EPA

Office of Research and Development

SAFE AND SUSTAINABLE WATER RESOURCES RESEARCH PROGRAM

Research Area 1, Output 4:
Methods to Identify and Quantify Micro/Nanoplastics in Environmental Matrices

Presented to the Board of Scientific Counselors

October 28, 2020

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Safe and Sustainable Waters Research (SSWR): Microplastics Research Program

Output 1.4

Methods to Identify and Quantify Micro/Nanoplastics in Environmental Matrices

Microplastics Definition

Plastic particles ranging in size from 5 mm to 1 nm[1,2]

[1] California State Water Board 2020

[2] European Chemicals Agency 2019

Safe and Sustainable Water Resources National Research Program (FY20)

Research Objective:

Methods for Collection, Extraction and Identification of Nano- and Microplastics for Surface Water and Sediments

Research Efforts:

1. Standardize extraction, identification and quantification methods for microplastics in sediment and surface waters.
2. Build capacity for MP method development in EPA laboratories nationwide.

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Progress Research Effort 1:

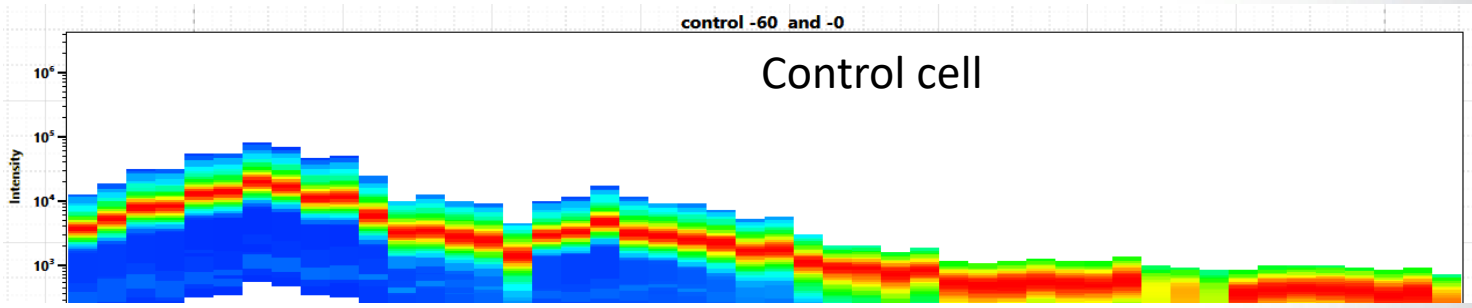
Methods for Collection, Extraction and Identification of Nano- and Microplastics for Surface Water and Sediments

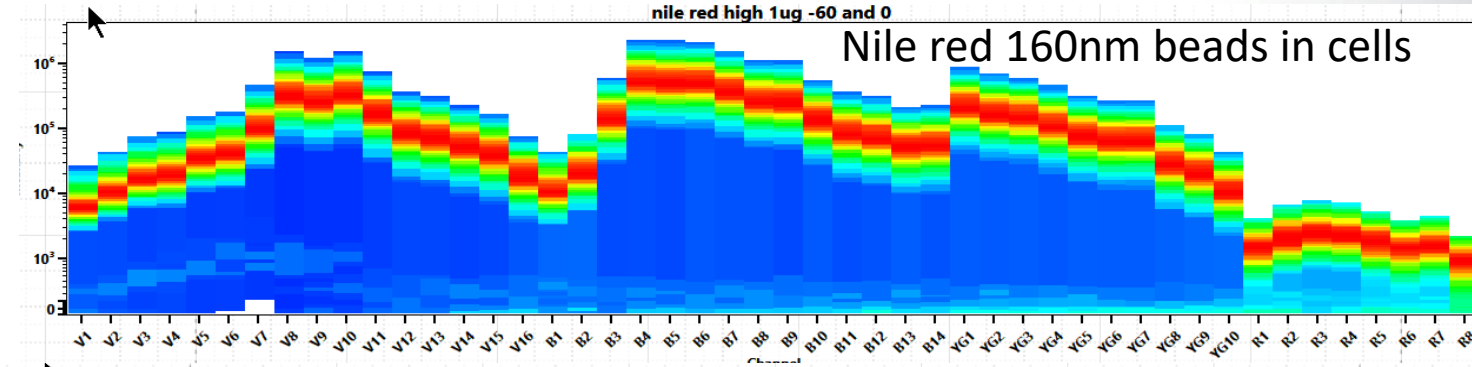
- Sediment and water methods for microplastics.
  - State of California - Drinking water methods by Fall 2020
  - Southern California Coastal Water Research Project (SCCWRP)
    - Methods for sediments and surface waters in the outyears
- Further standardizing sediment methods, using a hybrid method we developed, in a limited field survey in 2020.
  - National Coastal Condition Assessment (NCCA)
    - Collaboration with EPA Office of Water
- Development of new methods focusing on smaller microplastics and nanoplastics.
  - Likely to cross cellular membranes

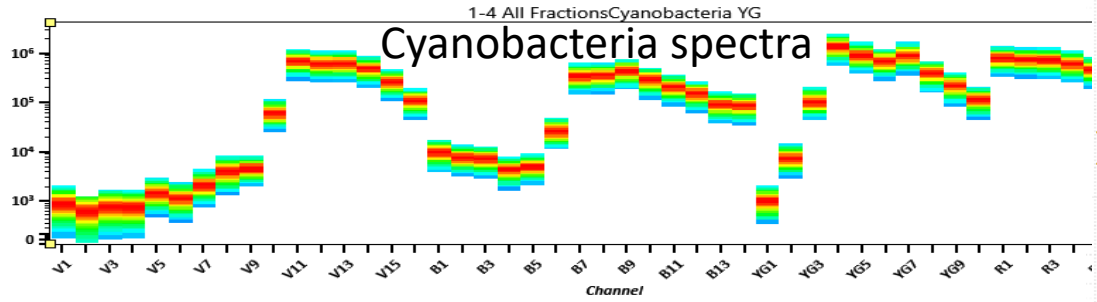
Development of Small Microplastics/Nanoplastics Methods

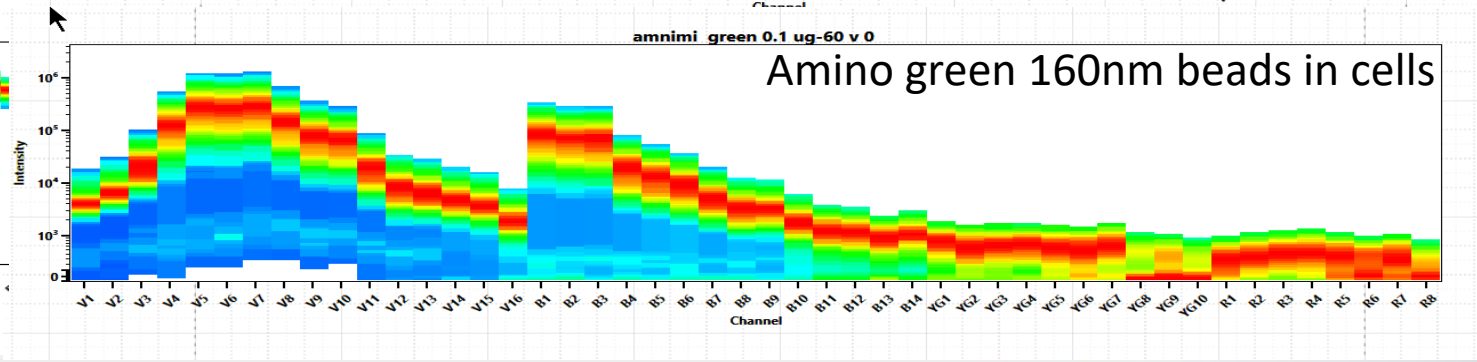
Flow cytometry, hyperspectral imaging and microscopy

Cytek aurora spectral flow cytometer 200nm-40um





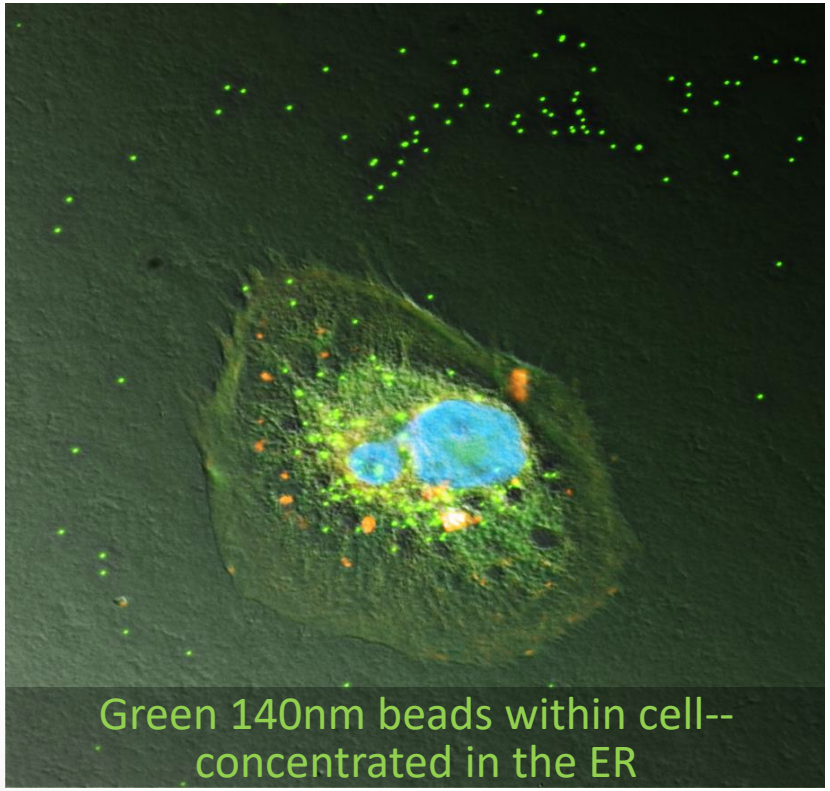




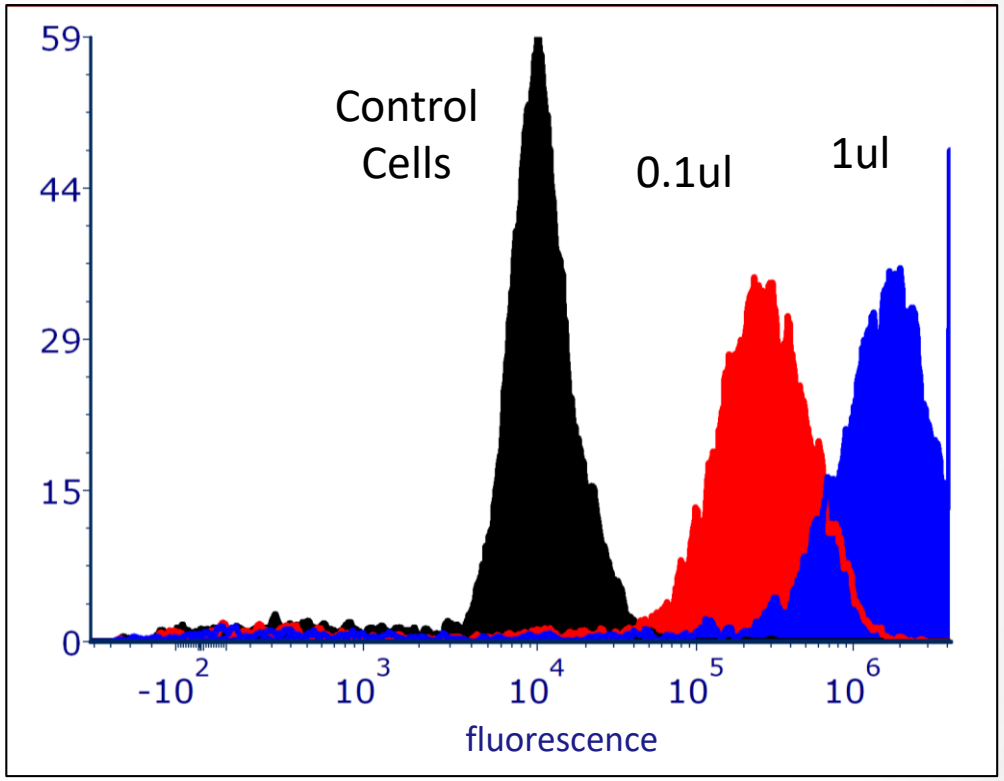
Development of Small Microplastics/Nanoplastics Methods

Visualization and Quantification of fluorescent 140 nm microbeads in cells

Microscopic visualization of fluorescent green 140 nm micro-beads in cells



Flow cytometer dose dependent detection of 140 nm microplastic beads within cells



Fluorescence Increase

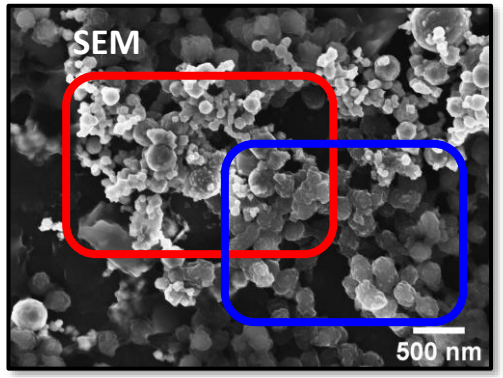
Development of Small Microplastics/Nanoplastics Methods

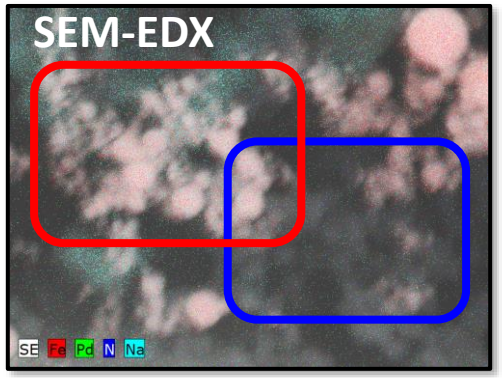
Extract, Concentrate and Characterize Nanoplastics

Adsorb nanoplastics to hydrophobic iron nanoparticles and extract using magnetic separation.

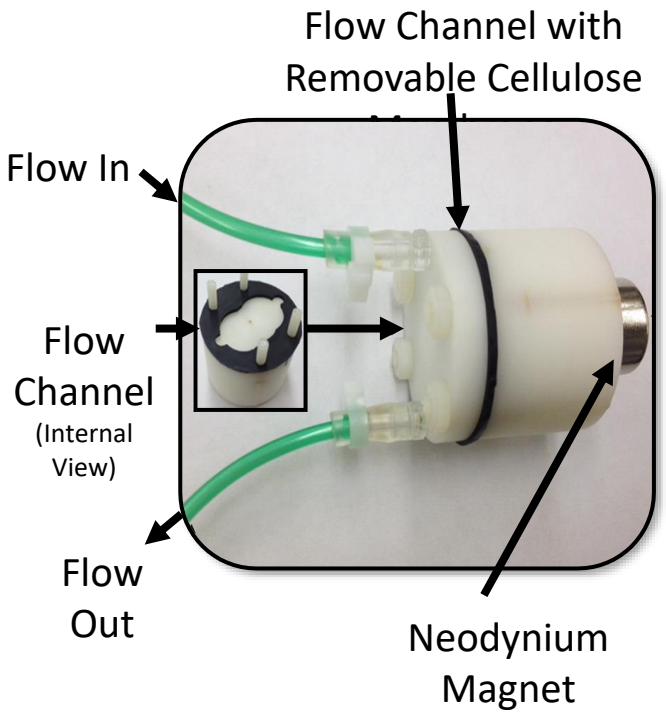
Iron Nanoparticles

Pd-Labelled Nanoplastics





Magnetic Flow-cell Extractor

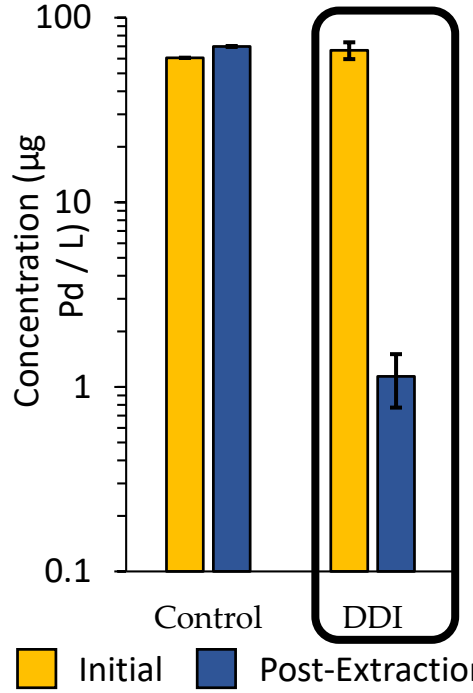


Post-Extraction Samples

Membranes with Extracted Particles

Results

Extraction Efficiency
= 98.3 ± 0.3%
(*n* = 12; ± 95% C.I.)



Development of Small Microplastics/Nanoplastics Methods

Generate Environmentally-Relevant Nanoplastic Particles

Characterize nanoplastic particles generated from relevant stock materials using a suite of techniques.

Environmentally-Relevant Feed Stocks

Marine Macroplastics

- LD- & HDPE
- PP
- PS
- PET
- Nylon

Collected from marine environment by NIST / HPU collaborator

Waste Electronic and Electrical Equipment (WEEE)

a.k.a. "Black Plastics"

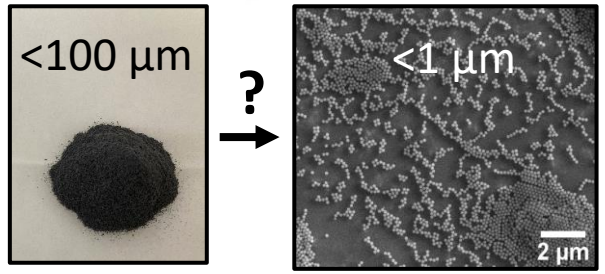
- Computer speakers
- Power cords
- Computer keyboard
- Wireless router

Process & Characterize Samples

Initial Processing of Feed Stocks via Cryomilling

Testing of Additional Processing Methods:

- Further cryomilling
- Sonication in KOH
- Aerosolization via 3D-Printer

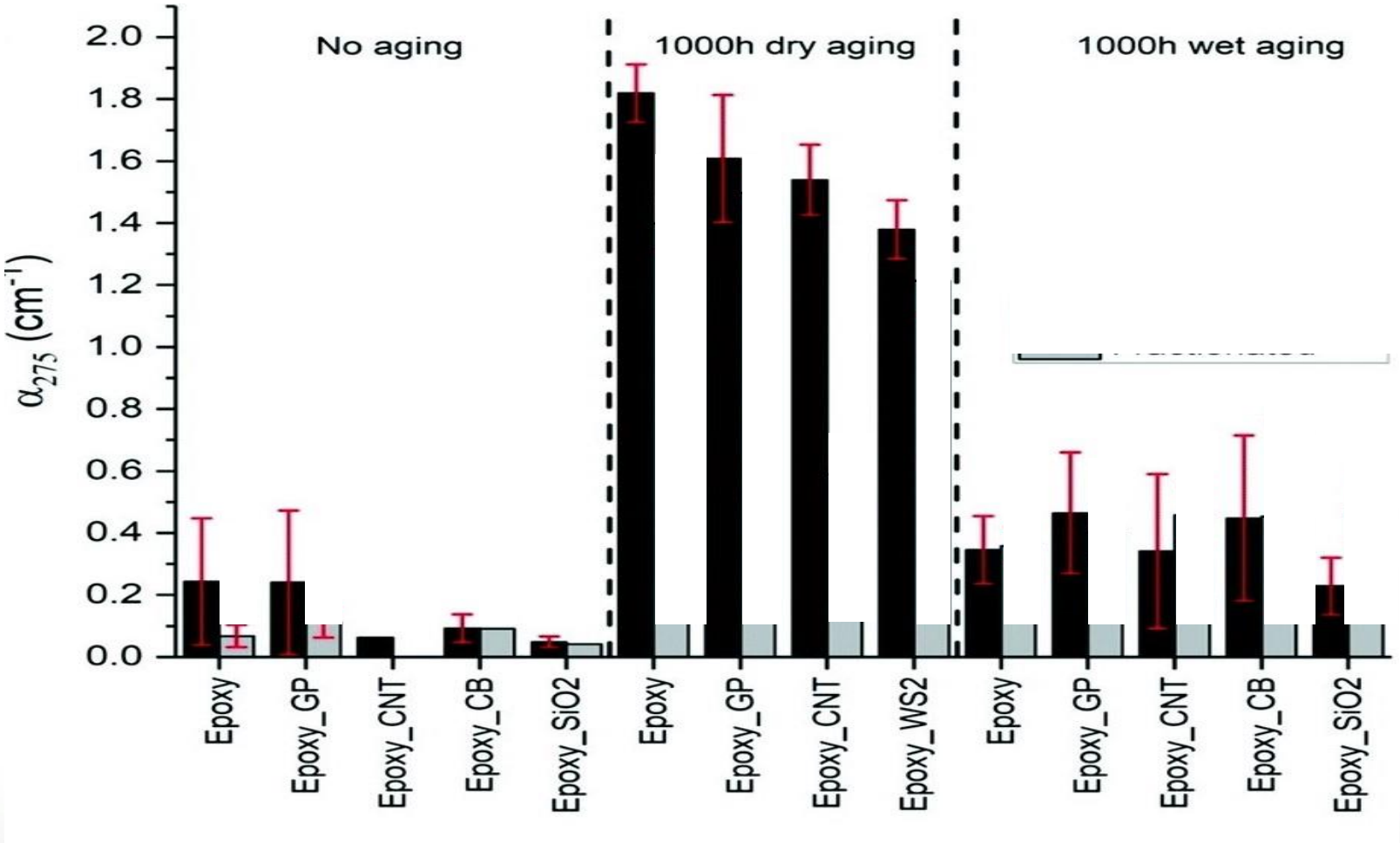


Characterize via:

- SEM / DLS / NTA
- pyro-GC-MS / µ-Raman / FT-IR

Development of Small Microplastics/Nanoplastics Methods

Measuring MP Weathering with UV-visible Spectroscopy



Zepp *et. Al*., Environ. Sci.: Nano, **2020**, *7*, 1742-1758

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Progress Research Effort 2:

Methods for Collection, Extraction and Identification of Nano- and Microplastics for Surface Water and Sediments

Build capacity in EPA labs nationwide for microplastics identification and quantification

- uFTIR : **Corvallis, Cincinnati, Athens:**
- Raman spectroscopy; **Narragansett, Athens:**
- Pyrolysis GC/MS: **RTP, Cincinnati, Narragansett**
- LDIR: **Cincinnati**
- Spectral Flow Cytometry, optical imaging, nanoparticle tracking analysis (NTA), flow field flow fractionation (AF4), SEM: RTP

Regional Applied Research Effort (RARE) in Conjunction with SSWR

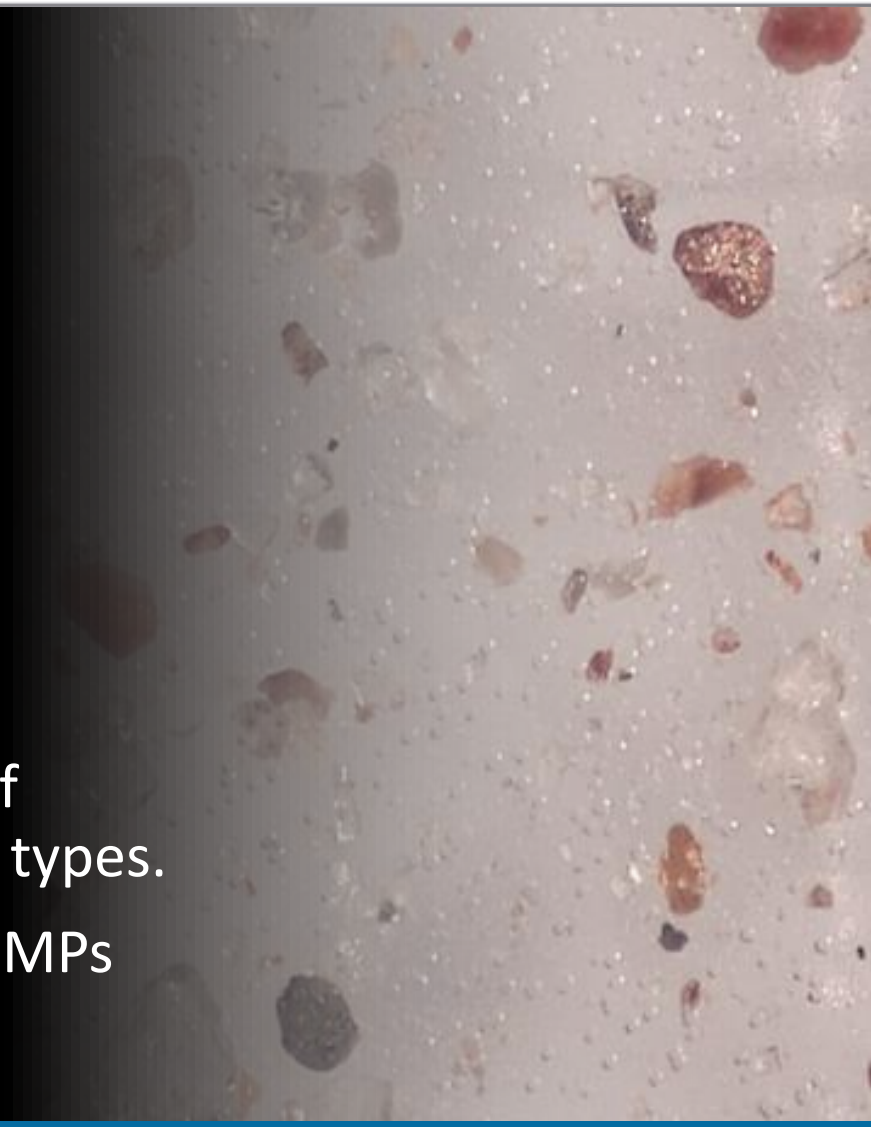
Quantification/Comparison of Sediment Extraction Methods

Background

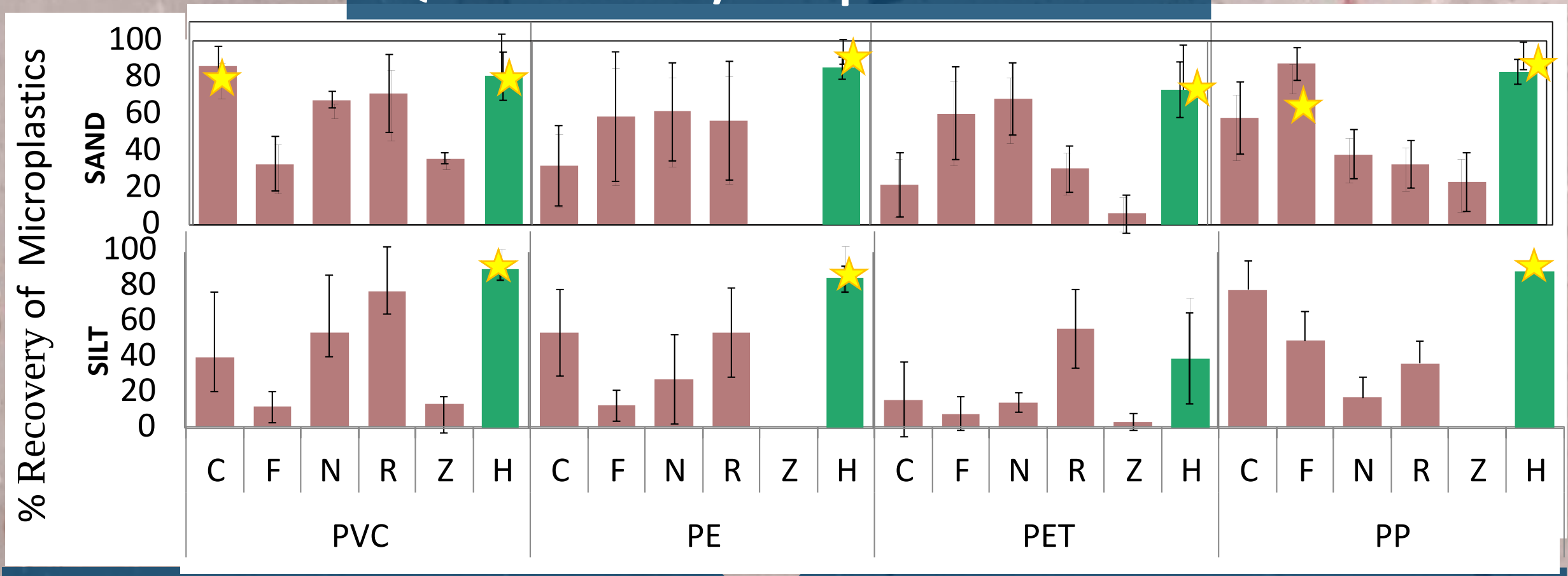
- Many existing methods, all different.
- Comparison of results among the methods is challenging.

Objective

- Assess 5 current methods for the extraction and isolation of microplastics from samples using 2 sediments and 5 plastic types.
- Based on initial findings, develop hybrid method to extract MPs from sediments.



Quantification/Comparison Results



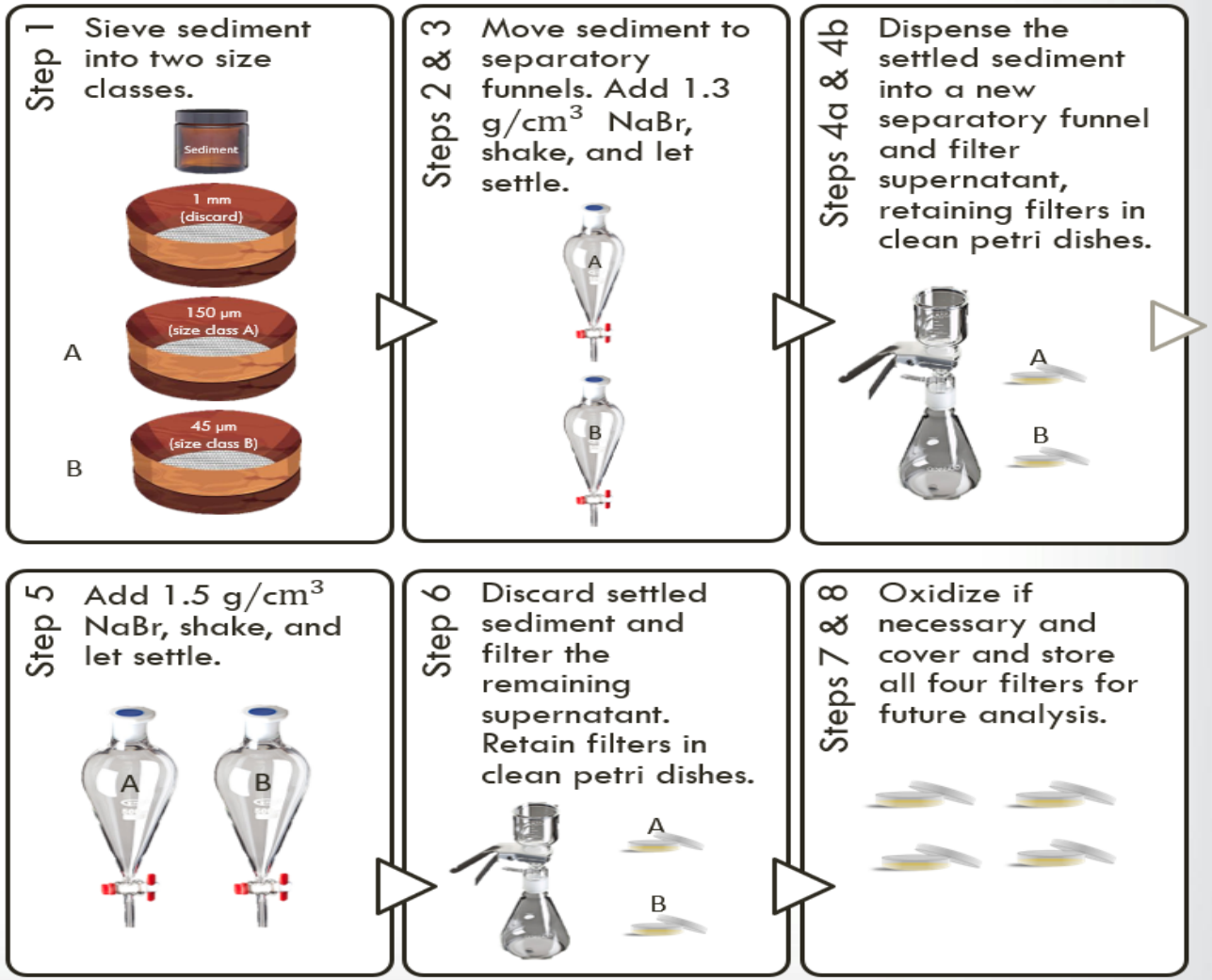
- No existing method consistently extracted >70% of each microplastic.
- Sediment, microplastics, and extraction methods all affect percent recovery.

- Hybrid method generally extracted >70% from both sediments and most microplastics.

Cashman, M. A., K. T. Ho, T. B. Boving, S. Russo, S. Robinson and R. M. Burgess (2020). "Comparison of microplastic isolation and extraction procedures from marine sediments." Marine Pollution Bulletin **159**: 111507.

Next Step: Hybrid Method

- Size fractionation
- 2 Density separations
- Oxidation
- 4 Filters / sample - Easier to identify each particle by raman spectroscopy
- Work with SCCWRP as part of an augmentation project for the round robin
- Use with NCCA samples



Regional Applied Research Effort (RARE) in Conjunction with ORD

Standardization of Water Methods via American Society for Testing and Materials (ASTM).

- Surface water methods for collection, extraction and identification are currently in ASTM sub-committee discussions.

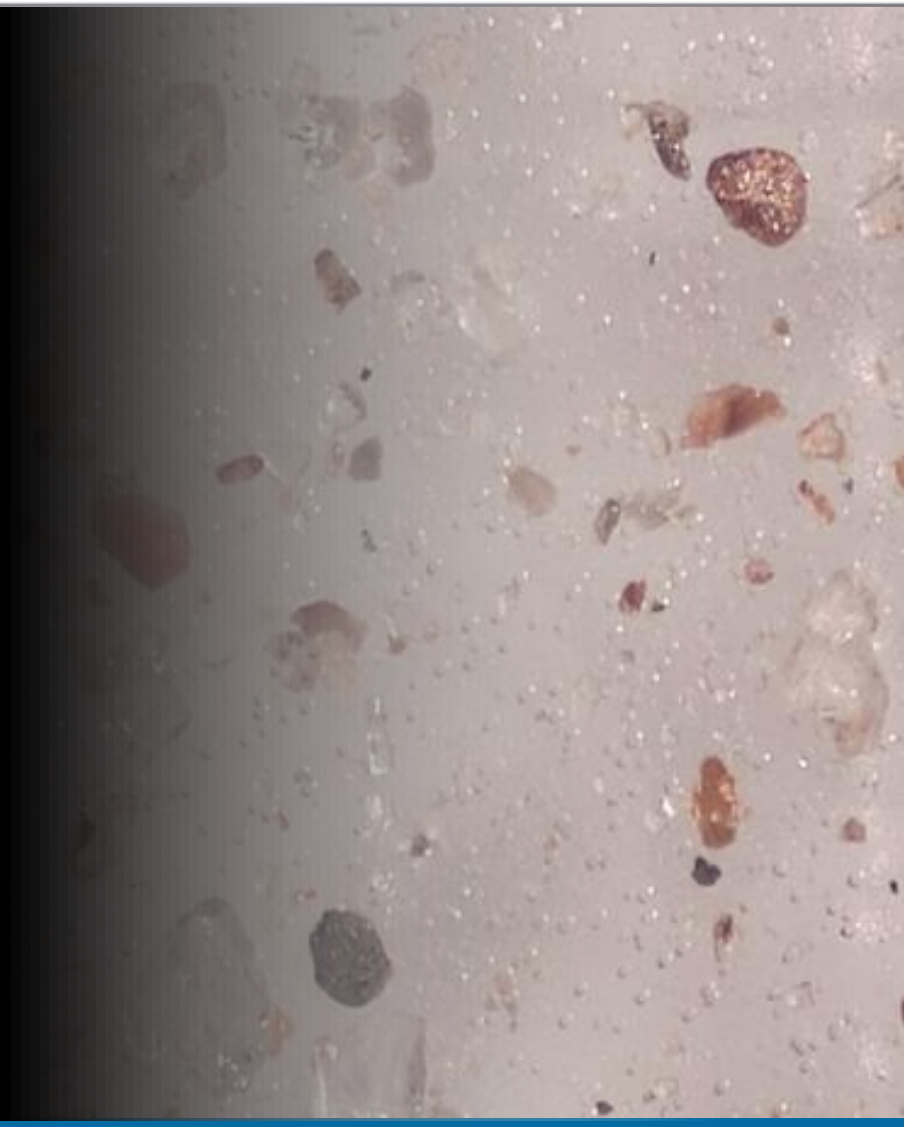
Image: USGS

Regional Applied Research Effort (RARE) in Conjunction with ORD

New RARE 2021 Microplastics Projects

- Assessing microplastics in freshwater urban watersheds Multi-regional surface water project. Work with hyperspectral cytometry and imaging at RTP.

- Combustion Alternative Treatment for Microplastics in the Environment (CAT ME) method for rapid determination of total plastics in sediments.



Citizen Science: Isn't there an easier way?

Citizen Science Techniques-Current methods for identifying plastics in sediments are time consuming, expensive and require a lot of training. Give polymeric identification.

- Provide types of plastics polymers, particle characterization

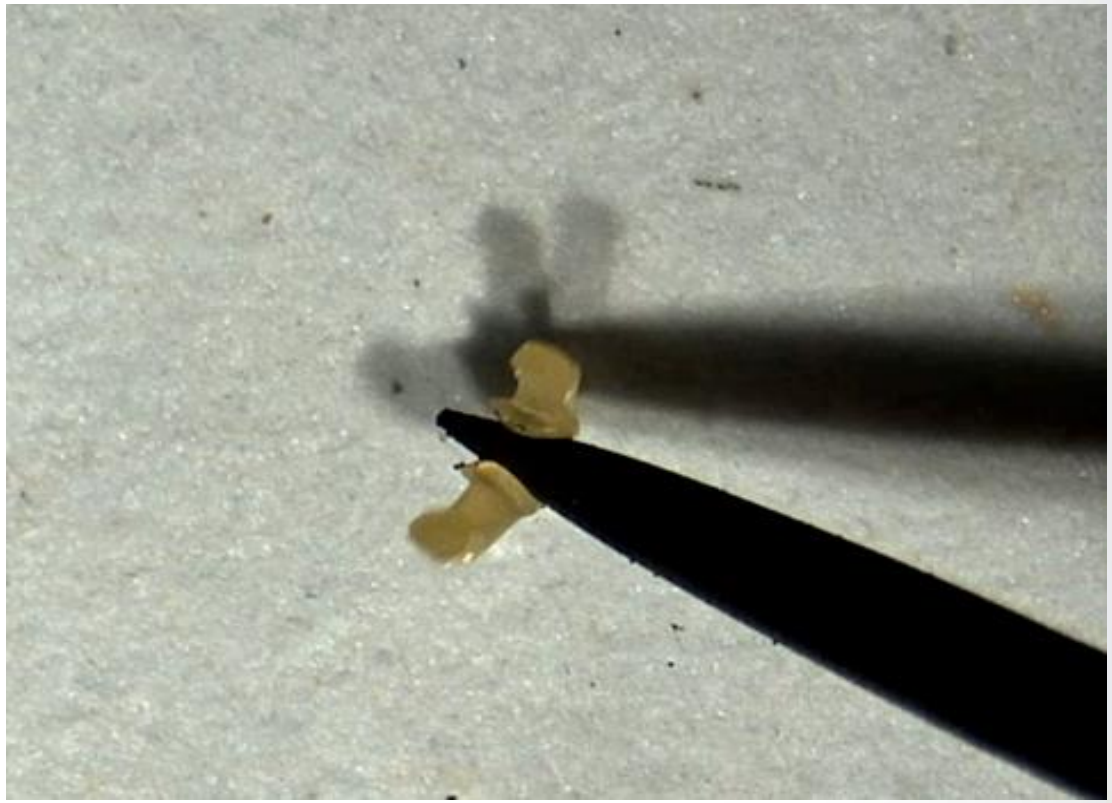
- Combustion Alternative Treatment for Microplastics in the Environment (CAT ME) method for rapid determination of total plastics in sediments - Total plastics by weight - Not polymer specific, no particle information
  - Loss on ignition method with low technology manipulations to separate plastic from non-plastic environmental particles
  - Provides total plastics (not polymer type)
  - Useful for screening, can identify areas of concern

Citizen Science: Isn't there an easier way?

Citizen Science Technique - Low technology, low cost, high speed method

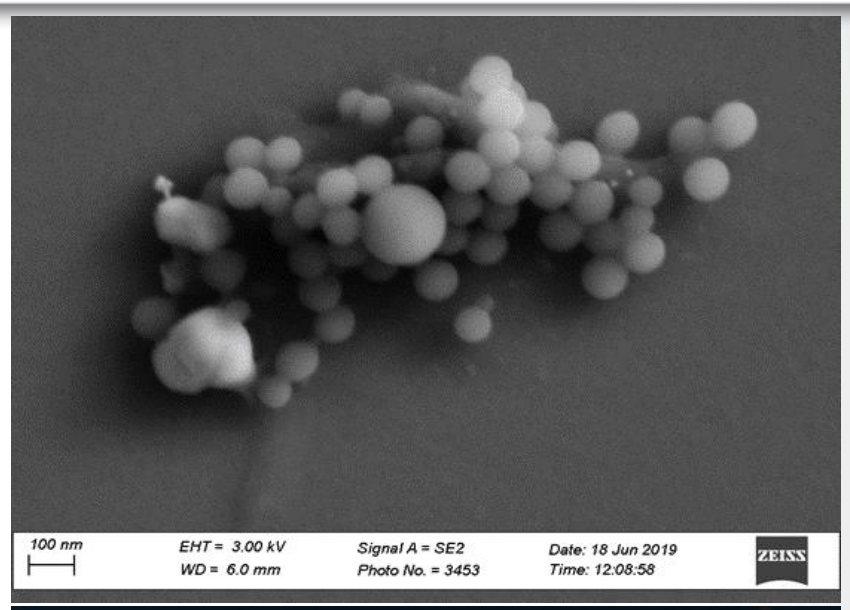
- Hot needle method
- Standardization and validation
  - ➢ Answers the question:

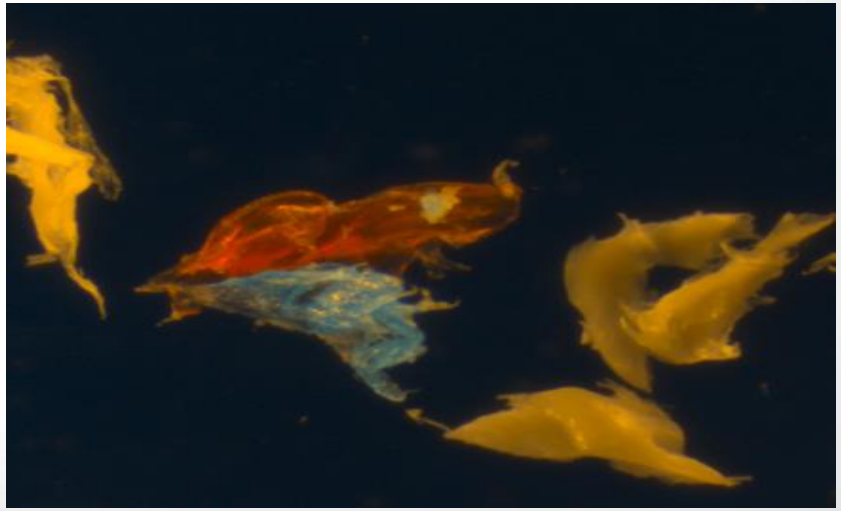
    " Is it a plastic?"
- Step-by-step on-line instructions, videos



Future Directions

- Continue method development, standardization process through publications, round robins, presentations, use with high profile samples (e.g., NCCA)
- Methods for smaller sized particles
- Move towards quantifying polymer concentration (pyrolysis GC/MS) rather than particle enumeration (spectroscopic methods (Raman, FTIR))
- Pyrolysis- polymer identification, nanoplastics, faster.
- Still need for particle characterization, early days!





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Questions?