# Microplastics in Lake Superior: Adventures in sampling and analysis of environmental samples

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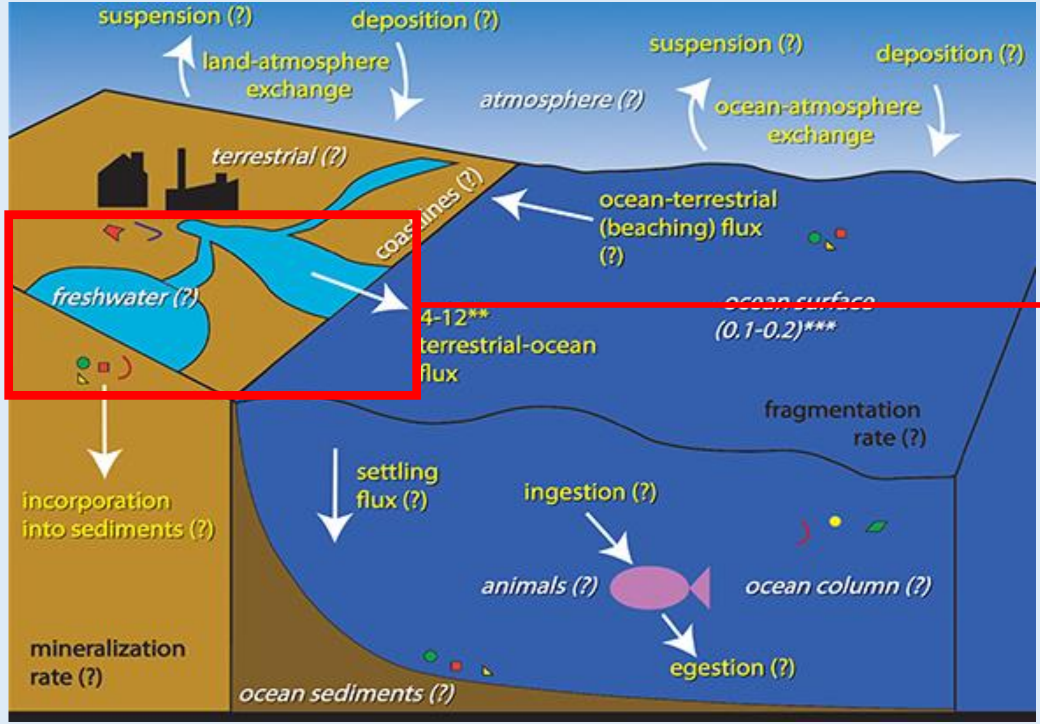
# Plastic Distribution through the Environment

Plastic Cycle




Zhu X (2021) The Plastic Cycle – An Unknown Branch of the Carbon Cycle. Front. Mar. Sci. 7:609243. doi: 10.3389/fmars.2020.609243

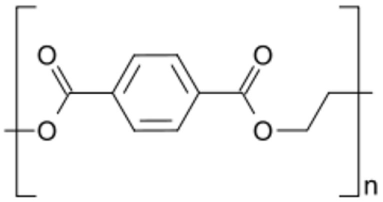
Size distributions?
Polymer distributions?

Important for informing toxicology studies, risk assessment, as well as understanding C cycling

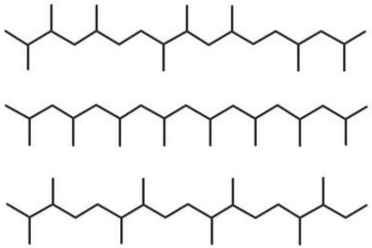
# Challenges: polymer diversity, sizes

- Plastic: a synthetic organic polymer that exhibits plasticity (the ability to deform under stress) at some point in its life cycle, can be molded.
  - Many polymers fit this definition. Often additives, too.

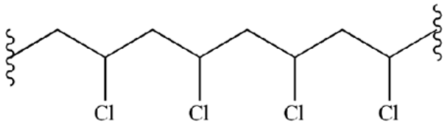
Polyester




Polypropylene



PVC




- Plastic size ranges
  - **Microplastics**: <5 mm. Lower size cut-off determined by analytical limitations (often >100 µm for microscopy, >20 µm for microFTIR)
  - **Nanoplastics**: variably defined size ranges, but are generally smaller than microplastics

# Water Sample Collection

Net towing: High volume sample. Prone to clogging. Collects one size (generally >300 or >100 um). Often sampling the air-water interface. Field blanks usually obtained by back-flushing net.

In situ pumping: 500L in ~ 2 hours. Allows sampling at different depths and size fractionation while sampling. Needs a relatively large stable platform for deployment. Getting field blanks is tricky.

Surface collection and sieving: diaphragm pump and hard-walled tubing onto metal sieves. ~260 L of water in ~ 1 hour from ~1 m depth. Can be used on small boats or docks or spigots for drinking water.

Photo: Minor lab

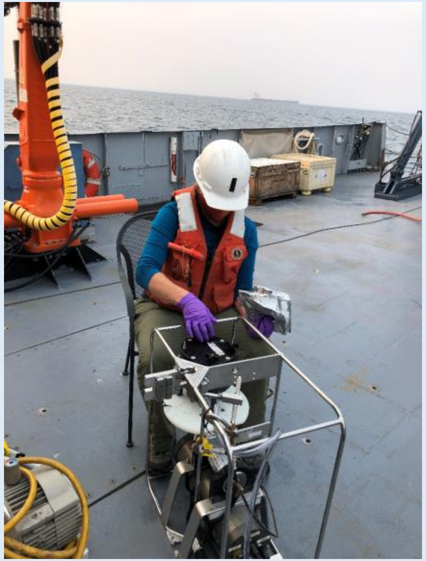


Photo: E. Minor

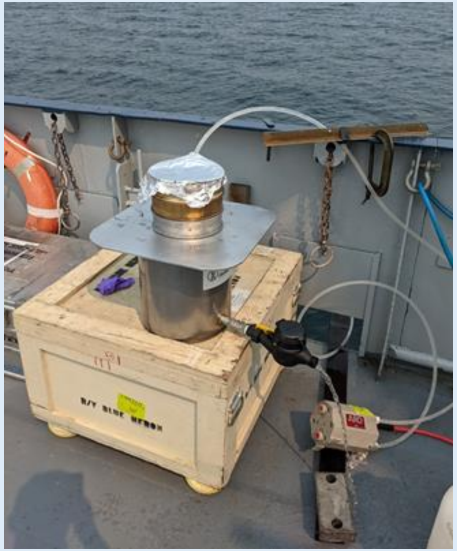
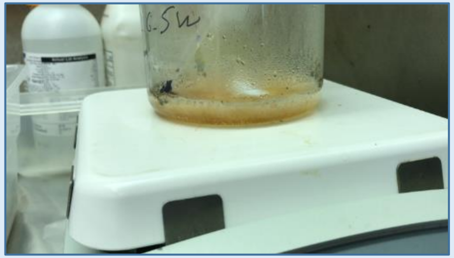


Photo: G. Schwoerer

# Sample preparation & analysis: time intensive

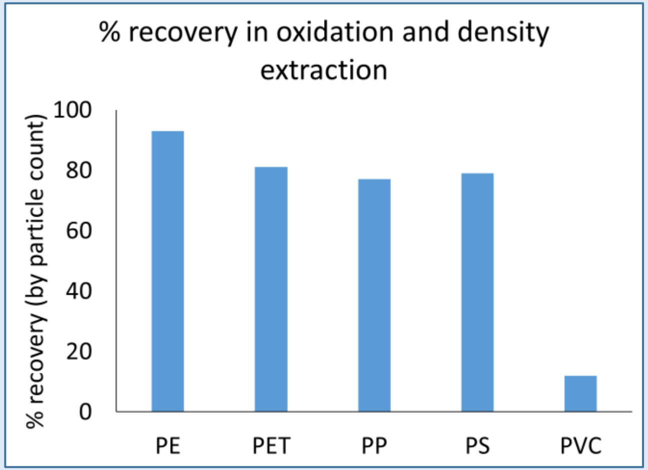
1. Sieving/filtering (~20-30 min/sample) and drying (overnight)
2. Removal of natural OM by Fenton oxidation (~2-4 hours for clear samples, up to 8 hours for organic-matter rich samples).
3. Density separation (2 to 8 hours)
4. Microscopy: melt testing, particle picking (4-8 hr/sample)
5. PyGCMS analyses (12 samples in 8 hour day).
6. Data analysis: 1 to 2 hours.

Note: If steps 4-6 are replaced with focal plane microFTIR and automated identification approaches, ~2 hours per sample for those steps.  Steps 1-3 are still necessary.



# QA/QC: positive controls with known plastic standards

- Sieve recovery test (using visual microscopy). Used PE spheres (600-710 um), PVC fragments (250 um), and PMMA spheres (85 um)
  - Samples resuspended from sieves & filtered for microscopy.
  - 68% recovery by particle number (sum of recovery on 300, 106 and 45 um sieves)
    - Some breakthrough of larger particles into smaller sieves (out of 8 total treatments large particles found in >106 um sample 3 times, and in >45 um sample 1 time).
- Oxidation and density extraction particle count recovery tests (by count) (from Hendrickson et al., 2018 on test fragments or powders and beads (generally 250-350 um size range):



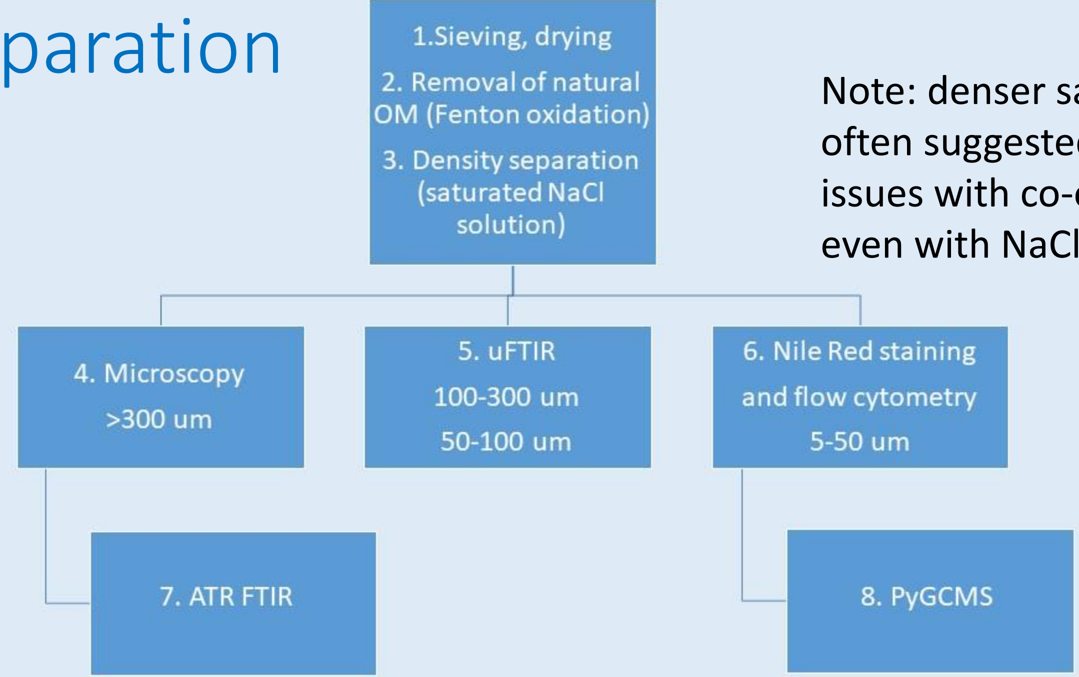
- Thermo uFTIR positive control test (directly filtered all provided volume on Anodisc)
  - PMMA = 85 um, spheres, MDPE = 350 um, powder/fragments, PA = 55 um, powder
  - Recovery data:
    - PA (0-5%).
    - PMMA (80-116%),
    - MDPE (15-38% when all PP, PE and poly(ethylene:propylene identifications combined)

  - Propagating across these for ~100 um particles, recovery ~54%

# QA:QC blanks

- Field and method blanks
- Ambient blanks

# Sample preparation & analysis




Note: denser salts than NaCl are often suggested but we have issues with co-occurring clays even with NaCl

Photo: E. Minor

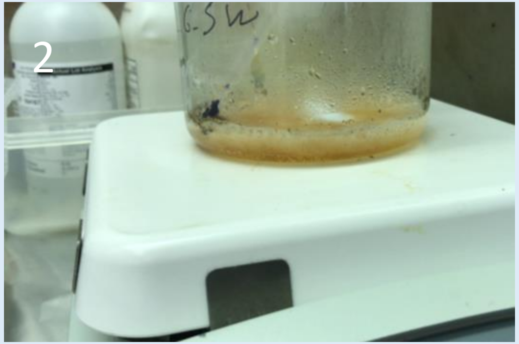


Photo: E. Hendrickson

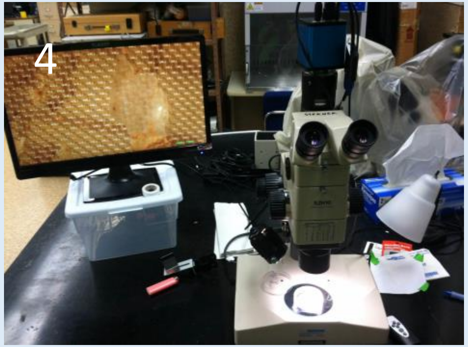
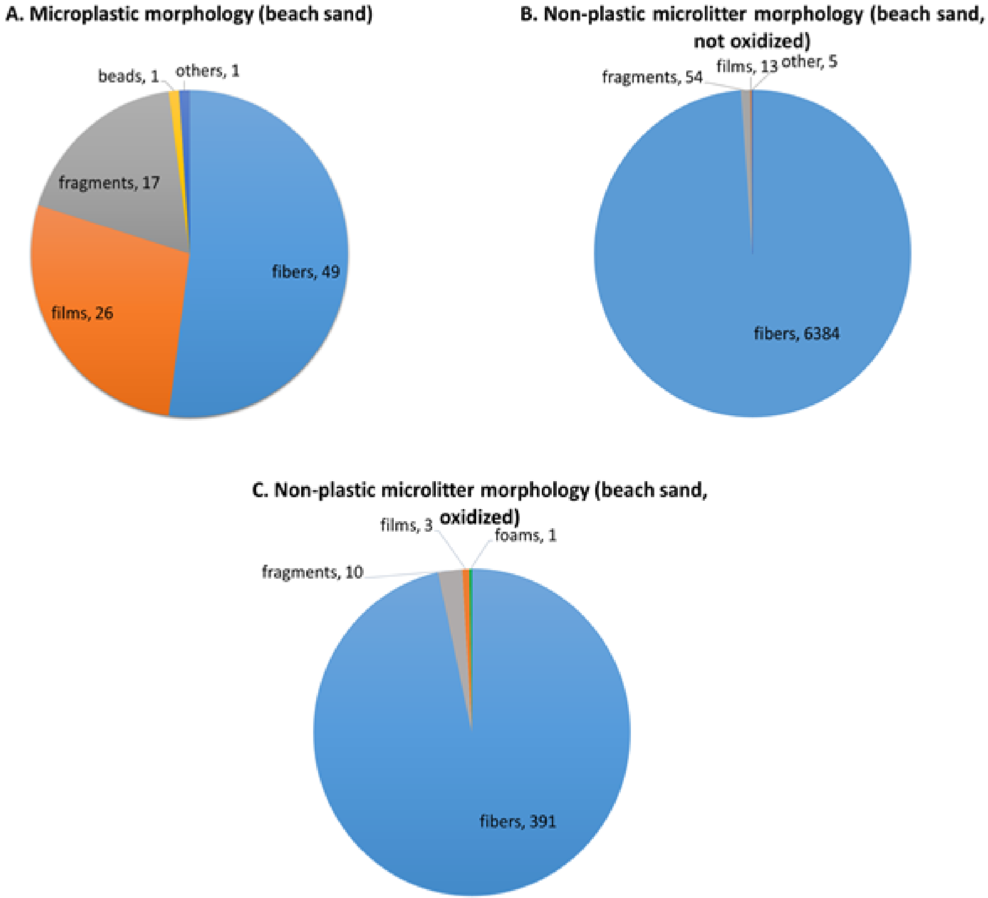


Photo: E. Hendrickson

Photo: E. Minor

# Study of sand from Lake Superior beaches:




For natural water and sediment/sand samples,
oxidation helps but is not a perfect matrix remover

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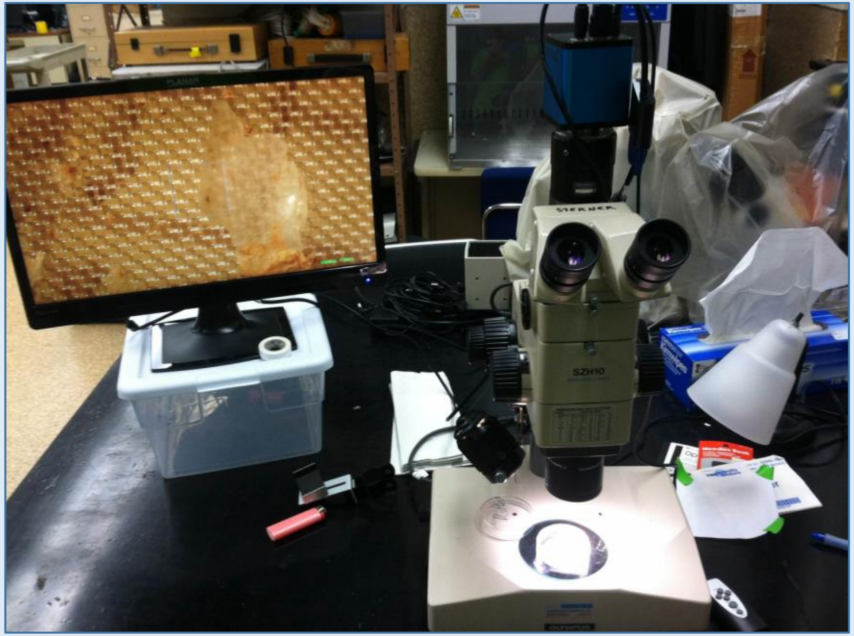
# Microscopy

Filters evaluated under dissecting microscope:

- 10x scanning.
- Measuring, color, morphology at 40x
- Questionable particles evaluated with “poke test” or “hot needle” test.
- Individual particles isolated into GC vials (pyGCMS) or onto filter grids (ATR FTIR).

Or do both microscopy and ATR-FTIR on Bruker LUMOS II—eliminates need for “hot needle test”



# Enumeration and analysis: uFTIR

Photo: E. Minor

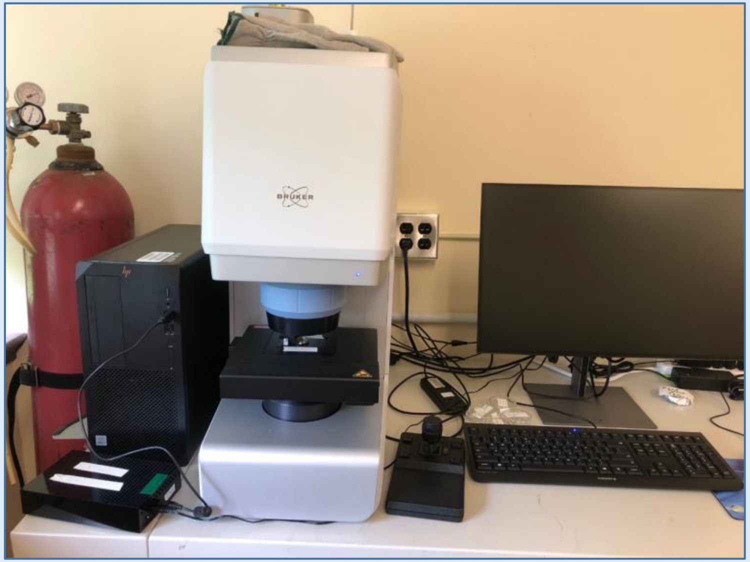
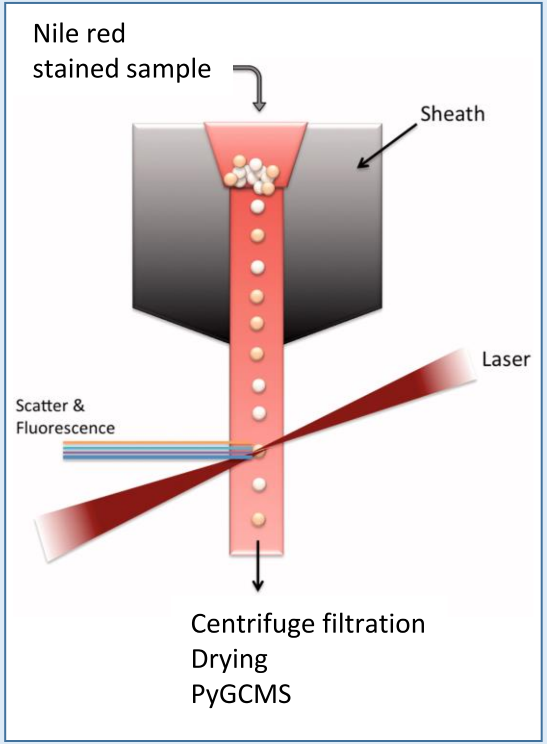


Photo: E. Minor

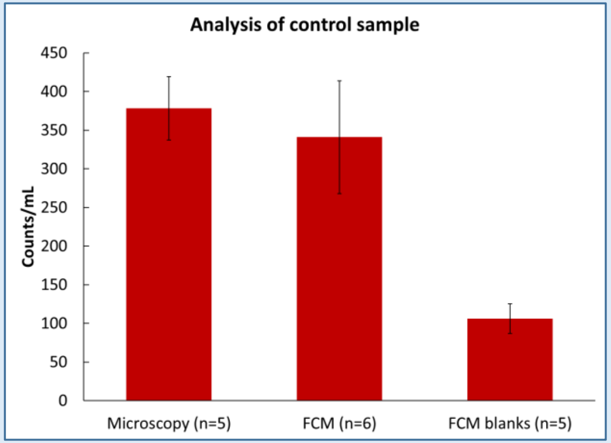
- Nicolet continuum infrared microscope coupled to a Nicolet iS50 FTIR spectrometer
- Detector is MCT (single point)
- Mainly transmittance mode, aluminum oxide filters

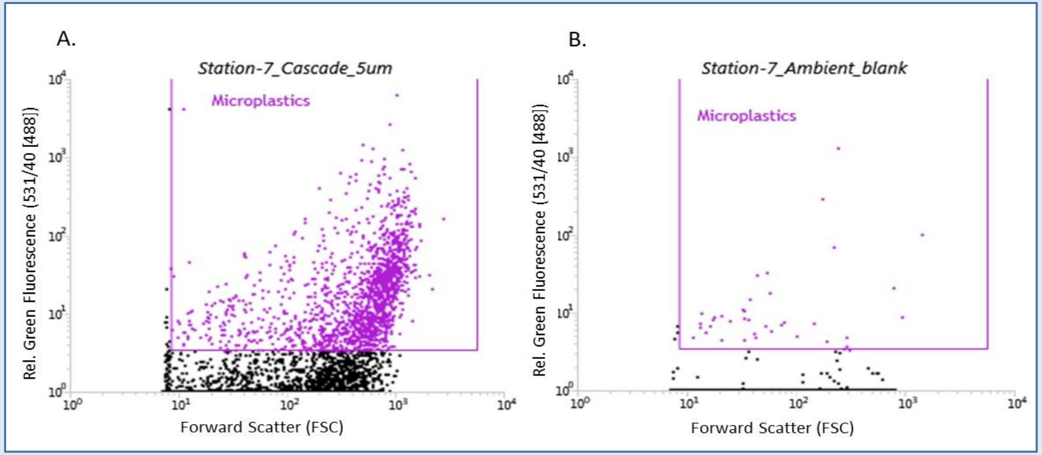
- Bruker LUMOSII infrared microscope
- Detector is FPA with 32 x 32 pixels
- Mainly transmittance mode on aluminum oxide filters
- Also ATR-FTIR for larger particles

# Enumeration and analysis: Flow cytometry (FCM)

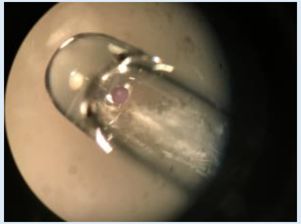


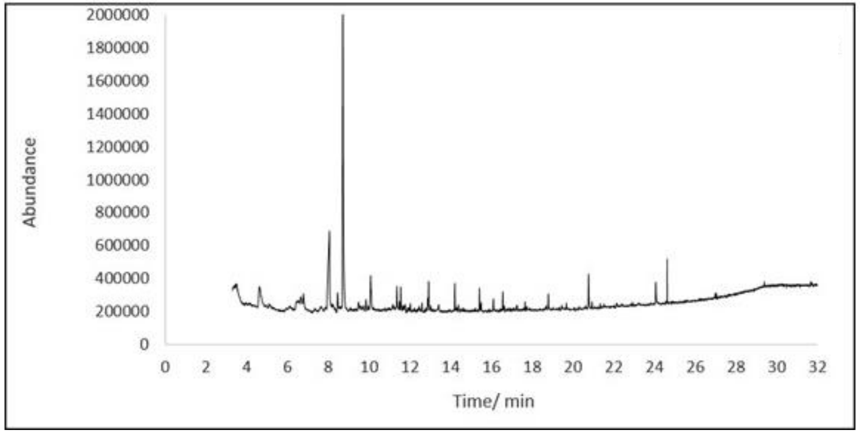

Modified from Adan et al (2017) Flow cytometry: basic principles and applications, Critical Reviews in Biotechnology, 37:2, 163-176, DOI: [10.3109/07388551.2015.1128876](https://doi.org/10.3109/07388551.2015.1128876)



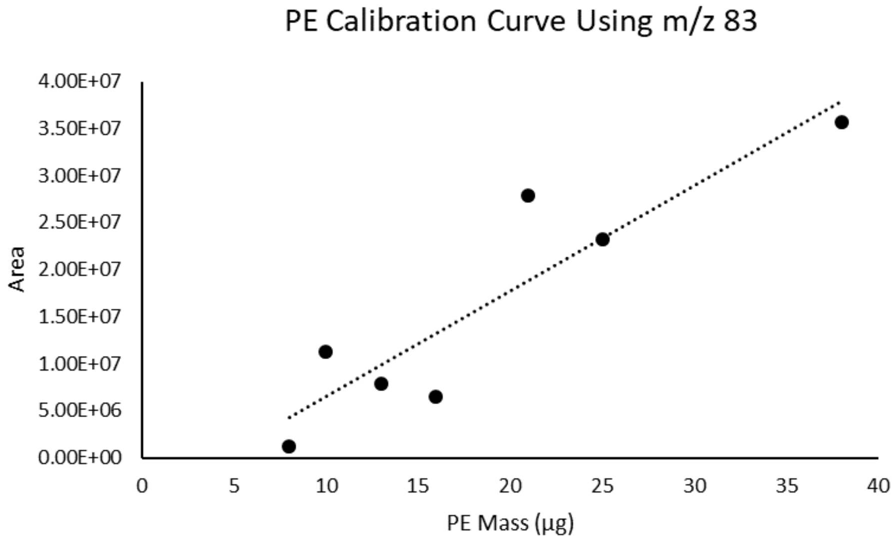
# PyGCMS

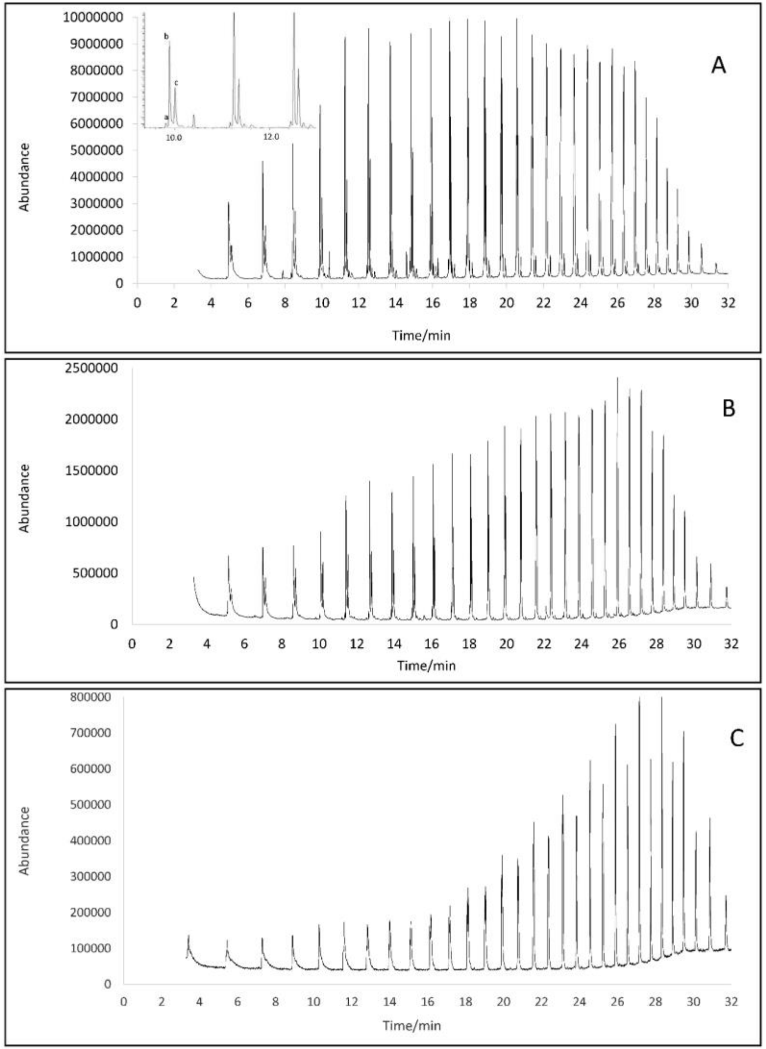





**Sample Prep & Analysis:**

- Pulsed pyrolysis at 550°C (Gerstel pyrolyzer and TDU)
- Agilent 7890B GC oven, initial temp 50°C; at 2 minutes begins ramp to 320°C at 10°C/minute. At 320°C, held for 3 minutes. Agilent HP-5MS column.
- MS EI+ (70 eV) on Agilent 5977A Mass Selective Detector



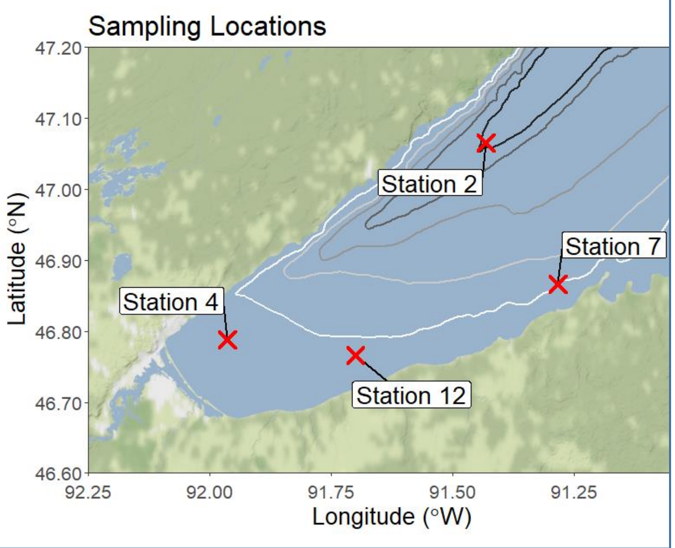



# For analyzing the smallest particles: Nile Red staining and flow cytometry do not affect PyGCMS analyses of test plastics.

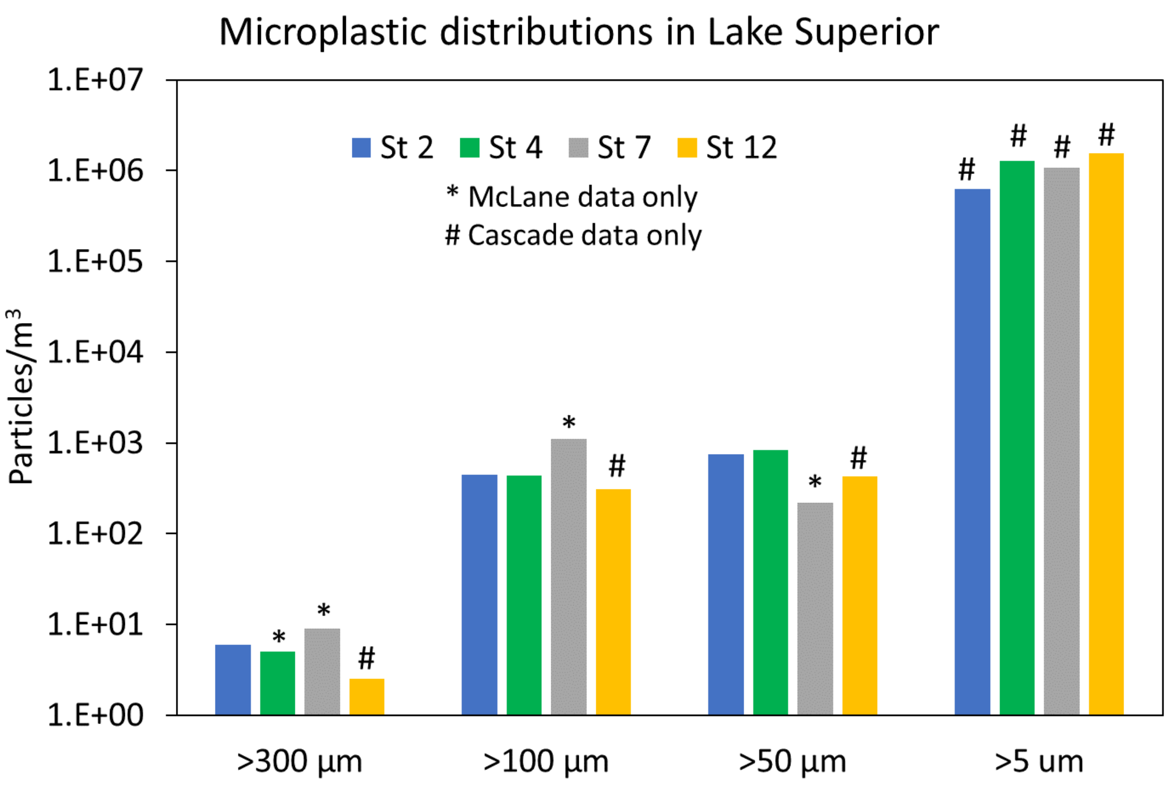
TIC from pyGCMS of PE particles (Cospheric, 10–45 µm).

A. unstained, 38 µg, insert shows the characteristic triplet (a. alkadiene, b, alkene, and c. alkane)
B. B. NR stained, 50 µg.
C. C. NR stained and FCM sorted PE particles (n=10,689, from flow cytometry enumeration); 29 µg.

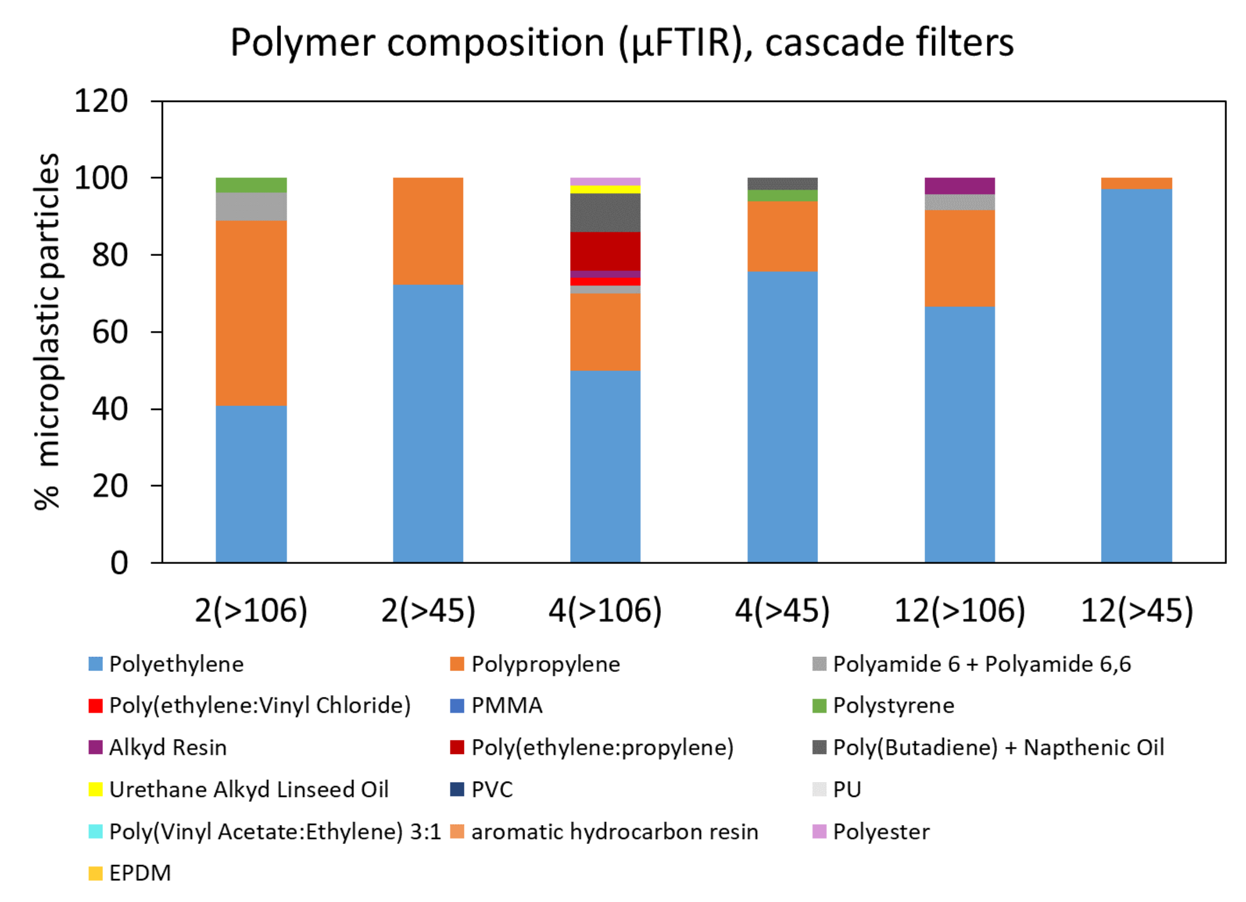
# In Lake Superior water, more microplastics appear in the smaller size ranges. At present we do not have mass distributions across such ranges.

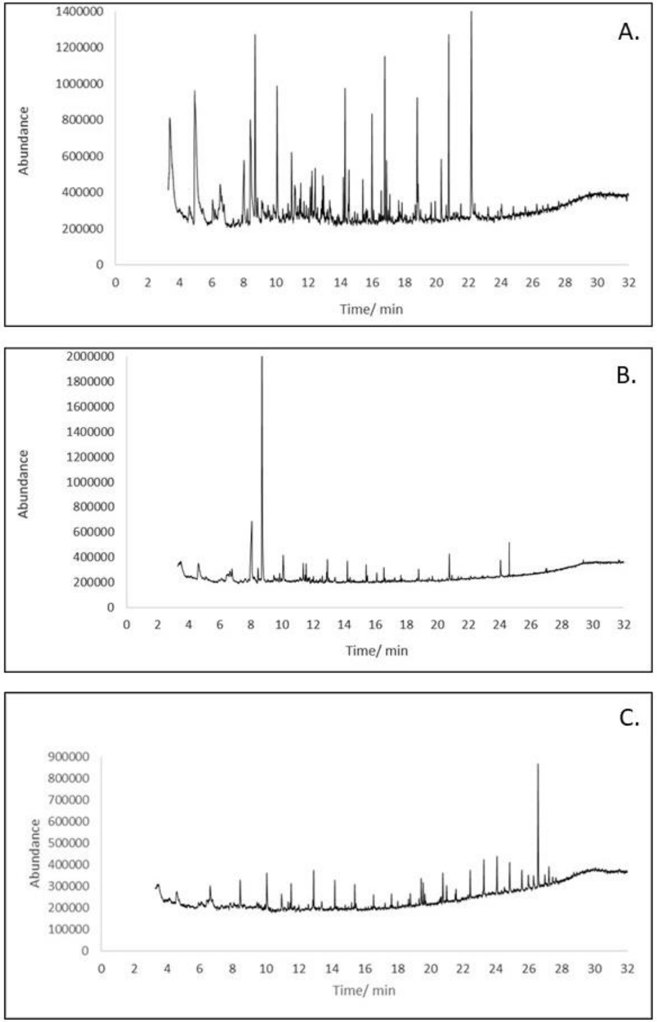



August 2021 sampling
From 1 to 2 m water depth



# 45-106 µm particles have a greater proportion of PE (in blue below) than 106-300 µm particles.  Site 4 (off Duluth entry) has more microplastic polymer types.






# Nile Red staining, FCM and PyGCMS analyses of 5-45µm particles from St 7 in Lake Superior yields a simpler more polymeric TIC but low signal

PyGCMS TIC:

A. Unsorted NR stained Station 7 sample,
B. FCM sorted material from the same sample (number of particles = 11,085), and
C. TIC of the method blank for centrifuge filtration step.

Testing for PE using characteristic ions shows 4.9 µg in A, 4.2 µg in B, 4.4 µg in C. LOD is 4.3 µg. Need to sort more particles for polymer characterization!

# Conclusions

- QA/QC critical
  - At the moment there is no nicely compounded standard
- Different sizes of plastics need different analytical techniques: how comparable are these?
  - Recoveries for different size classes via uFTIR vary a lot
- In lake samples more microplastics on a particle/volume basis from the smallest size class (5 – 45 µm)
  - Caveat: comparing results from different techniques.
  - Caveat: only have data from 1-2 m depth.
- µFTIR of 45 – 106 µm and 106 – 300 µm particles shows that the smaller size range has fewer polymer types and a higher proportion of PE
  - More weathered particles in smaller size class?
- Nile Red staining, FCM, and pyGCMS can characterize plastic polymers in the 5 – 45 µm size class
  - need longer FCM sorting times for clear signal relative to LOD and method blanks

# Acknowledgements

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