

Guidelines for River Microplastic Monitoring Methods

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1 Introduction

Plastics smaller than 5 mm in diameter (hereinafter referred to as "microplastics") are found in the ocean around the world, and 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 rivers, 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 based on the availability of scientific knowledges.

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 river microplastics, 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 rivers.

It is assumed that nets with about 0.3 mm mesh openings are used to collect microplastics. To reduce the effect of measurement errors caused by small particle of plastics depending on the laboratory (researcher), the plastics with a size of less than 1 mm are measured as supplemental references although they are collected with the net with about 0.3 mm mesh openings.

In addition, it is recommended to record results of plastic pieces with a size of 5 mm or more because such measurements can help in understanding the distribution of plastic in rivers.

2 Purpose

2.1 Purpose of survey

Purpose of the survey is to understand the distribution of microplastics in rivers 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 microplastics.

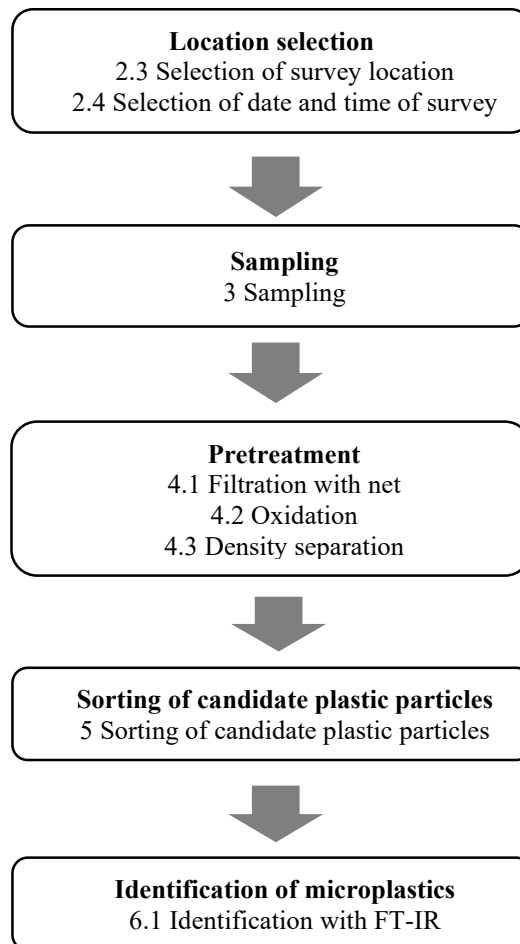


Figure 2-1. Survey procedure on river microplastics.

2.3 Selection of survey location

2.3.1 Survey location

1. The guidelines assume sampling with nets. Thus, locations where the velocity of water flow is 0.3 m/s or more and the water depth is 50 cm must be chosen as the survey location. Locations where the water flow fails to spread nets sufficiently or rotate flow meter screws must not be chosen.
2. The locations designated as reference points for water quality or water level can be the survey 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.¹

2.4 Selection of date and time of survey

1. As specified in the water quality survey procedure (in MoE water quality control No. 30, September 30, 1971), a day following a sunny streak should be chosen as a date of survey to ensure stable water qualities. Weathers at the survey location must be recorded for seven days before the survey.
2. When the survey 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 heavy rain

Since it is assumed that the amount of microplastics outflow will increase during or after heavy rain, there may be a case in which research will be conducted during this time. In such a case, the precipitation 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.

¹ 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)

3 Sampling

3.1 Outline

The samples containing microplastics are collected from rivers.

3.2 Equipment

3.2.1 Net

Plankton nets are used for sampling.

A flow meter and a bottom pipe (cod end) made of rubber or metal are installed to the mouth and the end of a commercial short-cone-shaped plankton net with 30 cm in diameter and 0.3 mm mesh openings that is used for collecting underwater plankton in rivers. A float is attached near the bottom pipe (cod end) for even spreads of the net.



Photo 3-1. An example of plankton net



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

[Reference] Square net

- A square net used to collect benthic organisms is modified for the survey.
- A net with about 0.3 mm mesh openings is laid on a square frame, and a metal bottom pipe 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 cm x 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 about 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 about 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. It is necessary to check that the rotor rotates normally with water flow and is not separated.

3.2.3 Specifications of flow meter

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

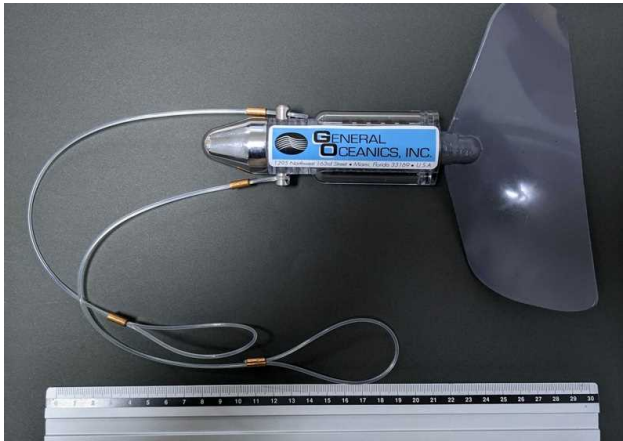


Photo 3-4. Flow meter and low flow velocity rotor

- 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 Case 1. An example of fixation of the flow meter with strings)

- 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)

- 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 by the stability and the low possibility of the 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 flowmeter with a metal frame

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 about 2 to 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's surface (so that the upper part of the mouth of the net lies just below the surface).

The guidelines are intended to collect microplastics in 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 in deep under the surface of the river.



Photo 3-7. Setting of net in stream

3.3.2 Fixation of net in stream at locations for sampling

The mouth of the net is fixed at three points with ropes.

In the case of hanging from bridges, the net is lowered to rivers with a rope.

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 3-8. 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 out at survey 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, sheets made of natural fiber cloth should be used, and sheets 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 impurities attached to them.

3.4.2 Measurement of flow velocity

Flow velocity is measured before collecting samples. The measurement time during a flow meter has 10 m³ or more 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. Measurement of flow velocity with flow meter

3.4.3 Sampling

The net is set in streams until the filtered water volume reaches 10 to 20 m³. 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 about 13 to 14 m³.

After this, the net is pulled up and 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.

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 river water 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 the field

Samples in the net are collected to the cod end by pouring washing to the net from the outside and transfer to storage containers. Samples that adhere to the cod end or bottom pipe are transferred to storage containers with a small amount of washing. When the samples are transferred to the storage containers, it should be careful to prevent contaminants in the container and not to leave any samples in the net. The photos below depict how the survey is conducted.



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 cod end to a storage container

Photos 3-12. Survey scenes

3.4.6 Transfer of samples from net to storage containers in laboratory

When multiple nets are available, it is recommended to transfer samples from the nets in a laboratory to avoid contamination in the field.

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.

In the laboratory, the nets are washed with tap water, and the samples are transferred to storage containers.



Photo 3-13. An example of cloth cover



Photo 3-14. An example of cloth bag



Photo 3-15. Collection of samples in a bottom pipe



Photo 3-16. Transfer of samples from a bottom pipe to a container

3.4.7 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.

3.4.8 Storage of containers

When no more survey work 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

Required items listed below must be measured and recorded in sampling. Recommended items are measured and recorded as far as possible.

Required items

1. Location name

Names of survey locations including sampling locations must be recorded.

(Example: Tama River, Maruko Bridge, Downstream, center of flow)

It is recommended to photograph the location and make records about it to ensure that the location where samples are collected is identifiable.

2. GNSS data for sampling positions

Data about latitude and longitude of sampling positions from Global Positioning System are obtained with mobile terminals or GNSS (GPS) receivers and must be recorded.

3. Water depth

Water depth of sampling locations must be measured and recorded to confirm safety at this location and 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 for calculations of filtered water volume.
6. Rotation number and filtered water volume
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 riverbanks or stepping into riverbeds must be recorded, and survey locations must be photographed.

Recommended items

1. Weather
To assess influences of rainfall, weather on a survey day and the number of preceding sunny days in a week around survey locations should be recorded.
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 survey 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
Condition of riverbeds should be recorded to confirm safeties on stepping into rivers for sampling and estimate possibilities of contamination by riverbed sediment.
5. Local circumstances
Local circumstances around survey locations that may affect distributions of underwater microplastics such as river works in upstream 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 normal weather and after rain), data for seven or more days about water level should be gathered from water level observatories near survey locations.

3.5.2 Precautions for sampling

1. At survey 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. Helmet must be also put at the places with risks of falling.
3. In the case of stepping in rivers for sampling, researchers must approach survey locations from downstream and be careful not to kick up gravel on riverbeds. After conditions of streams become stable, sampling should be commenced. 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 damage 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 river banks) and ensure their safety.

4 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.

4.1 Separation of solids from samples with nets

4.1.1 Outline

Solids from samples are separated with nets with 0.1 mm mesh openings.

4.1.2 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)

4.1.3 Procedure

1. Put a net on glass container A and carefully pour samples through the net to filter out impurities (Photo 4-1).
2. Remove impurities that are 5 mm or more in size with tweezers (Photo 4-2). 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 4-3).
4. Carefully pour filtered water in container A into container B through the net (Photo 4-4).
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 4-5).
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 4-6).



Photo 4-1. Filtration of impurities through a net



Photo 4-2. Removal of large impurities



Photo 4-3 . Putting of a net on container B

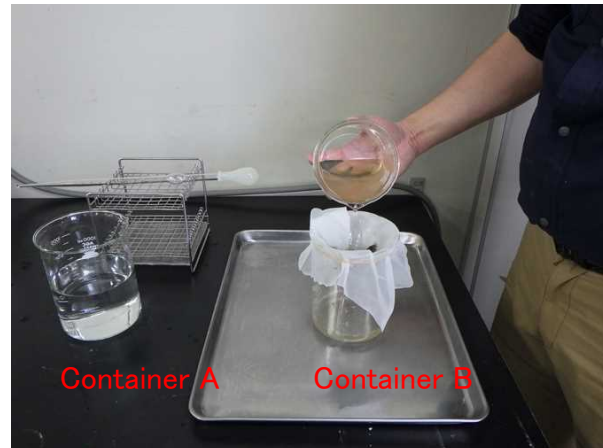


Photo 4-4. Filtration of filtered water



Photo 4-5. Putting of container A on the net with samples

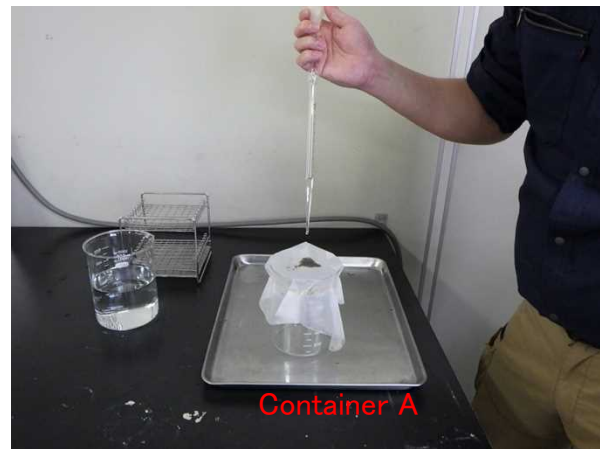


Photo 4-6. Drop of the samples sticking on the net into container A

4.2 Oxidation

4.2.1 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.

4.2.2 Reagents and equipment

- 1) Oven
- 2) Water bath (constant temperature water bath)
- 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

4.2.3 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 about 50 mL). A large amount of sample should be divided into additional containers.

- *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.
 - If water content in the dried samples is too high, oxidizing agent is diluted and reduced the effect of organic impurity decomposition.



Photo 4-7. Warming of containers in a oven



Photo 4-8. 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

Photos 4-9. 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 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.

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 warmed for one day.
4. Repeat Step 3 until the suspensions disappear.



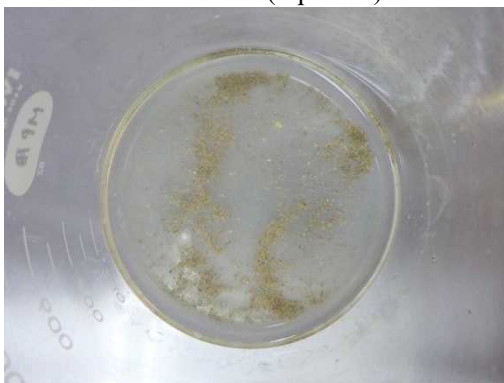
Photo 4-10. Warming of containers in a water bath



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)

Photos 4-11. 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 a glass containers or glass petri dishes.



Photo 4-12. 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.

4.3 Density separation

4.3.1 Outline

A large number of inorganic impurities such as soil particles is 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.

4.3.2 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
- 6) Glass container
- 7) Net with 0.1 mm mesh openings

4.3.3 Procedure

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



Photo 4-13. Securing of a tube tip

2. Add an appropriate amount of sodium iodide solutions to samples in a container. Stir it slowly with a glass rod.



Photo 4-14. Stirring of a sample with a glass rod

3. Pour solutions into the funnel.



Photo 4-15. Pouring of 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 about three hours.

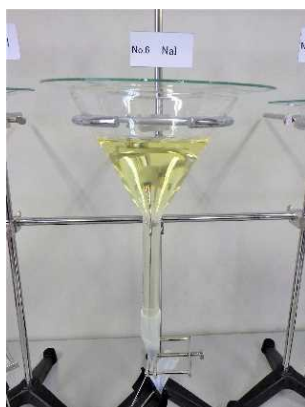
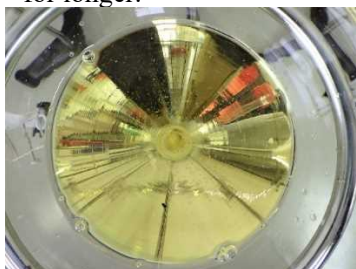


Photo 4-16. Leaving of solutions to stands

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



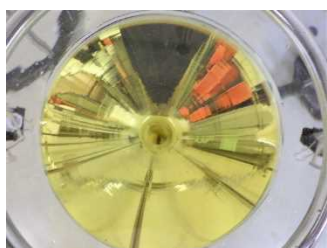
Top view



Upper and middle part
At the start of density separation



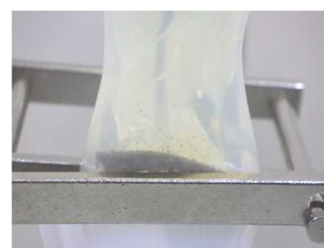
Lower part



Top view



Upper and middle part
At the end of density separation



Lower part

Photo 4-17. 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 the upper part one is remains.



Photo 4-18. Density separation

7. Rinse out the samples remaining in the upper part with purified water.



Photo 4-19. 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 4-20. 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.

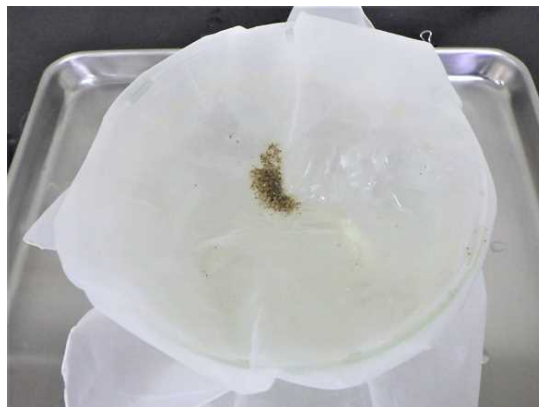


Photo 4-21. Precipitate in a lower part on a net after filtration

10. Transfer the filtered samples to glass petri dishes.



Photo 4-22. 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 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.

4.3.4 Check of contamination in test room

Before taking pretreatments written in section 4.1 to 4.3, a petri dish filled with purified water is placed at hand. 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 chapter 5 to 6. When identified contaminant particles are plastic and plastics which have the same components and characteristics as they have (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 11 and 12 on subsection 2 in section 8.2.

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

5. Sorting out of candidate plastic particles

5.1 Outline

From the treated samples, candidate plastic particles are sorted out. Then, the longest length of them are measured, and they are sorted by color and shape.

It is easy to sort them out by adding about 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.

5.2 Equipment

- 1) Stereomicroscope
- 2) Precision tweezers
- 3) Micrometer (for visual measurements of the longest length and other items)
- 4) Digital camera (for measurements of the longest length and other items with image processing)
- 5) Personal computer (for measurements of the longest length and other items with image processing)
- 6) Image editor application (for measurements of the longest length and other items with image processing)

5.3 Procedure

5.3.1 Visual measurement of the longest length 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 5.4.) Assign identification numbers to them and measure their length with micrometers.
2. Transfer the candidate plastic particles to another petri dishes.
3. Classify the candidate plastic particles by shape and color (for details, refer to section 5.5 and 5.6).
4. For the respective candidate plastic particles, make records about the longest length, shape, and color.



Photo 5-1. Work sceneries

5.3.2 Measurement of the longest length 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. For each of them, measure the longest length and the shortest length and area.
 5. For each of them, make records about the longest length and the shortest length, area, shape, and color (for details of classification by shape and color, refer to section 5.5 and 5.6).
- * The following treatments do not need for candidate plastic particles whose the longest length is over 5 mm.
- * Depending on shapes of plastic pieces, errors of the areas become big by calculating from the longest length and the shortest length. Thus, image processing software is required to evaluate the accurate areas.



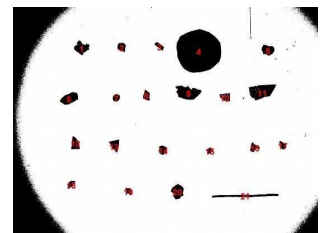
1



2



3



4

Photo 5-2. Work sceneries

5.4 Identification of candidate plastic particles

Photo 5-3 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.

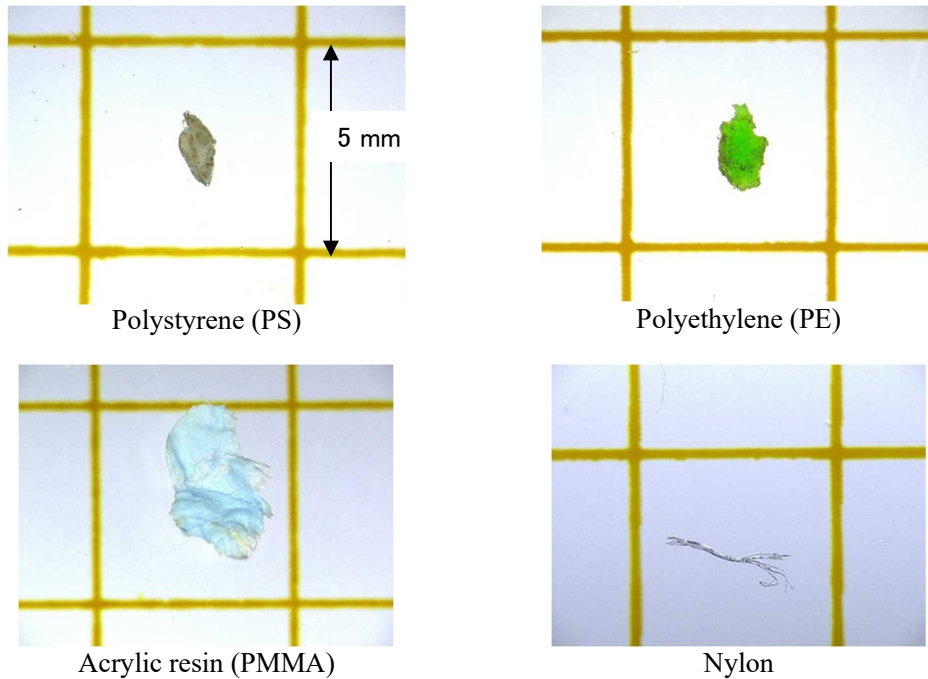


Photo 5-3. Examples of typical candidate plastic particle

Yellow frames are ruled into 5 mm squares.

(Photos above are examples. The same types of materials have different shapes and colors.)

5.5 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 The others

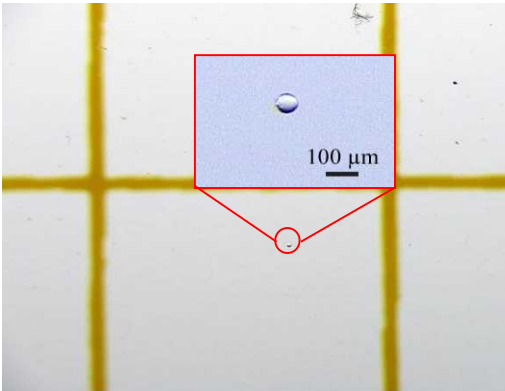
Photo 5-4 shows examples of plastic pieces by shape.



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): spherical and expanded pieces



5. Cylinder and sphere (pellet)



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

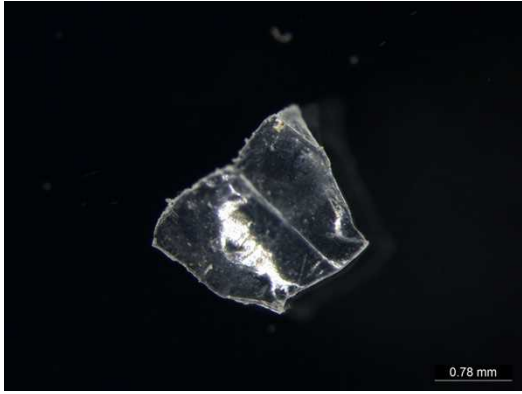
Photo 5-4. Examples of plastic pieces by shape

5.6 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 5-5 shows examples of plastic pieces by color.



1. Transparent



1. Transparent



2. White



2. White



3. Red



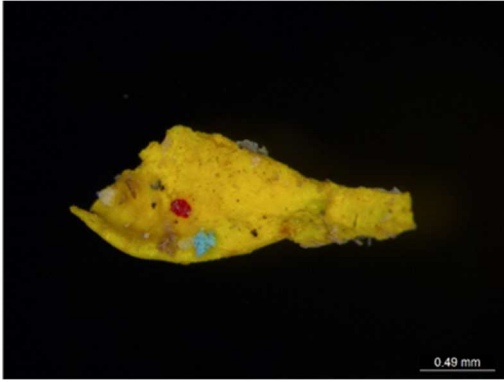
3. Red



4. Orange



4. Orange



5. Yellow



5. Yellow



6. Green



6. Green



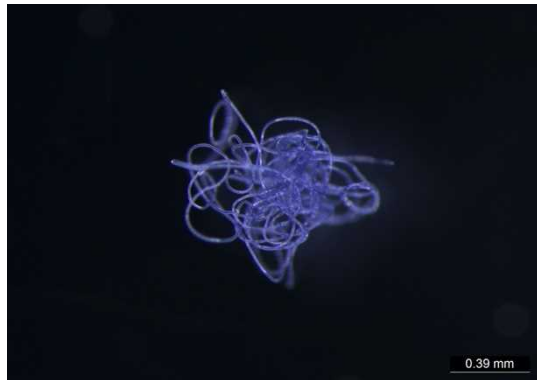
7. Blue



7. Blue



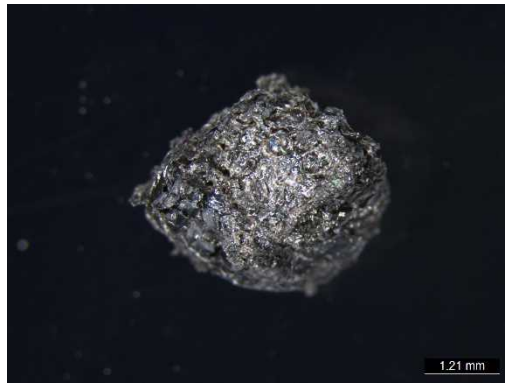
8. Purple



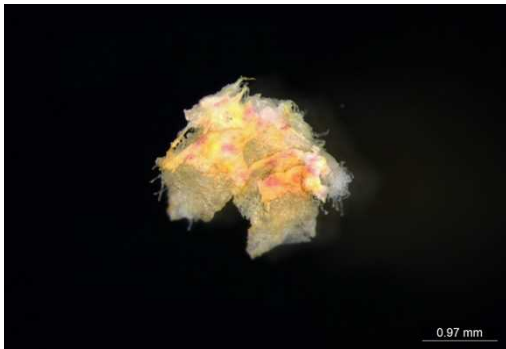
8. Purple



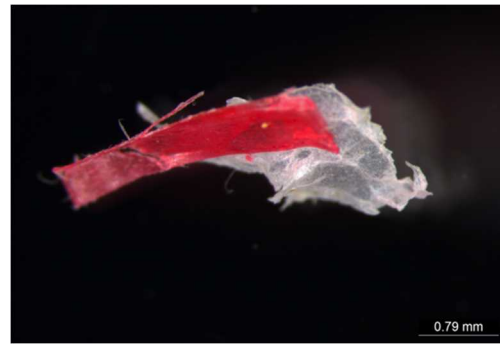
9. Black



9. Black



10. Compound (mixed color)



10. Compound (mixed color)

Photo 5-5. Examples of plastic pieces by color

[Reference] Addition-Recovery test with standard plastic particles

The accuracy of sorting of candidate plastic particles with microscopes are sometimes varied depending on researchers. When multiple researchers are involved in sorting, it is recommended to test the sort-out accuracy with standard plastic samples.

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 about 50, and recovery rates should be calculated.
- * Colors of standard plastic particles should be unusual colors in nature to distinguish them from collected samples.

6. Identification of plastic types

6.1 Identification of plastic types with Fourier transform infrared spectrophotometer

6.1.1 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.

6.1.2 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.

6.1.3 Procedure

1. Wipe sensors with paper wipes.
2. Configure measurement conditions.
 - * Measurements of microplastics are usually performed with the number of sample scans set to five or more and the conserved wave area set to 4000 to 400 cm^{-1} .
3. Perform background measurements.
4. Clamp candidate plastic particles sorted out in chapter 5 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.

6.1.4 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 survey, 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

- Photos 6-1 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 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) 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.

(g) Cellulose (natural fiber)

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

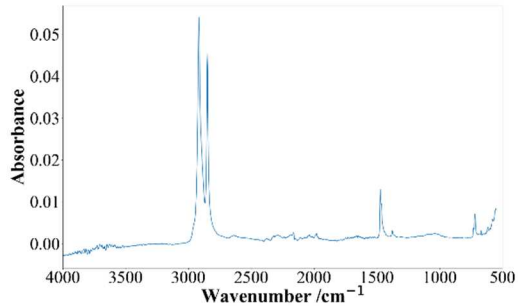
(h) Inorganic substances

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} .

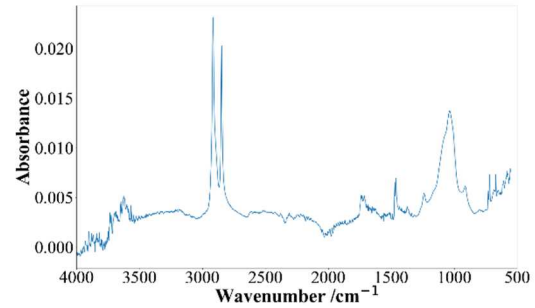
(i) Black substance

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

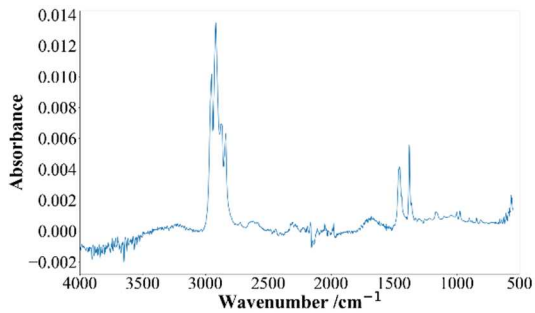
* In such black substances, their spectra are sometimes observed through ATR spectroscopy with germanium (Ge) prisms which have a higher refractive index.



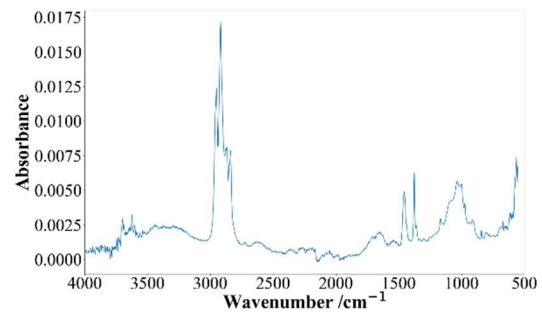
(a) Polyethylene (PE)



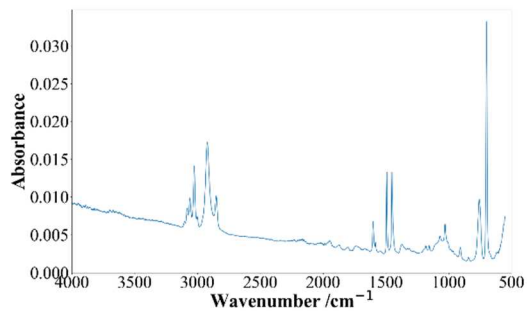
PE + impurities



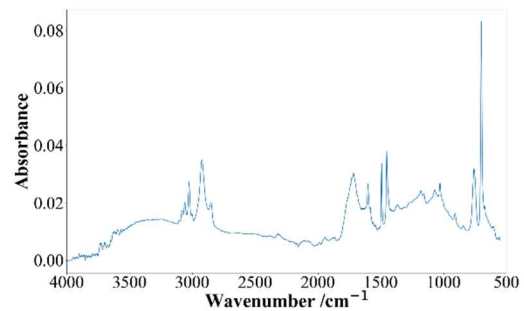
(b) Polypropylene (PP)



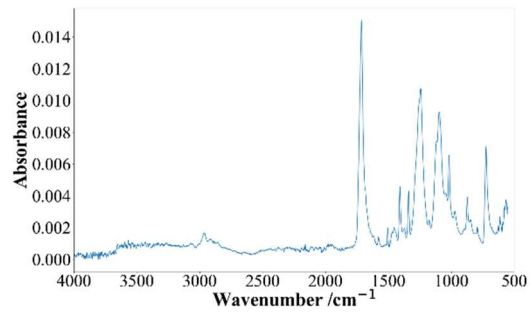
PP + impurities



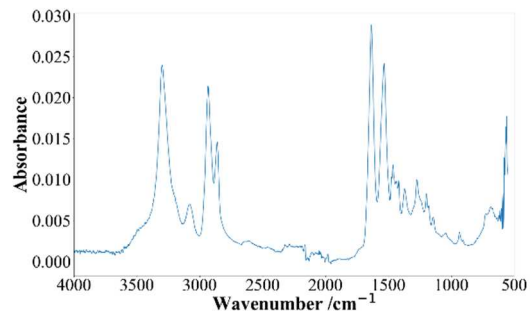
(c) Polystyrene (PS)



PS + impurities

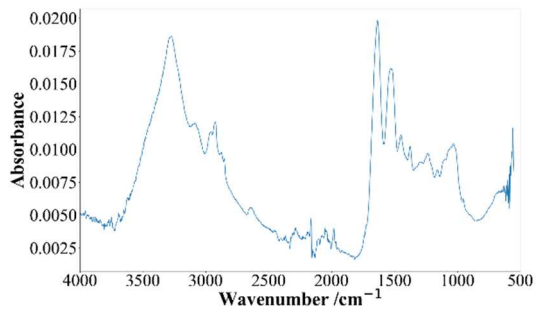


(d) Polyethylene terephthalate (PET)

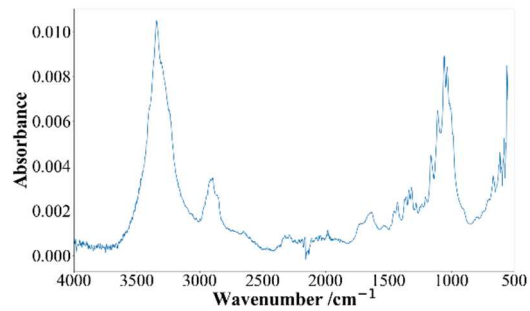
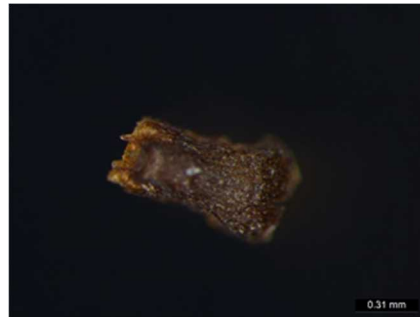


(e) Nylon

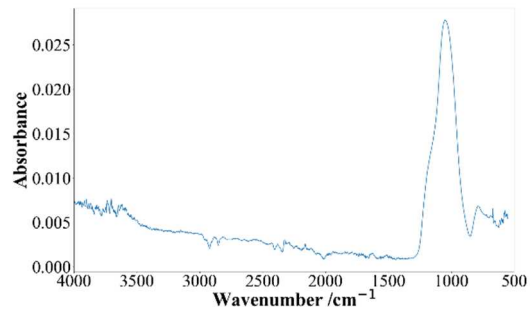
Non-plastic substances



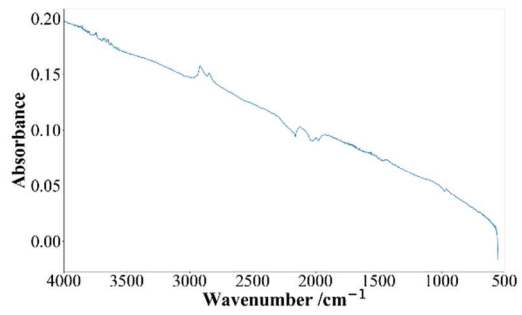
(f) Protein and its microscopic image



(g) Cellulose



(h) Inorganic substance



(i) Black substance and its microscopic image

Photos 6-1. Examples of infrared spectral shapes of plastics and non-plastics

7. Measurement of mass of microplastics

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

7.1.1 Outline

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

7.1.2 Equipment

Electronic balance

7.1.3 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 6.

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- μ g scale.

3. Procedure

Measure mass of plastic candidate particles sorted on the petri dish in chapter 5 with ultra-micro balances.

8. Sorting of measurement results

8.1 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. The longest length
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. The shortest length (for samples analyzed with image processing)
2. Area (for samples analyzed with image processing)
3. Thickness
4. Mass

8.2 Sorting of details of survey

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

1) Items for survey

1. Data and time
2. River and location for sampling
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 survey locations and samples

Required items

1. Location name
2. Sampling position (GPS data)
3. Water depth
4. Flow velocity (measured with flow meter)
5. Measurement time
6. Rotation number and filtered water volume of flow meter
7. Method of sampling (hanging from bridges or stepping into river)
8. Time duration which nets were underwater (start and end time)
9. Number of microplastics
10. Number density of microplastics
11. Number of candidate plastic particles that could not be identified as plastic

Recommended items

1. Weather (for about seven days prior to survey)
2. Air temperature and water temperature
3. Turbidity
4. Tides
5. Riverbed condition at measurement location (e.g. stones, sand and invisible)
6. Circumstances around survey location (e.g. river 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. Mass of microplastics
10. Mass concentration of microplastics
11. Percentage by shape
12. Percentage by color
13. Percentage by size
14. Percentage by type

8.3 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 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 the longest length classified at 0.1 mm intervals and the number of microplastics by shape are represented graphically by location.

The member of the investigation committee for the Microplastic Monitoring in Rivers in 2019

NIHEI Yasuo, Professor of Tokyo University of Science, Faculty of Science and Technology, Department of Civil Engineering

SUZUKI Go, Chief Senior Researcher of National Institute for Environmental Studies, Material Cycle and Waste Management Science and Engineering Research Section

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*The names are arranged in alphabetical order. Their posts are those in March 2020.

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

Supplementation

**The Standard Specification
of Automatic Sample Preparations
in Guidelines for River Microplastic Monitoring Methods**

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3	Solutions-----	6
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1 Introduction

The sample preparations in ‘Guidelines for River Microplastic Monitoring Methods (GRMMM)’ 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 lacustrine and marine surface samples.

2 Consideration for the automation

The oxidation (Chap. 4.2 in GRMMM) 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. 4.3 in GRMMM) was also automated by passing through the following process.

1. Wash the preparative container with a 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.

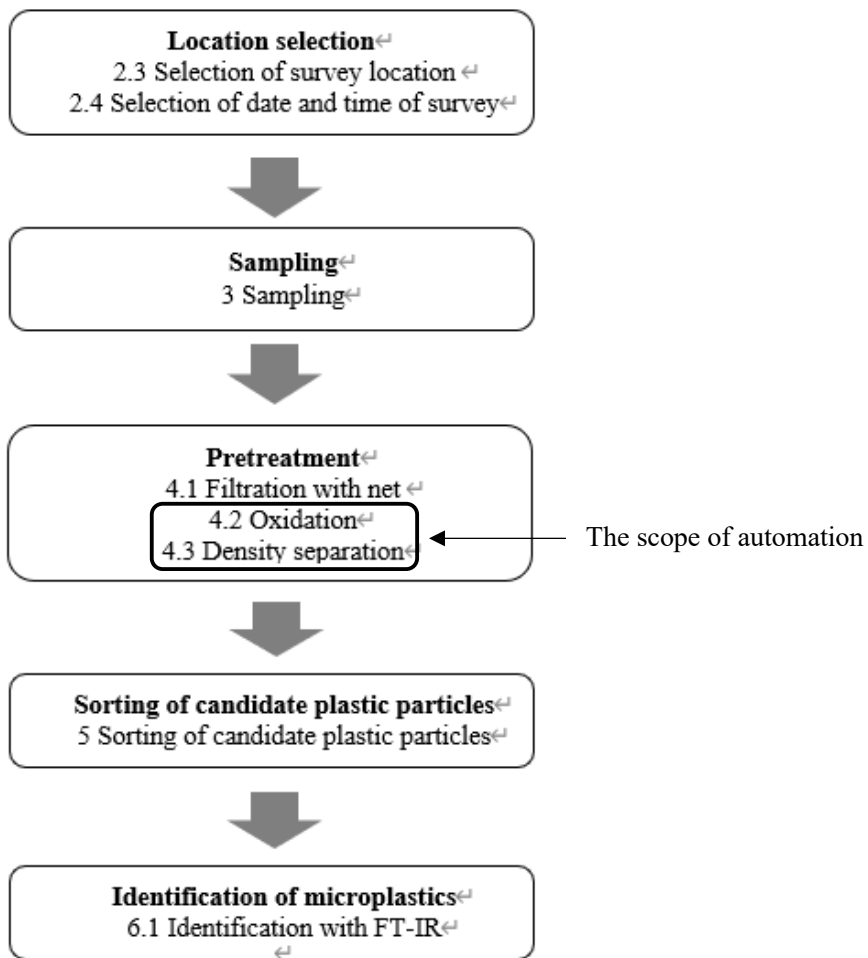


Figure 1. The procedure of the microplastic analysis indicated in GRMMM

3 Specification

(1) Samples

The samples taken by following GRMMM 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 °C with ± 5 °C, 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 enough 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 wetted parts inside the containers are made of the 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 is about 200 μm . The wetted parts are made of the 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 wetted parts are made of the 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 wetted parts are made of the materials which get less effects from reaction solutions and dense solvents.

(6) Drainage mechanism

The mechanism of ejecting solutions out of containers. The wetted parts are made of the

materials which get less effects from reaction solutions and dense solvents.

(7) Collection filter

The filters collect CPP from supernatants. The mesh openings is about 100 μm . The wetted parts are made of the materials which get less effects from reaction solutions and dense solvents.

(8) Chemical tank

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

(10) Piping

The wetted parts are made of the 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					

Extra document

This extra document explains the device 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

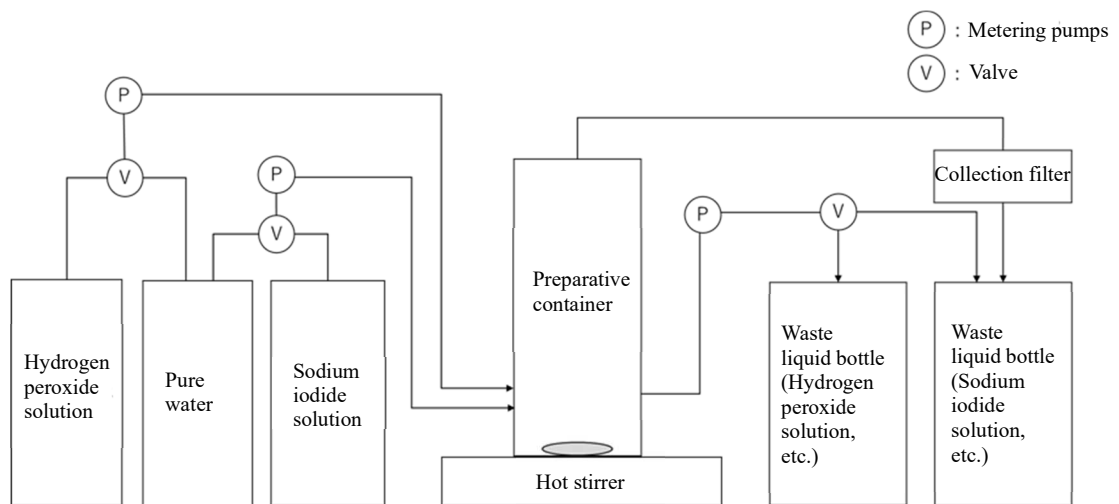


Figure 2. An example of the device constitution

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 the 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 caliber was put on the top of the device 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 that discharging capability was 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.

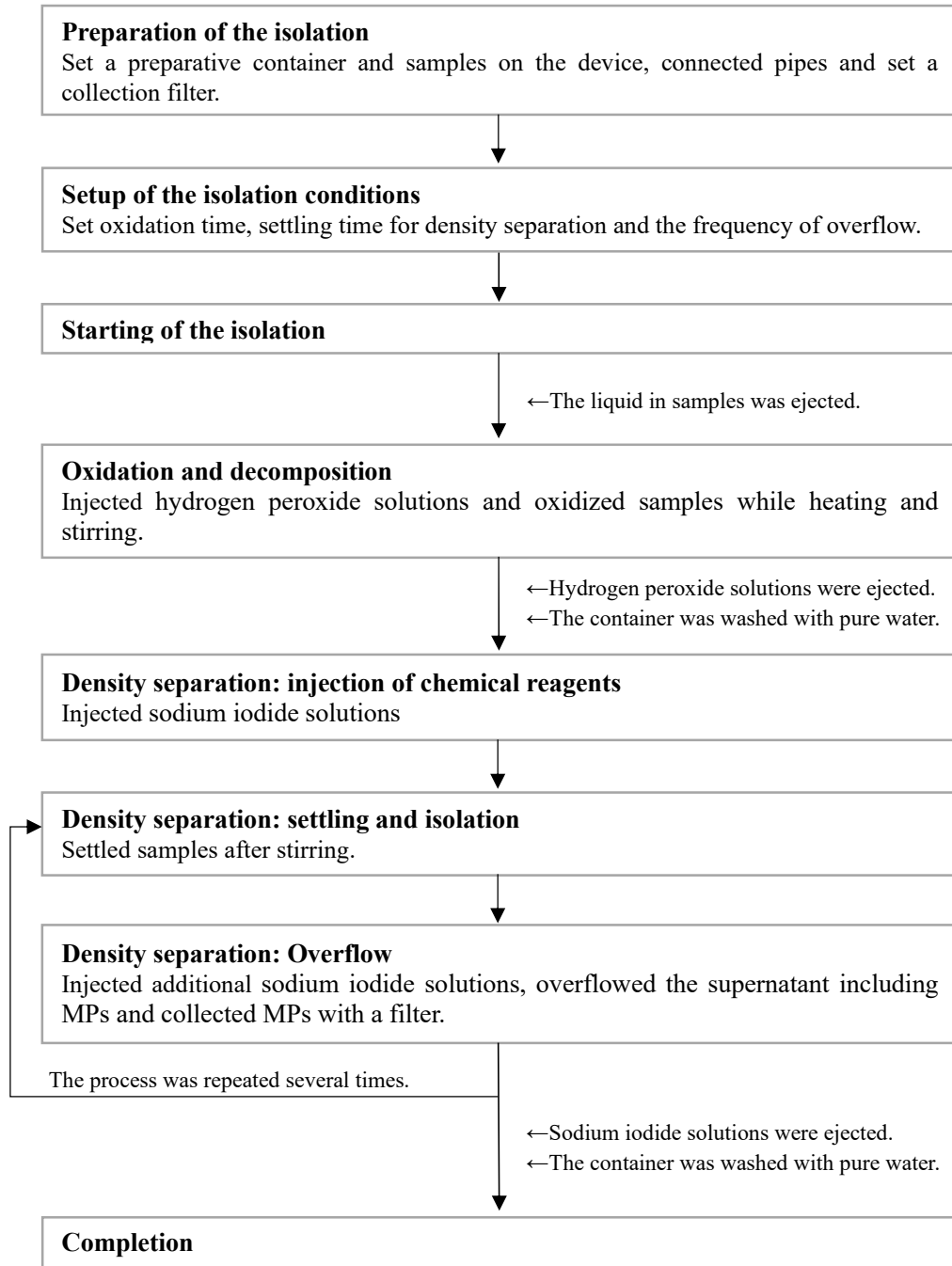
(10) Operation section

The time of oxidation and settling for density separation and the frequency of overflows were setover.

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 connected pipes.
- ③ Set a collection filter at the collecting section.
- ④ At the operation section, the oxidation time (day), settling time for density separation (hour) and the frequency of overflow were set.
- ⑤ Ejected the liquid from samples.
- ⑥ Injected 30 % hydrogen peroxide solutions slowly until it reached the set amount. The maximum amount was decided based on the oxidation time.
- ⑦ Kept the temperature of solutions at 55 °C to enhance the oxidation while stirred it slowly with a stirring bar. The temperature of solutions was 60 °C or less to avoid the degeneration of plastics.
- ⑧ Kept the above condition for maximum 8 days.
- ⑨ Stopped heating and a stirring bar.
- ⑩ Ejected hydrogen peroxide solutions from the preparative container to the drain bottle which was for hydrogen peroxide solutions only.
- ⑪ Injected pure water to the preparative container to wash and ejected it to the drain bottle which was for hydrogen peroxide solutions only. This process was repeated several times.
- ⑫ Injected sodium iodide solutions to the preparative container for density separation.
- ⑬ Stirred the solutions in the container for 90 seconds and settled it to collect CPP into supernatants.
- ⑭ After settling, injected sodium iodide solutions from the injection port and overflowed the solutions including CPP from the top of the preparative container.
- ⑮ Collected solid matters by passing the overflowed solutions including CPP through a collection filter.
- ⑯ Repeated the process from ⑬ to ⑮ in set frequencies.
- ⑰ Ejected sodium iodide solutions to the drain bottle which was for sodium iodide solutions only.
- ⑱ Injected pure water to the preparative container to wash and ejected 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 devise

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 °C
	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 devise 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

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