

INTRODUCTION

Microplastics (MPs) in waterbodies can negatively impact the health of all life. Evidence suggests that they especially cause damage to aquatic life. Unfortunately, MPs are found in marine systems, and as far as land-locked freshwater bodies. They have the ability to absorb toxins, act as sharp objects that scratches or punctures internal organs of aquatic organisms, and cause changes in metabolic functions¹. MPs range between 0.001mm to 5mm. They are either categorized as primary or secondary. Primary MPs are engineered plastics found in detergents, clothing, cosmetics, and more². Secondary MPs are those that have decomposed from other plastic sources such as litter³.



Figure 1. Types of Microplastics⁴

BACKGROUND

Most wastewater effluent is discharged into a freshwater streams where it is eventually treated for drinking water. Approximately 90 percent of MPs are found in wastewater sludge⁵. While observed that most MPs are removed in wastewater treatment processes, there remains a significant percentage of MPs in treated effluent. As noted by Pivokonsky et al., 2018, upwards of 600 particles/liter of MPs were found in treated wastewater. Moreover, the quality of most MP research is highly variable due to the lack of standardized analytical methods⁶. Therefore, it is crucial to develop standard methods to quantify and characterize MPs discharged from wastewater treatment plants (WWTPs).

RESEARCH QUESTIONS

- Which sampling method is better for collecting samples in wastewater treatment plants?
- Which method is most efficient in processing samples?
- Do MPs quantity and type differ among WWTPs receiving raw wastewater from different contributing service areas and using different treatment processes?

METHODOLOGY

I. Literature Review

- Completed literature review on sampling collection methods in order to determine which methods would be used for this project.

Sampling Technique	Number of Sources
Grab	7
Composite	9
Pump Filtration	5
Net Filtration	3

- Completed extensive literature review of **25 articles** to develop sample processing methods.

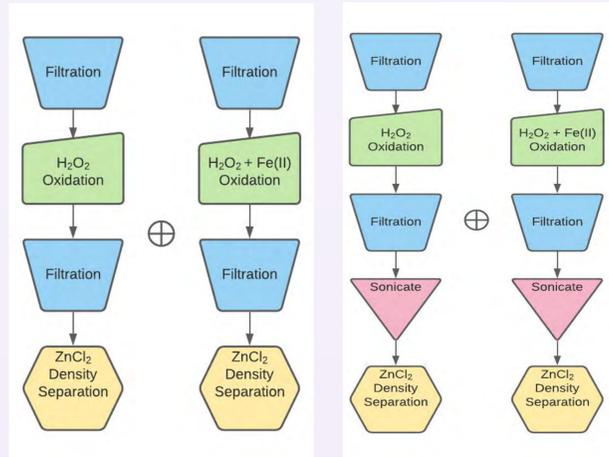


Figure 4. Proposed Sample Processing Methods

II. Reconnaissance of WWTPs on 07/22/2021

- Reconnaissance of Livingston and Cookeville's influent and effluent locations were accomplished to better prepare for future sampling.



Figure 5. Livingston, TN - Wastewater Influent (left) and Effluent (right)

RESULTS

I. Collection Methods

Grab, composite, and pump filtration were selected to gain representative data on efficient collection methods.



- Glass carboys were used to retain samples from grab, composite, and pump filtration.

Figure 6. AI foil cap With rubber stopper
Figure 7. Cleaned Carboys

- **Grab** sampling entails lowering a 15-gallon metal bucket connected to a stainless-steel chain into wastewater influent and effluent. Immediately transfer contents to glass carboy.
- **Composite** sampling was accomplished through two methods due to treatment plant restrictions.
 - **Method 1** (Influent): ISCO composite sampler, provided by Water Center, was deployed and collected 205 mL samples into a metal bucket every 30 minutes for 24 hours.
 - **Method 2** (Effluent): composite sampler, provided at location, took flow-based samples for 24 hours.
- **Pump filtration** device was built in-house. Sampling consists of pumping sample water through internal canister filters.



Figure 9. Devices used for collecting samples

II. Sampling of Wastewater Treatment Plants

- Grab and composite samples at the influent and effluent were collected on Friday, July 30, 2021, from Cookeville's WWTP.
- A 2L control was placed near the influent sampling location while a 20L control was centrally located.

Cookeville Sampling (07/30/2021)	Influent			Effluent		
	Grab #1	Grab #2	Composite	Grab #1	Grab #2	Composite
Carboy Volume (L)	Volume of Sample Collected (L)					
2	2.0	2.0	NA	2.0	2.0	NA
10	10.4	10.0	NA	10.0	10.3	NA
20	>20.0	>20.0	9.0	20.5	20.0	17.3

III. Sample Processing

- 1. Filtration:**
 - Phase 1:** Samples were filtered through a series of three sieves to remove larger materials creating four size fractions- the largest being 2.73mm.



Figure 10. Set of Sieves

Figure 11.

Phase 2: Once retained materials were collected from the sieves, each size fraction was filtered through a 10µm stainless steel wire cloth filter, **Figure 11**.

- 2. Oxidation** using 30% Hydrogen Peroxide for 24 hours at 50°C would ideally separate MPs from organic material in the sample matrix.

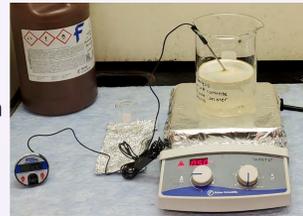


Figure 12. Oxidizing Sample

- 3. Filtration:** in preparing slides for analysis, the sample was filtered through a 0.2µm Membrane filter. By doing this, excess water was removed which allows for easier staining.

- 4. Preparation:** to prepare sample for analysis, a Nile Red dyeing mixture was used to stain the MPs.

Figure 13. Filter Tower

Figure 14. Nile Red Mixture

- 5. Analysis** using Epifluorescence Microscopy and Fourier Transform Infrared Spectroscopy will be used to quantify and identify, respectively, MPs in the sample.

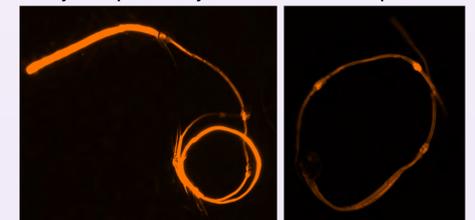


Figure 15. Potential MPs

CONCLUSIONS & FUTURE WORK

Through literature review, it is to be expected that the quantity and type of MPs in wastewater varies based on different contributing service areas and by using different treatment processes; however, additional work is necessary in order to make that determination. As samples are processed and analyzed, there will be a better understanding of the impacts of processing methods on MPs in samples. Therefore, additional sampling will take place at the Cookeville and Livingston WWTPs.

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