Contract No:

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Disclaimer:

LDRD-2020-00080
LDRD External Report Summary

Title of Project
Analysis of microplastics in bivalves along Fourmile Branch

Project Start and End Dates
Project Start Date: June 1, 2021
Project End Date: September 30, 2021

Project Highlight
Water and bivalves were collected at Fourmile branch sampling locations along the length of the branch to evaluate the formation of microplastics from site operations. Fourmile Branch is a site stream with historical and current industrial effluent from site operations and receives effluent from the site's wastewater treatment facility. This research is the first to explore microplastics environmental transport on the Savannah River Site, allowing SRNL to expand capabilities to a new field that is expanding as the deleterious effects of plastics on the environment is becoming more apparent.

Project Team
Principal Investigator: Wendy Kuhne
Team Members: Grayson Walker, George Larsen, Kaitlin Lawrence, Joseph Mannion, Heather Brant
External Collaborators: None

Abstract
Microplastics are commonly found near wastewater treatment facilities with the source originating typically from fibers associated with laundry detergents. Fourmile branch would have limited laundry associated effluent and does not receive any input from water originating from upstream industry sources, therefore microplastics would have originated from Site operations or through atmospheric deposition which are both unexplored pathways. In order to assess environmental inventory effects on the biota, water samples were collected from sampling locations along Fourmile Branch on the Savannah River Site using plankton nets and grab samples at Fourmile Branch locations (i.e., FM-2B, FM-A7, and FM-6). Fourmile Branch has a long history of receiving industrial effluents from site operations as well effluent from the site's wastewater treatment plant. Water samples and the debris collected in the nets were rinsed with deionized water and sieved to remove the larger fractions of plastics (4000-2000 µm) and retain fractions <500 µm. The water samples were analyzed for the type and size of plastics by µ-Raman, mass spectrometry, and Fourier Transform Infrared Spectroscopy (FTIR) methods. Previously collected bivalves were prepared into thin sections and analyzed using microscopy.

Objectives
- Develop a method to analyze and measure microplastics from SRS streams
- Analyze surface water samples collected along Fourmile Branch using plankton nets for the presence of microplastics.
- Analyze the tissue of previously collected bivalves from Fourmile Branch for microplastics taken into organism.

REVIEWS AND APPROVALS

1. Authors:

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Name and Signature Date

2. Technical Review:

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Name and Signature Date

3. PI's Manager Signature:

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Name and Signature Date

4. Intellectual Property Review:

This report has been reviewed by SRNL Legal Counsel for intellectual property considerations and is approved to be publicly published in its current form.

SRNL Legal Signature

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Name and Signature

Introduction

Plastics in the environment are recognized as a global environmental health problem. The majority of our daily one-use items (cups, food containers, and drink bottles) are made of monomers that are NOT totally biodegradable. Many of these items are not involved in recycling programs and end up in sanitary landfills or discarded to the local environment. Over time, these materials end up fracturing through processes of photodegradation and physical abrasion into smaller and smaller size fractions, going from macro scale (>5 mm)[1] to microplastic (1 µm to 5 mm)[2] and finally to nano-size particles (<1 µm)[3]. These particles are often referred to as micronizing plastics.

Fourmile branch (FMB) is a tributary on the upper coastal plain of South Carolina and historically received thermal effluent from C Reactor on the Savannah River Site from 1955 to 1985. It also received discharges from the F- and H-area Chemical Separations facilities and today from the Site's only sanitary wastewater treatment facility. To our knowledge levels of microplastics in SRS Site streams have not been investigated. This study would be the first to evaluate microplastics in FMB along three locations representing a clean "background" location (FM-2B), just below the wastewater treatment facility (FM-A7), and furthest away from site operations, but prior to entering the Savannah River (FM-6) (**Figure *1***).

Microplastics were evaluated in surface water collected from the locations using grab samples and plankton nets. Sieving was performed to remove larger fractions (4000-2000 µm) and to retain fractions <500 µm. Bivalves that had been collected previously were investigated for levels of microplastics in the tissue at these same locations (**Figure 2**). Both water and biota tissue samples were analyzed by microscopy and spectrometry (micro-Raman, FTIR, mass spectrometry) techniques to determine quantity, size, and type.

Approach

1) **Determine levels of microplastics in water collected from FMB**. Microplastics will be evaluated in previously collected surface water samples along the FMB (i.e., FM-2B, FM-A7, and FM-6). A 10 L grab sample and plankton nets were deployed to the main channel of the stream for collection times of ~4.5 h.
2) **Determine levels of microplastics in archived bivalves from FMB**. Filter feeder organisms are proving to be a valuable resource for understanding MPs in the environment. Histological sections of the Bivalve tissue were made and submitted for micro-Raman analysis.
3) Recovered microplastic particles from both water and bivalves were analyzed using bright field microscopy and spectrometry (micro-Raman, FTIR, mass spec) techniques to determine quantity, size, and type.

Accomplishments

- Water samples were collected at each of the three locations for an average collection time of ~4.5 h. The flow rate of the mainstream channel ranged from 1909 gallons per minute (gpm) at FM-2B, the upper most control location, to 7371 gpm at FM-A7, and finally a peak flow of 9777 gpm at FM-6 (**Figure 1**).

- Bivalve histological sections (**Figure 2**) could not be evaluated using micro-Raman due to intense background fluorescence levels.
- Polyamide bead standards were measured with micro-Raman for single particle measurements (**Figure 3**)
- Fluorescence microscopy was used with dye tagged polymer beads to investigate the utility of fluorescence microscopy for polymer imaging (**Figure 4**)
- Plastic particulates were found in the streams and FTIR was used to identify their chemical composition (**Figure 5**)
- Water samples were oxidized to remove biological material then filtered and imaged using bright field microscopy (**Figure 8** – **Figure 10**)
- Two-dimensional gas chromatography coupled with mass spectrometry (GCxGC-MS) data was measured using sorbent stir bars to identify the chemicals present in the water to help identify the types of plastics and chemicals found in the water streams (**Figure 6** and **Figure 7**). Chemical compositions were compared to a NIST library for positive chemical identification. Different styrene-based fragments were measured in FM-A7 (**Figure 7**). The sampling location for FM-A7 is near a roadway, where tire treads are one possible source of the styrene. The sample nets are made of nylon, the floats attached to the net and the grab sample carboy are both made of polyethylene.

Future Directions
Members of Atmospheric Technologies will continue to explore funding opportunities to investigate transport of microplastics through atmospheric pathways and levels of microplastics in other site streams.

FY 2021 Peer-reviewed/Non-peer reviewed Publications
None

Intellectual Property
None

Total Number of Post-Doctoral Researchers
None

Total Number of Student Researchers
None

References

1) Lebreton, L., Egger, M. & Slat, B. A global mass budget for positively buoyant macroplastic debris in the ocean. *Sci. Rep*. 9, 1–10 (2019).
2) Peeken, I. et al. Arctic sea ice is an important temporal sink and means of transport for microplastic. *Nat. Commun*. 9, 1505 (2018).
3) Wagner, S. & Reemtsma, T. Things we know and don't know about nanoplastic in the environment. *Nat. Nanotechnol*. 14, 300–301 (2019).

Figure 1. (Left) Fourmile Branch location FM-2B. Upper most location with the lowest flow rate 1909 gpm. (Right – Top) Location FM-A7, below the wastewater treatment facility with a stream flow with flow rate of 7371 gpm. (Right – Bottom) Location FM-A6 furthest location downstream before entering the Savannah River. Flow rate measured at 9777 gpm.

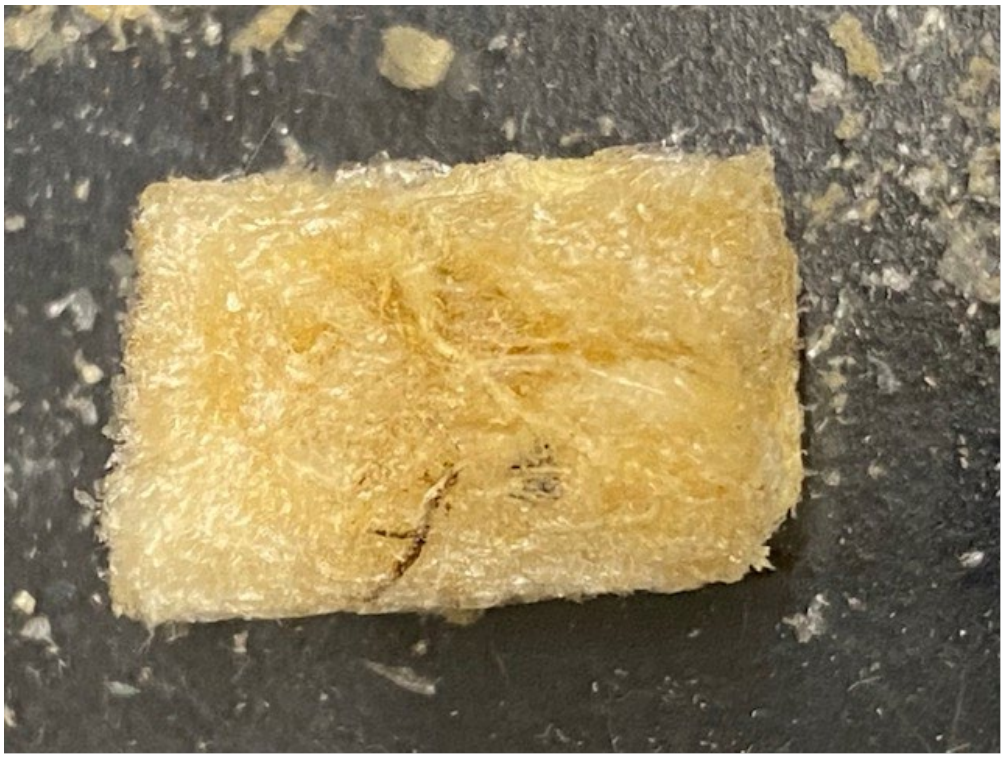

Figure 2. Tissue section from bivalve collected at FM-6

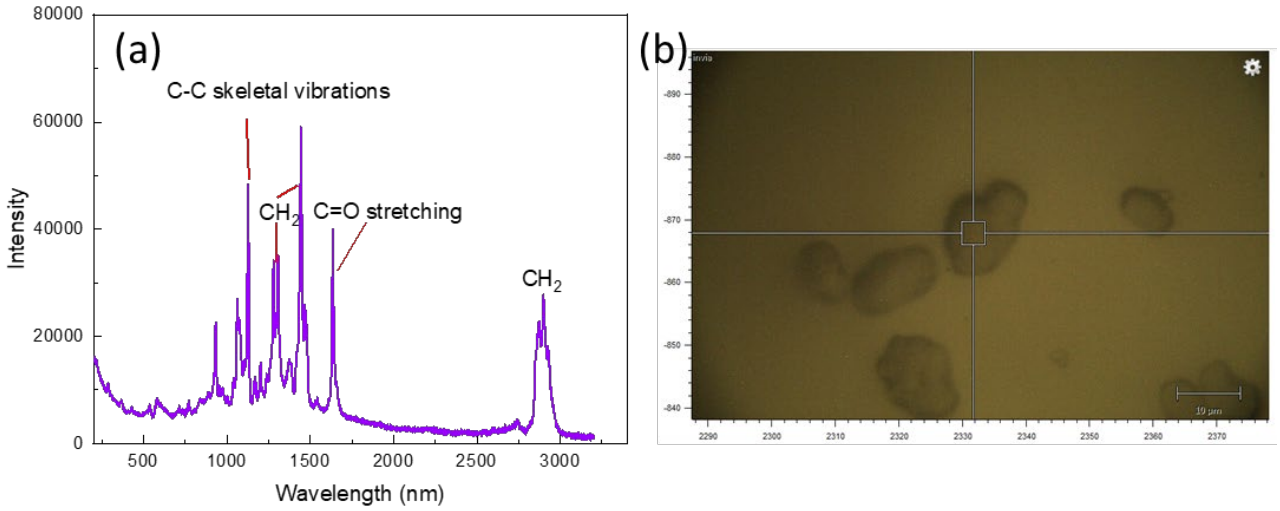

Figure 3. (a) micro-Raman of standard polyamide (b) single particle map for Raman measurements

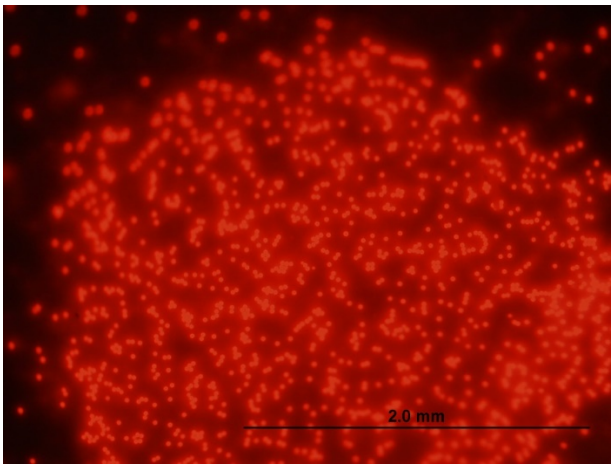

Figure 4. Fluorescence microscope image of dye tagged polystyrene beads

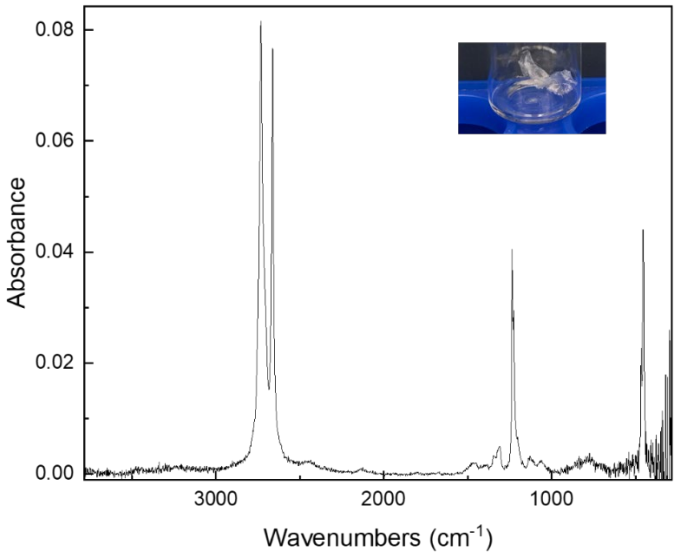

Figure 5. FTIR of plastic particulate found in FM2B, which was identified as polyethylene (inset: photo of collected plastic particulate)

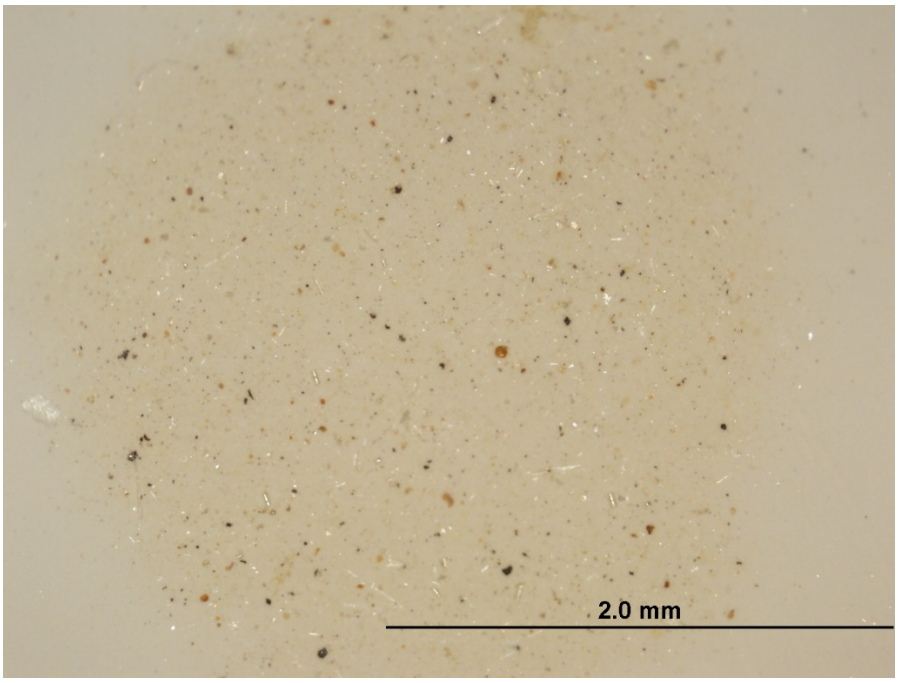

Figure 6. Bright field images of FMA7 after filtration and wet peroxide oxidation

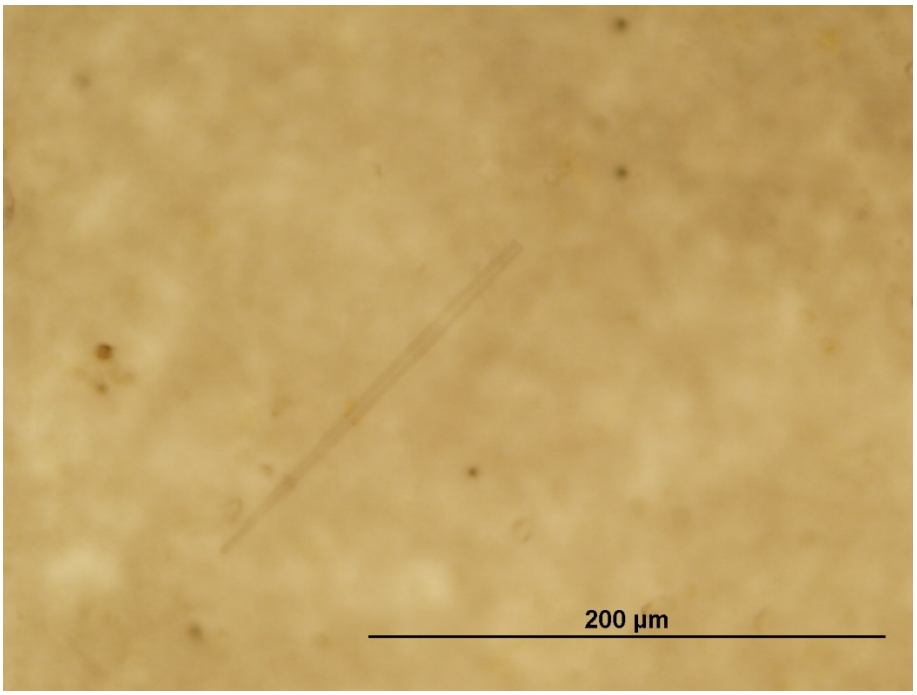

Figure 7..Brightfield image closeup of suspected microplastics from FM2B

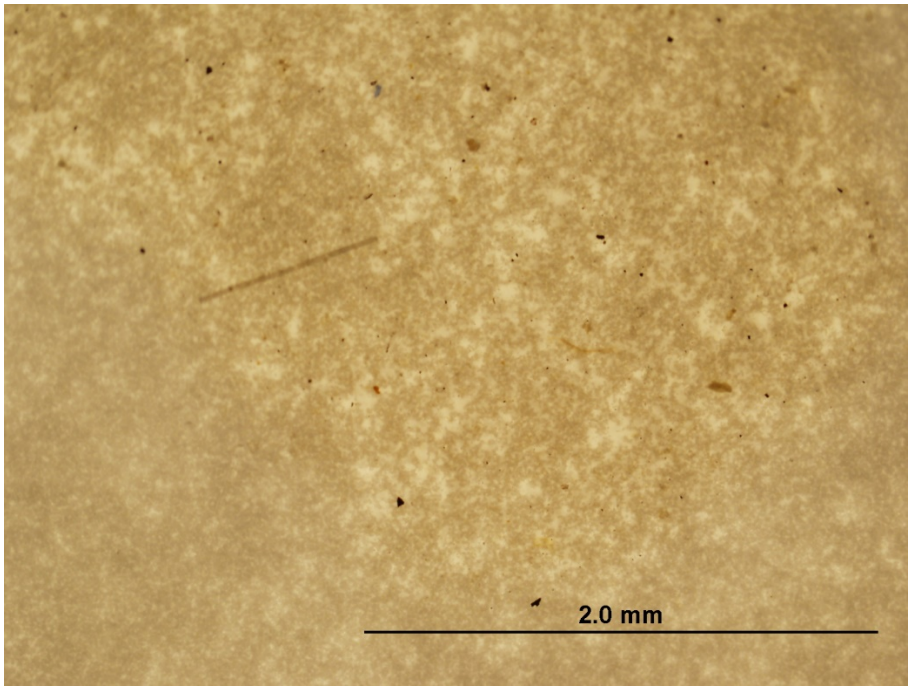

Figure 8. Brightfield image of FM6

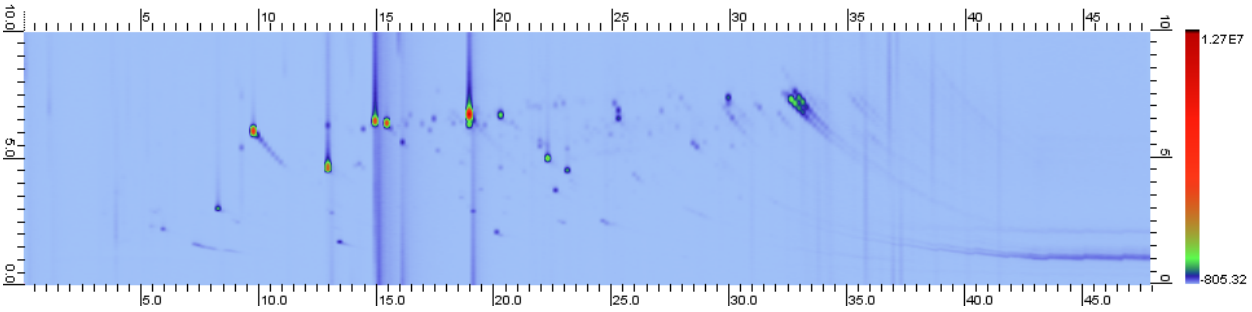

Figure 9. 2D GCxGC-MS for FM2B

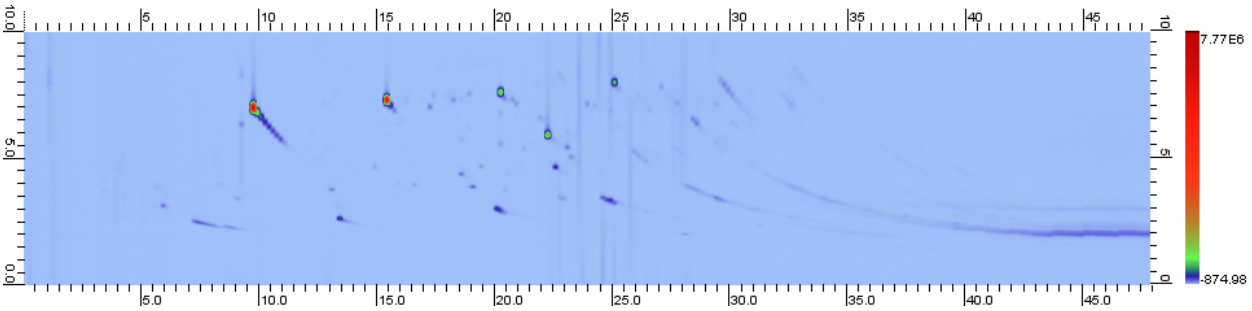

Figure 10. 2D GCxGC-MS data for FMA7