl chloride (PVC)

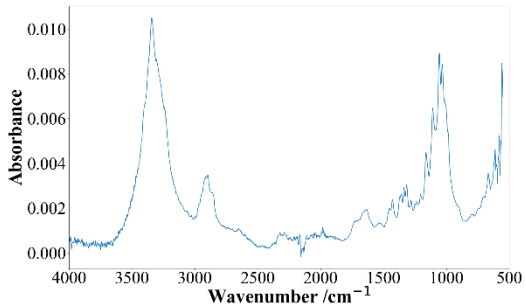


(h) Mixed PE and PP (PE/PP)

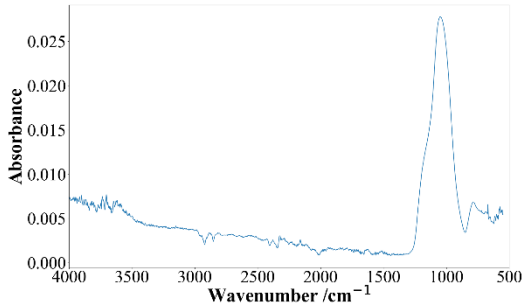
Non-plastic substances



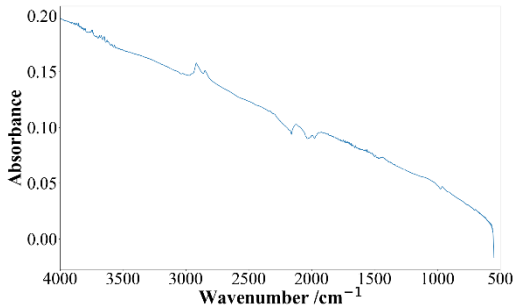
(i) Protein and its microscopic image

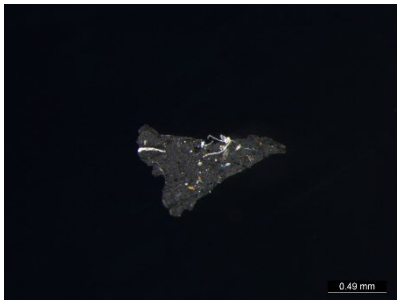


(j) Cellulose



(k) Inorganic substance





(l) Black substance and its microscopic image

Photo 7-1. Examples of infrared spectral shapes of plastics and non-plastics

8 Measurement of mass of microplastics

8.1 Measurement of total mass of microplastics in samples (optional)

8.1.1 Outline

Total mass of microplastics in samples are measured with electronic balances.

8.1.2 Equipment

Electronic balance

8.1.3 Procedure

1. From plastic candidate particles, collect microplastics identified in Section 6.1 by sample number.
2. Measure the mass of the collected microplastics with electronic balances.

[Reference] Measurement of mass of each microplastic

By identifying mass of microplastics by plastic type, it is possible to apply research results to determine types of plastics to be subject to microplastic reduction measures and evaluate to what extent the measures are effective. To understand distributions of plastics by type and mass, the mass measurements of each microplastic are effective.

1. Outline

Mass of each microplastic is measured with ultra-micro balances. It must be conducted before their identification written in chapter 7.

2. Equipment

- Ultra micro balance

* Some types of microplastics are extremely light; thus, it is recommended to use balance capable of measuring at 0.1-μg scale.

3. Procedure

Measure mass of plastic candidate particles sorted on the Petri dish in chapter 6 with ultra-micro balances.

[Reference] Geometric relationship between the projected surface area and mass of a plastic particle

The quantification of the mass of the meso/microplastic particles (MMP) is crucial for assessing the global inventory of ocean plastics and assessing environmental and human health risks; however, weighing individual samples is extremely laborious. A linear regression model between mass and projected surface area on a log scale was established by directly measuring the masses of 4390 MMP particles collected at 35 sites in 17 Japanese rivers with an ultramicrobalance (Kataoka et al., 2024). This model is applicable from 10^{-4} mm^2 to 10^2 mm^2 projected surface area. Furthermore, it can be applied to different shapes and material types.

$$Log10M = bLog10S + a$$

M: particulate mass, S: projected surface area
a:-1.12±0.01　b:1.14±0.01

- a and b are regression coefficients that were determined by the least squares method.
- Projected surface area was defined as the maximum visible area observed from above when each plastic particle was placed with its most stable plane facing downward under a stereoscopic microscope.
- There are two limitations of applying the geometric relationship: (1) large uncertainty in the mass estimation of particles with a major-axis length > 10 mm and (2) the quantification of microplastic particles <10 μm.

（Source: Geometric relationship between the projected surface area and mass of a plastic particle. Kataoka et al., 2024）

9 Sorting of measurement results

9.1 Sorting of measurement results of microplastics

The measurement results are sorted with reference to the items listed below:

Required items

1. Type of plastic
2. Maximum Feret's diameter
3. Shape
4. Color
5. Microscopic image
6. Hit quality in FT-IR measurement
7. Infrared spectrum data (csv and image data)

Recommended items

1. Minimum Feret's diameter (for samples analyzed with image processing)
2. The projected surface area (for samples analyzed with image processing)
3. Thickness
4. Mass
5. Wave range

9.2 Sorting of details of survey

Details of surveys for each location are sorted with reference to the items listed below:

1) Items for survey

1. Data and time
2. Locations for sampling in river and lake
3. Number of sunny days prior to survey
4. Specifications of nets
   - Mouth size
   - Mesh openings
   - Net length
5. Method of pretreatment:
   - Oxidation (implemented or unimplemented) (used reagents)
   - Density separation (implemented or unimplemented) (used reagents)
6. FT-IR device and library used to identify microplastics

2) Items for sampling locations and samples

Required items

1. Location name
2. Sampling position (GPS data)
3. Water depth
4. Flow velocity (measured with flow meter) (river)
5. Measurement time or towing duration
6. Rotation number and filtered water volume of flow meter
7. Towing distance (lake)
8. Boat wake (lake)
9. Boat velocity (lake)
10. Sampling position from a boat (lake)
11. Method of sampling (hanging from bridges or stepping into river (river), towing by boats (lake))
12. Time duration which nets were underwater (starting and ending time)
13. Wind direction and velocity and wave direction (lake)
14. Number of microplastics
15. Number density of microplastics
16. Number of candidate plastic particles that could not be identified as plastic

Recommended items

1. Weather (for approximately seven days prior to survey)
2. Air temperature and water temperature
3. Turbidity
4. Tides
5. Riverbed and lakebed condition at measurement location (e.g., stones, sand and invisible)
6. Circumstances around sampling location (e.g., river and lake work, drainage outlet, rainwater outlet, drifting garbage)
7. Flow rate (official or practically measured data)
8. Water level (for seven days or more prior to survey date to determine conditions of streams (e.g., streams during normal weather and after rain))
9. Information about rivers which flow into/out from lakes
10. Mass of microplastics
11. Mass concentration of microplastics
12. Percentage by shape
13. Percentage by color
14. Percentage by size
15. Percentage by type

9.3 Data analysis

Data are analyzed to identify distributions of microplastics along rivers. Examples of analysis are described below.

When the data are compared, it needs to consider that the amount of microplastics vary greatly. Samples collected at the same location and time sometimes show double or more differences.

1) Changes in the amount of microplastics from upstream to downstream of river

When multiple locations are surveyed in one river, the number density of microplastics should be represented graphically to show their changes from upstream to downstream. When mass measurements are included in the survey, the mass density should be also represented graphically.

2) Distribution of microplastics by particle size by location

To understand the characteristics of plastic by location and make comparisons between locations, the number of microplastics by maximum Feret's diameter classified at 0.1 mm intervals and the number of microplastics by shape are represented graphically by location.

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*The names are arranged in alphabetical order. Their posts are those in March 2024.

Appendix. 1

Standard Specification of Automatic Sample Preparations in Guidelines for River and Lake Microplastic Monitoring Methods

Contents

Extra documents

1 Introduction

The sample preparations in ‘Guidelines for River and Lake Microplastic Monitoring Methods (GRLMMM)’ have two problems: one is the deviations in results due to the variety of operators’ skills, and another is taking time for operations. Thus, the automatic sample preparations in accordance with GRMMM was considered, and its standard specification was prepared. This standard specification is expected to be applied not only to river samples, but also to lake and marine surface samples.

2 Consideration for the automation

The oxidation (Chap. 5.2 in GRLMMM) was automated by using a pump to supply a reaction solution to a glass preparative container, regulating heating temperature, controlling the reaction time and stirring. The density separation (Chap. 5.3 in GRLMMM) was also automated by passing through the following process.

1. Wash the preparative container with detergent.
2. Supply a dense solvent for the density separation to the container with pump.
3. Control the time of initial stirring and settling.
4. Supply an additional dense solvent with a pump after enough settling.
5. Take the supernatant out of the container.
6. Collect the candidate plastic particles (CPP) extracted from the supernatant with a filter.

The scope of automation in this consideration is indicated in Figure 1.

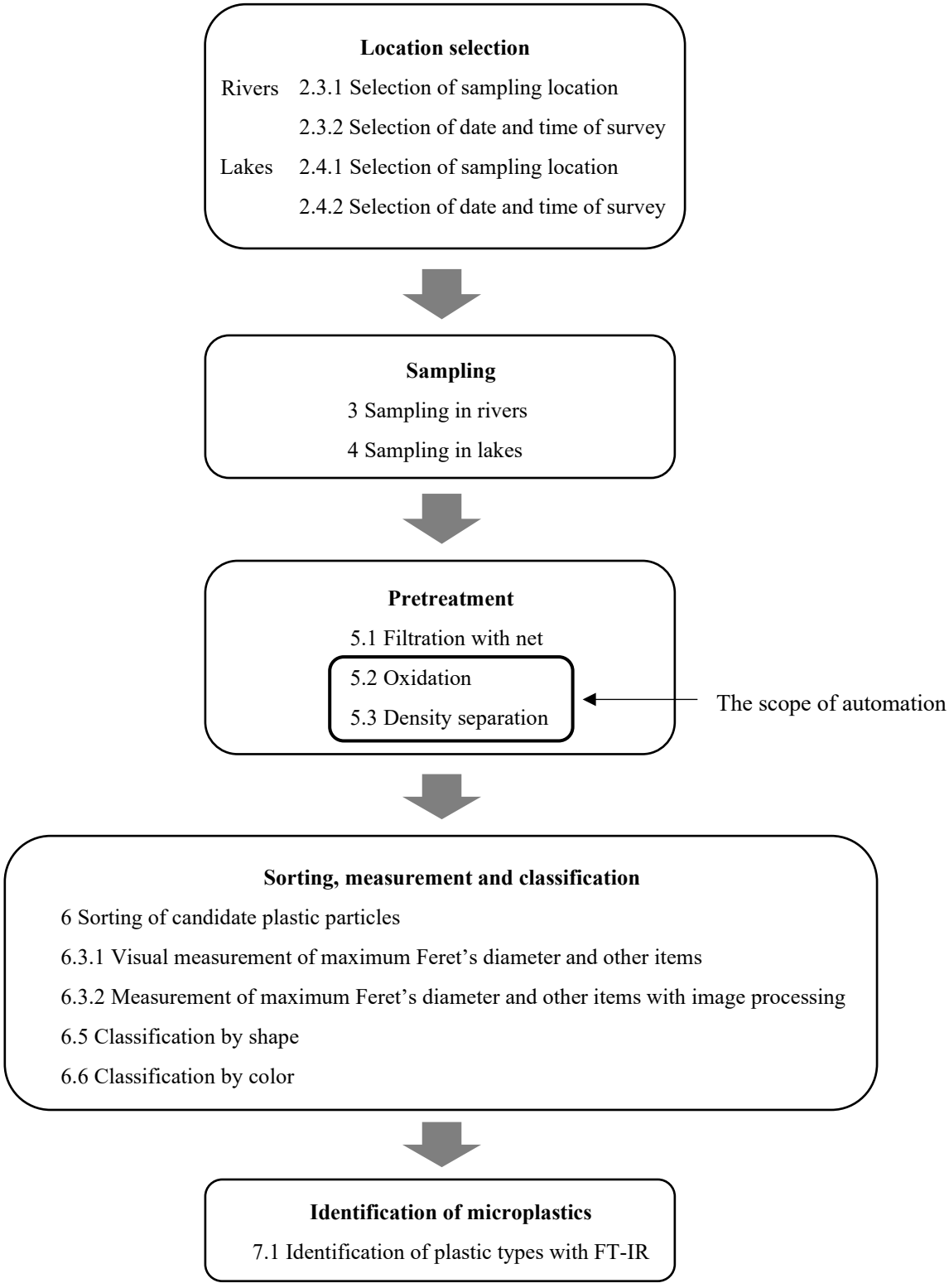


Figure 1. The procedure of the microplastic analysis indicated in GRLMMM

3 Specification

1. Samples

   The samples taken by following GRLMMM except those include many sand particles and algal fragments.

2. Size of plastic particles

   From 1 mm to 5 mm of the major axis.

   The particles which have less 1 mm of the major axis or 0.42 mm of the minor axis are considered as reference samples.

3. Method of removing organic impurities

   The oxidation with reaction solutions (e.g., hydrogen peroxide solutions).

4. Method of removing inorganic impurities

   The density separation with dense solvents (e.g., sodium iodide solutions)

5. Method of collecting CPP

   Collecting with filters from the supernatant of dense solvents

6. Recovery rate of CPP

   80 % or more in standard samples

3-1 Outline of the equipment functions

The equipment has the following functions.

1. Supply of water and reagents

   Supply detergents, reaction solutions and dense solvents to preparative containers with metering pumps.

2. Drainage

   Drain solutions out of preparative containers with metering pumps.

3. Washing

   Supply detergents to preparative containers and wash.

4. Heating and temperature control

   To keep the temperature of solutions in preparative containers ranging from the normal temperature to 55 ℃ with ±5 ℃, control the heating of preparative containers.

5. Stirring

   Stir up solutions in preparative containers.

6. Oxidation

   Oxidize samples by supplying reaction solutions. The oxidation time is set for eight days or less.

7. Density separation

   Supply dense solvents and separate CPP in supernatants with densities. The settling time is set

for 24 hours or less.

8. Overflow of CPP

Supply dense solvents and overflow CPP included in supernatants from preparative containers. The equipment is set to overflow supernatants multiple times.

9. Collection of CPP

Collect CPP from supernatants with filters.

10. Chemical tank

Keep the sufficient amount of reaction solutions for oxidation and dense solvents for density separations safety.

11. Waste liquid recovery

Recover the waste liquid of reaction solutions, dense solvents and detergents to bottles.

3-2 Outline of the equipment constitution

The equipment consists of the following parts.

1. Preparative containers

Preparative containers have injection ports to add chemical reagents and drain ports to eject solutions including supernatants from containers. The wet parts inside the containers are made of materials which get less effects from reaction solutions and dense solvents.

2. Strainer

Strainers separate water samples containing MPs into solid and liquid matter with their mesh. The mesh openings are approximately 200 μm. The wet parts are made of materials which get less effects from reaction solutions and dense solvents.

3. Heating and temperature control mechanism

Temperature-controlling mechanism heats solutions in preparative containers and keeps their temperature.

4. Stirring mechanism

The mechanism of stirring solutions in preparative containers with stirring bars. The wet parts are made of materials which get less effects from reaction solutions and dense solvents.

5. Liquid sending mechanism

The mechanism of supplying reagent chemicals to preparative containers. The wet parts are made of materials which get less effects from reaction solutions and dense solvents.

6. Drainage mechanism

The mechanism of ejecting solutions out of containers. The wet parts are made of materials which get less effects from reaction solutions and dense solvents.

7. Collection filter

The filters collect CPP from supernatants. The mesh openings are approximately 100 μm. The

wet parts are made of materials which get less effects from reaction solutions and dense solvents.

8. Chemical tank

The tanks keep the sufficient amount of reaction solutions for oxidation and dense solvents for density separations. The wet parts are made of materials which get less effects from reaction solutions and dense solvents.

9. Waste liquid recovery section

In this section, the waste liquid ejecting from preparative containers is collected in bottles. In the case of using both hydrogen peroxide solutions and sodium iodide solutions, they should be collected in separate bottles to avoid the energetic actions. The wet parts are made of materials which get less effects from reaction solutions and dense solvents.

10. Piping

The wet parts are made of materials which get less effects from reaction solutions and dense solvents.

11. Operation section

This section sets the time of oxidation and settling for density separation and the frequency of CPP overflows.

3-3 Quality assurance and quality control

1. Method

The addition-recovery test is conducted with standard plastic particles. Through this test, the basic functions of the equipment are checked without impurities.

2. The plastic particles used in this test

Two kinds of plastic particles are prepared. Their materials are polyethylene and sizes are 500-600 μm. The specific gravities are different: one is 1.0 and another is 1.35. The latter has metal materials inside it. The color should be separated to distinguish them.

3. Test condition

① The number of standard plastic particles: 25 in each kind of plastic particles

② Solutions for oxidation: 30 % hydrogen peroxide solutions

③ Oxidation time: for 15 minutes

④ Solutions for density separation: 5.3 mol/L sodium iodide solutions

⑤ Settling time of density separation: for 15 minutes

The test should be conducted three times or more under this condition.

4. Test procedure

① Add standard plastic particles to the equipment.

② Run the equipment under the condition explained in (3), and collect plastic particles with

filters.

③ Count the collected plastic particles, and calculate the recovery rate in each

$$\text{Recovery rate} = \frac{\text{The number of standard plastic particles collected by the equipment}}{\text{The number of add standard plastic particles}} \times 100 \ (\%)$$

④ Repeat the above processes.

5. Standards for judging

The recovery rate is over 80 % in each test.

Table 1. An example of the test data arrangement

Test number	Characters of standard plastic particles	The number of add standard plastic particles	The number of collected standard plastic particles				Recovery rate (%)
			Overflow First	Overflow Second	Overflow Third	Total	
First	SG: 1.00	25					
	SG: 1.35	25					
Second	SG: 1.00	25					
	SG: 1.35	25					
Third	SG: 1.00	25					
	SG: 1.35	25					

※SG means specific gravity

Extra document

This extra document explains the devise examined for establishing the standard specification in 2021.

1 Outline of the device

This device performs two processes, oxidization MPs samples and separation them by density in one glass preparative container. After that, it overflows the supernatants from the container, and it collects CPP with filters automatically.

2 Example of the device constitution

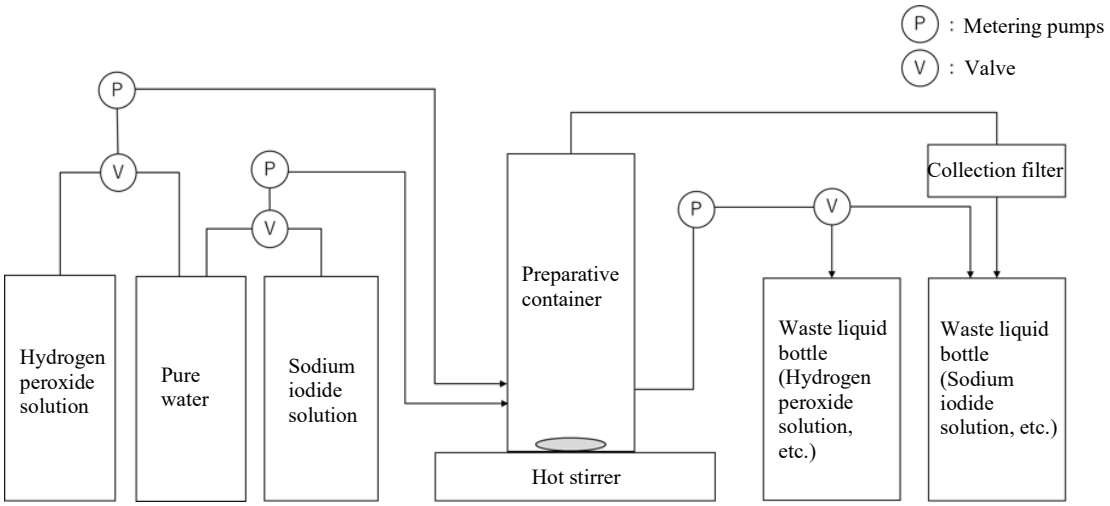


Figure 2. An example of the device constitution

3 Solutions

1. Pure water

   It was used to substitute and wash the inside of preparative containers.

2. Hydrogen peroxide solution

   It was used to oxidize and decompose organic impurities in samples. It was prepared at 30 %.

3. Sodium iodide solution

   It was used for density separations. It was prepared at 5.3 mol/L.

4 Functions in the device

1. Preparative container

   The container was made of glass, and its volume was 400 mL. It had upper and lower parts. A strainer was inserted into the lower part and held down by the upper part. It had separate injection ports and pipes of pure water, hydrogen peroxide solutions and sodium iodide solutions near the bottom to avoid the mixing of hydrogen peroxide solutions and sodium iodide solutions.

   To wash hydrogen peroxide solutions and sodium iodide solutions with pure water, its pipe was shared with them. The drain ports were also near the bottom. The shared drain ports and pipes were controlled with electromagnetic valves.

   A pipe of 6 mm caliver was put on the top of the devise to send the overflowed samples to collection filters.

2. Strainer

   It separated water samples containing MPs into solid and liquid matters. To avoid missing MPs (over 300 μm), a stainless-steel strainer with 180 μm mesh openings was used.

3. Heating and temperature control mechanism

   A hot stirrer with controlled temperature was used.

4. Stirring mechanism

   A hot stirrer was used to stir solutions in a container with a stirring bar.

5. Liquid sending mechanism

   Tube pumps with discharging capability of 120-140 mL/min were used. These tubes were made of thermoplastic elastomers and not affected by hydrogen peroxide solutions and sodium iodide solutions.

6. Drainage mechanism

   Tube pumps were used. These tubes were made of thermoplastic elastomers and not affected by hydrogen peroxide solutions and sodium iodide solutions.

7. Collection filter

   The stainless-steel filters with 90 μm mesh openings were used.

8. Waste liquid recovery section

   To avoid the mixing of hydrogen peroxide solutions and sodium iodide solutions, each waste liquid was collected in different bottles with electromagnetic valves from the drain ports near the bottom of a preparative container.

9. Piping

   Pipes were made of silicone and not affected by hydrogen peroxide solutions and sodium iodide solutions.

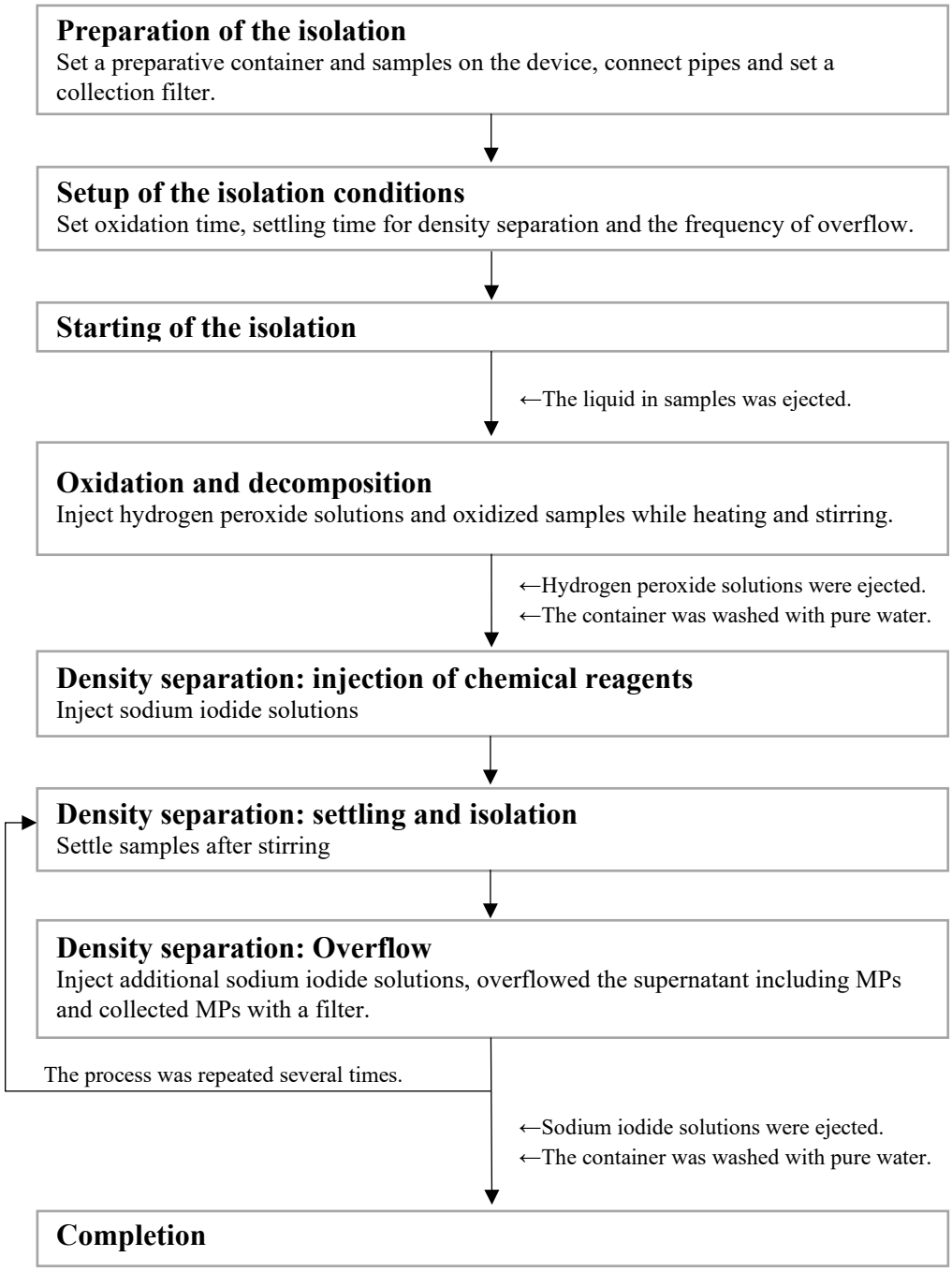
10. Operation section

The time of oxidation and settling for density separation and the frequency of overflows can be set.

5 Operation procedure

1. Put samples to a strainer in a preparative container. The sample was a few grams at one time. The sample with a lot of impurities was divided.
2. Put the preparative container on a hot stirrer and connect to pipes.
3. Set a collection filter to the collecting section.
4. At the operation section, the oxidation time (e.g. 3 days), settling time for density separation (e.g. 3 hours) and the frequency of overflow (e.g. 3 times) were set.
5. Eject the liquid from samples.
6. Inject 30 % hydrogen peroxide solutions slowly until it reached the set amount. The maximum amount was decided based on the oxidation time.
7. Keep the temperature of solutions at 55 ℃ to enhance the oxidation while stirred it slowly with a stirring bar. The temperature of solutions was 60 ℃ or less to avoid the degeneration of plastics.
8. Keep the above condition for maximum 8 days.
9. Stop heating and a stirring bar.
10. Eject hydrogen peroxide solutions from the preparative container to the drain bottle which was for hydrogen peroxide solutions only.
11. Inject pure water to the preparative container to wash and eject it to the drain bottle which was for hydrogen peroxide solutions only. This process was repeated several times.
12. Inject sodium iodide solutions to the preparative container for density separation.
13. Stir the solutions in the container for 90 seconds and settled it to collect CPP into supernatants.
14. After settling, inject sodium iodide solutions from the injection port and overflow the solutions including CPP from the top of the preparative container.
15. Collect solid matters by passing the overflow solutions including CPP through a collection filter.
16. Repeat the process from (13) to (15) in set frequencies.
17. Eject sodium iodide solutions to the drain bottle which was for sodium iodide solutions only.
18. Inject pure water to the preparative container to wash and eject it to the drain bottle which was for sodium iodide solutions only. This process was repeated several times.

6 Example of the operating process



7 Result of the device performance test

Table 2. The condition of the device

Process	Contents	Operating conditions in the performance test
Oxidation	Solution	100 mL of 30 % hydrogen peroxide solutions
	Heating temperature	Keeping the liquid temperature at 55 ℃
	Duration	0.2 hours (12 minutes)
Density separation	Solution	250 mL of 5.3 mol/L sodium iodide solutions
	Settling time	0.2 hours (12 minutes)
Collection	Overflow frequency	Three times

Table 3. The added plastic particles

Type (Cospheric made)	Size (diameter)	Material	Specific gravity	Shape	Color
PNKPMS-1.00	500-600 μm	Polyethylene	1.00	Sphere	Pink
WPMS-1.35	500-600 μm	Polyethylene (Core: Titanium)	1.35	Sphere	White

Table 4. The results of the device performance test

Characters of added plastic particles	Test number	The number of added particles	The number of collected particles				Residual number	Recovery rate
			Overflow frequency					
			First	Second	Third	Total		
Polyethylene 500-600 μm Specific gravity (SG※) [1.00 1.35]	First	25 (SG: 1.00)	25	0	0	25	0	100%
		25 (SG: 1.35)	25	0	0	25	0	100%
	Second	25 (SG: 1.00)	19	4	2	25	0	100%
		25 (SG: 1.35)	22	3	0	25	0	100%
	Third	25 (SG: 1.00)	17	8	0	25	0	100%
		25 (SG: 1.35)	20	3	0	23	1	95.8%
	Mean							99.3%

~~※SG means specific gravity~~

Reference　The investigation committee for the standard specification of automatic sample preparations in guidelines for river microplastic monitoring method

Members in 2020

KAMEDA Yutaka, Associate Professor of Chiba Institute of Technology,
Faculty of Creative Engineering, Department of Civil and Environmental Engineering

MORITA Masatoshi, Chief of Environmental Measurement and Quality Control Center,
a Specified Nonprofit Organization

NIHEI Yasuo, Professor of Tokyo University of Science, Faculty of Science and Technology,
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TANAKA Shuhei, Associate Professor of Kyoto University Graduate School of Global Environmental Studies, Environmentally Friendly Industries for Sustainable Development Field

*The names are arranged in alphabetical order. Their posts are those in March 2021.

Members in 2021

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Appendix. 2

Survey procedure manual for Microplastics in River by net towing

DRAFT ver. 1.0

XXXXXX XX, 2025

This procedure manual is developed based on insights gained through the Ministry of the Environment (MOEJ)'s technical cooperation projects for microplastics monitoring in developing countries. It outlines the procedures for conducting surveys using a net towing method with a boat in rivers where water flow is absent or insufficient for using . It consists of excerpts from relevant sections of the Guidelines for Microplastic Surveys in Rivers and Lakes by MOEJ, including Section 4.2 “Methodology: Trawling Method,” and supplementary information in the Guidelines.

- Contents -

1 INTRODUCTION (cf. the Guidelines section 1.2)

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

- Megaplastic (>1 m)
- Macroplastic (25 mm – 1 m)
- Mesoplastic (5 – 25 mm)
- Microplastic (0.3 – 5 mm).

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

In this document, the sampling and analysis target microplastic particles ranging in size of 0.3 – 5 mm.

The Guidelines are mainly targeting 1mm<d< 5mm. But other size (d < 1mm and 5<d<25mm) are recommended to record as a reference data .

2 DESCRIPTION OF NET TOWING METHOD (cf. the Guidelines section 4.2)

The riverine microplastic samples are collected by deploying a net from a boat on the river in case water flow is absent of insufficient. The greatest advantage of using a boat is to be able to sample actually floating plastics at any safe locations on a river and to estimate riverine plastic flux, and as well without being limited to suitable bridges on a river. The collected samples are stored and preserved in containers and brought to the laboratory for analysis.

2.1 Sampling/net towing location

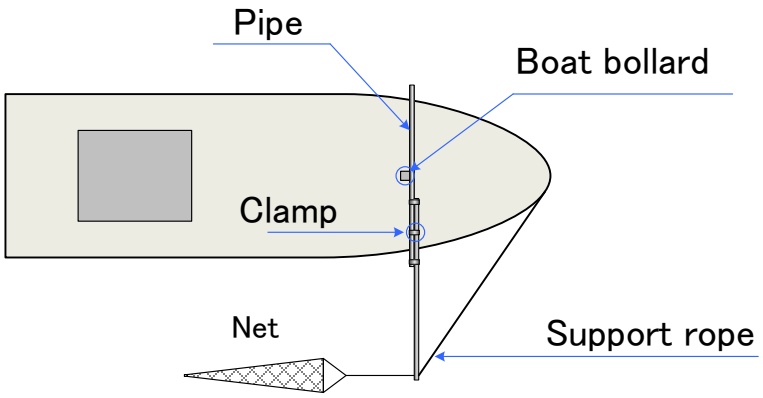
It is recommended to conduct sampling/net towing at three locations, i.e. the upstream, midstream and downstream (or estuary) along a river.

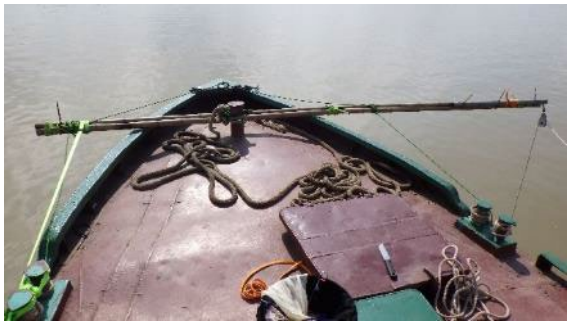
(Source: Google Earth)

Also, it is recommended to conduct sampling/net towing on the river along the left bank, the centroid and the right bank at each location mentioned above.

2.2 Net Towing Scheme (cf. the Guidelines section 4.2.3.2)

As a general rule, the net is typically set up on the starboard side (e.g. right side) of the boat. And for ensuring an ideal water filtration capacity (10-20 m^3), it is recommended to do towing at a speed of 2 knots for a distance of 200 meters in an upstream direction.





3 EQUIPMENT

3.1Sampling boat (cf. the Guidelines section 4.2.1.6)

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:

- Set an adequate length of the outfitting pipe which is long enough to fix a net and keep the net away from the body of the boat (in general a bow of the boat). Also, in order to avoid impact from undertows especially for towing wave and to avoid plastics from boat cover entering into the net, the net fixed at the end of the pipe equipped on the boat should be positioned as far as possible to the boat (about 1.5-2.0 m). This is also effective for prevention of contamination. It is suggested to adjust the distance between the boat and the net according to the actual situation, as towing wave vary depending on the speed and shape of the boat.
- The pipe should be fixed at a safe and stable location where the boat strength is high such as boat bollards.

- In order to prevent damage to the mounting hardware, stabilize the edges of the pipe by tension using support ropes.

3.2 Tow net (cf. the Guidelines section 3.2.1)

A plankton net is standardized for all sampling locations with the following specifications:

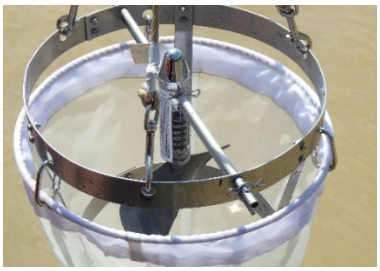
Net mouth size	Diameter 30 cm
Mesh size	0.3 mm
Length	75 cm

3.3 Flow meter (cf. the Guidelines section 3.2.3)

A flow meter is attached to the tow net to measure the tow distance.

3.4 Frame

A custom-made stainless frame can be used to fix the plankton net and the flow meter.



3.5 Pump and sample containers

A pump is used to wash the samples of microplastic down to the cod end of the plankton net.

Containers (2 L) are used to store and preserve samples which will be brought to a laboratory for analysis. The material of the container should not be composed of plastic in order to avoid sample contamination. If a plastic container is used, the container should be a new product.

3.6 Other

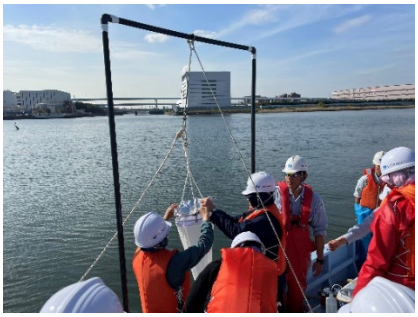
The other equipment includes a float to be fixed to the cod end of the plankton net, an onboard or handheld GPS, and dispensing bottles (referred in the Guidelines). Also a portable multiple water quality meter, a handheld electromagnetic current meter, an anemometer, a cool box, a digital camera, and materials for rigging such as steel/bamboo pipes, winches and strings will benecessary.

4 SAMPLING (cf. the Guidelines section 3.2.4)

In general, sampling should be conducted at time period when seawater does not flow upstream, for example at a time of low tide. The sampling is conducted according to the following steps:

4.1 Preparation (cf. the Guidelines section 3.2.4)

- Checking all required equipment and their conditions.
- Attach a flow meter and a float. A flow meter should be fixed at the center of the net frame. A float should be attached at the end of the cod end.
- Bring all required equipment on the boat.
- Fix a steel/bamboo pipe to the starboard side (right side) of the boat for towing a net. Set up a frame with pipes or attach a pipe to the existing frame on the boat for hanging the net.
- Fix the net to a pipe (steel pipe/bamboo pipe).




(Left: a frame to hang the net, Right: a pipe fixed to the existing frame)

4.2 Sampling

4.2.1 Cleaning the plankton net before use (supplement part to the Guidelines)

Wash a bucket with water filtered through a plankton net with a mesh size of 0.1mm.

Before using the plankton net and the stainless frame for fixing the plankton net, they should be cleaned 3 times onboard the boat. This can be done by placing the net or the frame into the bucket filled with water that has been filtered through a plankton net with a mesh size of 0.1mm. After cleaning, fix the plankton net (mesh size of 0.3mm) to the frame and cover it with a piece of aluminium foil to avoid contamination.

4.2.2 Blank test (supplement part to the Guidelines)

The blank test should be conducted daily using the plankton net that has been cleaned but have not been used for towing yet. It is conducted by washing a plankton net in the same manner as after regular towing, and then collect the particles from the cod end into a sample container. Before washing, release the frame from the net and cover the plankton net mouth with a piece of aluminium foil.

4.2.3 Field observation (supplement part to the Guidelines)

Observe and measure the water quality and wind speed, etc., according to the 3.5.1 the Guidelines.

4.2.4 Determining centroid

Before towing a net, it is necessary to decide the centroid of a river at upstream, midstream and downstream. It can be decided by towing a direct reading current meter

while moving a boat across the river in a pitch of 50 m. The location which has the maximum flow rate is determined to be the centroid.

4.2.5 Start towing

Take off the aluminium foil covering the net mouth. Fix the net to the frame. Read and record the number of the flow meter.

Start towing by dropping the net gently to the river surface after the boat reaches a pre-determined speed. And for ensuring an ideal water filtration capacity (10-20 m^3), it is recommended to do towing at 2 knots (cf. the section 2.2 of this Annex). Confirm a smooth towing by checking the orderly alignment from the net mouth to the cod end and the net completely submerging just under the water surface.

Record the time, GPS coordinate of starting towing point.

4.2.6 Towing (cf. the Guidelines section 4.2.3.2)

Continue to tow at a certain speed and monitor the floating substances in the net and on the river surface. When net clogging or other malfunction is detected during towing, find the reason for the problem by lifting up the net. If the net is clogged, either collect the samples or change the net and resume the towing.

4.2.7Lifting net (cf. the Guidelines section 4.2.3.2)

Once the planned towing distance (200 meters) has been reached, haul the net and lift it up slowly.

Record the time, GPS coordinate of the ending towing point.

4.2.8 Net cleaning/sample collection (cf. the Guidelines section 3.4.4 and 3.4.5)

(1) Take off the frame and cover the net mouth with a piece of aluminum foil. It will be convenient to hang it to a pipe which has been fixed before. During and at the end of this process, wash the net from the outside to wash down the particles inside to the cod end. During this process, use water filtered through a plankton net with a mesh size of 0.1mm.

(2) Transfer the particles piled up in the cod end to the sample container by opening the outlet of the cod end. Before using a container, wash it three times by using water filtered through a plankton net with a mesh size of 0.1mm.

(3) Repeat washing and transfering mentioned above twice. In total, conduct washing and transfering, for example, 3 times. If necessary, identify large pieces of rubbish inside the net for removing. Washing those pieces before removing with a dispensing bottle containing 0.1mm filtered river water.

(4) Add formalin of about 5% of the volume of the sample in the container, if necessary.

(5) Label the container. Containers can be labeled before conducting the sampling.

(6) Seal and store the container. It is recommended to store the container in a cool box and, if possible, transport it to a laboratory in cold storage.