Interim Report for Mitacs Accelerate Grant

Possible Methods for Detection and Removal of Microplastics Contamination in Municipal Compost

Overview:

Initial compost samples from Grassland Organics of one day at the facility have been collected. The same pile will be used for the duration of the experiment as it continues to go through the composting process.

Initial samples have been prepared and used for microscopy and spectroscopy analysis.

Sample Preparation:

Multiple different solvents were tested to dissolve the organic matter in the compost samples to isolate only the plastic fragments. First, all samples were dried at 60°C for about 24 hours.

Samples were then chemically digested in the four different solvents (100% ethanol, 99% isopropanol, 30% hydrogen peroxide and 10% potassium hydroxide) and allowed to digest for 24 hours and then centrifuged for one minute at max rpm. Some key points to consider for the future is when using KOH, digestion should not be longer than 48 hours or else the plastic components may degrade as well as the organic matter. The supernatant of the samples were used for microscopic imaging as well as UV-Vis analysis.

Fluorescent imaging uses Nile Red dye to stain the plastic fragments by binding to the hydrophobic plastic carbon chain. The fragments will absorb light at about 488nm and emit light at around 509nm giving the fluorescent glow. A drop of the liquid digested samples were placed on a microscope slide and heat-fixed through a flame. Then the Nile Red dye was added to the slide and allowed to dry. No rinsing of the dye was done to avoid any loss of the sample.

Another sample preparation technique was also tested. This technique involved grinding the samples after the drying processes. To grind the samples they were first sublimated using dry ice for 4-24 hours, then placed in a coffee grinder and pulsed until they were in a fine powder. These samples were not digested in any solvent listed above. Instead these samples were used for ICP-MS analysis.

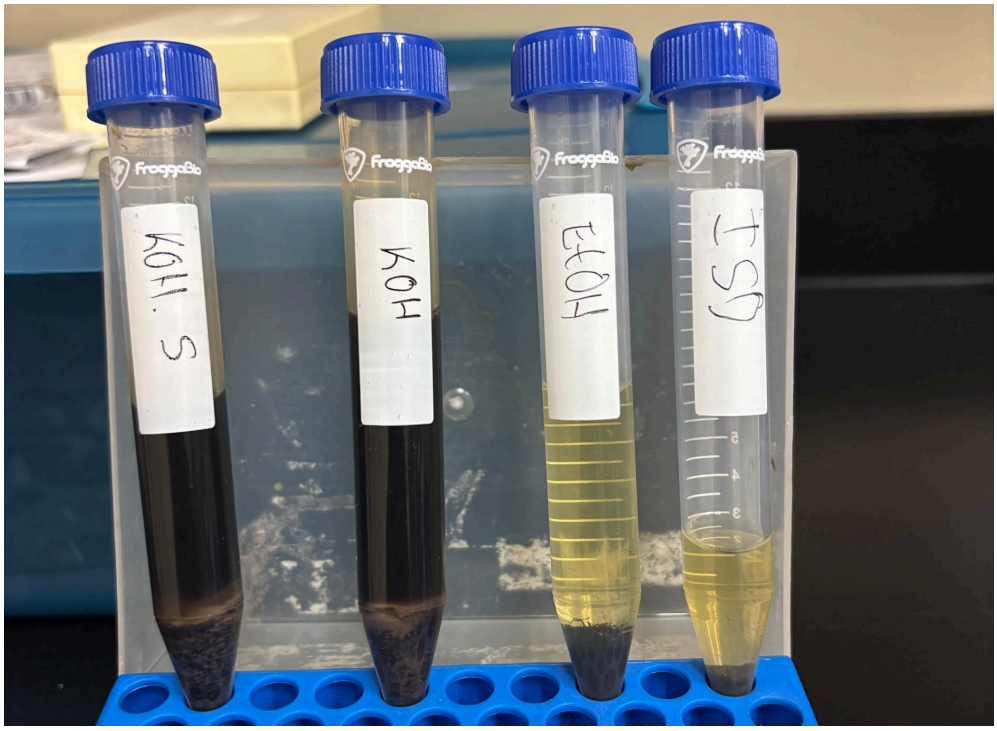

Figure 1. Supernatant used for fluorescent microscopy and UV-Vis.
The first vial on the left contains digested compost in 10%KOH and is sonicated before visualization. The second vial contains digest compost in 10% KOH, third vial is compost digested in ethanol, and the fourth vial is composted digested in isopropanol.

Microscopy Results:

- Different chemical degradation solvents were tested to obtain the best images
- Found that under fluorescens, 10% KOH gave the most clear images
- Under optical microscopy 30% hydrogen peroxide gave the most clear images
- Solvent that will be used for further microscopy will be 10% KOH
- Nile Red dye is used for fluorescent microscopy
- Image results suggest that microplastics are present in the compost
- Suspected microplastics have a bright green glow on the outsides of the plastic fragments
    - These results confer with other literature results (Tsuchiya, 2025)

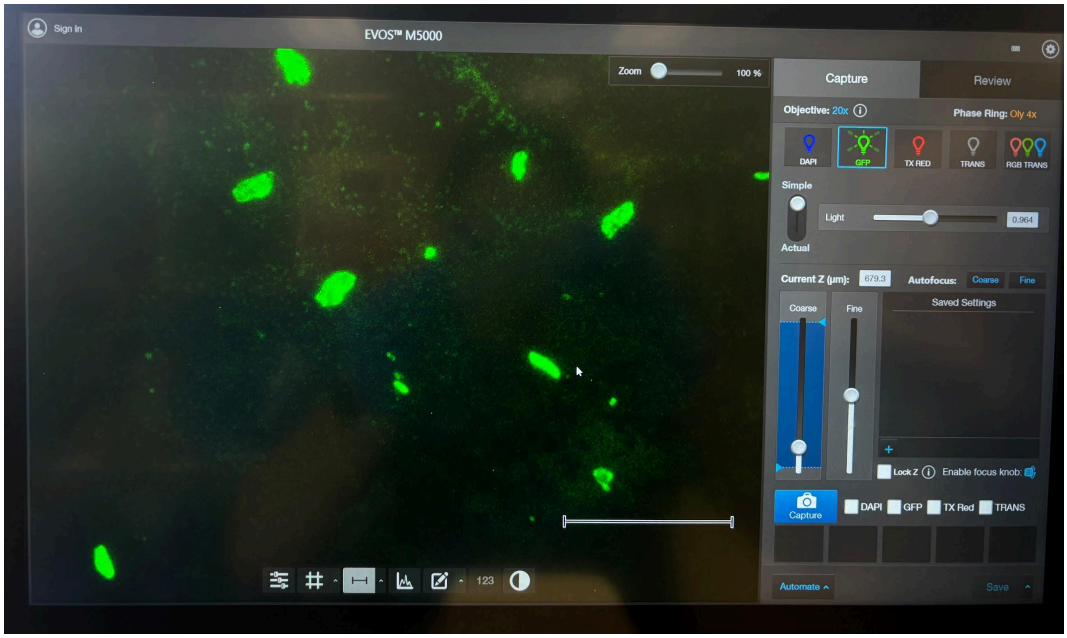

Figure 2. Fluorescent microscopy using Nile Red dye under Green Fluorescent Protein light using 4x magnification, digested with 10% KOH.
Green specks are suspected to be microplastic fragments.

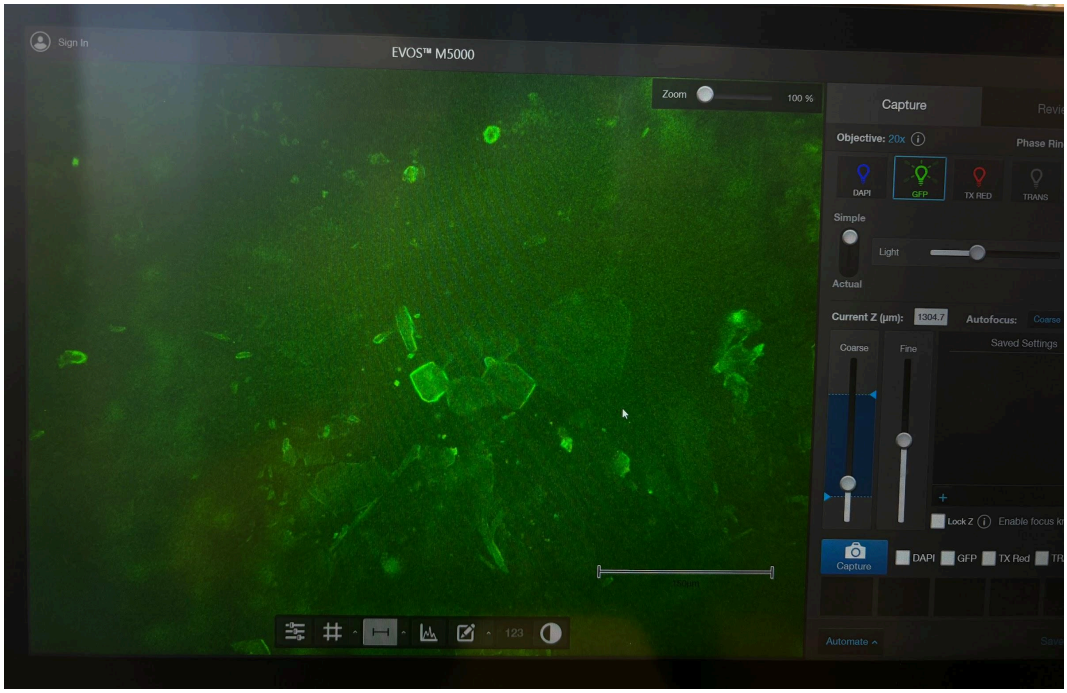

Figure 3. Stained microplastic fragments under x10 magnification of the fluorescent microscope, digested with 10% KOH.

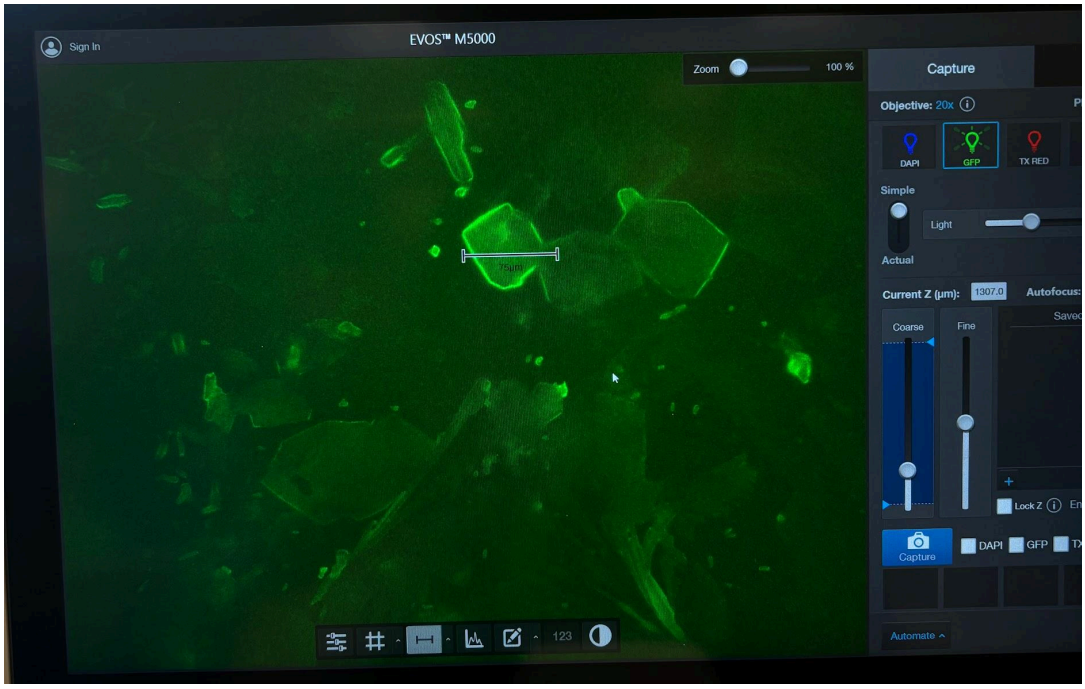

Figure 4. Stained microplastic fragments under x20 magnification of the fluorescent microscope, digested with 10% KOH..

Taken from the same spot as Figure 2. The fragment has a width of about 75µm.

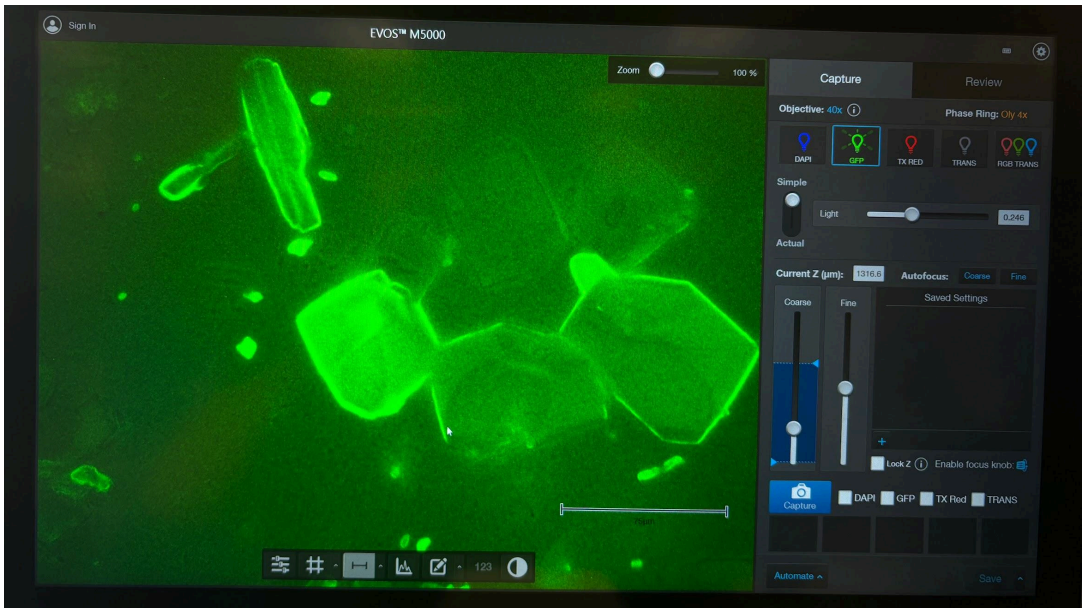

Figure 5. Stained microplastic fragments under x40 magnification of the fluorescent microscope, digested with 10% KOH.

Taken from the same spot as Figure 2 and 3.

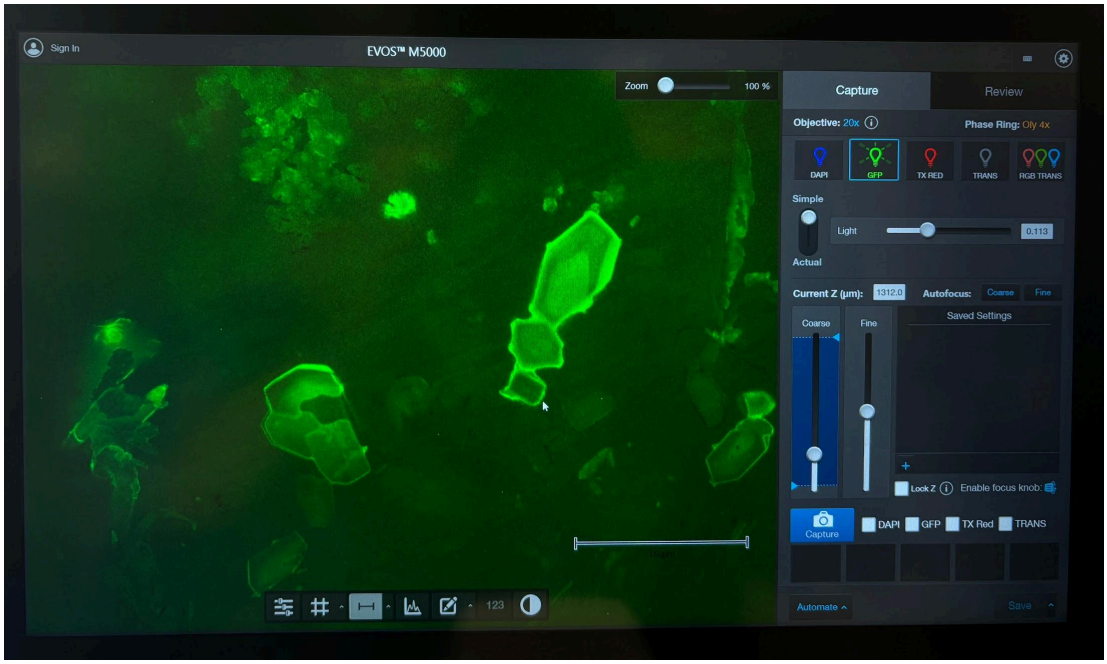

Figure 6. Stained microplastic fragments under x20 magnification of the fluorescent microscope, digested with 10% KOH.
Microplastics are outlined in green whereas the organic matter is filled in with the glowing green.

<u>Polypropylene standard microscopy results:</u>

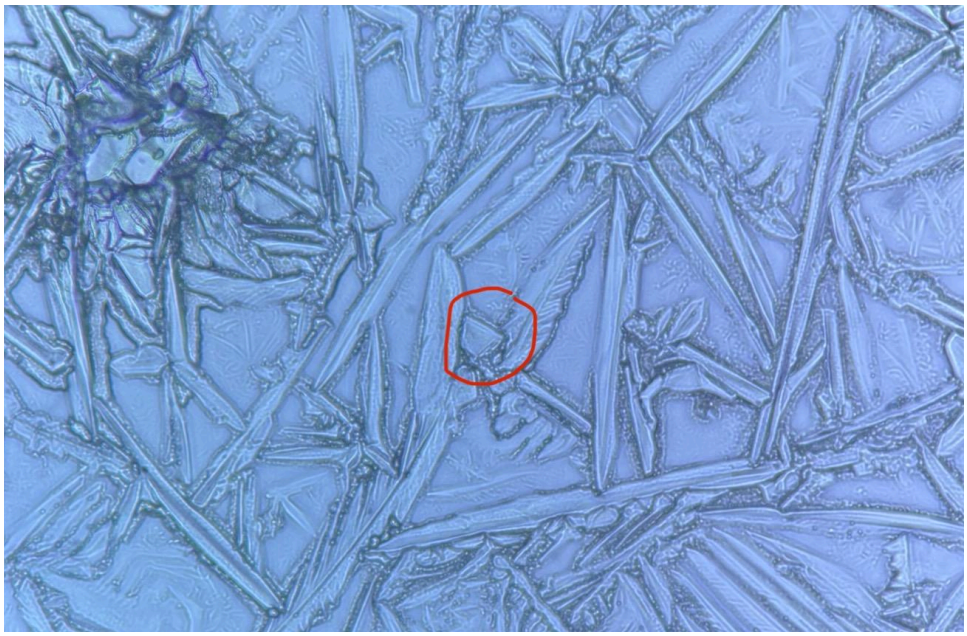

Figure 7. Optical microscopy of polystyrene standard diluted with KOH under 40x magnification.
Potential microplastic is circled in red.

Figure 8. Trans microscopy of polypropylene standard diluted with KOH at 40x magnification. *Potential microplastic is circled in red.*

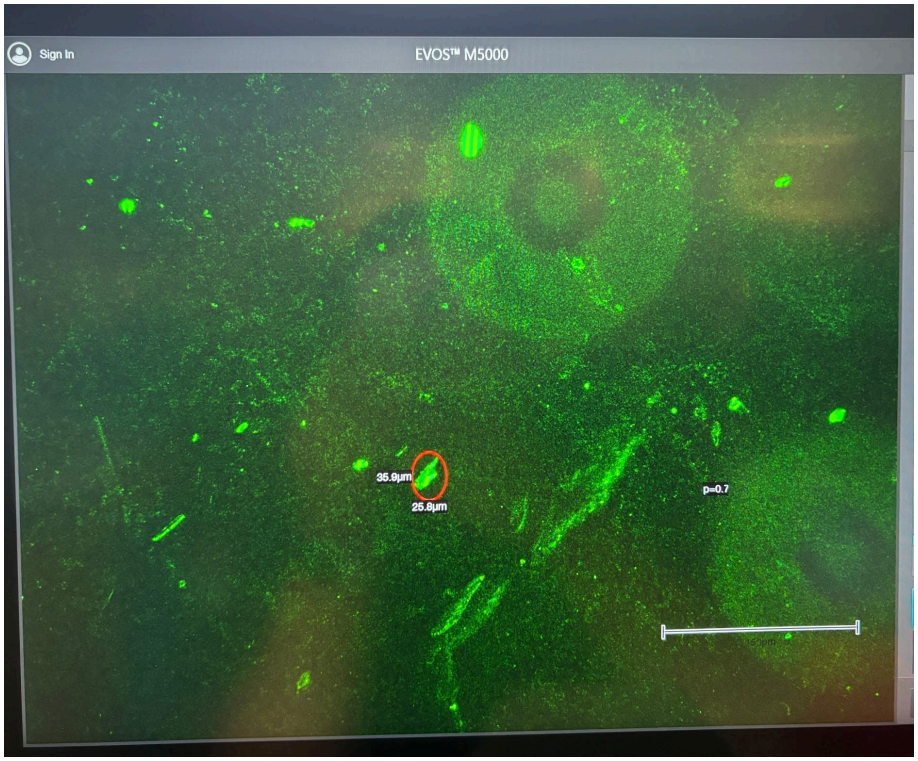

Figure 9. Fluorescence microscopy of a potential microplastics under x10 magnification. *Samples have been stained with Nile Red and the potential microplastic is circled in red.*

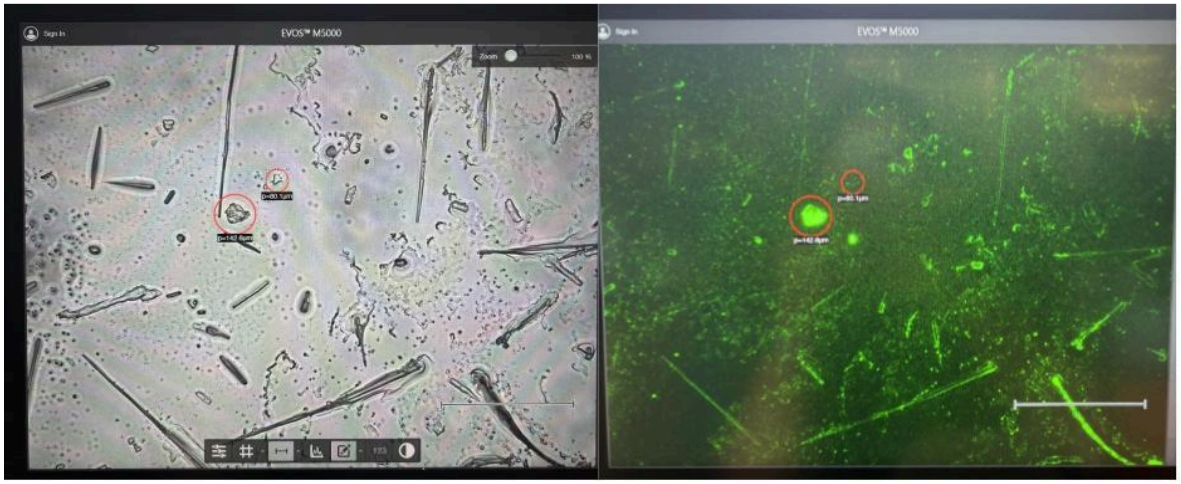

Figure 10. Fluorescence microscopy comparison of Trans and GFP fluorescent light under 20x magnification.
Circled in red are the two fragments under analysis. Both appear to fluoresce under the GFP light.

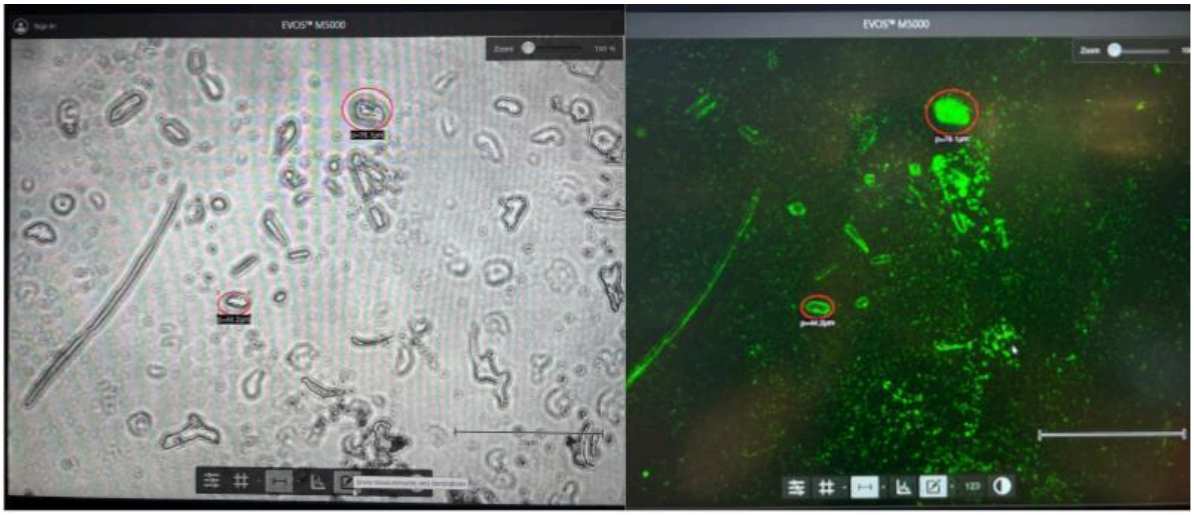

Figure 11. Fluorescence microscopy comparison of Trans and GFP fluorescent light under 40x magnification.
The left (Trans) photo includes red circles showing the fragment of interest. In the right (GFP) photo, the top red circled fragment is fully green, whereas the bottom red circled fragments are only outlined in the green.

Plastic Standards Sample Prep:

Six different types of plastic were bought from grocery stores that mimic plastic contamination that is common at the Grasslands Organics Composting facility. These six plastics are, hard plastic food container and lid, compostable plastic bags, plastic bottles and cap, mesh fruit/veggie bags, suran warp, and the inner plastic lining of Back to Earth composting bags. All

plastics used can be seen in Figure 12. To prepare these plastics for fluorescent microscopy and UV-Vis analysis they needed to be ground up into microplastics. Liquid nitrogen was used to flash freeze the plastic pieces, once frozen they were ground up in a coffee grinder until in small enough pieces. Plastic pieces were sieved through a 5mm sieve to ensure the pieces are microplastics in size. Microplastic pieces were then digested in 10% KOH for 24 hours the same as the compost preparation. After 24 hours samples were plated on a microscope slide and stained with Nile Red for fluorescent microscopy, as well as a small portion was used for UV-Vis.

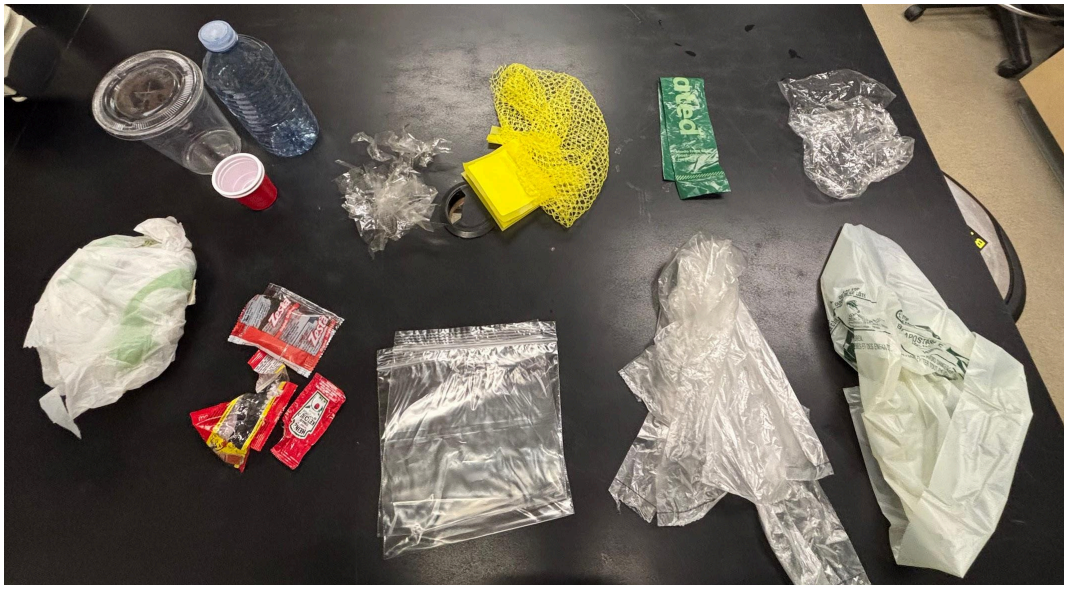

Figure 12. Hard plastic food container and lid, compostable plastic bags, plastic bottles and cap, mesh fruit/veggie bags, suran warp, and inner plastic lining of Back to Earth composting bags standards used for microscopy and spectroscopy.

Plastic Standards Florence Microscopy Results:

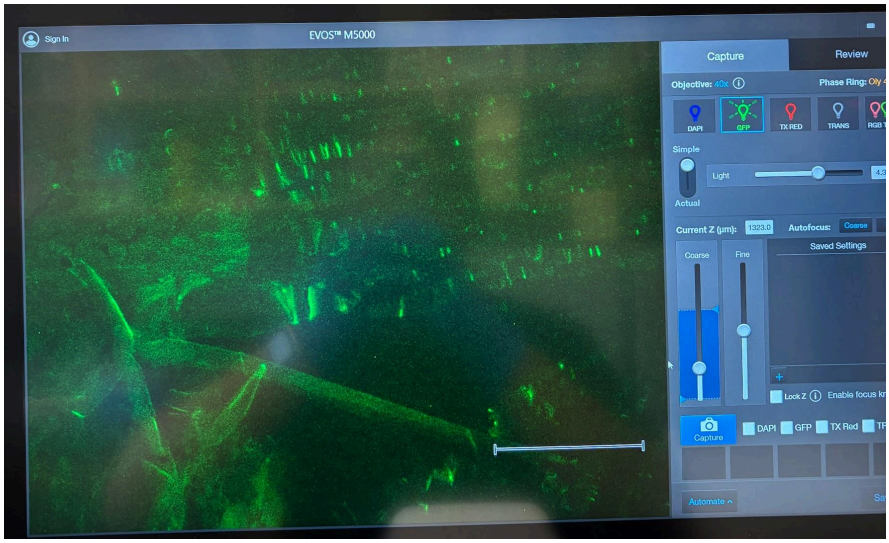

Figure 13. Fluorescent microscopy of hard plastic container and lid at 40x magnification.

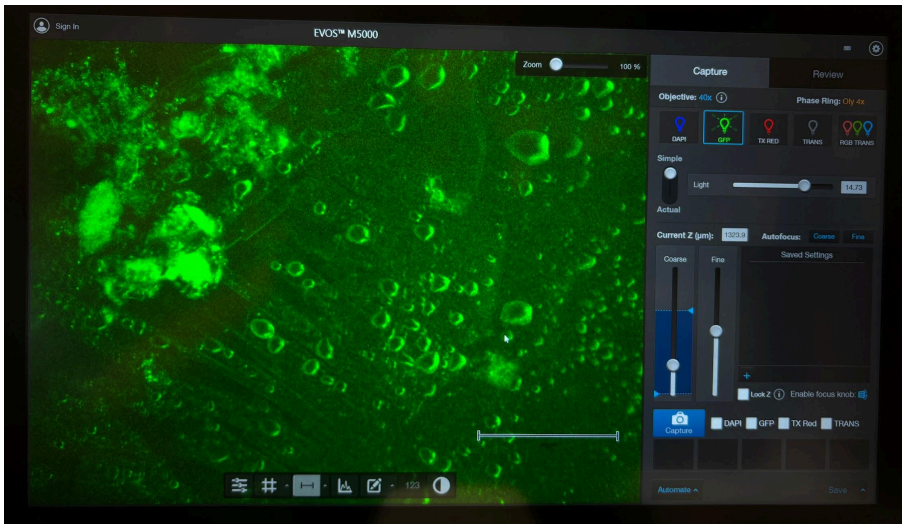

Figure 14. Fluorescent microscopy of compostable utensils at 40x magnification.

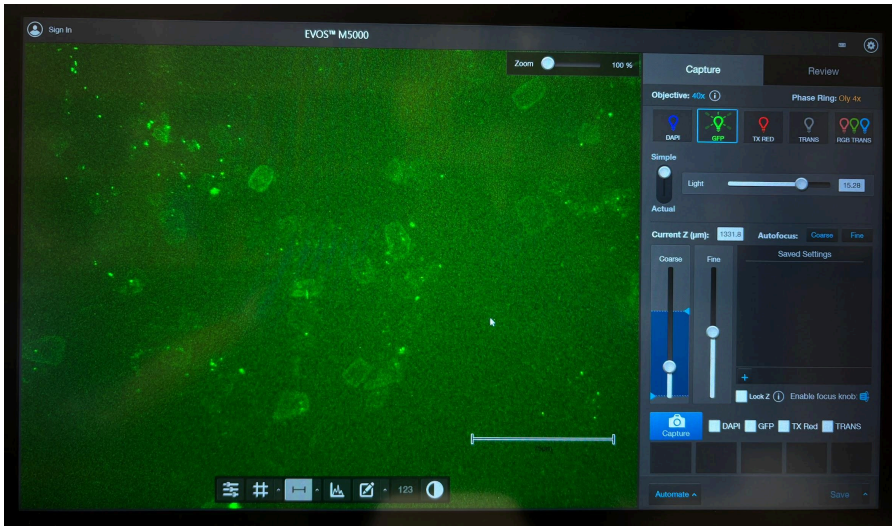

Figure 15. Fluorescent microscopy of compostable plastic bag at 40x magnification.

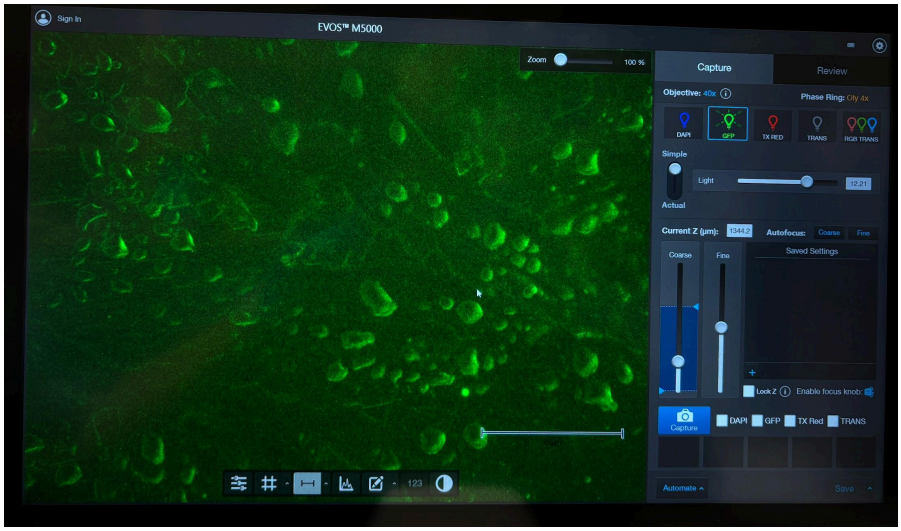

Figure 16. Fluorescent microscopy of saran wrap at 40x magnification.

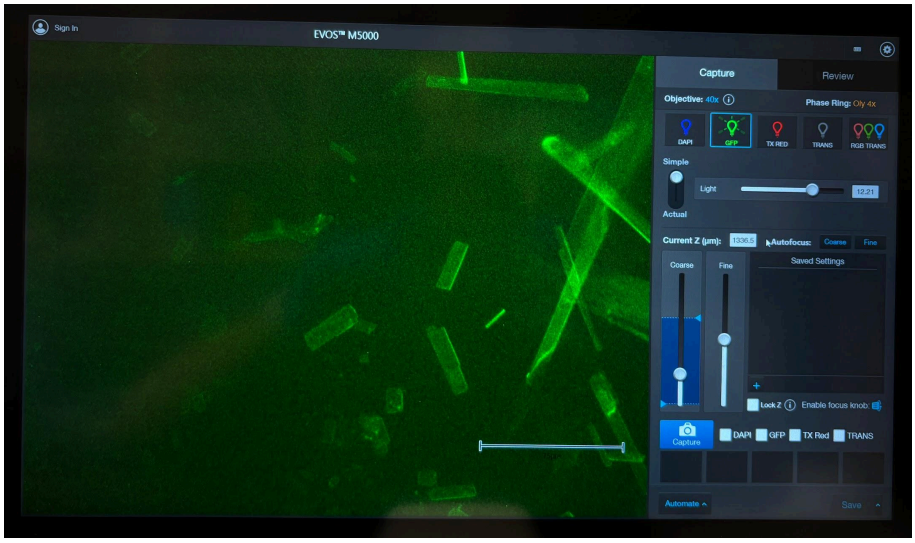

Figure 17. Fluorescent microscopy of plastic bottle and cap at 40x magnification.

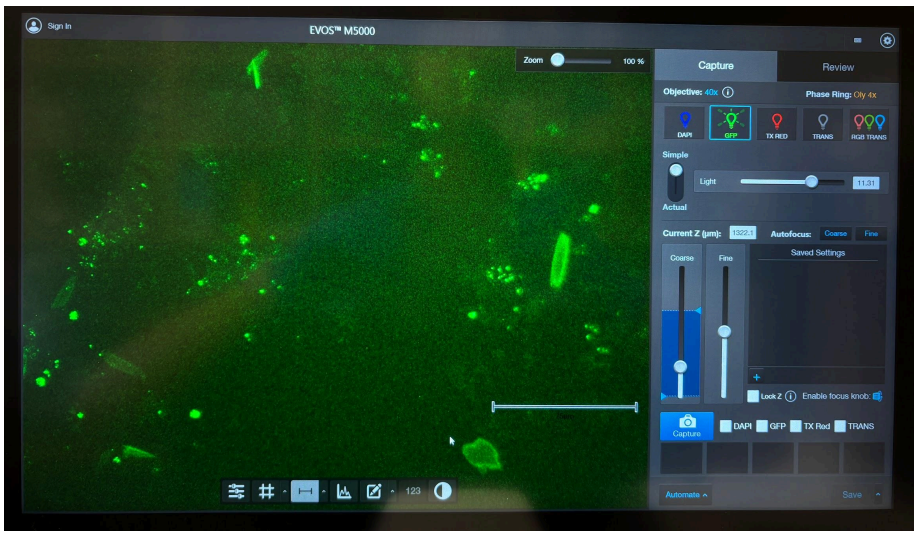

Figure 18. Fluorescent microscopy of inner lining of Back to Earth compostable bag at 40x magnification.

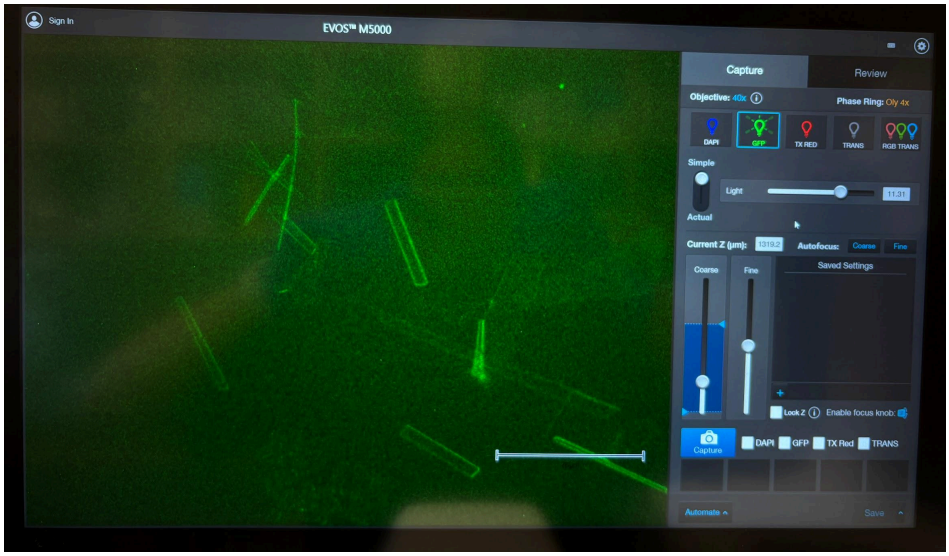

Figure 19. Fluorescent microscopy of mesh fruit bag at 40x magnification.

Summary:

Optical and fluorescent microscopy has been completed on compost and polypropylene where microplastics fragments have been identified. Under fluorescent microscopy after Nile Red staining, microplastics are outlined in a green glow, whereas excess organic matter is fully stained green.

Spectroscopy Results:

UV-Vis:

- UV-Vis was run on all four different test solvents for organic matter degradation
- Peaks were found for all samples and will be compared to plastic samples to identify if what is in the compost plastic or not

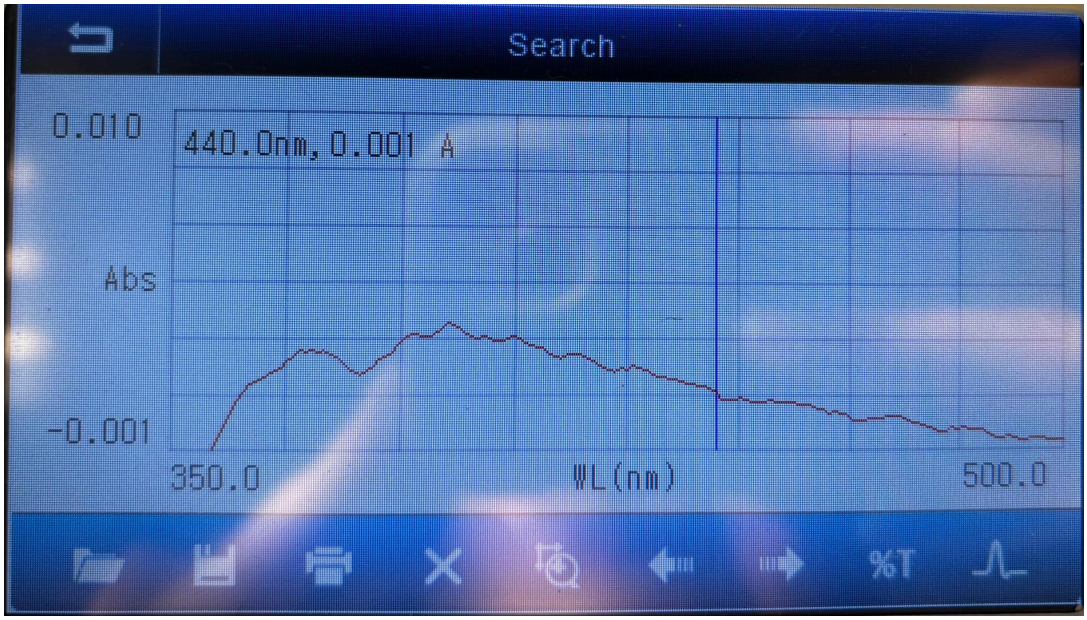

Figure 20. UV-Vis results of polypropylene standard diluted with KOH. Wavelength ranges from 350nm to 500nm.

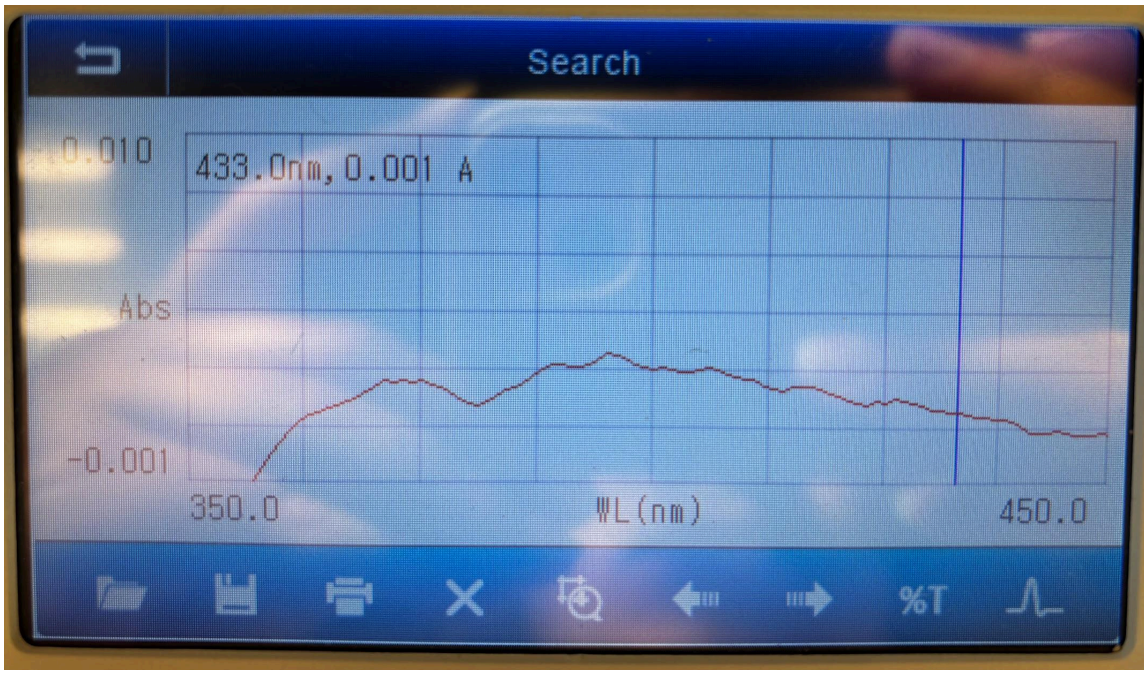

Figure 21. UV-Vis results of polypropylene standard diluted with KOH. Wavelength ranges from 350nm to 450nm.

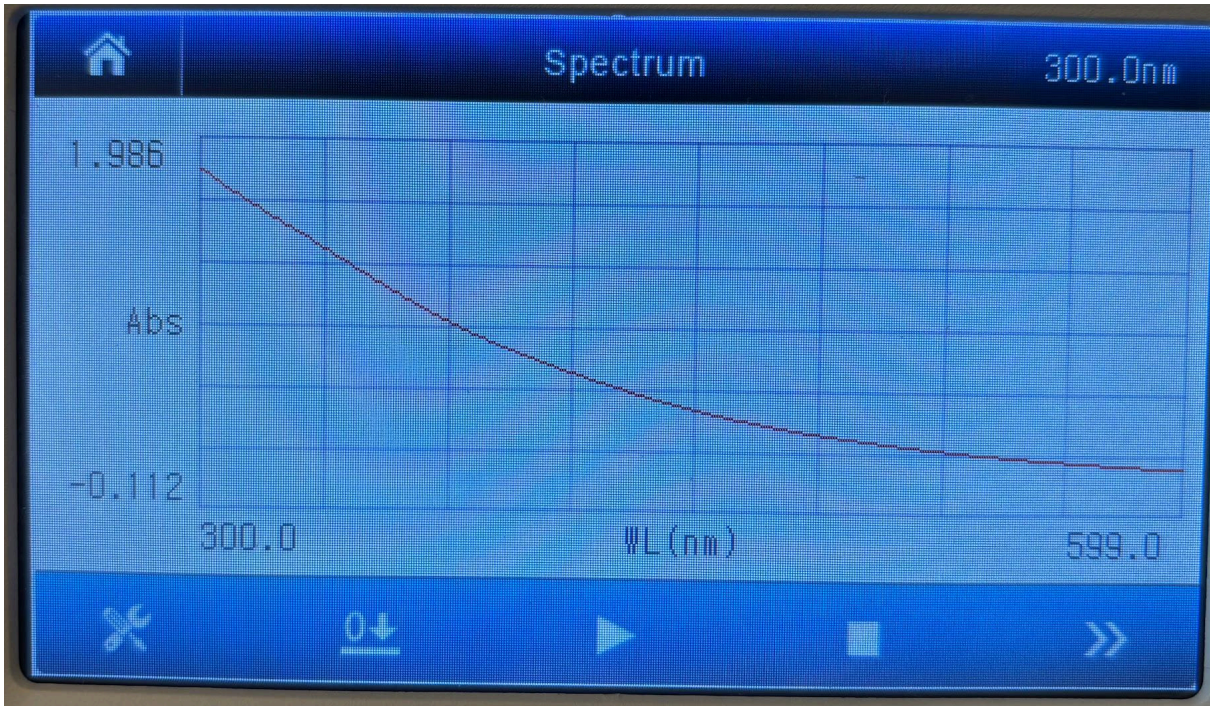

Figure 22. UV-Vis results of initial compost samples digested in KOH. Wavelength ranges from 300nm to 599nm.

Summary:

Initial readings have been collected. Samples will be run again to confirm any comparison in wavelength of the polypropylene standards and the compost samples. In addition, other plastics types will be tested and used to compare wavelengths to the compost samples.

ICP-MS:

- Samples have been dried, sublimated (dry ice), and ground up
- Samples have been digested for ICP-MS analysis
- Samples will be tested on September. 3

Next Steps:

- Run ICP-MS samples to get a chemical analysis
- Grind up plastics to use as baseline data for ICP-MS and UV-Vis
- Redo fluorescence microscopy and UV-Vis using the same dry ice method as the ICP-MS samples
- Try preparing samples using the same method but without drying the samples first to enhance the dry ice process

References:

M, Tsuchiya. (2025). Rapid detection and quantification of Nile Red-stained microplastic particles in sediment samples. *National Library of Medicine.* doi:10.7717/peerj.19196. https://pmc.ncbi.nlm.nih.gov/articles/PMC11967427/.