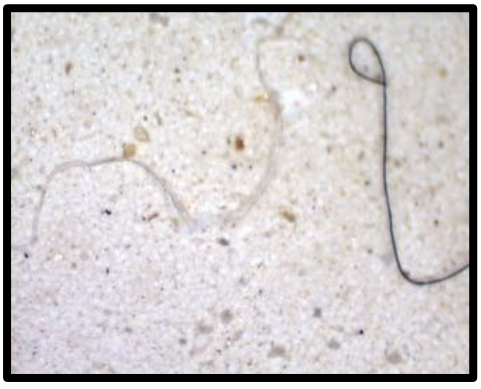
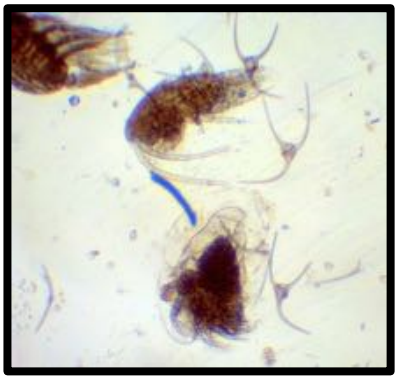
Guide to Microplastic Identification





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This guide is intended as an introduction to microplastic identification. It is not meant to be a guaranteed reference for each and every piece as microplastics are extremely diverse and can vary tremendously. When identifying, use your best judgment and try to identify as many plastic characteristics as possible. If you are unsure about a piece, do not count it as plastic. While counts should be as accurate as possible, it is better to have a conservative estimate. Further reading of scientific articles is recommended.

I. Microplastic Characteristics (Hidalgo-Ruz *et al*., 2012)

1. Small size (largest dimension ≤5mm)
2. No cellular or organic structures visible
3. Fibers should be equally thick throughout their entire length
4. Particles should exhibit clear and homogeneous color throughout (Please see Section IV for rule variations)

II. Equipment

1. Dissecting microscope, compound microscope, slides, cover slips, filters (ideally gridded)
2. Petri Dishes (ideally glass)
3. Tweezers and probe for prodding pieces
4. Clean working area with no contamination (See Section VI)
5. Data Sheets

III. How to Read a Filter

1. Examine the fragments within the filtration perimeter. The perimeter will be defined by the shape of your filtration piece and by varying intensities of coloration (i.e: brown, yellow, green, or slightly discolored white). Some are easier to see with the naked eye than others. Underneath the dissecting microscope, even light colored perimeters are easily distinguishable.

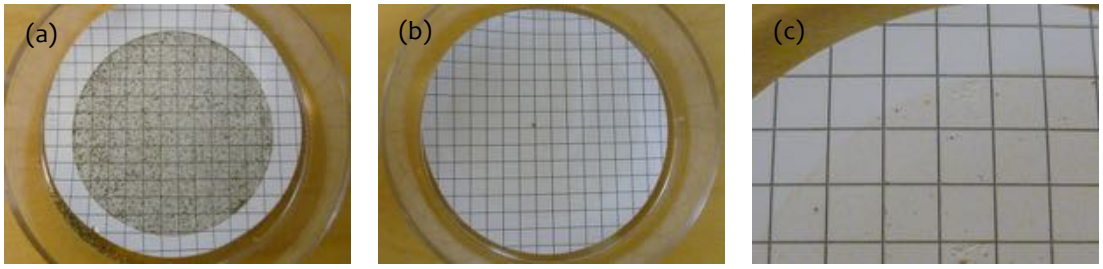


Figure 1: Different colored filters: (a) Brown; (b), (c) Yellow.

2. If filters are curved and too difficult to examine under the microscope, glue them directly to the petri dish using two or three very small drops of glue on the filter edges.

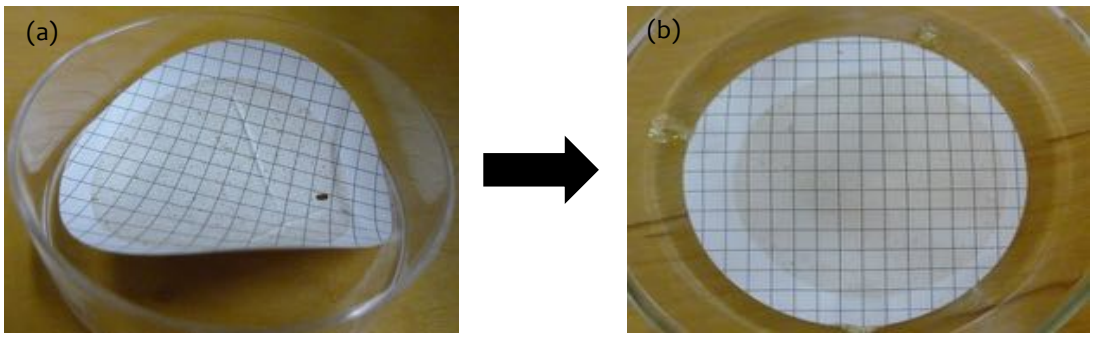


Figure 2: Glue procedure: (a) Curved filter; (b) Glued filter.

3. Read each filter from left to right, then move down one row, and read from right to left. A grid is helpful to ensure pieces are not double counted.

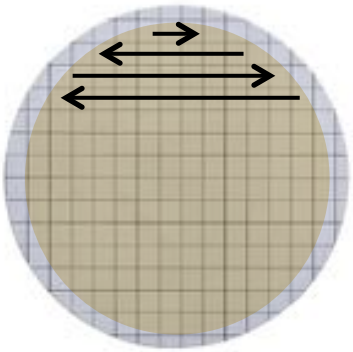


Figure 3: Filter reading procedure.

IV. Identifying Microplastics

A. Microscope Inspection

Inspect filters under a dissecting microscope at 4.5x magnification. Filters need to be dry, as wet filters reflect the light of the microscope. Typically, covered filters will dry after 24 hours at room temperature, but depending on the moisture of the filter and temperature in the lab, it may take longer.

B. Prodding Pieces and Texture

Most plastic pieces are somewhat flexible and will not break when prodded. Tweezers and probes will allow you to poke at individual pieces. Plastic pieces will often bounce or spring when prodded. If a piece breaks when touched, do not count it as plastic.

C. Examining Filters with High Debris Loads

Detritus and salt piles may cover or make it more difficult to see microplastic pieces underneath them. Carefully pick through and move aside debris in order to make sure you don't miss any microplastics.

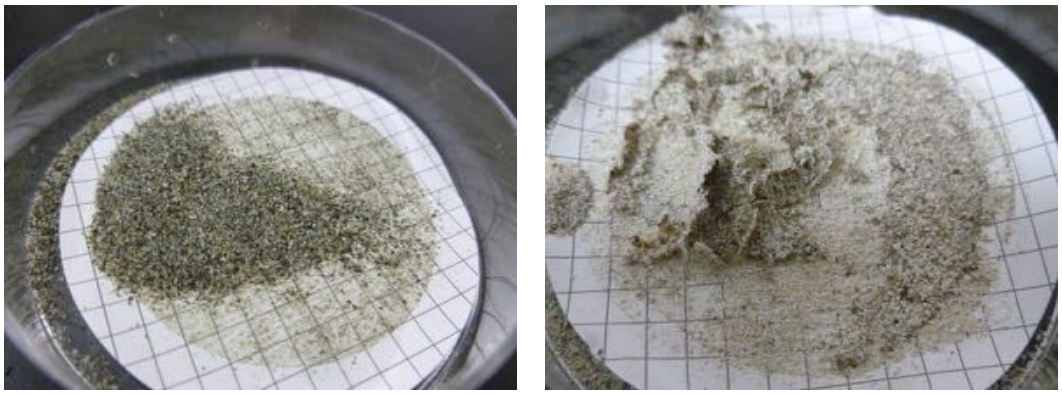


Figure 4: Detritus on filters.

D. Alternative Identification Methods

i. The Hot Needle Test (based on De Witte *et al*., 2014)

This test is useful in cases where you are not able to distinguish between plastic pieces and organic matter. In the presence of a very hot needle, plastic pieces will melt or curl. Biological and other non-plastic materials will not. The hot needle test works well when your fragments are spread apart. However, when many pieces are in close proximity, this test can be difficult to conduct. When using this technique, be sure your needle is very hot and held as close as possible to the piece in question (without blocking your view). If the needle is not hot enough, you will see no movement, even if the piece is plastic. This test should be used in conjunction with knowledge of other characteristics of plastic pieces. If you find yourself unsure, remove the piece in question and inspect it under a compound microscope.

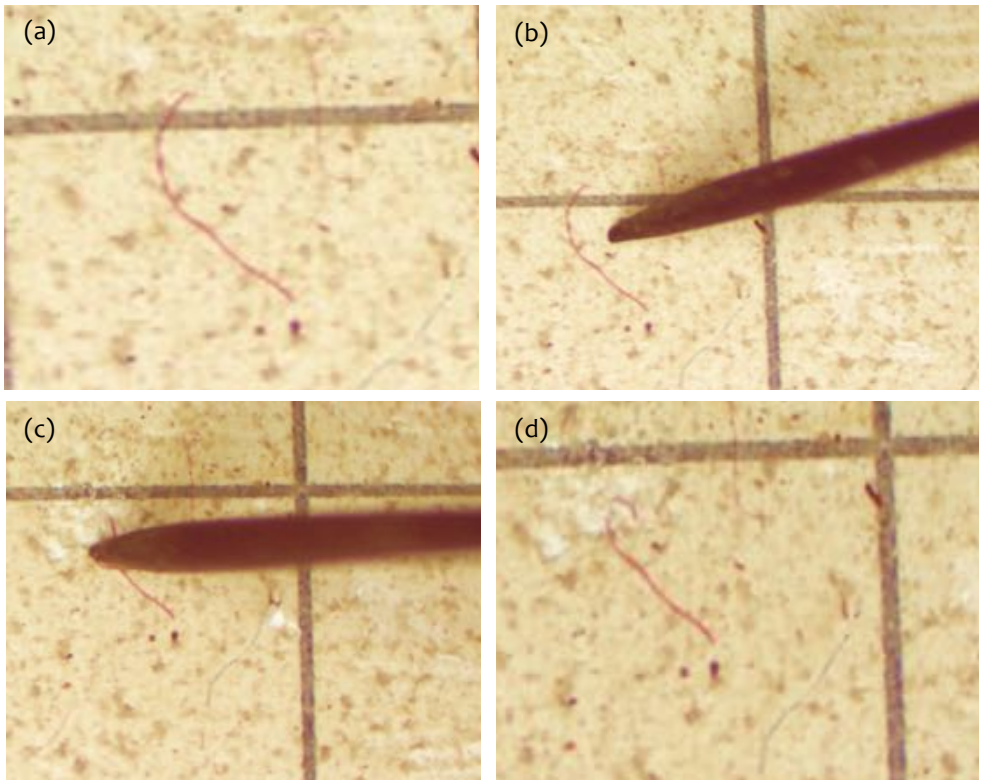


Figure 5: Hot needle test on red filament: (a) Pre-test; (b) Approach of hot needle; (c) Hot needle in close proximity; (d) Final result – filament reacted and curled due to heat.

ii. Compound Microscope Inspection

If the composition of a piece is questionable, carefully remove it and further inspect under a compound microscope where biological structures are more readily visible. It is beneficial in training to examine filtered algae under the microscope so you are confident identifying algal pieces versus plastic pieces with biofouling (i.e. growth of algae on plastics surface).

E. Variations to Look for When Using the Hidalgo-Ruz Rules

The Hidalgo-Ruz *et al*. (2014) rules will help you identify most microplastics you encounter. However, you may come across pieces with variations on these rules. Identify as many characteristics as possible for accurate categorization.

i. Rule 1: *No Cellular or Organic Structures Visible*

Variation: Microplastics will never have cellular or organic structures. However, biofouling can alter the appearance of a piece of plastic. Organic material may be visible just on the surface of the plastic. Take care to note if organic structures are present throughout a piece, or just on one portion or the surface.

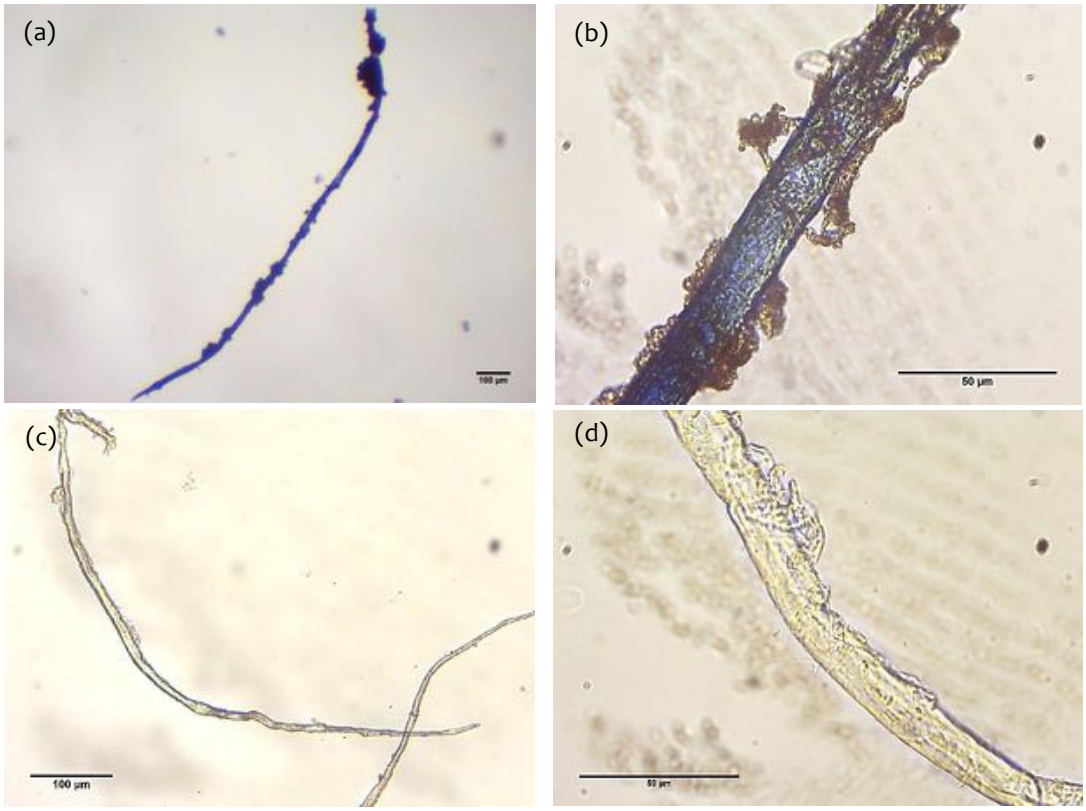


Figure 6: Biofouled microplastic fibers: (a), (b) Blue biofouled fiber; (c), (d) Translucent biofouled fiber.

ii. Rule 2: *Fibers Should be Equally Thick Throughout Their Entire Length*

Variation: Generally always true for fibers. However, sometimes splitting or fraying is seen.

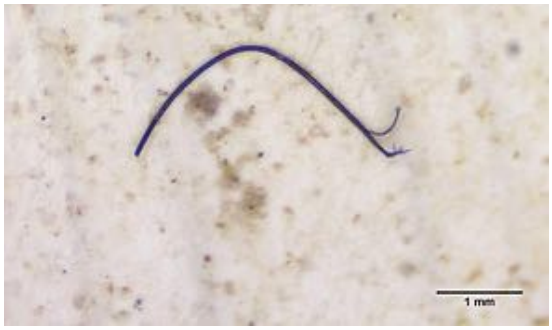


Figure 7: Frayed microplastic fiber.

iii. Rule 3: *Particles should exhibit homogeneous color throughout*

Variation: Some plastics are not homogenous in color. You may find patterns or stripes. Additionally, biofouling can potentially disguise color, or part of the fiber may be bleached.

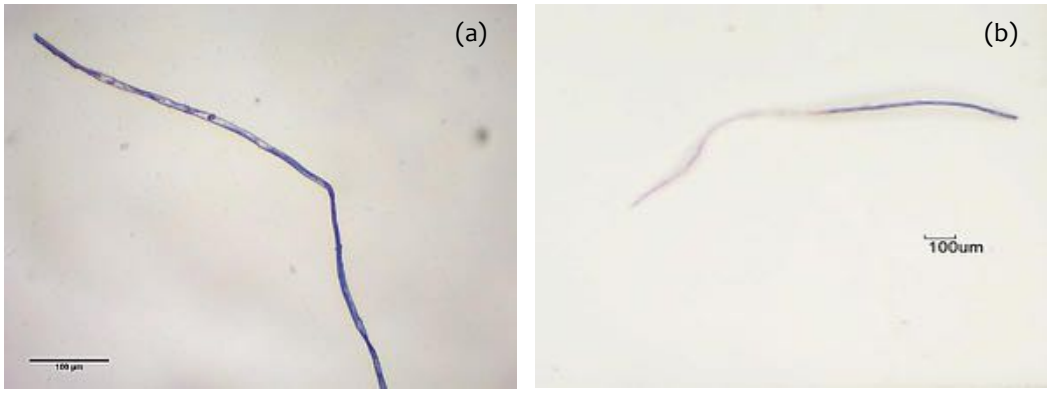


Figure 8: Exceptions to the rules: (a) Partially bleached blue fiber; (b) Red, white and blue fiber.

F. Other Materials You May Find on Your Filter

i. Algae

Algae, especially translucent pieces, can sometimes be difficult to distinguish from plastic underneath the dissecting microscope. Even when prodded, these biological pieces may not break. If you are unsure, remove the piece in question and inspect it under a compound microscope. Look for cellular structures *throughout* the piece. If there are cellular or organic structures just on one portion, or just on the surface, it may be due to biofouling (See Section D, part i).

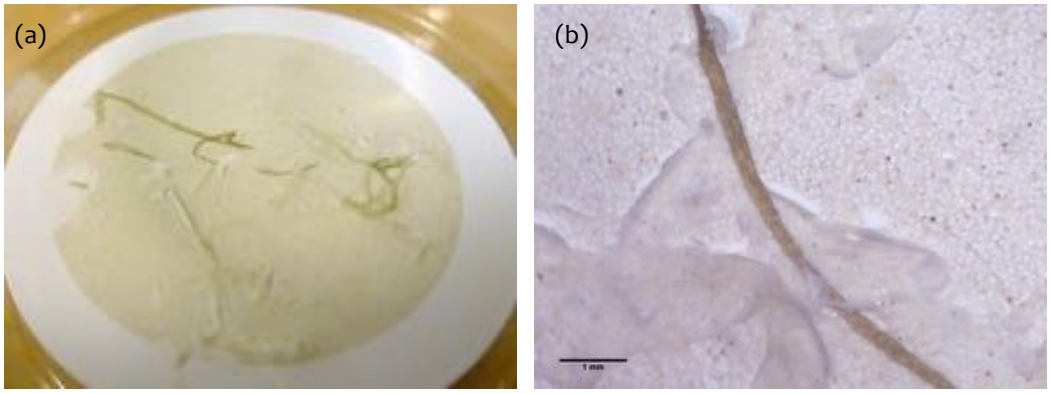


Figure 9: Algae examples: (a) *Ulva* algae on a filter after filtration; (b) The translucent film from the algae may look as a plastic film, but is very brittle and breaks apart when prodded.

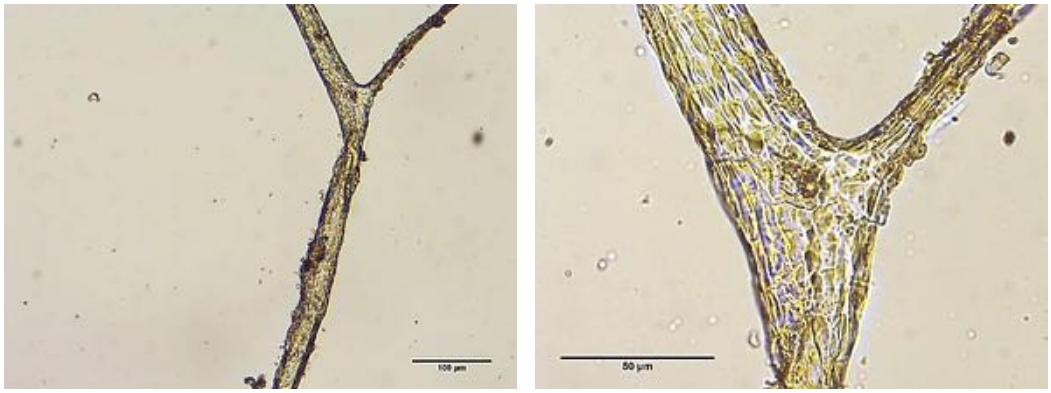


Figure 10: A piece of algae. The forking seen here is usually indicative of algae. Notice the cellular structure seen throughout the piece.

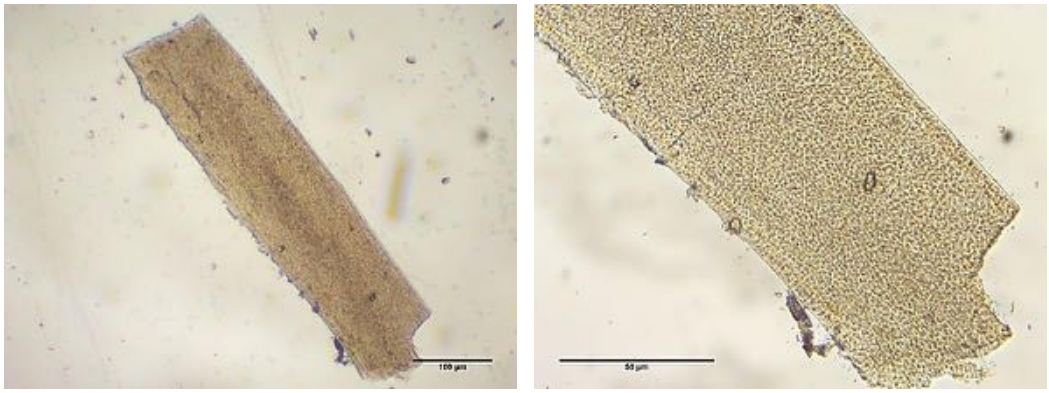


Figure 11: A piece of algae. Notice cellular structure seen throughout.

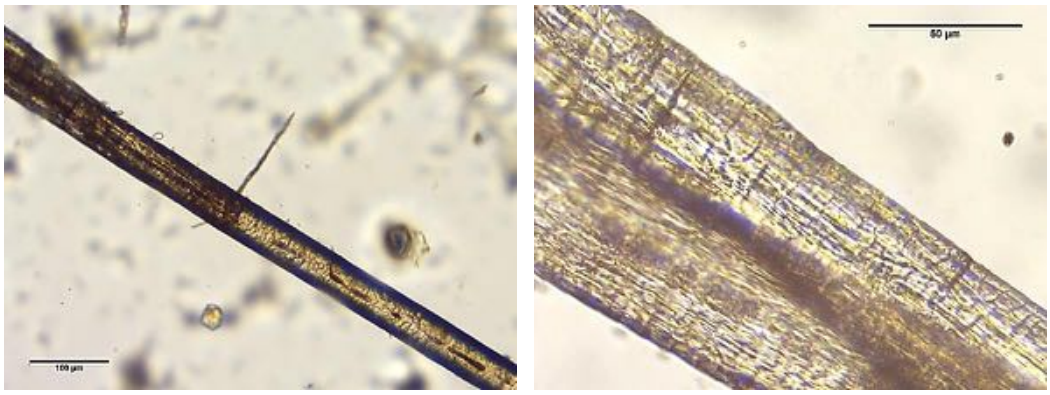


Figure 12: A piece of algae. Notice cellular structure seen throughout.

ii. Salt Crystals and Sand

Salt and sand pieces may also sometimes look similar to plastic. However, when prodded, salt crystals will break apart and sand will break and sound like breaking glass. If you are having difficulty distinguishing sand from microplastics, consider using the hot needle test. Sand will not respond to the heat from the needle, but microplastics will.

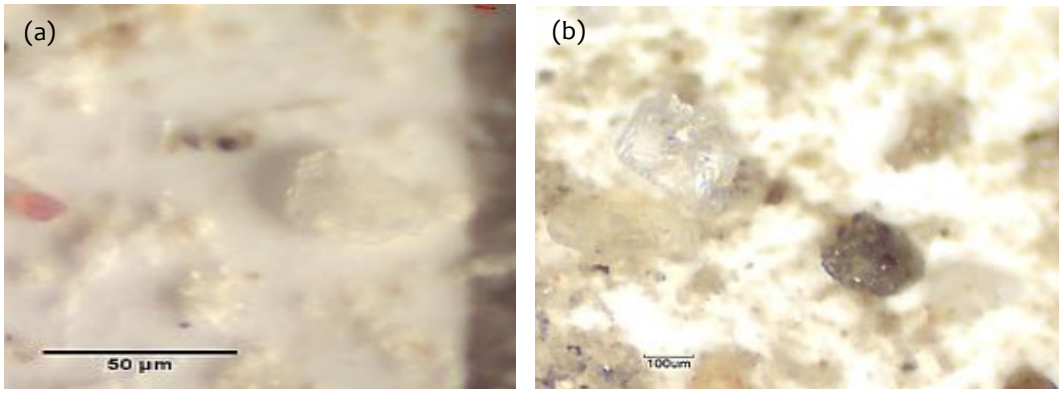


Figure 13: Non-plastic examples: (a) Salt crystal; (b) Sand grain.

iii. Animals, Animal Parts and Shells

You may find animals, shells, phytoplankton, zooplankton, etc., in your sample. These will usually break apart or lose much of their structural integrity when prodded.

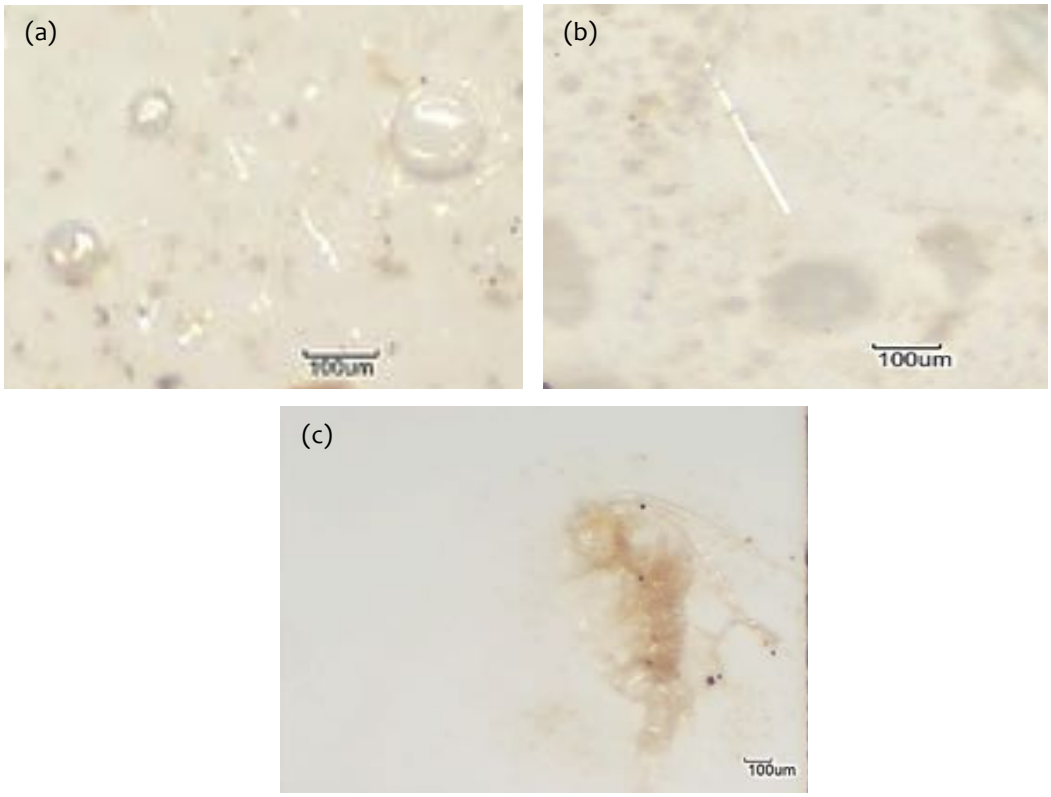


Figure 14: Plankton. Might be present in the form of geometrical shiny structures or easily recognized as individual animals: (a), (b) Diatoms; (c) Copepod.

Figure 15: Trichome. Looks like plastic, and will spring right off of the filter when prodded.

iv. Metal Paint and Aluminum Foil

You may see pieces of metal paint or aluminum foil in your samples. Metal paint can scrape off a boat. Aluminum pieces can flake off of foil lined sample lids or equipment. You can identify these pieces due to their intense reflectivity and shininess. They will not display the characteristic traits of most plastic pieces such as the flexibility.

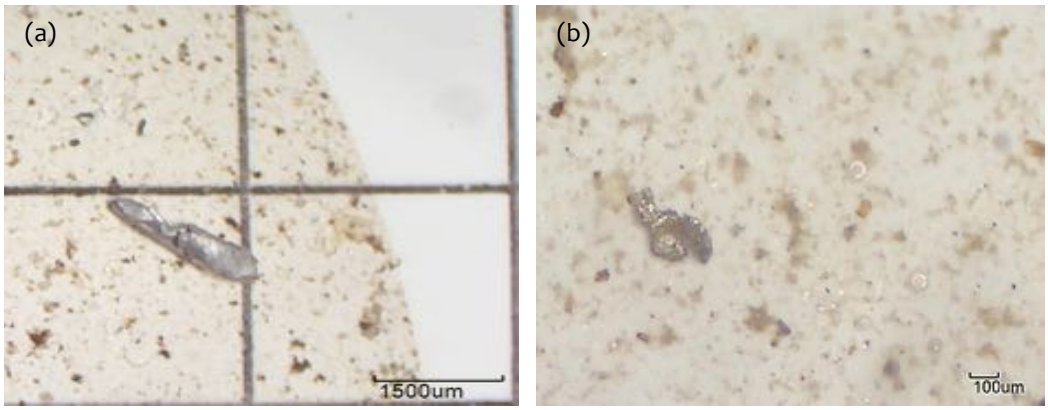
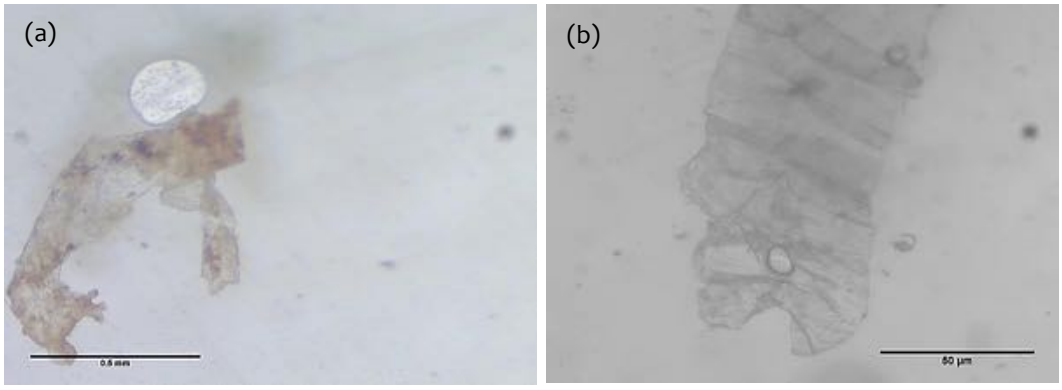


Figure 16: Examples of aluminum pieces: (a) Large piece; (b) Smaller piece in detail.

V. Categories of Microplastics

MERI identifies microplastic pieces based on color and shape. If possible, it is also beneficial to measure the length or area of each piece. Software programs such as Image J and MoticCam both have this capability. Make sure that your equipment is calibrated so measurements are more accurate. Rough estimates of plastic size (e.g. length equals # squares) can also strengthen your analysis.



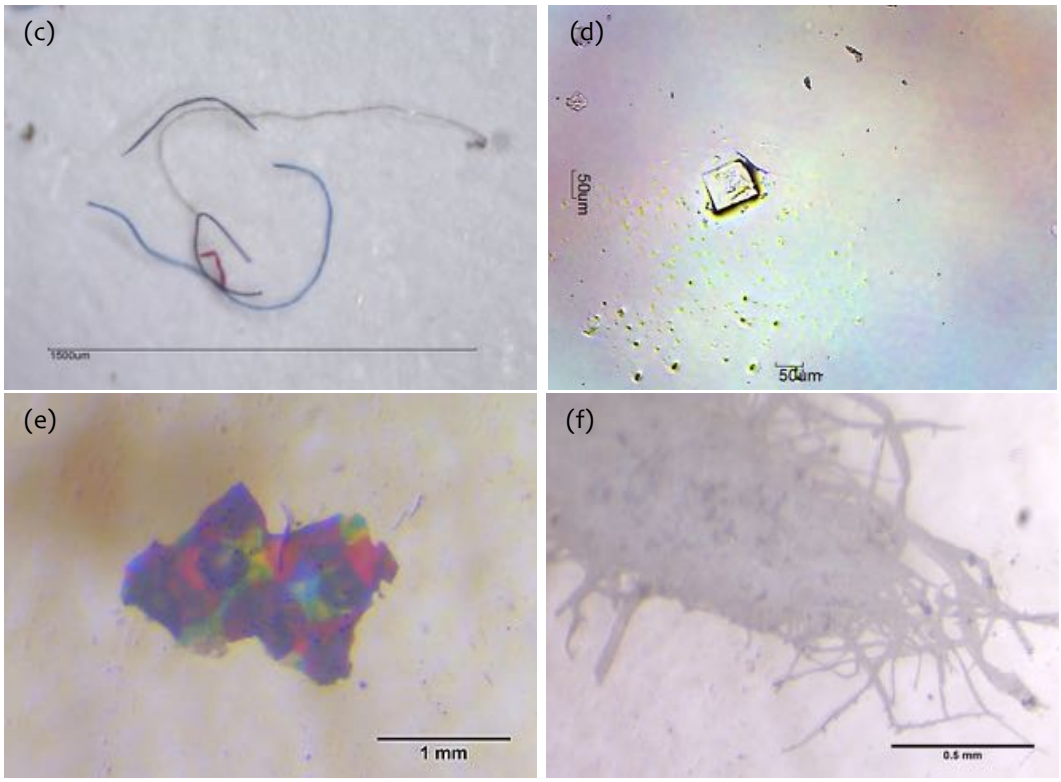


Figure 17: Different shapes and colors of Microplastics: (a), (b) Translucent fragment; (c) Filaments; (d) Angular; (e) Multi-colored fragment; (f) Clump of white fibers.

A. Categories Used to Describe Microplastics

Some studies further categorize pieces, as seen in the chart below. Without FT-IR analysis (i.e. plastic polymer identification), it is extremely difficult to confidently pinpoint the source of microplastics in your sample.

CATEGORIES	DESCRIPTION
SOURCES	- Consumer product fragments (e.g. fishing net) - Raw industrial pellets
TYPE	- Plastic fragments, pellets, filaments, plastic films, foamed plastic, granules and styrofoam
SHAPE	- For pellets: cylindrical, disks, flat, ovoid, spheruloids - For fragments: rounded, subrounded, subangular, angular - General: irregular, elongated, degraded, rough and broken edges
EROSION	- Fresh, unweathered, incipient alteration, and level of crazing (conchoidal fractures), weathered, grooves, irregular surface, jagged fragments, linear fractures, subparallel ridges and very degraded
COLOR	- Transparent, crystalline, white, clear-white-cream, red, orange, blue, opaque, black, grey, brown, green, pink, tan, yellow and pigmentation

Source: Hidalgo-Ruiz *et al*., 2012

VI. Contamination Considerations

Airborne fiber contamination is recurrent in many studies. It is necessary to be very aware of this and to take precautions to reduce it.

A. Methods to Reduce Contamination

1. Keep your filter covered whenever possible. If you are not looking at it under the microscope, it should be covered.
2. Store filters in glass petri dishes. Plastic petri dishes will work, but glass is better to reduce possible contamination from the dish itself.
3. Wipe down all surfaces before inspecting each sample. A brightly colored sponge is recommended. Any pieces sourced from the sponge will be more easily identified as contamination if it is a unique color.
4. Rinse all tweezers, probes, and your hands three times under a heavy stream of water before opening a petri dish for inspection.
5. Wear cotton or natural fiber clothes. Avoid bringing any synthetic materials into lab.
6. Minimize traffic in your lab or working space, if possible. The smaller the number of people in and out of your work space, the smaller the chance of contamination.

B. Contamination Examples

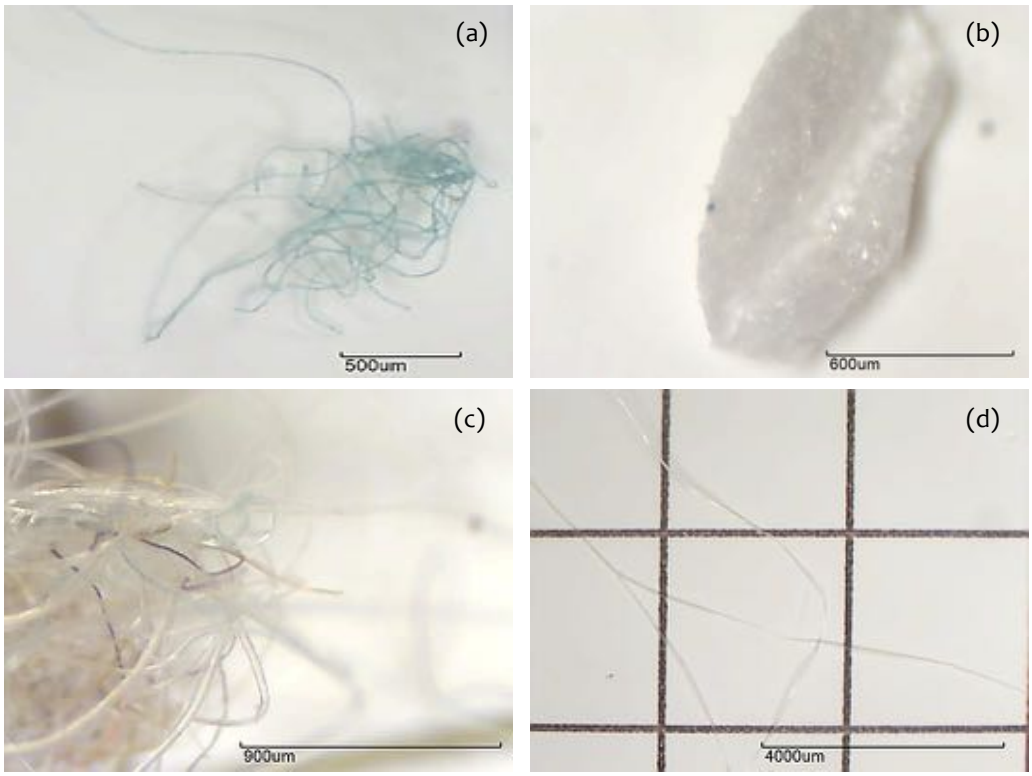


Figure 18: Examples of different types of lab contamination: (a) Cotton clothing fibers; (b) Styrofoam piece; (c), (d) Wool clothing fibers.

VII. References

De Witte, B.; Devriese, L.; Bekaert, K.; Hoffman, S.; Vandermeersch, G.; Cooreman, K.; Robbens, K. (2014) – Quality assessment of the blue mussel (*Mytilus edulis*): Comparison between commercial and wild types. Marine Pollution Bulletin, 85(1):146-155.
DOI: 10.1016/j.marpolbul.2014.06.006

Hidalgo-Ruz, V.; Gutow, L.; Thompson, R.C.; Thiel, M. (2012) – Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. Environmental Science & Technology, 46:3060-3075.
DOI:dx.doi.org/10.1021/es2031505

VII. Appendix

A. Microplastics Data Sheet

	Blue	Red	Transparent/White	Black	Green	Other Colors	Total per Filter	Pieces/Liter
Round								
Filament								
Angular								
Other Shape								
Total								

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