Microplastic Extraction Protocol – Summary (Mussels & Anchovies)

Introduction

Microplastics (<5 mm) are found in Mediterranean fish. Mussels (Mytilus galloprovincialis), being filter feeders, and anchovies (Engraulis encrasicolus), often consumed without being eviscerated, are key species for exposure studies. Recent scientific research has developed specific methods useful for aAccurate extraction from biologic tissues, avoiding secondary contamination [https://doi.org/10.1038/s41598-022-25631-2].

Materials

- Laminar flow hood, analytical balance, thermostat, magnetic stirrer, vacuum filtration setup
- Glassware: cylinders, beakers, flasks, Petri dishes
- Filters: 0.45 µm (solutions), 8 µm (digested samples)
- Tools: steel scissors, tweezers, spatulas
- Reagents: KOH, NaCl, 30% H_2O_2

Procedure

First, prepare a saturated NaCl solution by dissolving salt in water until a density of 1.2 g/cm^3 is reached. Then, prepare a 10% KOH solution using 50 mL per sample. Record morphometric data: for mussels, measure shell length and soft tissue weight; for anchovies, weight the whole fish and measure its length. Proceed with dissection: open mussel shells to extract tissue, and cut anchovies to isolate the gastrointestinal tract. Digest samples by placing them in 10% KOH at 45°C overnight. For density separation, stir the digested sample for 10 minutes, let settle, and collect the supernatant; repeat once. Filter the liquid to isolate microplastics. A second digestion step with 15% H_2O_2 (a few drops per filter) is performed at 45°C overnight to remove organic residues. Finally, observe filters under a microscope to count and classify microplastics by shape and color.

To reduce contamination, glassware should be rinsed three times with distilled or filtered water before and between sample use. Avoid using paper or cotton cloths to dry equipment; instead, use compressed air. Laboratory staff should wear cotton lab coats, and samples should be covered with aluminum foil when not in use. It's recommended to include procedural blanks in parallel with sample digestion to evaluate contamination from the lab environment and operators.

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