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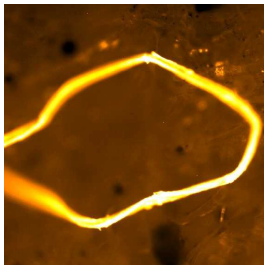
Non-destructive microplastic isolation from water and sediment samples



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We use this protocol and it's working

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Abstract

Microplastics in aquatic ecosystems serve as unique habitats for diverse microbial communities, collectively referred to as the plastisphere. Investigating these microbes using both culture-dependent and culture-independent techniques (e.g., 16S rRNA gene sequencing, metagenomics) requires a method that preserves the integrity of the plastic and associated microbiota. This protocol outlines a non-destructive isolation technique for microplastics from water and sediment samples using Nile Red dye, thus allowing for a comprehensive study of the microplastic microbiome and its functional potential without compromising the plastic matrix or the microbial communities it harbors.

Image Attribution

Jonas Stadfeld and Sneha Suresh

Nile Red Solution (2 µg/mL)

1 Add 50 mg of Nile Red to a Falcon Tube.

2 Using a serological pipette, carefully add 50 mL of molecular-grade acetone to the Falcon Tube.

3 Screw the Falcon tube cap and vortex vigorously to dissolve the Nile Red dye (00:00:30). 30s

4 Pick up the dissolved Nile red dye into a 50 mL syringe by pulling the plunger.

5 Filter sterilization: Attach a sterile 0.22 µm Luer-lock syringe filter to the syringe and push the Nile red into a new sterilized 50 mL falcon tube.

6 Label the tube as concentrated Stock solution ([M] 1 mg/mL), cover it in aluminum foil, and store at 4 °C

7 In a sterilized 500 mL Nalgene Glass media bottle (orange cap), add 499 mL of Milli Q water.
Tip: Add 500 mL using a measuring cylinder and remove 1 mL using a pipette.

8 Add 1 mL of filter-sterilized Nile red stock solution ([M] 1 mg/mL). Close the lid and shake gently.
Cover it in Aluminum foil and label.

9 Store in the dark at 4 °C for long-term storage.

Microplastic Isolation - Water

10 NOTE: Use only metal and glass equipment. Tubing should be silicone.

11 Filter at least 40L of water through a 20µm Cellulose filter (47 mm).

Note: Depending upon the murkiness, the filter may clog before 40L is filtered. In that case, multiple filters can be combined to form ONE replicate 40L sample.
The Filter can be stored at -20 °C if used for DNA extraction.

12 Place the filter in a petri dish and add 1 mL of Nile Red solution ([M] 2 µg/mL).

13 Incubate the filter in dark at 4 °C for 24:00:00 1d

14 Place the filter in a fluorescence microscope under a 529 nm wavelength filter

15 Using sterile forceps, pick the red fluorescing microplastics and transfer them to a 1.5 mL microcentrifuge tube containing 100 µL sterile 1X PBS.
Note: Nile Red can stain roots and other biological materials. In background noise, microplastics fluoresce strongly, lacking compartments and multicellular structure.

16 Optional step: Rinse the microplastics with 100 µL 1X PBS to remove environmental contaminants by pelleting the plastics with centrifugation (1000 xg for 00:01:00), discarding the supernatant and repeating the step twice with fresh sterile 1X PBS. 1m

Microplastic Isolation - Sediment 12h

17 Freeze dry the 0.5 kg Sediment Sample at 80 ˚C to remove all water.
Note: The duration of freeze-drying varies based on the water content of the sample. Expect 24 hours to three days.

18 While the freeze dryer runs, prepare 900 mL of Calcium Chloride Solution (final density of 1.4 Kg/L) for each sample.

18.1 Weigh the 400 g of CaCl2 and transfer it to a sterile 1L Nalgene media bottle.

18.2 Add a sterile stir bar and 1 L of MilliQ water and stir until dissolved.

19 Optional Step: weigh the freeze dried sediment to compare microplastics across various samples *

20 Using as sterile spatula, transfer the freeze-dried sediments to a 1 L sterile beaker.

21 Add 300 mL of the CaCl2 solution and mix vigorously using the spatula.

22 Cover with aluminum foil and let the sample stand for 12:00:00 . 12h

23 Decant the CaCl2 solution into a clean, sterile beaker and store the decanted solution at 4 °C .

24 Repeat Steps 19-21 two more times with 12:00:00 and 24:00:00 of sedimentation time for Step 21. 1d 12h

25 Filter all the decanted CaCl2 solution through a 20 µm cellulose filter.

26 Use Steps 11 -15 (Microplastics Isolation - Water) to isolated sediment microplastics.