Handbook for Quantifying Plastics in the Marine Environment

Microbial Oceanography Laboratory

Handbook for Quantifying Plastics in the Marine Environment

Microbial Oceanography Laboratory, Marine Science Institute,
University of the Philippines Diliman

Acknowledgements

The development of this handbook is funded by:

PlasMics (Plastics in the marine environment, trophic systems, and aquaculture in the Philippines)
Department of Science and Technology - National Research Council of the Philippines

PlastiCount Pilipinas
Department of Science and Technology - Philippine Council for Industry, Energy and Emerging Technology Research and Development

MicroSEAP
UK Research and Innovation - Natural Environment Research Council

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The printing of this handbook is funded by:
The Circular Explorer Project
Holcim Philippines, Inc.

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Introduction

Plastic pollution has been affecting different environments worldwide, with most ending up and accumulating in coastal and marine environments. Plastic wastes remain afloat at sea or sink down the seafloor. Majority of these wastes were estimated to accumulate in coastal regions (Lebreton et al. 2019), leaving coastal areas and sediments at risk to plastic pollution (Barnes et al. 2009; Pinnell and Turner 2019). Plastics accumulating in the environment break down into smaller fragments, categorized as microplastics, which are small plastic particles less than 5mm in size (Arthur et al., 2009). The oceans are increasingly becoming contaminated with microplastics, posing a serious threat to marine life and ecosystems (Hale et al., 2020). Effective sampling, processing, and quantification of macro- and microplastics in different compartments (e.g., water, sediment, biota) are crucial to understand the extent and nature of their distribution in the marine environment, and for developing appropriate strategies to mitigate their impact (Prata et al., 2019).

This toolkit outlines methods for macro- and microplastics analysis. The methods for macroplastics survey will determine the debris density (# of plastic pieces per unit area) and the type of debris materials. This toolkit also provides methods to sample and analyze microplastics in water, sediments, and biota from the marine environment, and a guide for data submission to the PlastiCount Pilipinas online portal. The procedures are designed to be accessible and replicable by researchers, policy makers and citizen scientists given the proper resources. While several methods are already available for plastics research, suitable technologies and approaches are required to harmonize the efforts and provide more robust data. These methodologies must be rolled out in localities with the same environmental setting to allow comparability of data, and hence, produce more accurate information on plastic pollution.

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Macroplastics Quantification: Field Survey

Site Selection

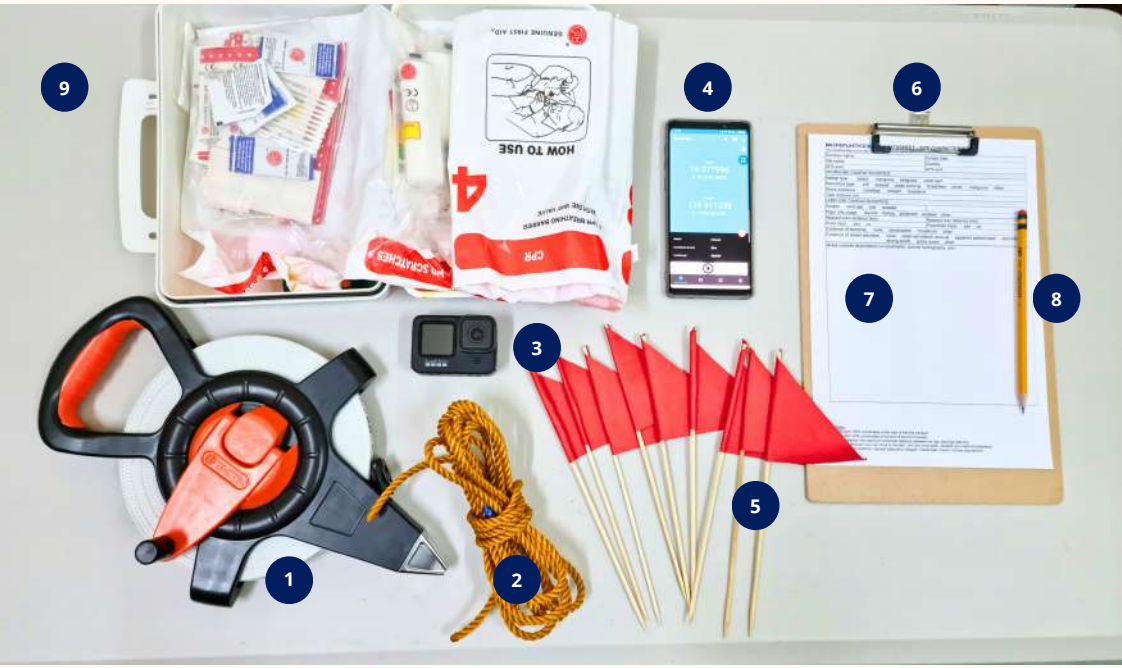
The beach sites for macroplastics survey should be selected according to the following criteria:

San Rafael III, Noveleta, Cavite

- **Sandy or pebble shoreline**
- **A minimum length of 100 m parallel to the water**
- **Low to moderate slope (15 to 45 degrees)**
- **Clear access to the sea (no breakwater or jetties)**
- **Accessible to survey teams year round**
- **Must not be part of a clean-up program (if possible)**

Materials

1. Transect tapes, at least 30 m (x 3)
2. Rope, 4 m
3. Digital camera
4. Phone (with apps for GPS and wind compass)
5. Flag markers
6. Clipboard for each surveyor
7. Data sheets
8. Pencils for data recording
9. First aid kit



Field Survey

Adapted from "Microbial transformation of plastics in SE Asian seas: a hazard and a solution" (MicroSEAP)

1. **For beach sites, lay three 30 m transects at the strandline.**
    a. **The strandline is the part of the beach where debris accumulate.**
2. **The transects are laid parallel to the shoreline (Fig. 1). The minimum distance between two consecutive transects should be at least 2 m apart or wider. For larger study sites, the distance between two consecutive transects can be larger.**
    a. **For smaller sites that cannot fit 3 x 30 m transects, shorter transect length (e.g., 15 or 20 m) may also be adopted, but the number of transects at each tidal zone should still be three.**

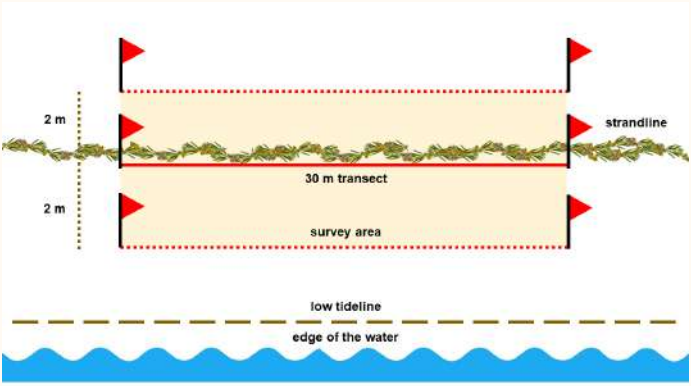


Figure 1. Survey area (30m x 4m) along beach strandline

Field Survey

Adapted from "Microbial transformation of plastics in SE Asian seas: a hazard and a solution" (MicroSEAP)

3. **To mark the survey area, use the 4 m rope to measure 2 m to the left and right of the strandline.**
4. **Place flaglets to mark the edges of the survey area.**
5. **For sampling macro-litter (> 25 mm in size, bigger than a bottle cap), record the following information for each litter item found within 2 m to the left and 2 m to the right of the transects (i.e., 4 m total width x 30 m total length belt transect).**
6. **Record debris counts while walking across the survey area in a pattern (Fig. 2). If you find litter items that are not in the list of debris types in the datasheets, take a photo for documentation.**
7. **Fill-out the datasheet for other information on site characteristics after the field survey.**

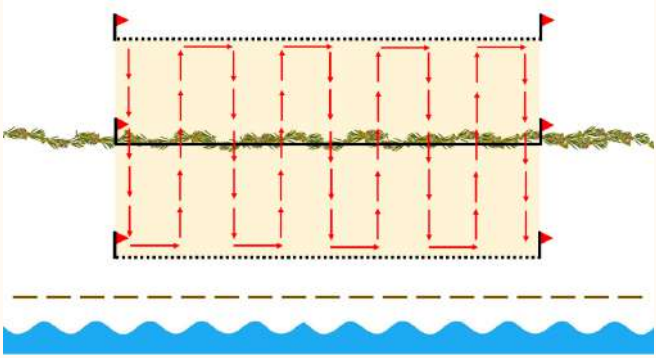


Figure 2. Suggested field survey walking pattern

Field Survey

Adapted from "Microbial transformation of plastics in SE Asian seas: a hazard and a solution" (MicroSEAP)

The macrodebris item concentration (number of debris items/sq-m) per transect is calculated as follows:

$$C = \frac{n}{wl}$$ (Lippiatt et al., 2013)

Where

C = concentration of debris items (# of debris items/square meter)
n = # of macro-debris items observed
w = width (m) of shoreline section (i.e. transect width)
l = length (m) of the shoreline sampled = 30

For a given sampling event, take the mean concentration at each transect to calculate an overall site concentration (± standard deviation) for that date.

Field Survey (Schematic Guide)

1 Lay 30 m transects at the strandline.

2 Mark survey area using a 4 m rope.

3 Place flaglets at the edges of the survey area.

4 Record macroliiter found within area.

5 Record debris counts in the datasheet.

6 Fill out information on site characteristics.

Microplastics Quantification: Sampling Methods

Site Selection

The beach sites for microplastics survey should be selected according to the following criteria:

Inagawan, Puerto Princesa City, Palawan

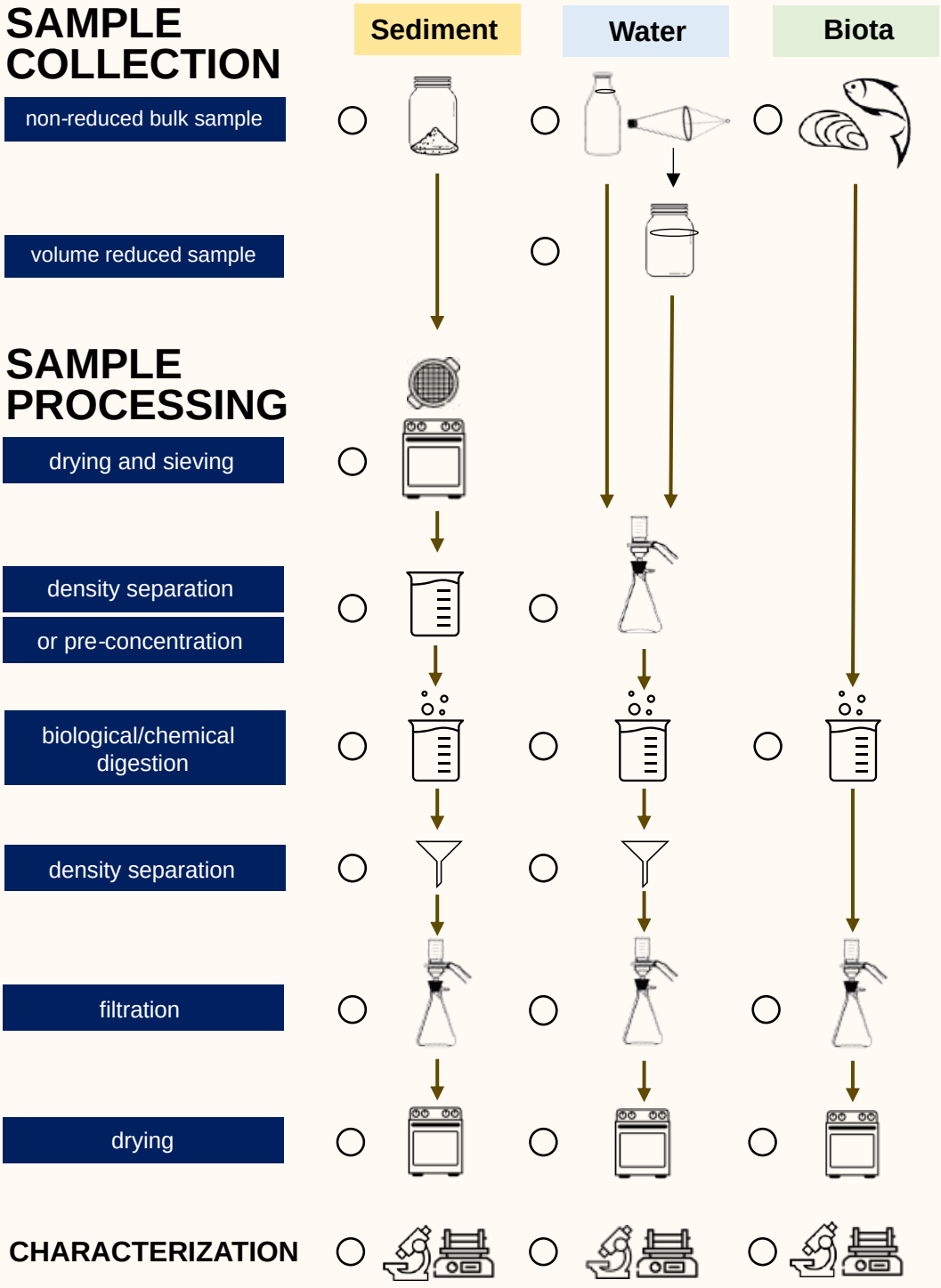
- **Sandy or pebble shoreline**
- **A minimum length of 100 m parallel to the water**
- **Low to moderate slope (15 to 45 degrees)**
- **Clear access to the sea (no breakwater or jetties)**
- **Accessible to survey teams year round**
- **Must not be part of a clean-up program (if possible)**

Note that these criteria are guidelines to allow smooth conduct of fieldwork. However, not all sites exactly align to these criteria and any deviations should be noted.

Recommended Attire

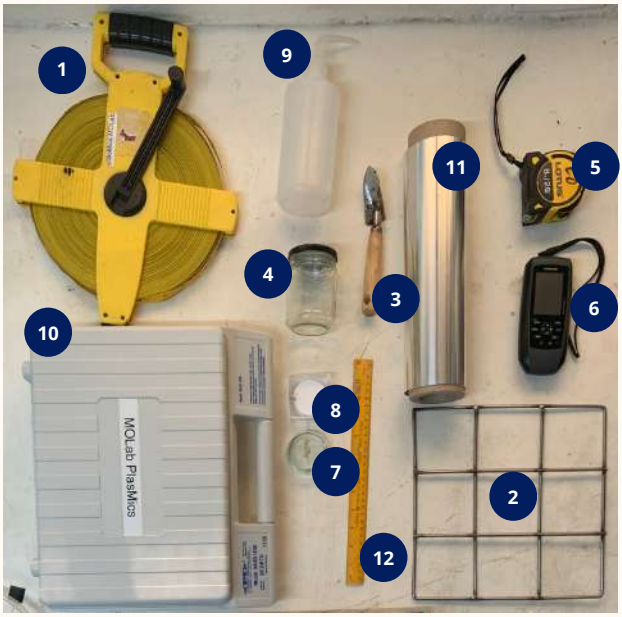
- **Cap / Hat**
- **Rash guard / arm sleeves (Cotton)**
- **Long pants (cotton)**
- **Closed non-slip shoes (preferably non-synthetic)**
- **Cotton safety gloves**
- **Sunscreen**

The use of natural-fiber based clothing decreases the amount of microplastics contamination in samples.


SAMPLE COLLECTION
Sediment
Water
Biota
non-reduced bulk sample
volume reduced sample
SAMPLE PROCESSING
drying and sieving
density separation
or pre-concentration
biological/chemical digestion
density separation
filtration
drying
CHARACTERIZATION

Materials (Sediments)

1. Surveyor's Tape
2. Quadrat (25 x 25 cm)
3. Scoop / Trowel
4. Sampling Bottle (250 mL)
5. Tape Measure
6. GPS
7. Petri dish (3 pieces)
8. Filter paper (GF/C)
9. Wash bottle with filtered distilled water
10. Multiparameter meter
11. Aluminum foil
12. Ruler
13. Wind app and randomizer app on phone



Sediment Sampling

Adapted from "Microbial transformation of plastics in SE Asian seas: a hazard and a solution" (MicroSEAP)

1. **For beach sites, lay three 30 m transects at the strandline.**
    a. **The strandline is the part of the beach where debris accumulate.**
    b. **Lay transects during periods of low tide to maximize study area.**
2. **The transects are laid parallel to the shoreline (Fig. 1, in red). The minimum distance between two consecutive transects should be at least 2 m apart or wider. For larger study sites, the distance between two consecutive transects can be larger.**
    a. **For smaller sites that cannot fit 3 x 30 m transects, shorter transect length (e.g., 15 or 20 m) may also be adopted, but the number of transects at each tidal zone should still be three.**

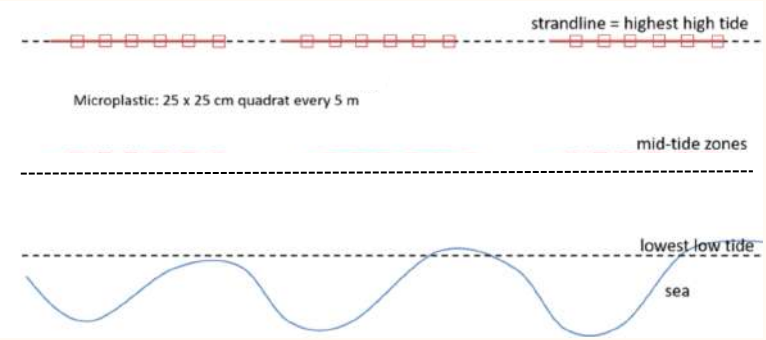


Figure 1. Survey area (30m x 4m) along beach strandline

Sediment Sampling

Adapted from "Microbial transformation of plastics in SE Asian seas: a hazard and a solution" (MicroSEAP)

3. **Prepare air contamination controls: place a GF/C filter inside the petri dish and dampen with filtered distilled water from the wash bottle. Close when not in use.**
4. **One air contamination control per transect should be used.**
5. **Lay one 25 cm by 25 cm quadrat every 5 meters of the transect line.**
6. **Randomly select 1 grid out of 9 from the quadrat (Figure 2).**
7. **Sample coastal sediment equal to the volume obtained by the 8 cm x 5 cm corer or approximately 250 cm^3 of the upper 5 cm of the sediment surface.**
8. **Place sampled sediment in 250 mL glass jars.**
9. **Cover the glass jar mouth with clean aluminum foil before covering it with a metal lid.**
10. **Fill-out datasheet for other information on site characteristics.**

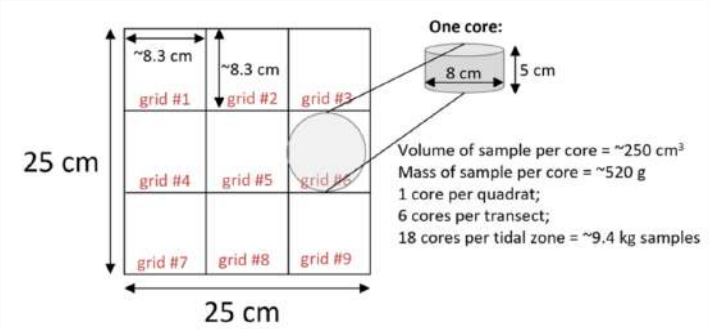


Figure 2. Quadrat layout and numbering

Sediment Sampling (Schematic Guide)

1 Lay 30 m transects at the strandline.

2 Set up air contamination controls.

3 Place quadrats after every 5 meters.

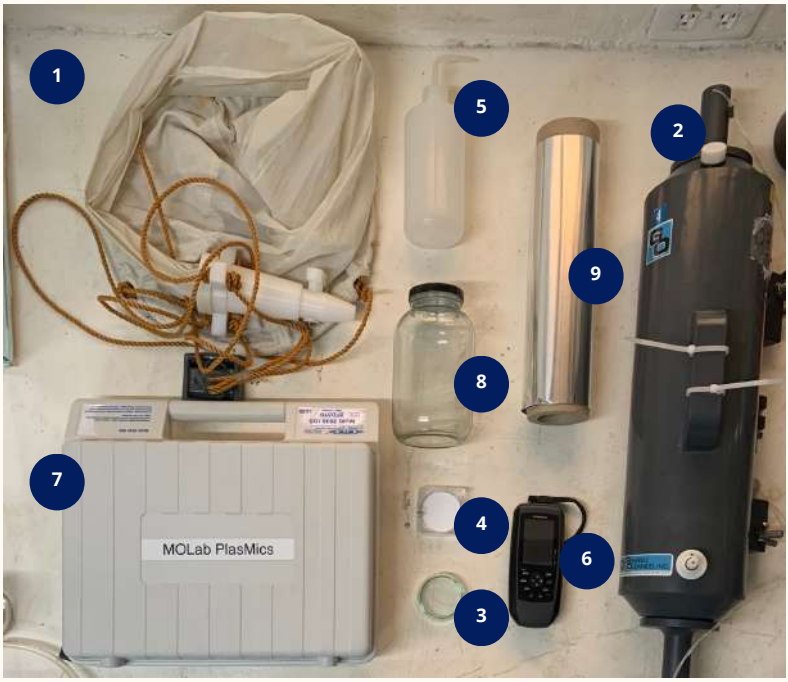
4 Randomly select one grid and collect sediment.

5 Continue for a total of 6 samples.

6 Fill out information on site characteristics.

Materials (Water)

1. Sampling net / Tow sampler
2. Niskin bottle / Grab sampler
3. Petri dish
4. Filter paper (GF/C)
5. Wash bottle with filtered distilled water
6. GPS
7. Multiparameter meter
8. Sampling bottles (1 L)
9. Aluminum Foil
10. Wind app and stopwatch on phone



Water Sampling

Adapted from "Microbial transformation of plastics in SE Asian seas: a hazard and a solution" (MicroSEAP)

1. **Wash net and discard content from cod end three times.**
2. **Prepare air contamination controls: place a GF/C filter inside the petri dish and dampen with filtered distilled water from the wash bottle. Close when not in use.**
3. **Place three air contamination controls near the sampling point.**
4. **Record starting coordinates using GPS.**
5. **Collect 1 L of grab sample at the starting location.**
6. **Deploy net at the side of the vessel.**
7. **Tow net for 10 mins at a relatively slow, constant pace.**
8. **Bring net overboard and transfer contents of cod end to 1 L sampling bottle.**
9. **Wash net with sample water and transfer contents of cod end twice.**
10. **Collect 1 L of grab sample at the ending location.**
11. **Record ending coordinates using GPS.**

Water Sampling (Schematic Guide)

1 Record starting coordinates.

2 Set up air contamination.

3 Collect grab sample at start position.

4 Tow net for 10 minutes and collect sample.

5 Collect grab sample at end position

6 Record end coordinates.

Materials (Biota)

1. Pre-filtered distilled water
2. Aluminum foil
3. Rubber bands
4. Ziplock
5. Oyster knife
6. Dissecting materials
7. Vernier caliper

Biota Sampling and Preparation

Adapted from AMAP Litter and Microplastics Monitoring Guidelines, Version 1.0, 2021.

1. **Collect at least 30 individuals from each sampling site.**
Note: Collection method may vary depending on the type of biota to be sampled and type of sampling site. (e.g., market sampling, hand collection, trawl nets)

2. **Wash the samples with pre-filtered water then wrap with aluminum foil and rubber bands to prevent contamination.**

3. **Store in an ice box and transfer to a -20°C freezer until lab processing.**



Biota Prep (Schematic Guide)

1 Thaw samples and wash with distilled water.

2 Measure sample length with a caliper.

3 Weigh clean 500 mL beakers.

4 De-shell or dissect to extract target tissue.

5

Place tissue samples in beaker for weighing.

Next Steps

1. **Inspect that water and sediment sample jars and biota foil wraps are tightly sealed to avoid air contamination.**
2. **Seal petri dishes properly to avoid accidental opening during transport.**
3. **Identify laboratories that are capable of processing samples for microplastics. A list of laboratories are uploaded onto the PlastiCount portal (https://plasticount.ph/) for reference.**

Microplastics Quantification: Laboratory Procedures

Contamination Control

Provisions for contamination control must be followed to assure that ambient microplastics in the laboratory environment do not affect the count from environmental samples.

- All materials, equipment, and laboratory surfaces to be used should be thoroughly rinsed and cleaned with pre-filtered water and stored under clean air conditions.
- Reagents and solutions should be pre-filtered before treatment.
- Sample handling and processing should be conducted in clean air facilities. If clean air conditions cannot be fully achieved, use of negative controls needs to be established.
- Triplicate negative controls need to be included in the treatments for each batch of sample processed.
- For additional controls, clean filter papers in Petri dishes or glass slides should be placed around the area for sample processing to check for airborne contamination.
- Samples should be covered with aluminum foil before, during, and after processing. Polymer-free gloves and laboratory coat made of 100% cotton material should be worn during analysis to prevent contamination.

Preparation of Spiked Samples

- **Microplastics**

(Methods adapted from Nuelle et al. 2014; Kuhn et al. 2018; Selonen et al. 2020)

Materials/Equipment

- Beakers/glass containers
- Cutter/Scissors
- Cutting mat
- Plastics:
  - PET – water bottle
  - PP – straw
  - LDPE – ice bag and plastic bag
  - HDPE – shampoo container
  - PVC – pipe
  - PS – yogurt cup
- Fibers:
  - Fish net

Procedure

- Cut plastics into smaller pieces using a cutter or scissors, less than 25 mm.
- Store cut plastics in glass containers.

- **Organic matter**

(Methods adapted from Isobe et al. 2019)

Materials/Equipment

- Glass containers
- Wood chips
- Seaweed
- Grinder

Procedure

- Dry wood chips and seaweed in the oven at 60°C overnight or until brittle. Mechanically fragment organic matter using a grinder.
- Store in glass containers.

Preparation of Spiked Samples

- **Sediments**

(Modified from Shim et al. 2016)

Materials/Equipment

- Beach sand
- Sieve (5.6-mm)
- Furnace
- Furnace-friendly container for sand combustion/aluminum pans
- Prepared microplastics (MPs) for spiking

Procedure

- Collect natural sand from the beach.
- Sieve with a 5.6-mm mesh size to remove larger particles. Store in aluminum tray.
- Pre-combust cleaned sand for six hours or overnight at 550 °C to remove plastics and organic matter.
- Add known amount of MPs to spike 100 g of the pre-combusted sand in a beaker. Also add organic matter into the mixture. Mix.
- Analyze this sand and plastic mixture along with samples to test for extraction efficiency.

- **Water**

(Modified from Isobe et al. 2019)

Materials/Equipment

- Glass filtration set-up
- Vacuum pump
- Rubber tubing
- Seawater
- Glass microfiber filter (GF/F)
- 1 L glass bottle containers
- Prepared microplastics (MPs) for spiking

Procedure

- Filter seawater with GF/F and wash sample bottles three times before filling. Make sure there are no plastic fragments in the water and in the sample bottles.
- Add known amount of MPs per type to the sample bottle containing filtered seawater.
- Add organic material to the sample bottle. Mix.
- Analyze this mixture along with samples to test for extraction efficiency.

Preparation of Spiked Samples

- **Biota**

(Modified from Dehaut et al. 2016)

Materials/Equipment:

- Target biota samples
- Dissecting kit
- 500-mL beaker
- Prepared microplastics (MPs) for spiking

Procedure:

- Collect 3 to 5 individuals of target species.
- De-shell or dissect the sample and obtain the target component (e.g. gastrointestinal tract).
- Measure wet weight of dissected sample and add tissue to a clean 500-mL beaker.
- Add known amount of MPs per type to the beaker containing the target tissues.
- Analyze this tissue and plastic mixture along with samples to test for extraction efficiency.

Extraction of MPs from Samples

Reagent Preparation

For sediment and water samples

Zinc chloride ($ZnCl_2$) salt solution with density of 1.5 g/mL

NOTE: Irritant, toxic, and harmful to the environment. Handle with precaution, use gloves, mask, and goggles.

1. Mix 750 g of $ZnCl_2$ in approximately 700 mL of distilled water in a 1000-mL beaker, stirred (with a stir bar) and heated on a hot plate until no solids appear in the solution. Adjust the density to 1.5 g/mL by checking the mass of 1 mL of the solution. If solution is less than 1.5 g, add more $ZnCl_2$.
2. If white precipitate is observed, add concentrated HCl until the solution turns clear (pH of solution should be <5).
3. Let solution cool and transfer to 1000-mL volumetric flask. Dilute to mark with distilled water. Shake to mix.
4. Filter using 0.45-µm filter papers to remove impurities.
5. Store in pre-washed (with the solution) reagent bottle or media bottle.

0.05 M Iron (Fe(II)) solution

NOTE: Light-sensitive solution, prepare inside the hood. Harmful if swallowed, irritant. Handle with precaution, use gloves and mask.

1. Dissolve 7.5 g of $FeSO_4 \cdot 7H_2O$ (MW= 278.02 g/mol) in distilled water in a 250-mL beaker.
2. Transfer contents to a 500-mL volumetric flask, making sure the beaker is thoroughly washed with distilled water. Cover volumetric flask with aluminum foil.
3. Add 3mL of concentrated sulfuric acid to the solution.
4. Dilute to mark with distilled water. Shake to mix.
5. Filter using 0.45-µm filter papers to remove impurities.
6. Store in pre-washed (with the solution) amber reagent bottle or media bottle.

30% Hydrogen peroxide (H_2O_2)

NOTE: Strong oxidizer, corrosive, and irritant. Handle with precaution, use gloves, mask, and goggles.

1. Depending on the initial concentration of the hydrogen peroxide, calculate the volume to be measured using $C_1V_1 = C_2V_2$ to prepare a 1000 mL solution. In this case, $V_1 = ((0.30)(1000\ mL))/C_1$.
2. Measure the appropriate volume using a graduated cylinder. Transfer the liquid to a 1000-mL volumetric flask.
3. Dilute to mark with distilled water. Shake to mix.
4. Filter using 0.45-µm filter papers to remove impurities.
5. Store in pre-washed (with the solution) reagent bottle or media bottle.

Extraction of MPs from Samples

Sediments

Materials / Equipment

- Drying oven (80°C)
- Small aluminum pans
- Aluminum foil
- CD markers
- 5.6-mm stainless steel sieve (No. 3.5)
- Top loading balance
- 250-mL glass beaker
- 500-mL tall glass beaker
- 800-mL glass beaker
- Metal scoop or spatula
- 50-mL graduated cylinder
- 100-mL graduated cylinder
- 500-mL graduated cylinder
- Glass rod
- Wash bottle containing filtered distilled water
- Wash bottle containing filtered saturated solution ($ZnCl_2$)
- Glass filtration set-up
- Vacuum pump with motor oil
- Rubber tubing
- Glass microfiber filter (GF/C)
- Glass microfiber filter (GF/F)
- Bowl for waste jar during filtration
- Metal forceps or tweezers
- Laboratory hot plate
- Cotton gloves
- Glass spatula
- Density separator, which is assembled using the following method:
  - A glass funnel (122 mm in diameter), fitted with a 50-mm segment of latex tubing on the bottom of the stem and a pinch clamp attached to control liquid flow from the funnel
  - Retort stand
  - O-ring
- 60-mm petri dish
- Dissecting microscope (40X magnification)
- Carboys for waste disposal

Extraction of MPs from Samples

Sediments

Procedure

1. **Transfer sediment samples to aluminum pans or trays. Cover with aluminum foil and label accordingly.**
2. **Dry sediment at 80°C for at least 48 h or until sample dryness.**
3. **Sieve the sediment sample through a 3-inch 5.6-mm metal sieve on top of a 500-mL tall beaker. Weigh 100 g of the sieved sediment sample.**
4. **Prepare spiked samples and procedural blanks (no sediment). Analyze this together with the samples. Also prepare air contamination blanks with wet glass microfiber filters in petri dishes.**
5. **Conduct density-separation method:**
   a. **Prepare $ZnCl_2$ salt solution**
   b. **Add salt solution at a ratio of 100 g dried sediment to 300 mL salt solution.**
   c. **Mix sediment and salt solution thoroughly for 2 min using a glass rod.**
   d. **Cover the beaker with aluminum foil and let the particles settle at least 5 hours or overnight.**
   e. **Gently pour only the supernatant to a clean 800-mL beaker.**
   f. **Add another set of 300 mL salt solution to the beaker with the sediments. Repeat steps c to e, pooling the second collected fraction to the beaker in step e.**
   g. **Filter the supernatant through a glass microfiber filter (GF/C) using a glass filtration set-up. Thoroughly wash the beaker with filtered distilled water to ensure total transfer of particles.**
   h. **Rinse the filter paper with enough distilled water to remove the salt solution.**
   i. **Transfer the particles on filter paper into a 500-mL beaker with as little distilled water as possible. Cover the glass beaker with aluminum foil, but one edge is slightly opened.**
   j. **Dry the beaker at 80°C in the oven for 24 h or longer until sample dryness.**

Extraction of MPs from Samples

Sediments

Procedure

6. **Conduct oxidative digestion:**

a. **Prepare 0.05 M Fe (II) solution.**
b. **Add 20 mL of Fe(II) solution and 20 mL of 30% H_2O_2 solution into the beaker (Fenton's reagent: corrosive, wear personal protection).**
c. **Stir the solution at room temperature. Then place on hot plate, keep the temperature around 40°C. If there's violent bubbling, put in ice bath or add filtered distilled water into solution.**
d. **If the solution is still brownish in color, add more H_2O_2 (repeat step c and d).**
e. **Let the solution cool down.**
f. **Filter the supernatant through a glass microfiber filter (GF/C) using a glass filtration set-up. Thoroughly wash the beaker with filtered distilled water to ensure total transfer of particles.**
g. **Rinse the filter paper with enough distilled water to remove the residual solution.**
h. **Transfer the particles on filter paper into the 500-mL beaker with as little distilled water as possible. Cover the glass beaker with aluminum foil, but one edge is slightly opened.**
i. **Dry the beaker at 80°C in the oven for 24 h or longer until sample dryness.**

Extraction of MPs from Samples

Sediments

Procedure

7. **Conduct another round of density separation:**

   a. **After the solution has dried, add 75 mL of zinc chloride to the solution. Transfer this solution to a density separator. Cover with aluminum foil and allow to stand for at least four hours or overnight.**
   b. **Collect the fraction with the settled debris (D) into a 250-mL beaker. Collect the top fraction (F) into a separate 250-mL beaker. Wash the funnel with saturated zinc chloride solution to ensure full transfer of particles. Set aside F and cover with aluminum foil. Resuspend D fraction for another round of density separation. Collect the top fraction and pool it into the F fraction collected previously.**
   c. **Filter the F fraction through glass filtration using a glass microfiber filter (GF/F). Thoroughly wash the beaker with filtered distilled water to ensure full content transfer.**
   d. **Place the filter paper in a glass petri dish.**

8. **Count the microplastics on the filter paper and record.**

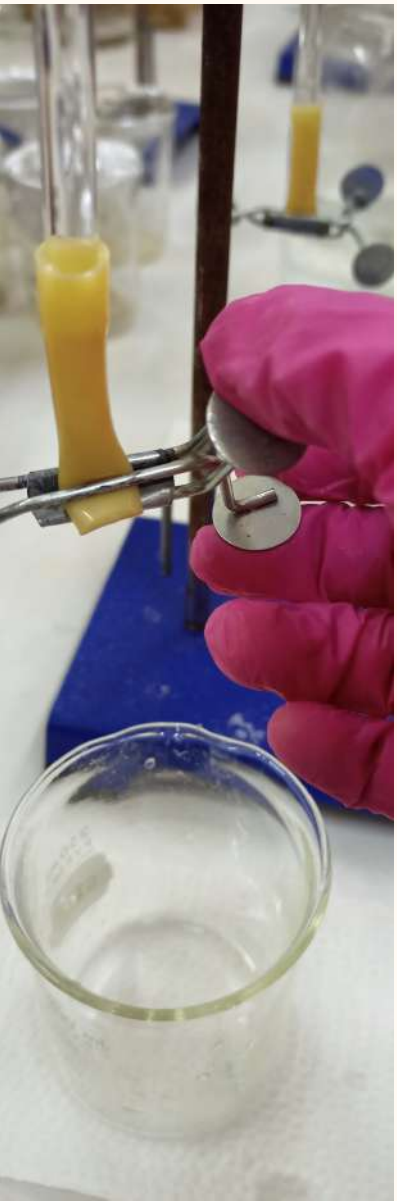
Waste Disposal

- Excess zinc chloride ($ZnCl_2$) – $ZnCl_2$ waste jar
- Excess Fe (II) solution – Fenton's reagent waste jar
- Excess hydrogen peroxide (H_2O_2) – Fenton's reagent waste jar
- Sediments with zinc chloride ($ZnCl_2$) – Contaminated solids ($ZnCl_2$) waste jar
- Waste during oxidative digestion – Fenton's reagent waste jar
- Waste zinc chloride ($ZnCl_2$) – $ZnCl_2$ waste jar

Extraction of MPs from Samples

Water

Materials / Equipment



- Tap water
- Glass sampling bottles
- Aluminum foil
- Glass filtration set-up
- Vacuum pump
- Rubber tubing
- 0.45 µm membrane filter
- Forceps
- Wash bottle
- Filtered distilled water for washing
- Glass microfiber filters (GF/C and GF/F)
- 250-mL glass beakers
- Laboratory hot plate
- 50-mL graduated cylinder
- Cotton gloves
- 100-mL graduated cylinder
- Glass spatula
- Drying oven (80°C)
- Density separator, which is assembled using the following method:
  - A glass funnel (122 mm in diameter), fitted with a 50-mm segment of latex tubing on the bottom of the stem and a pinch clamp attached to control liquid flow from the funnel.
  - Retort stand
  - O-ring
- 60-mm petri dish
- Dissecting microscope (40X magnification)
- Carboys for waste disposal

Extraction of MPs from Samples

Water

Procedure

1. **Prepare spiked samples and procedural blanks. Procedural blanks are prepared by filling 1 L glass bottles with filtered tap water. These are analyzed along with spiked and actual samples.**
2. **Filter all samples, including spiked samples and procedural blanks through vacuum filtration with a glass microfiber filter. Transfer residue to a clean 250-mL beaker using distilled water.**
3. **Conduct oxidative digestion by adding 20 mL of 0.05 M Fe(II) solution and 20 mL of 30% hydrogen peroxide (Fenton's reagent: corrosive, wear personal protection). Let the solution stand for 10 minutes then heat on a hot plate to 40°C. Heat the solution until the solution violently bubbles. Remove from the hot plate carefully and if there are still organic matter present, add 20 mL more of 30% hydrogen peroxide. Wait until the solution bubbles again. When the solution becomes yellow instead of the initial brown color, let the solution heat for 20 min. After 20 mins, allow the solution to cool before vacuum filtration.**

Extraction of MPs from Samples

Water

Procedure

4. **When the solution has cooled, filter the solution through glass filtration, and transfer all contents of the filter paper to a 250-mL beaker. Allow the solution to dry at 80°C.**
5. **After the solution has dried, add 75 mL of zinc chloride to the solution. Transfer this solution to a density separator. Cover with aluminum foil and allow to stand for at least four hours or overnight.**
6. **Collect the fraction with the settled debris (D) into a 250-mL beaker. Collect the top fraction (F) into a separate 250-mL beaker. Wash the funnel with saturated zinc chloride solution to ensure full transfer of particles. Set aside F and cover with aluminum foil. Resuspend D fraction for another round of density separation. Collect the top fraction and merge it into the F fraction collected previously.**

Extraction of MPs from Samples

Water

Procedure

7. **Filter the F fraction through glass filtration using a glass microfiber filter. Thoroughly wash the beaker with filtered distilled water to ensure full content transfer.**
8. **Count the microplastics on the filter paper and record.**

Waste Disposal

- Excess zinc chloride ($ZnCl_2$) – $ZnCl_2$ waste jar
- Excess Fe (II) solution – Fenton's reagent waste jar
- Excess hydrogen peroxide (H_2O_2) – Fenton's reagent waste jar
- Sediments with zinc chloride ($ZnCl_2$) – Contaminated solids ($ZnCl_2$) waste jar
- Waste during oxidative digestion – Fenton's reagent waste jar
- Waste zinc chloride ($ZnCl_2$) – $ZnCl_2$ waste jar

Extraction of MPs from Samples

Reagent Preparation

For biota samples

10% Potassium hydroxide (KOH) 

NOTE: Corrosive and irritant. Handle with precaution, use gloves, mask, and goggles. Preferably made fresh for each batch as bases can etch glass.

1. **Mix 200 g of KOH in approximately 800 mL of distilled water in a 1000-mL beaker and stirred (with a stir bar) on a hot plate until no solids appear in the solution.**
2. **Transfer to 2000-mL volumetric flask. Dilute to mark with distilled water. Shake to mix.**
3. **Filter using 0.45-µm filter papers to remove impurities.**
4. **Temporary store in pre-washed (with the solution) reagent bottle or media bottle.**

Saturated sodium chloride 

NOTE: Handle with precaution, use gloves and mask.

1. **Dissolve 380 g of NaCl (MW= 58.44 g/mol) in approximately 700 mL of warm distilled water in a 1000-mL beaker, stirred (with a stir bar) and heated on a hot plate until solids no longer dissolve in the solution.**
2. **Transfer contents to a 1000-mL volumetric flask, making sure the beaker is thoroughly washed with distilled water.**
3. **Dilute to mark with distilled water. Shake to mix.**
4. **Filter using 0.45-µm filter papers to remove impurities.**
5. **Store in pre-washed (with the solution) reagent bottle or media bottle.**

Extraction of MPs from Samples

Biota

Materials / Equipment

- **Sterile filtered distilled water**
- **Aluminum foil**
- **Ice box**
- **Ziplock bags**
- **Rubber bands**
- **Dissecting kit (scalpel, forceps, needle, scissors)**
- **Dissecting pans**
- **Stainless steel caliper**
- **Stainless steel shucking knife**
- **Metal spatula**
- **Beakers (250 ml, 600 ml)**
- **Petri dishes (60 mm, 100 mm)**
- **Glass microfiber filters (1 µm, 47 mm)**
- **Buchner funnel**
- **Buchner flask**
- **Stopper and rubber tubing**
- **Vacuum pump**
- **Stereomicroscope**
- **Blue LED lamp (450 nm)**
- **Top loading balance**
- **Carboys for waste disposal**

Extraction of MPs from Samples

Biota

Procedure

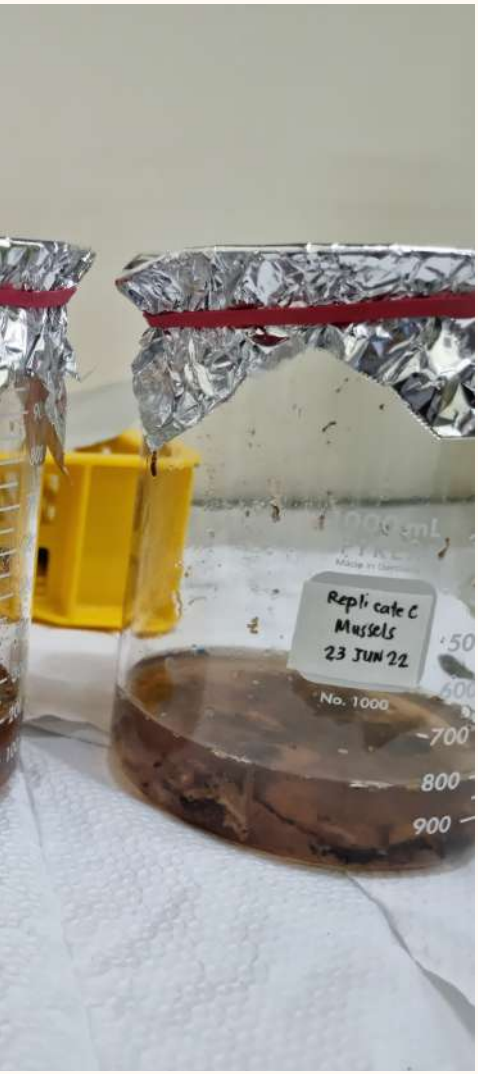
Bivalves

1. **From storage, thaw samples at room temperature and rinse the shells with pre-filtered distilled water.**
2. **Measure shell length with a stainless steel vernier caliper.**
3. **Using a stainless-steel shucking knife, open the shells and empty tissue mass into a clean 500-mL beaker. Individuals per species can be pooled per replicate depending on the number of samples collected per site (e.g. 5 mussels per replicate, 3 oysters per replicate).**
4. **Measure wet weight of the pooled tissue samples on a top loading balance.**
5. **Prepare triplicates of spiked samples and triplicates of procedural blanks to be processed along with the samples.**

Extraction of MPs from Samples

Biota

Procedure



6. **Add 10% KOH three times more than the tissue volume, cover the beaker opening with foil, and stand at 80°C for 48 hours. Agitate the set-up manually every 12 hours.**
7. **If there are residues in the digestate, proceed to density separation. Add 150 mL saturated NaCl into the digested solution and mix. Allow to settle overnight.**
8. **If the tissues are fully digested, proceed to microplastics extraction by vacuum filtration. Filtration is done using a 20-µm quantitative ashless filter paper in a Buchner funnel and flask set-up.**
9. **Store the filter paper in a sterile petri dish. Residual organic matter on the filter may be digested by adding 2-3 drops of 30% H_2O_2 onto the filter.**
10. **Dry the filters in the oven at 60°C overnight. Wrap petri dish in foil for storage until MP quantification.**

Extraction of MPs from Samples

Biota

Procedure

Fish

1. **After thawing, rinse the exterior of the fish thoroughly before placing in the dissection area to reduce particle contamination during processing.**
2. **Using a sterile scalpel blade, make an incision 1 mm in front of the rectum and cut towards the anterior part of the fish.**
3. **In order to estimate ingested microplastics accurately, the entire gastrointestinal tract should be examined. Cut through the esophagus while keeping the entire stomach intact and cut through the gut approximately 2-3 mm before the anus. Record the weight of the stomach and gut.**
4. **Place the excised stomach and gut in a clean 1L beaker. Add 10% KOH three times more than the tissue volume, cover the beaker opening with foil, and stand at 80°C for 48 hours. Agitate the set-up manually every 12 hours.**

Extraction of MPs from Samples

Biota

Procedure

5. If there are residues in the digestate, proceed to density separation. Add 150 mL saturated NaCl into the digested solution and mix. Allow to settle overnight.

6. If the tissues are fully digested, proceed to microplastics extraction by vacuum filtration. Filtration is done using a 20-µm quantitative ashless filter paper in a Buchner funnel and flask set-up.

7. Store the filter paper in a sterile petri dish. Residual organic matter on the filter may be digested by adding 2-3 drops of 30% H_2O_2 onto the filter.

8. Dry the filters in the oven at 60°C overnight. Wrap petri dish in foil for storage until MP quantification.

Waste Disposal

- Excess potassium hydroxide (KOH) – base waste jar
- Excess saturated sodium chloride (NaCl) – sink with copious amount of running water
- Waste during oxidative digestion – base waste jar

Quantification and visualization

Reagent Preparation

For staining

Nile Red stock solution (1 mg/mL or 1000 g/mL)

NOTE: Flammable, toxic, and irritant. Work under a fume hood or in a well-ventilated room. Handle with personal protective equipment (gloves, mask, and goggles)

1. Dissolve Nile Red (NR) by dissolving 100 mg of NR powder in 100 mL acetone. Filter the solution using a glass syringe through a syringe membrane filter. Note: NR is light-sensitive, please minimize exposure to direct light.
2. Store filtered solution in (pre-washed with filtered NR solution) amber reagent bottle and wrap in aluminum foil. Label the bottle properly. Store in 4°C.

Nile Red working solution (10 µg/mL)

NOTE: Flammable, toxic, and health hazard. Work under a fume hood or in a well-ventilated room. Handle with personal protective equipment (gloves, mask, and goggles). Can cause blindness.

1. Add 1 mL stock solution to 100 mL of filtered methanol to make 10 µg/mL working solution. Recalculate if you plan to make a smaller batch of working solution.

Materials/Equipment

Nile Red stain
Filtered acetone
Distilled water
Filtered methanol
Glass syringe
Syringe filter
100 mL amber reagent bottles
100 mL volumetric flask
Pasteur pipette

Waste Disposal

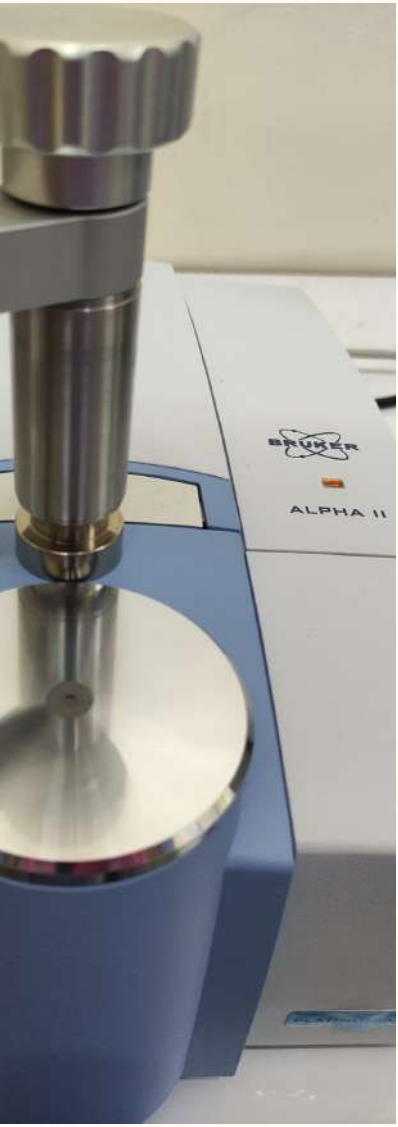
- Excess acetone with NR – acetone waste jar or non-halogenated organic waste jar
- Excess methanol with NR – methanol waste jar

Procedure

1. Drop around 600 µL of the working solution onto each filter paper containing microplastics, carefully making sure that all surface has been covered with the solution.
2. Cover the petri dish with aluminum foil, and allow to rest for 24 h for excess moisture to evaporate.
3. Place each sample under the microscope stage.
4. Shine blue light (450-510 nm) and observe the sample through an orange filter (529 nm).
5. Take a photo.
6. Quantify the fluorescing microplastics by manual counting or feeding the images to an artificial intelligence (AI) software.

Characterization

(Adapted from Harshvardhan and Jha (2013), Jung et al. (2018)



Materials/Equipment
Oven
Fourier Transform infrared spectrophotometer with attenuated total reflectance
Computer
Forceps
Kimwipes

Reagents
70% Ethanol

Procedure

1. **Among the identified MPs, random samples will be selected from each size category and will be further verified through FT-IR spectroscopy.**
2. **Dry samples overnight in oven at 60°C.**
3. **Collect spectra from plastic samples from 4,000 cm^{-1} to 450 cm^{-1} with a data interval of 1 cm^{-1}, with the resolution set to 2 cm^{-1}.**
4. **Clean the ATR diamond with 70% ethanol before and in-between samples.**
5. **Perform background scans between samples.**
6. **Each sample is compressed against the diamond with the minimum force recommended by the manufacturer to ensure good contact between the sample and the ATR crystal.**
7. **The resultant spectra will be identified using the spectra library in the software and will be use to determine polymer types.**

Appendix A:
Field Sampling Data Sheets

MACRO- AND MICROPLASTIC DATASHEET – SITE CHARACTERISTICS

Surveyor name:	Survey date:
Site name:	Country:
GPS start:	GPS end:
SHORELINE CHARACTERISTICS	
Habitat type: beach mangrove seagrass coral reef	
Backshore type: cliff seawall urban building forest/tree shrub mangrove other:	
Shore exposure: cove/bay straight headland	
Tidal distance (m):	
ENVIRONMENTAL CONDITIONS (three readings per site)	
Weather: clear rain/storm overcast drizzle	
Wind speed (m/s):	Wind direction:
Sea surface temp (°C):	Salinity (psu):
Dissolved O_2 (mg/L):	Total suspended solids (mg/L):
Nitrate (mg/L):	Phosphate (mg/L):
LAND-USE CHARACTERISTICS	
Access: vehicular trail isolated	
Major site usage: tourism fishing protected isolated other:	
Nearest town distance (km):	Nearest river distance (km):
River input: yes no	Pipe/drain input: yes no
Evidence of dumping: none construction household other:	
Evidence of recent activities: none clean-up/rubbish removal apparent spilled trash storm/flood strong winds public event other:	
Notes (*include descriptions on landmarks, coastal hydrography, etc*):	

Notes:
GPS start: GPS coordinates of the start of the first transect
GPS end: GPS coordinates of the end of the third transect
Tidal distance: the maximum horizontal distance between the low- and high-tide line
Access: vehicular (you can drive to the site), trail (you must walk), isolated (you need a boat/plane)
Nearest town distance: nearest distance to villages / residences / towns / human populations

MACRO- AND MICROPLASTIC DATASHEET (v20230401)

Surveyor name:	Survey date:
Site name:	Country:
Transect number: out of	Transect size (length × width):
Distance from strandline (m):	Depth (m):
Sampling zone: strandline mid-tide reef crest	
Substratum type: sand mud boulder seagrass seaweed coral other:	

Main category	Specific litter category	Fragment	Whole	Note*
Plastic	Beverage bottles < 1 L			
	Beverage bottles ≥ 1 L			
	Buckets / jerry cans / drums			
	Caps / lids / covers			
	6-packs rings / drink package rings			
	Straws / pipettes			
	Clear cups / bowls / food containers			
	Foamed cups / bowls / food containers			
	Knives / forks / spoons			
	Bags			
	Thin plastic wraps / labels / packagings			
	Thick plastic wraps / sacks			
	Lighters / matches			
	Cigarette tips / butts / filters			
	Ropes / strings / strapping bands			
	Pipes / hoses			
	Fishing lines / nets / rods			
	Buoys / floats			
	Shampoos / shower gels / toothbrushes			
	Fragments (hard plastic)			
	Fragments (soft plastic / films / sheets)			
	Fragments (foamed)			
	Masks/gloves/PPE			
	Sanitary pads/diapers			
	Lollipop sticks/earbuds			
	Nurdles/plastic pellets			
	Medicinal packaging			
	Others (please indicate):			
Rubber	Slippers / flip-flops / shoes / gloves			
	Tires			
	Balloons, balls			
	Rubber bands			
	Other:			

Notes:
Distance from strandline: the horizontal distance between the strandline and and transects
Depth: Depth of transects; for coral reef sites only
If the macro-debris cannot be weighed because it is too big / heavily encrusted / soaked, record its size (length, width, height) for mass estimation.

Main category	Specific litter category	Fragment	Whole	Note*
Metal	Aluminium / tin / aerosol cans			
	Bottle caps			
	Buckets / drums			
	Nails / irons			
	Fishing related (lures, hooks, sinkers)			
	Other:			
Glass	Bottles / jars			
	Light bulbs / tubes / globes			
	Fragments			
	Other:			
Glass	Bottles / jars			
	Light bulbs / tubes / globes			
	Fragments			
	Other:			
Wood	Cigarette packs			
	Lighters / matches			
	Paper / newspaper / pieces of papers			
	Crates / boxes / cardboards			
	Fishing traps / pots			
	Ice cream sticks / chopsticks / toothpicks			
	Fragments			
	Other:			
Cloth	Clothes / towels / rags			
	Sacking / gunny sacks / canvas			
	Fabric pieces			
	Other:			
Other	Batteries			
	Appliances, electronics			
	Furniture			
	Contraceptives / condoms			
	Syringes			

WATER GRAB SAMPLING DATASHEET

Surveyor name(s):		Sample date:
Site name:		Country:
Water sampler:	Volume (L):	Sampling depth (m):
ENVIRONMENTAL CONDITIONS (three readings per site)		
Weather: clear rain/storm overcast drizzle		
Sea surface temp (°C):		Salinity (psu):
Dissolved O_2 (mg/L):		Total suspended solids (mg/L):
Nitrate (mg/L):		Phosphate (mg/L):
LAND-USE CHARACTERISTICS		
Major site usage: tourism fishing protected isolated other:		
Nearest town distance (km):		Nearest river distance (km):
River input: yes no		Pipe/drain input: yes no
Evidence of dumping: none construction household other:		
Evidence of recent activities: none clean-up/rubbish removal apparent spilled trash storm/flood strong winds public event other:		
Notes (*include descriptions on landmarks, coastal hydrography, etc*):		

Sample details	#1	#2	#3
Wind speed (m/s)			
Wind direction			
Latitude			
Longitude			
Time			
Note			

PLANKTON TOW DATASHEET

Surveyor name(s):					Tow date:			
Site name:					Country:			
Net type:			Mesh size:			Net mouth diameter:		
ENVIRONMENTAL CONDITIONS (three readings per site)								
Weather: clear rain/storm overcast drizzle								
Sea surface temp (°C):					Salinity (psu):			
Dissolved O_2 (mg/L):					Total suspended solids (mg/L):			
Nitrate (mg/L):					Phosphate (mg/L):			
LAND-USE CHARACTERISTICS								
Major site usage: tourism fishing protected isolated other:								
Nearest town distance (km):					Nearest river distance (km):			
River input: yes no					Pipe/drain input: yes no			
Evidence of dumping: none construction household other:								
Evidence of recent activities: none clean-up/rubbish removal apparent spilled trash storm/flood strong winds public event other:								
Notes (*include descriptions on landmarks, coastal hydrography, etc*):								

Tow details	#1	#2	#3
Wind speed (m/s)			
Wind direction			
Start latitude			
Start longitude			
Start time			
Start flow meter count			
End latitude			
End longitude			
End time			
End flow meter count			
Average boat direction			
Average depth (m)			
Note			

Appendix B:
Data Submission

Data Submission

1. To submit your datasheet, encode it onto a spreadsheet (offline or online)
2. Go to the submission portal via https://plasticount.ph and click on the submit data button on the header

or go directly to: https://forms.gle/Ecgtqq8eaFt7r7k79

3. Accomplish the contributor's information section and then choose to upload field/monitoring sheet option.
4. Upload a copy of your datasheet or you may also share it via a link.

5. Fill out the field details of the collection and accomplish the rest of the form to submit your data.

Note: Your data will take time to process. You may receive updates via email.

Appendix C:
Microplastics Analysis Datasheets

Names: _____ Date: _____
Group No.: _____

Microplastics Data Sheet

Recovery Test for QA/QC

Sample Name	Fraction	Red sando bag (PE)	Blue straw (PP)	Blue fish net (PA nylon)	White styro (PS)	White ProYo bottle (HDPE)	Total Count
Spike 1	Initial						
	Final						
	% Recovery						
Spike 2	Initial						
	Final						
	% Recovery						
Spike 3	Initial						
	Final						
	% Recovery						

Notes

Names: _____ Date: _____

Group No.: _____

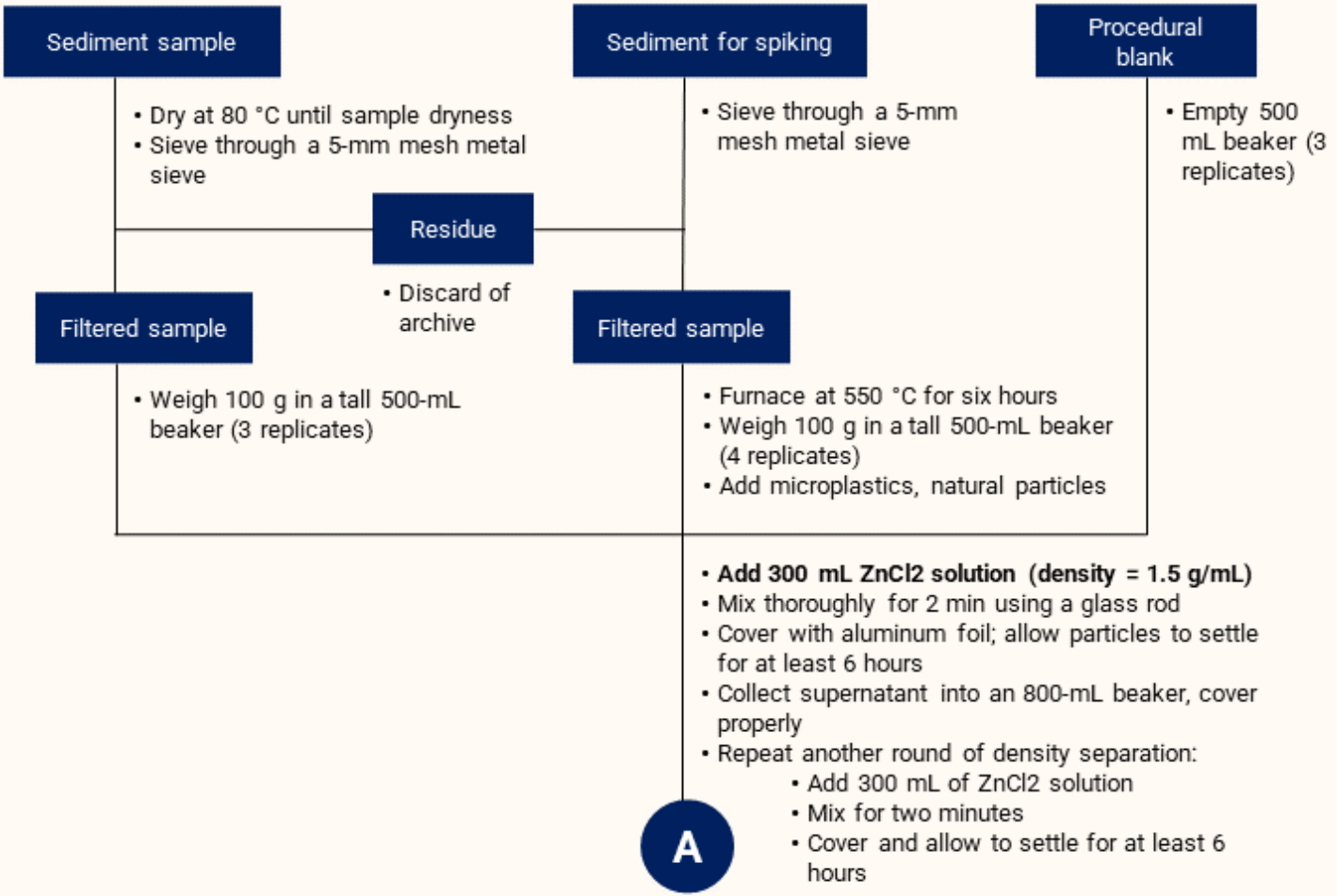
Microplastics Data Sheet

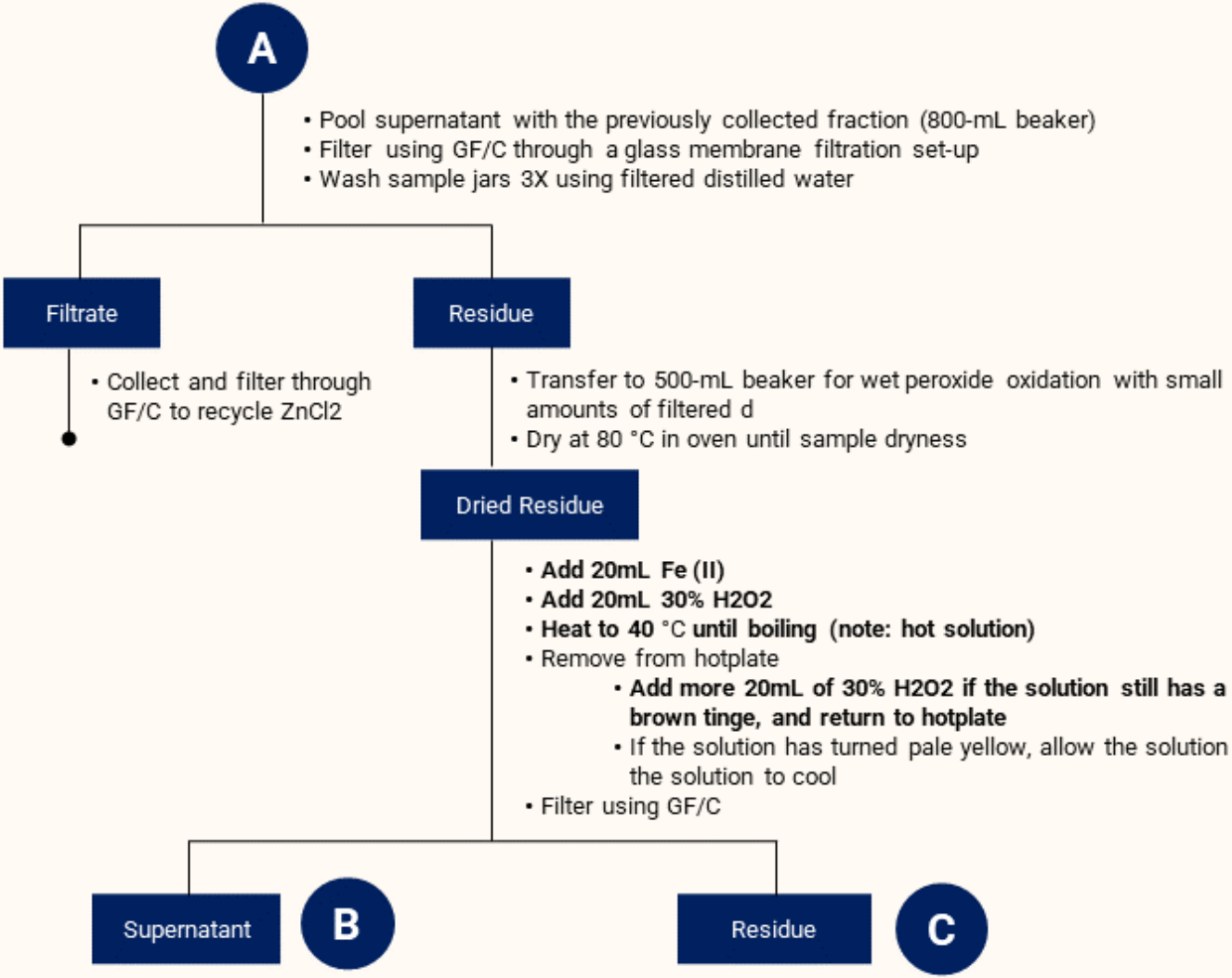
Blanks, Samples, and Air Contamination

Sample Name	Fibers	Fragments	Films/Sheets	Granules/Pellets	Foams	Total Count
Blank 1						
Blank 2						
Blank 3						
Air Contam 1						
Air Contam 2						
Air Contam 3						
Sample 1						
Sample 2						
Sample 3						

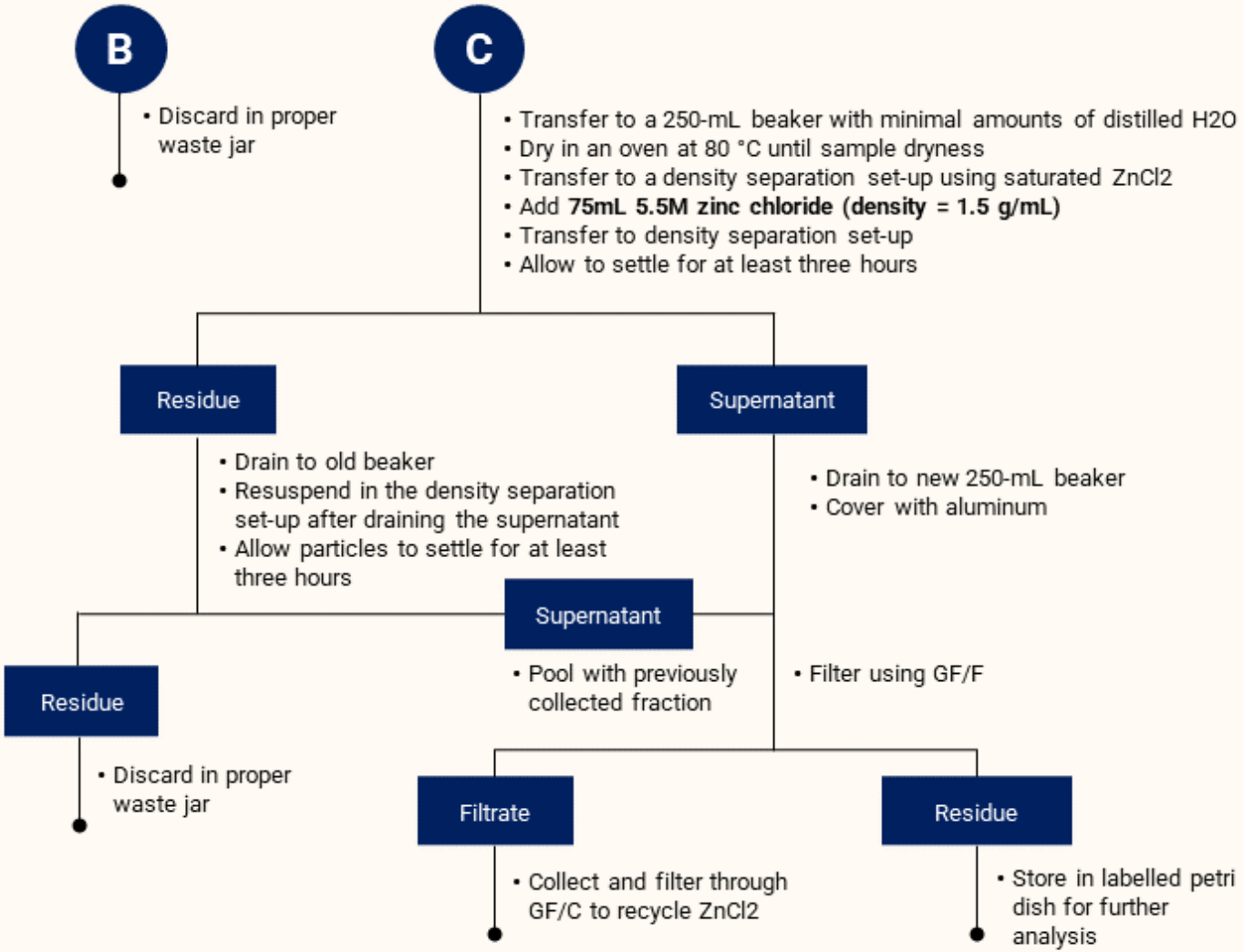
Appendix D:
Workflow Diagrams

Microplastics Extraction from Sediments



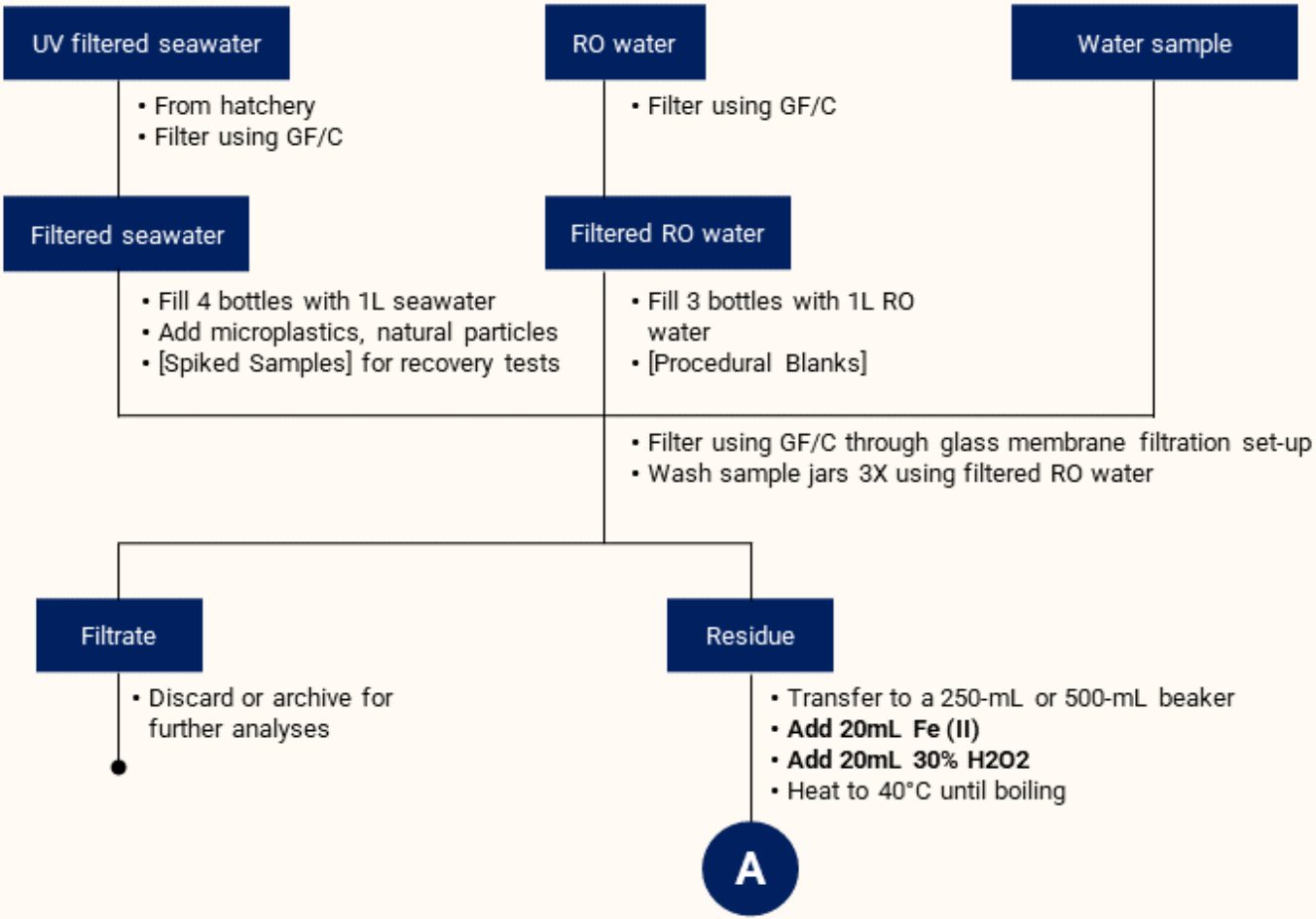


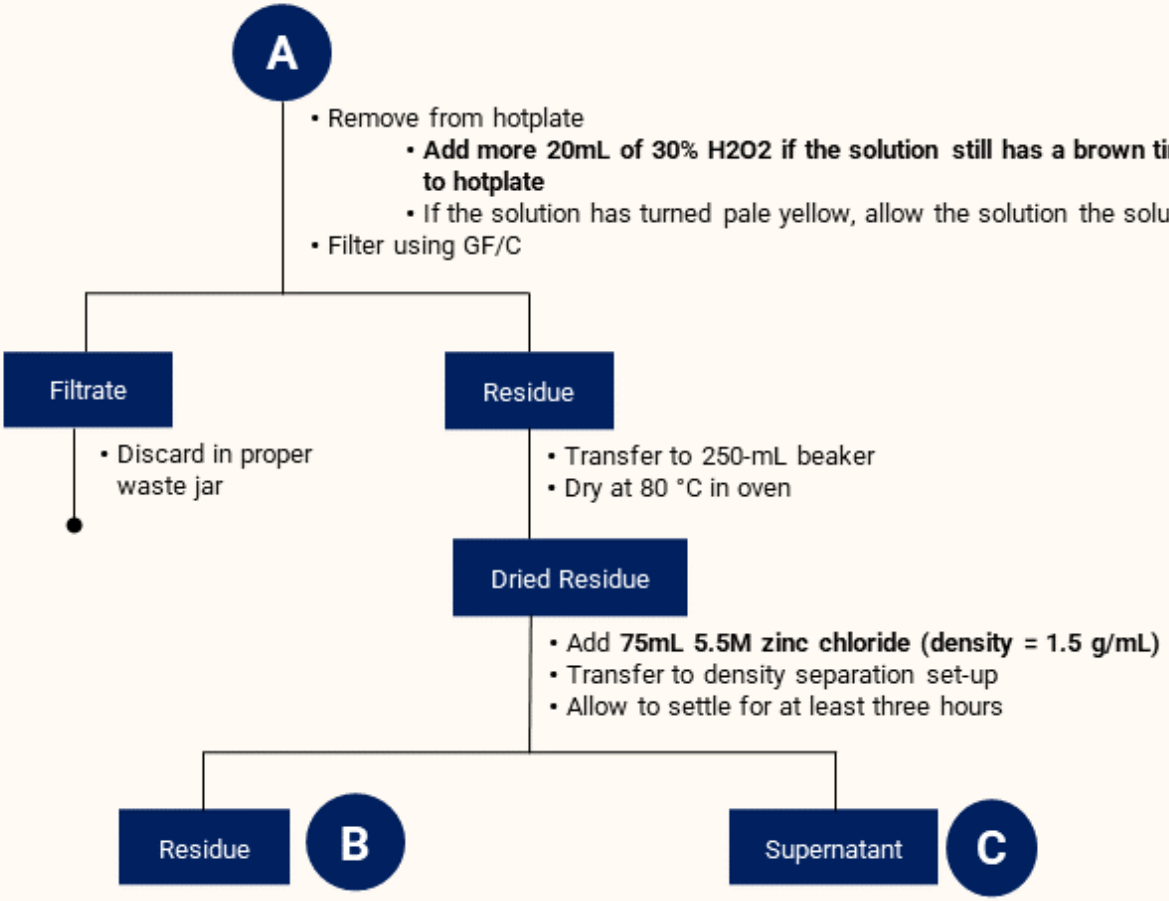
Microplastics Extraction from Sediments



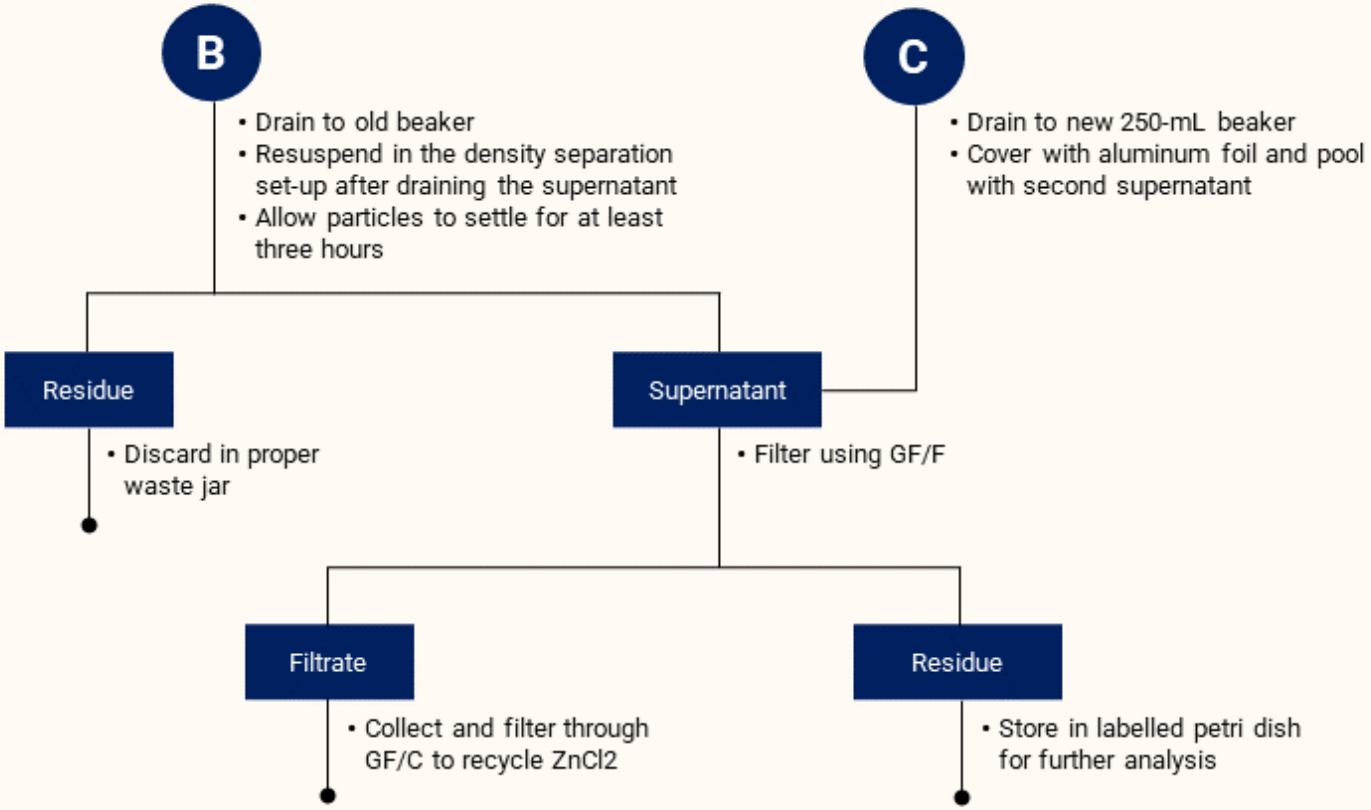
Microplastics Extraction from Sediments

Microplastics Extraction from Water



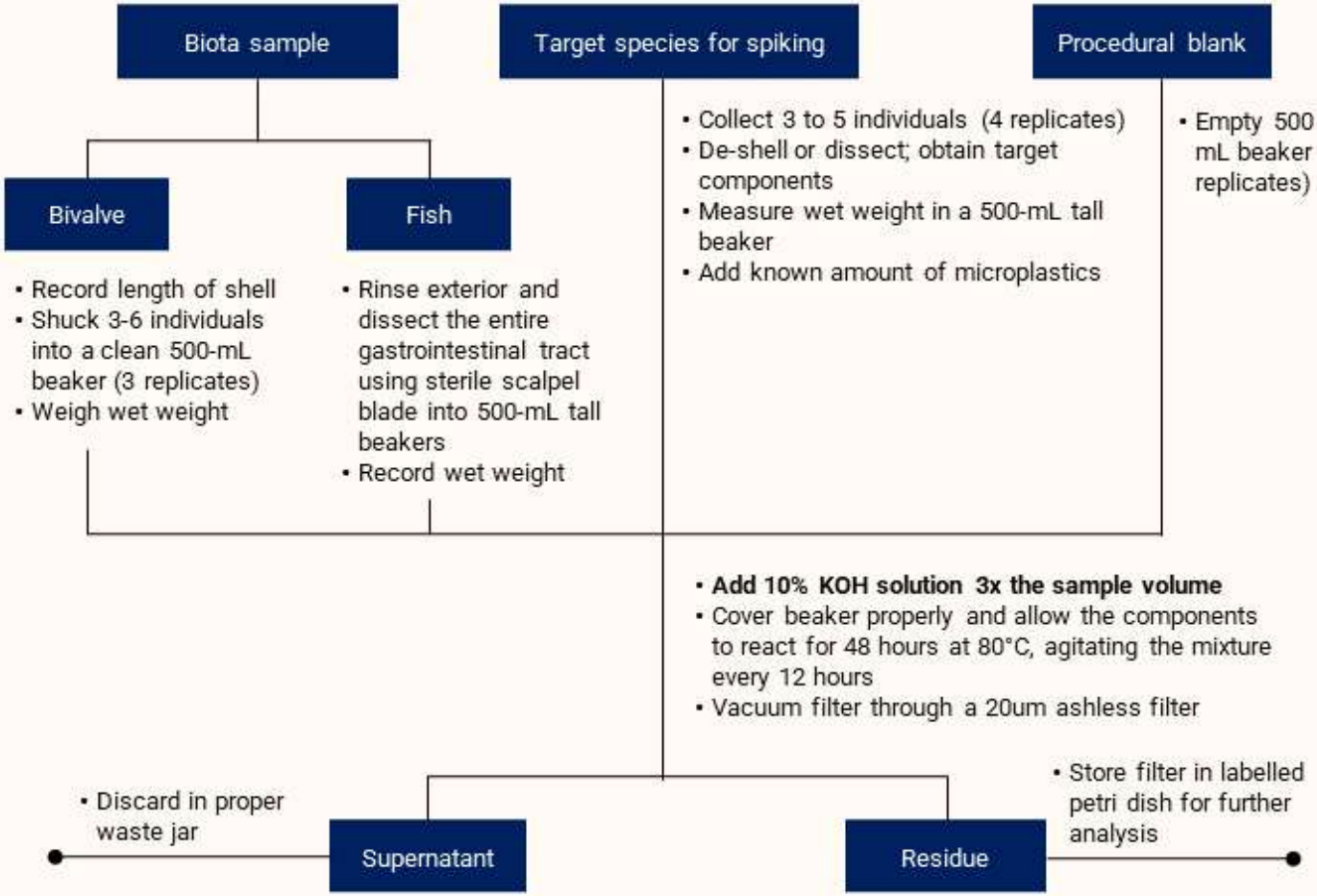


Microplastics Extraction from Water



MicroplasticsExtraction from Water

Microplastics Extraction from Biota



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Notes

Notes

About the Projects

PlasMics

This project investigates the fate of plastics in select bays and seas in the Philippines through (A) a standardized data gathering and survey of prevalence and abundance of micro- and macroplastics in Manila Bay and the West Philippine Sea; (B) employment of various -omics tools to look at plastics-associated microbial community and biodegradation potential of microbes; (C) conduct monitoring of select aquaculture species for prevalence of microplastic ingestion; and (D) bring awareness of the impacts of plastic pollution to the Filipino people, scientific community, and government agencies.

PlastiCount Pilipinas

PlastiCount Pilipinas aims to increase local capacity for monitoring plastics pollution in the coastal and marine environments by adopting technologies implemented in Japan and United Kingdom and making the data available to the public for use in policy, advocacy, and education Through this project, PlastiCount Pilipinas aims to make the public aware of the extent of plastics, especially in marine environments, and what we can do to help through a whole-of-nation approach.

MicroSEAP

The objective of the project is to reduce the impact of marine plastic pollution in Southeast Asia through understanding the role of microorganisms living on the plastic surface on the pollution threat and investigate the potential of these microorganisms in creating a solution to this problem. MicroSEAP aims to determine the loading of plastics in three marine environments: beach, mangroves and coral reefs. The project seeks to quantify ecosystem plastic loads to establish baseline information on the occurrence of plastic debris, identify types of plastic polymers, and estimate annual proportions of polymers based on historical data obtained from collaborators.

The printing of this handbook is funded by:

The Circular Explorer Project
Holcim Philippines, Inc.