1. Methods for the Analysis of Microplastics in Water Samples

1.0.1. Water samples. The method can be used for the analysis of plastic debris as suspended solids in water samples collected by a surface net. Plastics include hard plastics, soft plastics (e.g., foams), films, line, and sheets. The method involves the filtration of solids obtained by 330 or 335 micron surface net, like a Manta net, through 5.6-mm and/or 0.3-mm sieves to isolate the solid material of the appropriate size. The sieved material is dried to determine the solids mass in the sample. The solids are subjected to wet peroxide oxidation (WPO) in the presence of an Fe(II) catalyst to digest labile organic matter. The plastic debris remains unaltered. The WPO mixture is subjected to density separation in NaCl(aq) to isolate the plastic debris through flotation. The floating solids are separated from the denser undigested mineral components using a density separator. The floating plastic debris is collected in the density separator using a custom 0.31-mm filter, air dried and weighed. Plastic material is removed and collected to determine the microplastics concentration.

1.0.2. An overview of the analysis of microplastics in water is shown in Figure 1.

Prior to gravimetric analysis, the material collected by filtration following density separation is examined under a microscope (40X power).

1.0.3. The method is applicable to the determination of many of the common plastics including polyethylene, polypropylene, polyvinyl chloride, and polystyrene.

1.0.4. The plastics debris analyzed by this method is considered microplastics, which range in size from 5-mm to 0.3-mm.

1.0.5. Microplastics debris is operationally defined by this method as any solid material in the appropriate size range that 1) is resistant to wet peroxide oxidation, 2) floats in 5 M NaCl or lithium metatungstate (d=1.62 g/mL) solution, and 3) passes positive visual inspection under a microscope at 40X power.

1.0.6. A preliminary visual inspection of the sample must be made to determine if best to process using low or high sample mass procedure. Any sample high in woody debris should be processed using the high sample mass method. Woody debris is not efficiently removed using WPO and further separation is needed for quantifying microplastics. The 2 g threshold for the low to high mass must be determined upon visual inspection of the initial sample. Typically, any sample container (200 mL jar) that is greater than half-full of solid, woody material is considered a high mass sample.

1.1. Apparatus and materials

- U.S. standard stainless steel sieves (8-in diameter, 2-in deep) in 5.6-mm (No. 3.5), 1-mm (No. 18) and 0.3-mm (No. 50) stainless steel mesh sizes (Fisher cat. 04-881-10_)
- Customized small sieves (59-mm diameter) of 5-mm, 1-mm and 0.3-mm mesh sizes fabricated from polypropylene Buchner funnels (Fisher cat. 10-362B) and nylon mesh (5-mm, 1-mm and 0.3-mm). The Buchner funnel bottoms were removed and the nylon mesh was glued to the modified funnel. –OR- 3” PVC pipe cut into 1” lengths with meshes glued with gel-type superglue
- Hot plate (up to 90^{o}C)
- 800-mL, 500-mL, 200-mL, 150-mL, 100-mL and 80-mL glass beakers

- Retort stand (Fisher cat. S47806)
- O-ring (Fisher cat. 14-050CQ)
- 2” spring clamp
- 30% Hydrogen peroxide (Fisher cat. BP2633-500)
- Glass funnel (122-mm dia., Fisher cat. 10-372A) fitted with latex tubing (50-mm segment) fixed to the bottom of the stem, and a pinch clamp (Fisher cat. 05-849B) to control liquid flow from the funnel. The apparatus served as a density separator
- 0.05 M Fe(II) solution (prepared by adding 7.5 g of $FeSO_4 \cdot 7H_20$, FW = 278.02 g/mol (Fisher cat. 1146-500, CAS 7782-63-0), to 500 mL of water + 3 mL of concentrated sulfuric acid (CAS 7664-93-9))
- Watchglass
- Sodium chloride (commercial table salt is sufficient)
- Metal spatula (Fisher cat. 14-375-10)
- 4-mL glass vials (Fisher cat. 14-955-327)
- Plastic squirt bottle containing distilled water
- Drying oven (75^{o}C)
- Analytical balance (precise to 0.1 mg)
- Dissecting microscope (40X magnification)
- Stir bar
- Forceps

1.2. Surface Net Samples

- The 300 or 335 micron surface net sample is provided in a glass jar with ~200 mL of water + solids

1.2.1. Wet Sieving

- Pour tow sample (contained in glass jar) through stacked arrangement of two custom sieves (5-mm over 0.3-mm)
- Rinse sample bottle with distilled water and transfer all residual solids to the sieve set (repeat as necessary)
- Rinse sieve set thoroughly using distilled water (ensure all material has been well washed, drained and sorted)
- Discard material retained on 5-mm sieve

1.2.2. Transfer solids in the 0.3-mm sieve into a separate 500-mL beaker

- Pre-weigh a clean and dry 500-mL beaker to nearest 0.1 mg
- Transfer solids collected in the 0.3-mm sieve into the tared beaker using a spatula with rinsing using a squirt bottle containing distilled water
- Ensure all solids are transferred into the beaker
- If water volume in the beaker exceeds 10 mL, boil off residual water on a hotplate with a stir bar
- Remove and rinse stir bar, and then place beaker in 75^{o}C drying oven for 24 hours or longer to sample dryness

1.2.3. Determine mass of total solids in the 0.3-mm fraction

- Determine the mass of each beaker + solids using an analytical balance to nearest 0.1 mg
- Subtract mass of tared beaker to provide mass of total solids collected on the sieve

1.2.4. Wet peroxide oxidation (WPO) of the 0.3-mm size fraction

CAUTION: this mixture is highly reactive

- Add 20-mL of aqueous 0.05 M Fe(II) solution to the beaker containing the 0.3-mm size fraction of collected solids
- Add 20-mL of 30% hydrogen peroxide (caution: this solution can boil violently if heated >75^{o}C); let mixture stand on lab bench at room temperature for 5 minutes prior to heating
- Add stir bar
- Heat to 75^{o}C on a hotplate (cover with a watchglass)
- As soon as gas bubbles are observed at the surface remove from hotplate and place on bench top in fume hood until boiling subsides; add distilled water to slow reaction, if needed
- Heat to 75^{o}C for additional 30 minutes
- If natural organic material is visible, add another 20-mL portion of 30% hydrogen peroxide and repeat heating step; repeat until no natural organic material is visible
- Add ~7 g of salt (NaCl) per-20 mL of sample to increase the density of the aqueous solution
- Heat mixture to 75^{o}C until all the salt dissolves

1.2.5. Low sample mass procedures (<2 g total solids)

1.2.5.1. Density separation for low sample mass

- Transfer WPO solution from step 1.2.4. to the density separator
- Rinse the WPO beaker with distilled water to transfer all remaining solids to the density separator
- Allow solids to settle for 60 min
- Visually inspect settled solids for any microplastics; if any present remove after draining using forceps
- Drain settled solids from separator and discard
- Collect floating solids in a clean, tared 0.3-mm custom sieve
- Rinse the density separator several times with distilled water to transfer all solids to the 0.3-mm sieve
- Allow sieve to air dry while loosely covered with aluminum foil away from drafts for 24 hours

1.2.5.2. Determine mass of microplastics in 0.3-mm sieve for low sample mass

- Pre-weigh a clean and dry 4-mL vial
- Using a dissecting microscope at 40X magnification, collect identifiable microplastics from 0.3-mm sieve with forceps into tared vial
- Weigh the mass of the vial and microplastics to the nearest 0.1-mg

- Subtract the mass of the vial from the combined mass to determine the total mass of microplastics in the sample

1.2.6. High sample mass procedures (>2 g total solids)

1.2.6.1. Density separation for high sample mass

- Transfer WPO solution from 1.2.4. to the density separator
- Rinse the WPO beaker with distilled water to transfer all remaining solids to the density separator
- Allow solids to settle for 60 minutes
- Drain settling solids from separator and discard
- Collect floating solids in clean, tared dual sieves of a 1-mm stacked on a 0.3-mm small custom sieve. Be sure to not lose any sample from the 1-mm sieve due to clogging
- Rinse the density separator several times with distilled water squirt bottle to transfer all solids to the 1-mm over 0.3-mm sieve stack
- Allow sieves to air dry while loosely covered with aluminum foil away from drafts for 24 hours

1.2.6.2. Determine mass of microplastics in the 1-mm fraction for high mass samples

- Pre-weigh a clean and dry 4-mL vial
- Using a dissecting microscope at 40X magnification, collect identifiable microplastics from 1-mm sieve with forceps into tared vial
- Weigh the mass of the vial and microplastics to the nearest 0.1-mg
- Subtract the mass of the vial from the combined mass to determine the total mass of microplastics in the sample

1.2.6.3. Determine mass of microplastics in the 0.3-mm fraction for high mass samples

- Using a dissecting microscope at 40X magnification, collect identifiable microplastics from 0.3-mm sieve with forceps into tared vial from 1.2.6.2.
- Weigh the mass of the vial and microplastics to the nearest 0.1-mg
- Subtract the mass of the vial from the combined mass to determine the total mass of microplastics in the sample

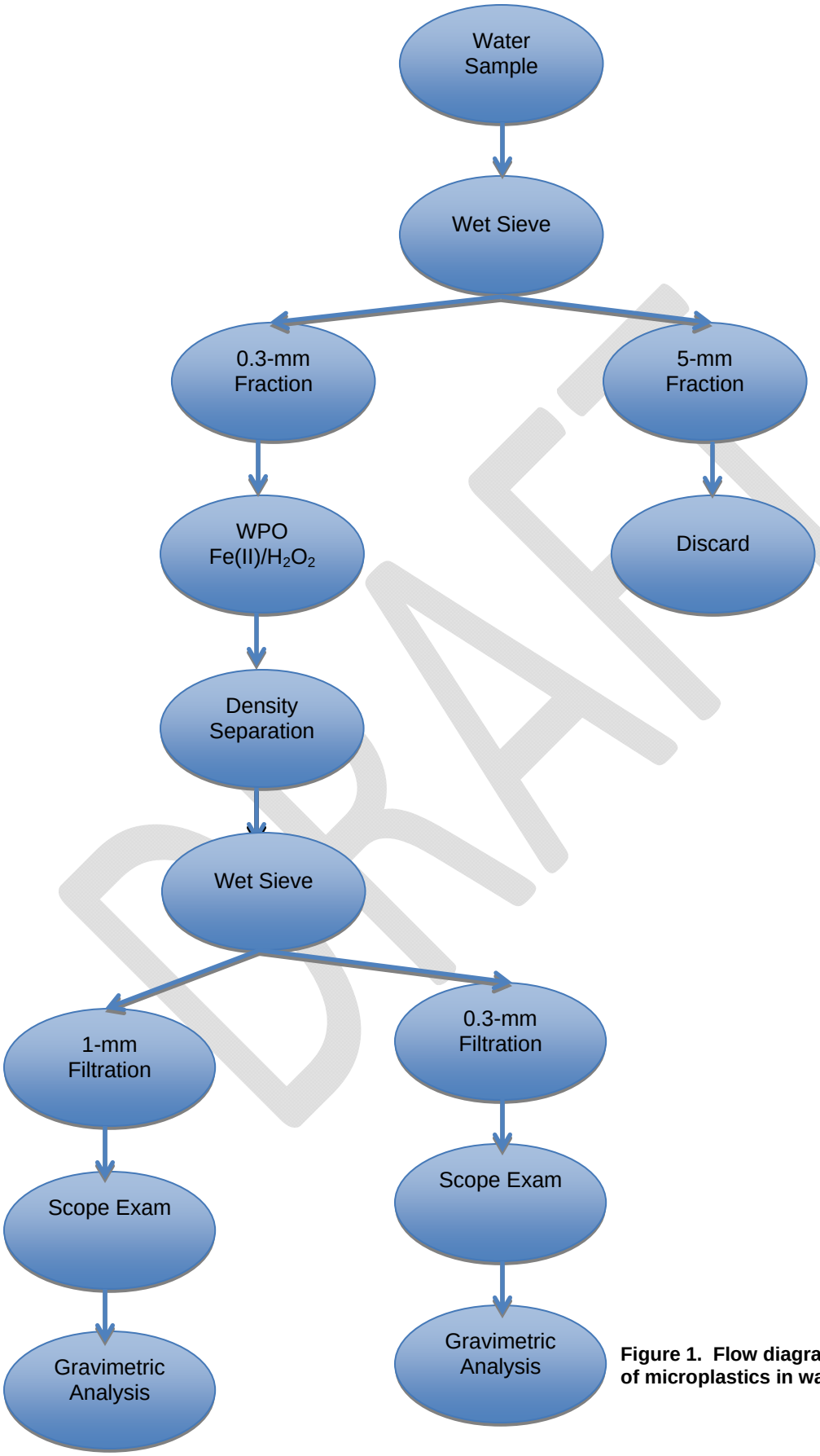

Figure 1. Flow diagram for the analysis of microplastics in water samples.