APPLIED RESEARCH

NAIT

Assessing the Prevalence of Microplastics in the Yukon River

February 12, 2025

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Background

The goal of this project is to provide technical and analytical support to the Yukon government's Water Resource Branch (WRB) by collaboratively conducting a preliminary quantitative assessment of microplastic occurrence in the Yukon River. NAIT Applied Research has guided the sampling process, following ASTM D8332. NAIT has helped WRB adapt this method for winter conditions, including sampling through ice, which, to our knowledge, has never been done before. WRB collected large volume samples (greater than 5,000L) and submitted them to NAIT Applied Research on November 26, 2024, for testing. Microplastics were extracted from the samples using ASTM D8333 as a reference method, with some modifications. Microplastic particles were identified and quantified individually using µFTIR.

Methods

Operation	Method	Notes
Sampling	ASTM D8332	High volume samples, >5,000L
Extraction	ASTM D8333, MOD	Density separation, chemical and biological digestion
Analysis	NAIT Custom	Particle by particle analysis using µFTIR

Sample Collection

ASTM D8332 was used to collect freshwater samples from the Yukon River, by WRB, using a custom high-throughput sampling system, as described in Bryksa et al., 2024. A battery-powered Jabsco 12V centrifugal pump was positioned on the shoreline, and multiple 4-foot lengths of ¾-inch (outer diameter) stainless steel tubing were connected with compression fittings to sample > 5,000 L of moving water. The water was passed through a cascading sieve stack composed of 5 mm, 500 µm, 125 µm, and 45 µm sieves until desired volume was filtered. The collected particles were rinsed into a 1L (pre cleaned) wide-mouth glass jar with an aluminum lid and then stored in a refrigerator at 4°C until shipped and/or processed in the laboratory.

Table 1. Project Samples. Field blank samples were submitted to NAIT as a sieve, wrapped in tin foil. Lab blanks were processed by NAIT with each batch of samples.

Sample	Type	Notes
24-0918-TakhiniYK-FB	Field Blank	45 µm sieve open for entire sample collection
24-0913-BurmaYK-FB	Field Blank	45 µm sieve open for entire sample collection
Lab Blank-1	Lab Blank	NAIT internal QC sample
Lab Blank-2	Lab Blank	NAIT internal QC sample
23-0213-Takhini-US	Environment Sample	**8,800 L**
24-0911-Livingston	Environment Sample	**8,200 L**
24-0912-MarshDam	Environment Sample	**5,400 L**. Contained H_2S
24-0913-Burma	Environment Sample	**8,600 L**. Contained H_2S
24-0918-Takhini	Environment Sample	**8,220 L**

Sample Extraction

Samples submitted to NAIT were processed in 2 batches, each containing a lab blank. Samples were filtered over a 3-inch, 45 µm stainless-steel sieve to isolate particles, which were then rinsed with saturated sodium iodide. The particles were transferred to a 250 mL separatory funnel using sodium iodide, and density separation was performed in three rounds, each lasting at least an hour. After each round, the floating particles were transferred to the sieve, and the bottom layer was discarded. A fourth round removed any remaining sediment by resuspending the particles in the sieve.

The isolated particles were rinsed with water and 30% hydrogen peroxide, then transferred to a 1 L beaker for wet peroxide oxidation. The beaker was covered with tin foil and left overnight. The following day, 30 mL of a catalyst (0.05M Iron (II) Sulfate heptahydrate) was added to initiate the Fenton reaction, with additional peroxide if needed. After another overnight reaction, the contents of the beaker were transferred back to the sieve and rinsed with Schweizer's reagent (saturated copper (II) hydroxide in ammonium hydroxide) to remove water. The sieve was soaked in the reagent for 5 minutes, then rinsed with ammonium hydroxide and water.

Next, TRIS-HCl (pH 8) was used to remove water and transfer the particles to a 250 mL beaker. Protease (7.5 mL) and Lipase (2.5 mL) were added, and the beaker was incubated at 45°C with agitation overnight. After incubation, the contents were transferred back to the sieve, rinsed with water, and excess enzymes were removed with concentrated HCl. Ethanol was used to remove water and prepare the sample for centrifugation.

The sample was transferred to a 100 mL centrifuge tube and spun at 1000 rpm (290 RCF) for 5 minutes, concentrating the particles at the bottom. The pellet was transferred onto a 0.2-µm silver membrane for IR microspectroscopy analysis. This process was repeated for a minimum of three rounds. 2 samples (2024 Livingston, 2023 Takhini) were split onto 2 filter papers due to high particle counts. Field blanks underwent this step only and were not subjected to the entire extraction process.

Sample Analysis

The *Thermo Scientific Nicolet iN10MX* was used for sample analysis, with samples placed on a 0.2-µm, 25 mm silver membrane substrate. IR spectra were collected in transflectance mode using Thermo Scientific's OMNIC Picta, scanning one-quarter to half of the substrate per pass with the cooled MCT detector. The full sample was analyzed by combining scans of the entire substrate.

Using the automated stage, spectra were collected in a 2 cm x 2 cm area with a 22 µm step size, creating a hyperspectral image of IR-absorbing particles. The hyperspectral image was further processed by creating a correlation profile to reduce noise, allowing for easier identification of target polymer materials. This was done by comparing individual polymer reference spectra to the entire map, which adjusted the image's color intensity based on the % match to the selected reference spectra, aiding in the identification of target polymers.

Results and Discussion

Samples were processed in three (3) batches. Each sample batch had an associated lab blank. The lab blank mirrors the sample processing conditions and is treated exactly like a sample. The field blanks were processed separately and did not undergo the full procedure. Field blanks were submitted by WRB.

Batch 1
24-0912-MarshDam
24-0913-Burma
Lab Blank-1

Batch 2
23-0213-Takhini-US
24-0911-Livingston
24-0918-Takhini
Lab Blank-2

Batch 3
24-0918-TakhiniYK-FB (Field Blank-1)
24-0913-BurmaYK-FB (Field Blank-2)

Targeted analysis was performed for 8 polymer materials: polypropylene (**PP**), polyethylene (**PE**), polyethylene terephthalate (**PET**), polyamide (**PA**), acrylonitrile butadiene styrene / polystyrene (**ABS/PS**), poly(methyl methacrylate) (**PMMA**), polycarbonates (**PC**), and polyvinyl chloride (**PVC**). Using correlation mapping with reference standards, library spectra of target polymers are matched to every particle analyzed in the sample. Matches above 70% are considered significant, with some matched 60%-70% included with manual spectra review from NAIT technicians. Only particles above 40 µm in size are included in results.

Table 2. Quality Control Results. Results are reported as particle counts, >40µm.

Sample	Type	PE	PP	PA	PET	ABS/PS	PMMA	PC	PVC
Field Blank-1	Field Blank	4	8	7	2	0	1	0	0
Field Blank-2	Field Blank	2	3	0	1	0	0	0	0
Lab Blank-1	Lab Blank	1	5	1	3	0	0	0	0
Lab Blank-2	Lab Blank	4	3	0	3	0	1	0	0

Table 2 presents the results for two field blanks and two laboratory blanks. Notably, no individual polymers were detected above a threshold of 10 particles in any blank sample, with single digit microplastic counts generally considered acceptable by NAIT. This shows there is no single contamination source, indicating the field and lab approach are adequate for controlling microplastic contamination.

Neither ABS/PS, PC, nor PVC were found in any of the lab or field blanks, indicating no contamination from these plastic types. ABS and PS are grouped together because distinguishing between these two polymers based solely on FTIR spectra is challenging due to overlapping functional groups and similar spectral features. For example, both materials share aromatic structures containing styrene.

PA and PET (commonly known as nylon and polyester, respectively) were detected as contaminants in both field and laboratory blanks. Interestingly, these particles predominantly exhibit a fragment morphology. Given that nylon and polyester are commonly used in clothing and field gear, contamination from clothing would typically be expected to appear as fiber-shaped particles. Furthermore, if contamination were sourced from clothing, any color variation could potentially provide clues to the origin of the contamination, linking it to specific field gear or clothing worn on the day of collection. However, the results indicate minor fibrous particle counts with no distinct color pattern suggests that clothing was not the source of contamination (**Figure 1**). This aligns with the precautionary measures taken to avoid wearing synthetic clothing materials in both the lab and the field.

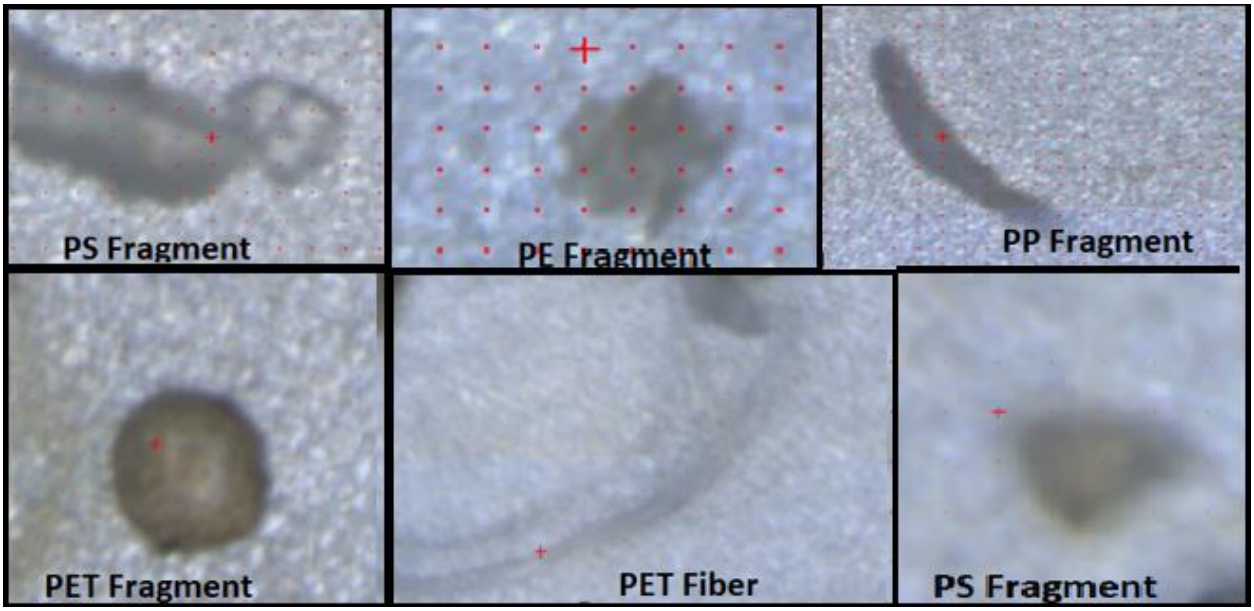

Figure 1. Particle Morphology

The average particle size for field blanks was 82 ± 47 µm, while the average particle size for laboratory blanks was 127 ± 100 µm. Particle size distributions are presented in **Figures 2** and **3**, with **Figure 3** further subdividing by polymer type.

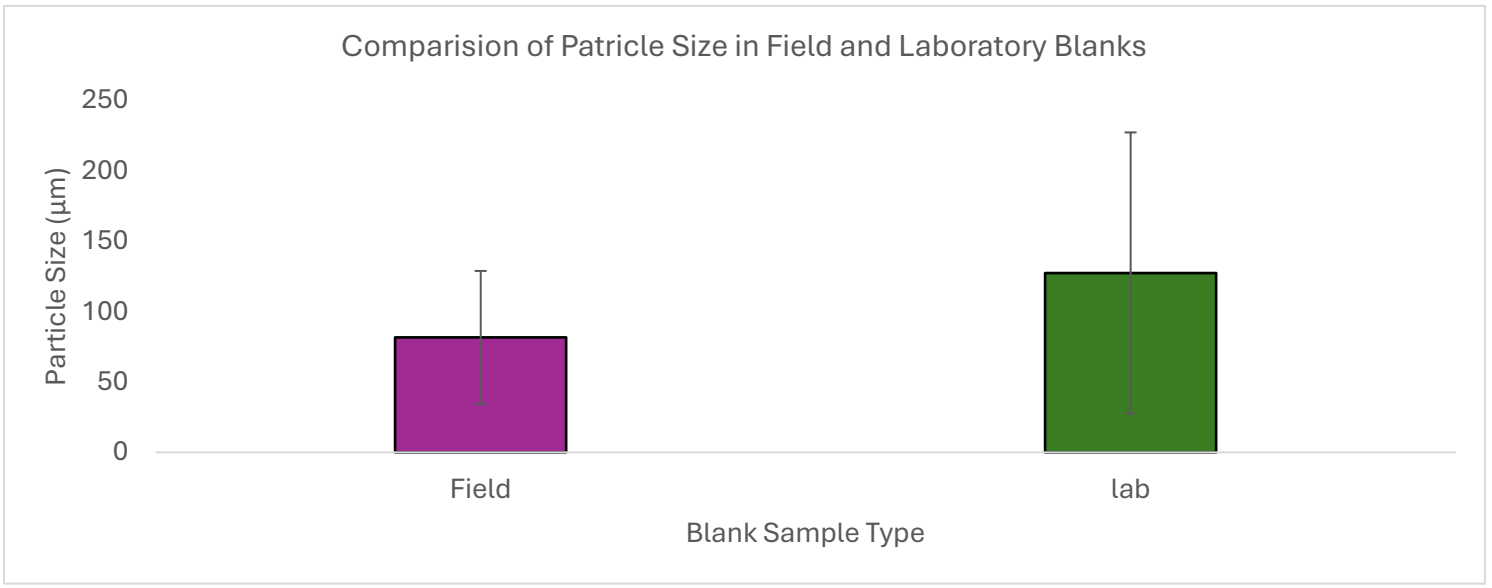

Figure 2. Comparison of Particle Size in Field and Laboratory Blanks

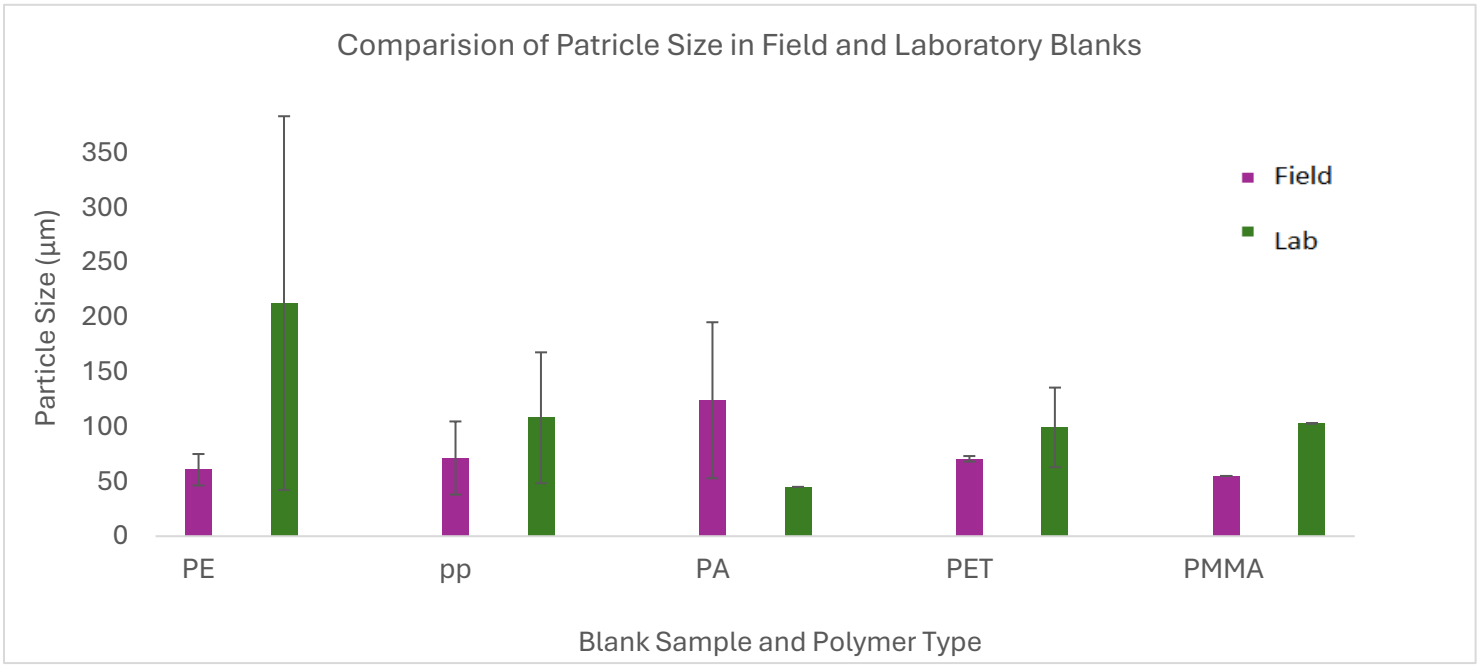

Figure 3. Comparison of Particle Size in Field and Laboratory Blanks.

Table 3. Sample Results. Results are reported as particle counts, >40µm.

Sample	PE	PP	PA	PET	ABS/PS	PMMA	PC	PVC
23-0213-Takhini-US	9	4	4	5	0	1	0	0
24-0911-Livingston	5	4	2	1	2	1	0	0
24-0912-MarshDam	3	4	1	0	0	0	0	0
24-0913-Burma	2	9	6	5	0	0	0	0
24-0918-Takhini	4	4	0	3	1	0	0	0

Microplastics were detected in all samples from the Yukon River (**Table 3);** however, concentrations were found to be very low. Despite taking special care to sample large volumes (5,400-8,800 L), and employing strict quality control procedures and protocols, the results indicate that microplastics, exist at exceedingly low concentrations within the river (if even present at all).

A key challenge in interpreting these results lies in referencing contamination (although minimal) during both field and laboratory processes and how those values compare to environmental samples. This is critical for distinguishing concentrations above background levels and determining their significance. In fact, the sample results generated from this work even fall within the same order of magnitude as those of the blanks, for each polymer type, **necessitating caution in drawing conclusions from this sample data.**

Referencing literature, polypropylene (PP) and polyethylene (PE) are common types of microplastics found in environmental studies and were also identified in this investigation. **Figure 4** and **Figure 5** illustrates the microplastic counts for PE and PP in comparison to both field and laboratory blank counts.

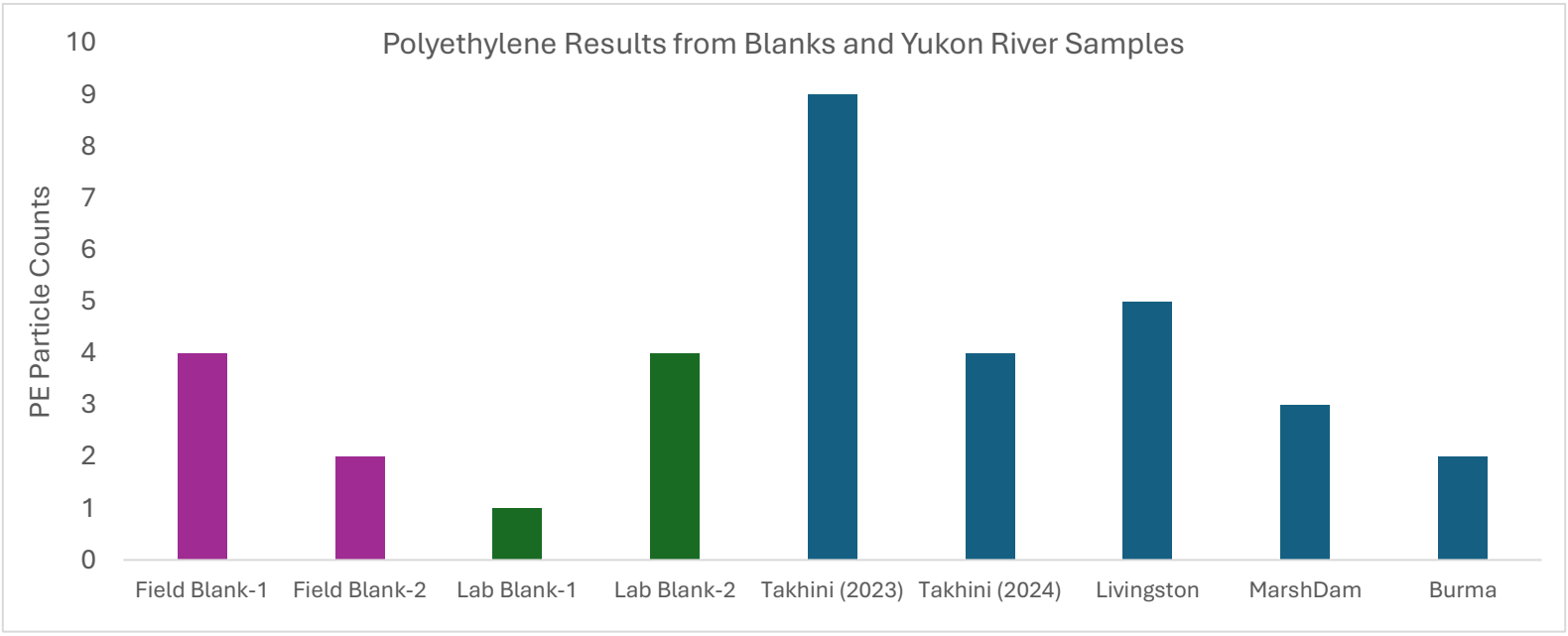

Figure 4. Polyethylene Results from Blanks and Yukon River Samples

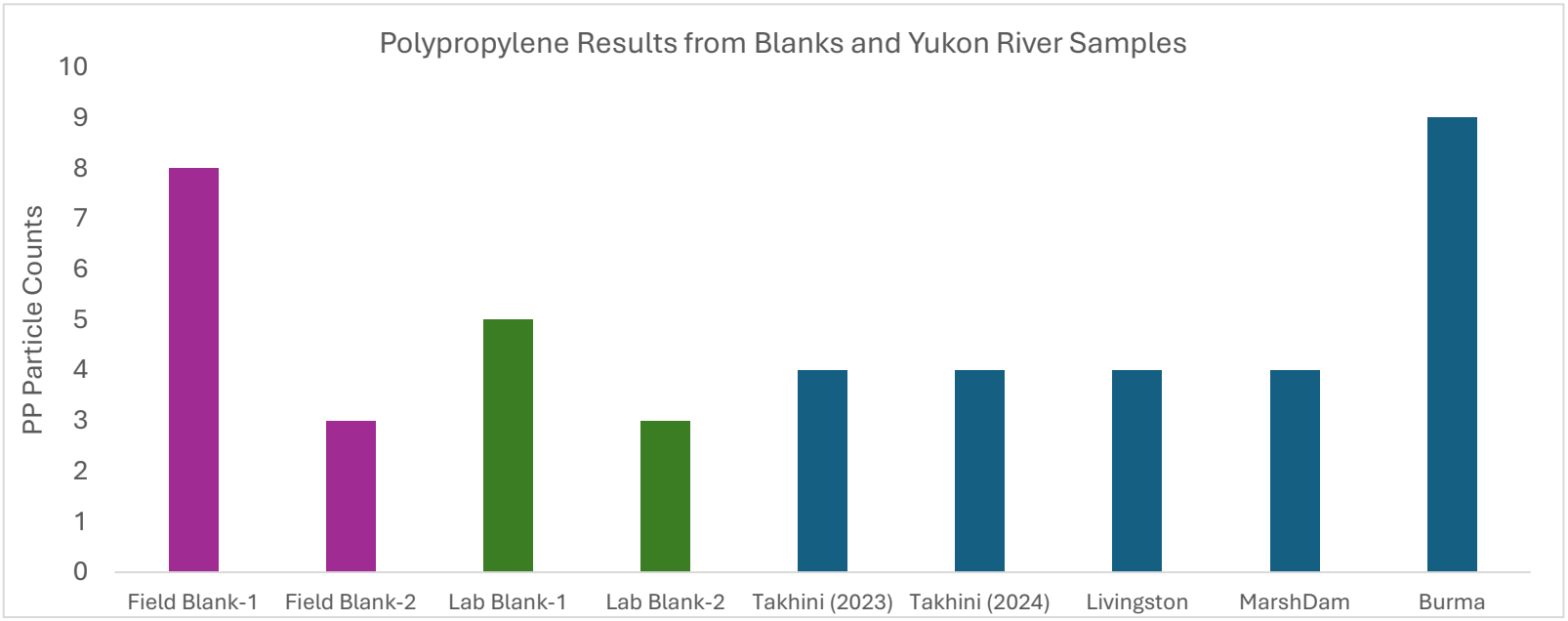

Figure 5. Polypropylene Results from Blanks and Yukon River Samples

Polycarbonate (PC) and polyvinyl chloride (PVC) were not detected in the sample results. Polyethylene terephthalate (PET) and polyamide (PA) were present, but at concentrations lower than those observed in the blank samples. PA presents additional challenges due to its molecular similarities with a variety of natural organic compounds, including proteins, lipids, and carbohydrates, which are commonly found in substances such as pollen and skin cells.

ABS/PS was not observed in any of the blanks (not even one particle) but present in 2 samples: Livingston (2 particles) and Takhini 2024 (1 particle). These detected microplastic counts, while potentially genuine, are exceptionally low, particularly when considering the large volumes of samples collected. With an increased number of blank samples, it is likely that random occurrences of polystyrene (PS) and acrylonitrile-butadiene-styrene (ABS) would be observed, further suggesting that the sample results may not be statistically significant.

To analyze the sample data in relation to lab and field contamination, each sample was paired with its corresponding blank, creating a direct blank-sample relationship. For example, the 24-0918 Takhini sample, processed in batch 2, was paired with both the lab blank from batch 2 and the field blank from that site. The results from the field and lab blanks were combined and paired with the Takhini sample. Similarly, the 24-0913 Burma sample had both a lab blank and a field blank associated with it. All other samples were paired with lab blanks, but did not have a field blank from the same site. For these samples, we used the average of the two field blanks from the project to ensure that each sample had a corresponding blank value.

Microplastic results are displayed in **Figures 6-9**, paired with blank values.

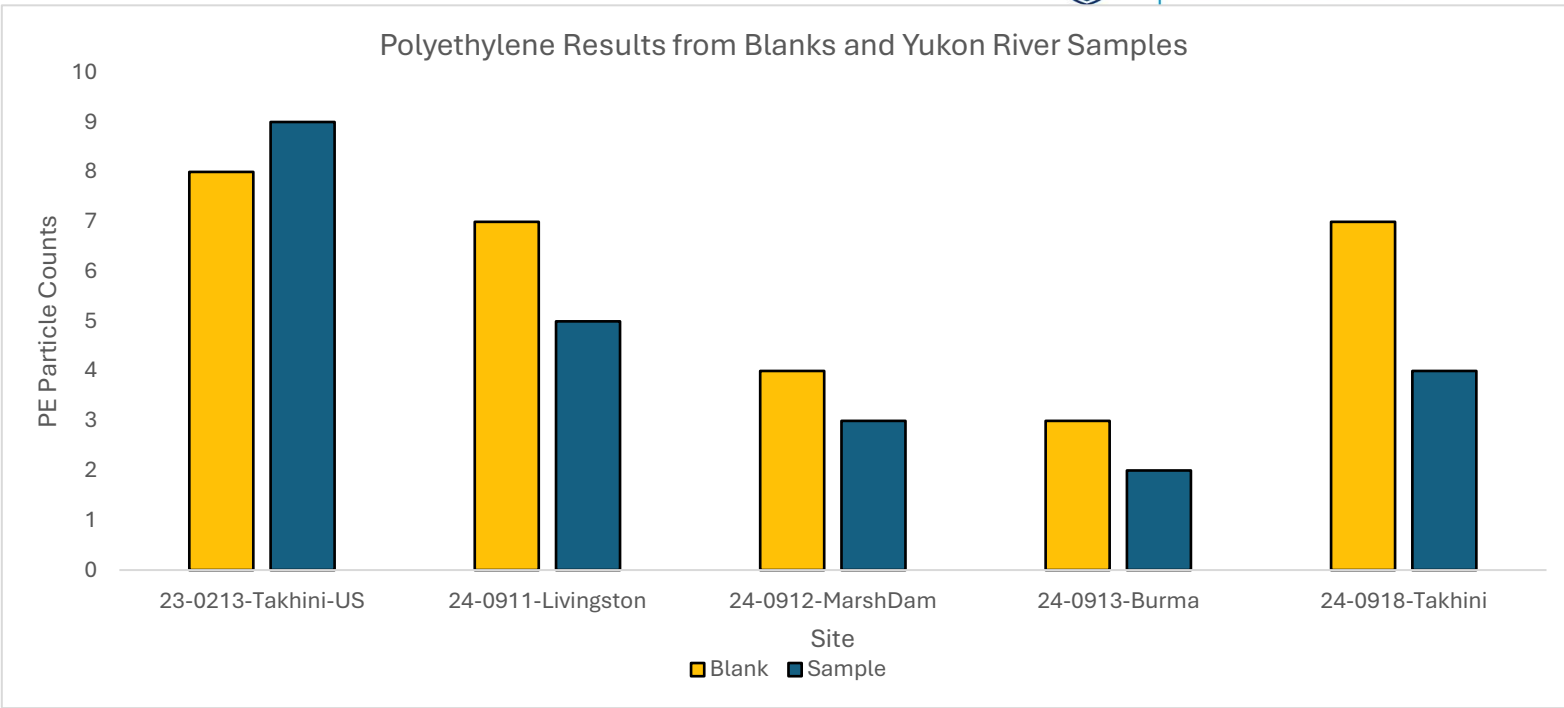

Figure 6 Polyethylene Yukon River Results Paired with Blanks

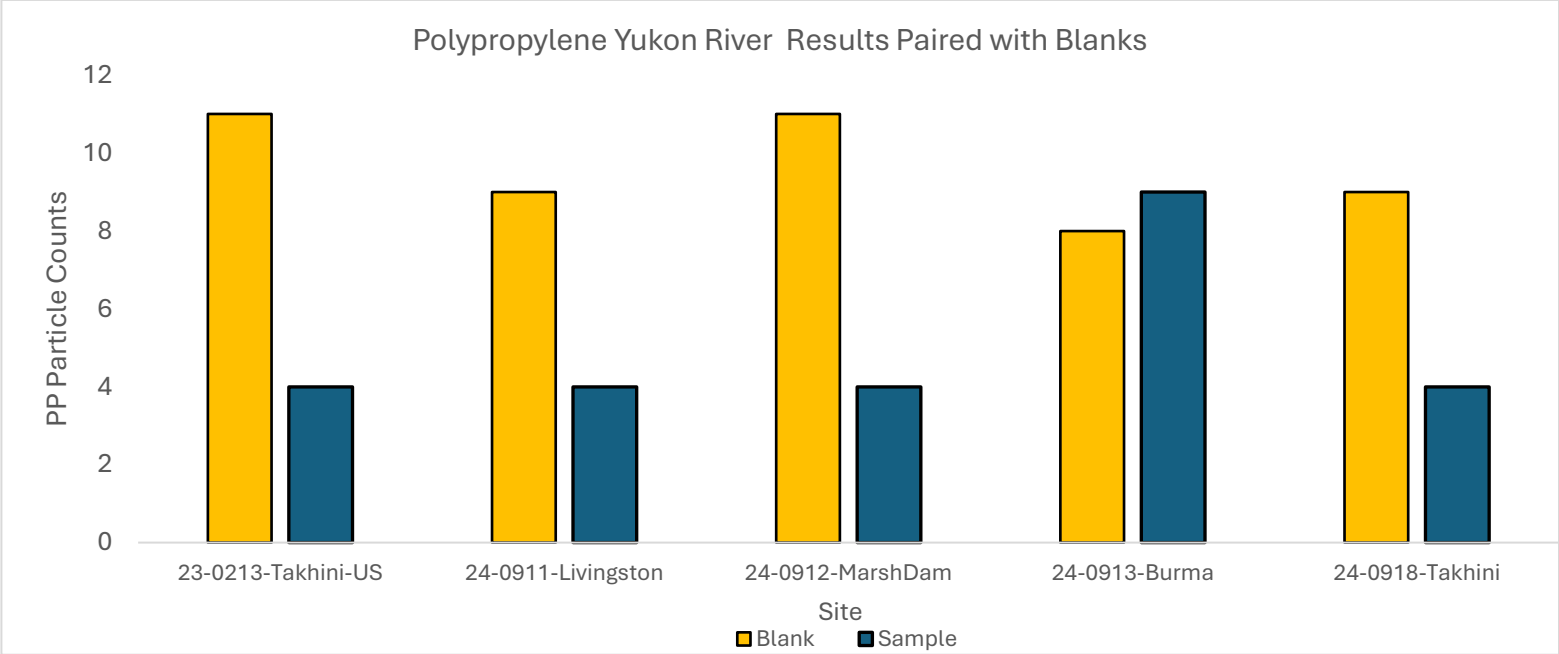

Figure 7. Polypropylene Yukon River Results Paired with Blanks

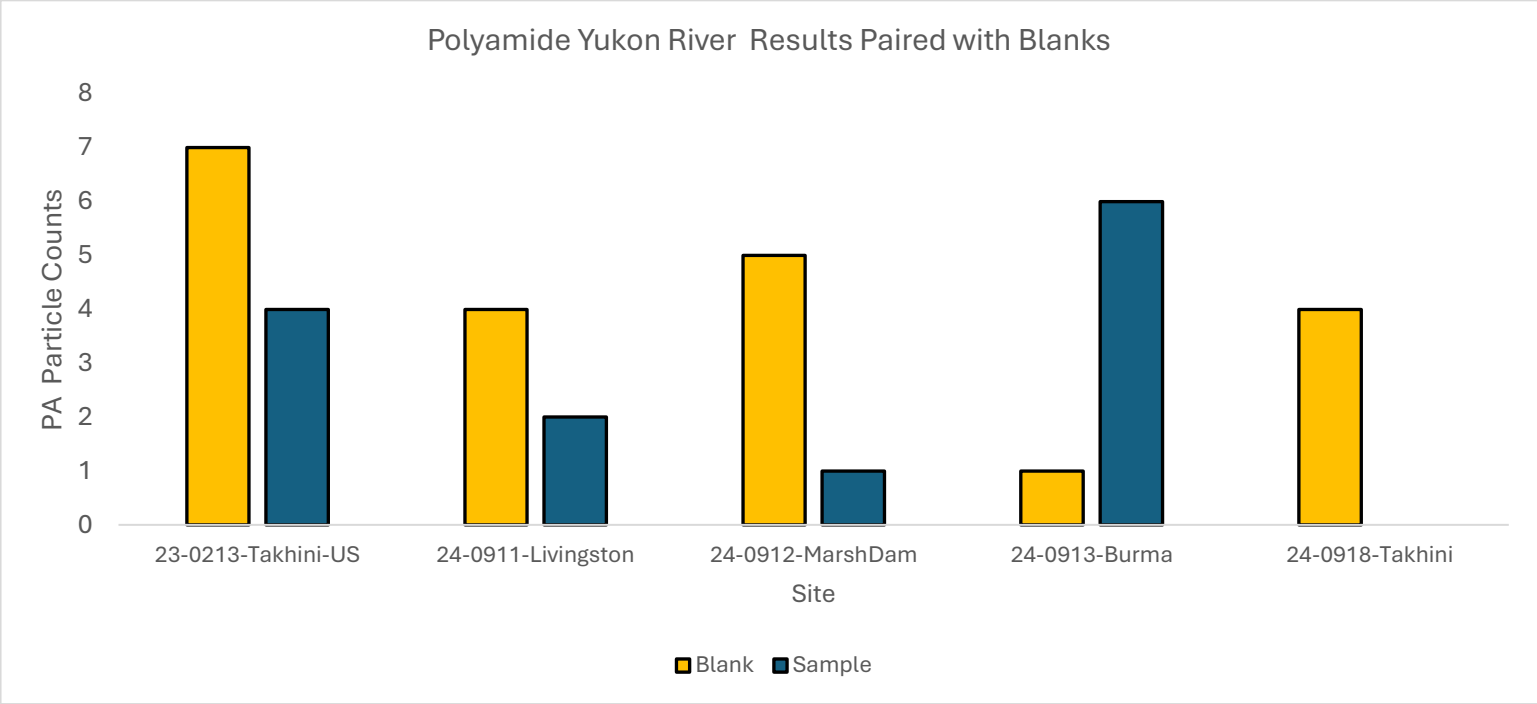

Figure 8. Polyamide Yukon River Results Paired with Blanks

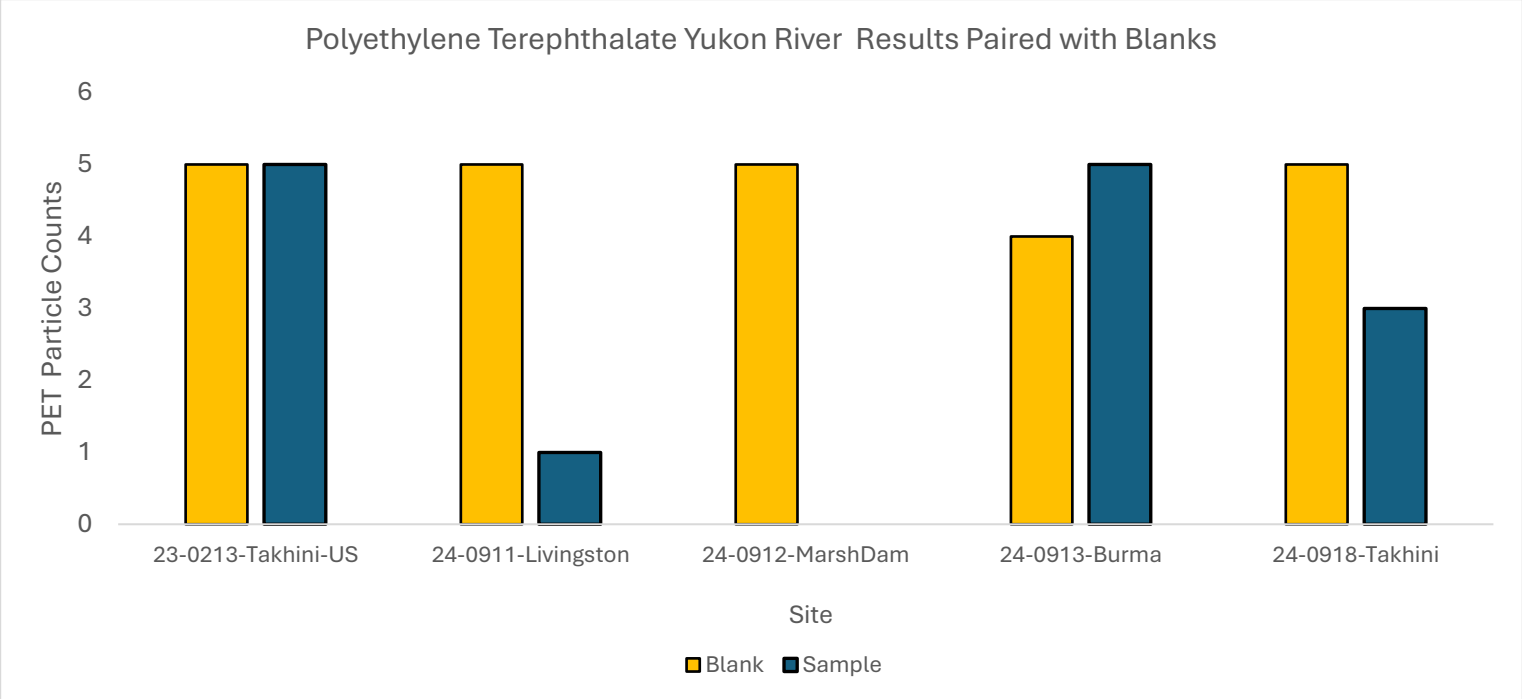

Figure 9. Polyethylene Terephthalate Yukon River Results Paired with Blanks

Conclusion

In this study, large volumes of Yukon River water were filtered down to 45 µm using ASTM D8332 for microplastic testing. The samples were submitted to NAIT for processing, which included the extraction of microplastics from other microparticles, using modified ASTM D8333, and identification of potential microplastic materials through µFTIR analysis, comparing reference spectra to determine polymer matches.

The data generated from this work makes it challenging to definitively confirm the presence of microplastics in the Yukon River, as the sample results are within the same order of magnitude as the contamination levels observed in both field and laboratory blanks. Given the extremely low contamination levels to begin with, we believe there is little that can be done to further reduce contamination during field or laboratory processes. Consider the laboratory extraction process; the procedure spans five business days and involves multiple chemical reagents and particle transfer steps. With such an intricate workflow, it would be nearly impossible to pinpoint the source of only the few particles that unintentionally enter the blanks.

The highest counted microplastic in the sample set was polyethylene (PE) in the Takhini 2023 sample. However, given that this value is close to the blank values paired with that sample it should not be considered above background, thus caution should be exercised in drawing definitive conclusions about the presence of microplastics in the Yukon River. Additionally, since no ABS/PS contamination was observed in the blanks, a small number of particles could be considered detectable and quantifiable. However, if this work progressed further, with an increased number of quality control samples, its likely that a random inclusion of a single particle in the blanks would render these results insignificant.

This suggests that microplastics, if even present at all, exist at extremely low concentrations in the Yukon River, **possible < 0.003 microplastics / L** for each polymer type. Despite the rigorous quality assurance protocols and the filtration of large volumes of freshwater, microplastic counts above the detectable limit were rarely observed.

Supplementary

24-0918-TakhiniYK-FB			
Polymer	**Size (µm)**	**Library Match %**	**Morphology**
PE	55	64	FRAGMENT
PE	43	88	FRAGMENT
PE	68	82	FRAGMENT
PE	58	70	FRAGMENT
PP	47	70	FRAGMENT
PP	88	71	FRAGMENT
PP	70	75	FRAGMENT
PP	41	72	FRAGMENT
PP	98	85	FRAGMENT
PP	52	75	FRAGMENT
PP	156	84	FRAGMENT
PP	57	91	FRAGMENT
PA	162	60	FRAGMENT
PA	231	65	FRAGMENT
PA	196	72	FRAGMENT
PA	64	66	FRAGMENT
PA	85	63	FRAGMENT
PA	79	76	FRAGMENT
PA	52	64	FRAGMENT
PET	71	72	FRAGMENT
PET	68	71	FRAGMENT
PMMA	55	63	FRAGMENT

24-0913-BurmaYK-FB			
Polymer	**Size (µm)**	**Library Match %**	**Morphology**
pe	55	81	FRAGMENT
pe	85	81	FRAGMENT
pp	74	79	FRAGMENT
pp	57	70	FRAGMENT
pp	45	84	FRAGMENT
pet	73	69	FIBER

Lab Blank-YK-1			
Polymer	**Size (µm)**	**Library Match %**	**Morphology**
pe	96	70	FRAGMENT
pp	75	89	FRAGMENT
pp	42	89	FRAGMENT
pp	49	68	FRAGMENT
pp	207	63	FRAGMENT
pp	88	75	FRAGMENT
pa	45	63	FRAGMENT
pet	65	67	FRAGMENT
pet	72	66	FRAGMENT
pet	63	95	FRAGMENT

Lab Blank-YK-2			
Polymer	**Size (µm)**	**Library Match %**	**Morphology**
pe	224	80	FRAGMENT
pe	103	84	FRAGMENT
pe	504	90	FRAGMENT
pe	137	63	FRAGMENT
pp	184	79	FRAGMENT
pp	115	73	FRAGMENT
pp	105	78	FRAGMENT
pet	136	67	FIBER
pet	122	75	FRAGMENT
pet	138	72	FRAGMENT
pmma	103	73	FRAGMENT

23-0213-Takhini-US			
Polymer	**Size (µm)**	**Library Match %**	**Morphology**
Pe	99	71	FIBER
Pe	204	90	FIBER
Pe	270	92	FRAGMENT
Pe	114	88	FRAGMENT
Pe	373	77	FRAGMENT
Pe	72	92	FRAGMENT
Pe	263	80	FRAGMENT
Pe	111	94	FRAGMENT
Pe	56	91	FRAGMENT
Pp	91	78	FRAGMENT
Pp	75	75	FRAGMENT
Pp	159	71	FIBER
Pp	319	63	FRAGMENT
Pa	181	61	FRAGMENT
Pa	71	69	FRAGMENT
Pa	92	64	FRAGMENT
Pa	58	66	FRAGMENT
Pet	91	72	FIBER
Pet	134	72	FRAGMENT
Pet	63	72	FRAGMENT
Pet	74	82	FRAGMENT
Pet	80	61	FIBER
pmma	155	78	FRAGMENT

Polymer	Size (µm)	Library Match %	Morphology
pe	121	79	FRAGMENT
pe	120	92	FRAGMENT
pe	92	91	FRAGMENT
pe	57	63	FRAGMENT
pe	130	75	FRAGMENT
pp	90	81	FRAGMENT
pp	122	85	FRAGMENT
pp	88	75	FRAGMENT
pp	98	78	FRAGMENT
pa	125	62	FRAGMENT
pa	150	61	FRAGMENT
pet	80	64	FRAGMENT
abs/ps	97	80	FRAGMENT
abs/ps	69	62	FRAGMENT
pmma	256	67	FRAGMENT

24-0912-MarshDam			
Polymer	**Size (µm)**	**Library Match %**	**Morphology**
pe	247	82	FRAGMENT
pe	89	93	FRAGMENT
pe	44	95	FRAGMENT
pp	89	79	FRAGMENT
pp	58	73	FRAGMENT
pp	89	80	FRAGMENT
pp	574	80	FRAGMENT
pa	63	79	FRAGMENT

24-0913-Burma			
Polymer	**Size (µm)**	**Library Match %**	**Morphology**
Pe	68	85	FRAGMENT
Pe	45	74	FRAGMENT
Pp	110	72	FRAGMENT
Pp	60	73	FRAGMENT
Pp	137	83	FRAGMENT
Pp	49	76	FRAGMENT
Pp	95	68	FRAGMENT
Pp	181	75	FRAGMENT
Pp	72	86	FRAGMENT
Pp	52	80	FRAGMENT
Pp	63	69	FRAGMENT
Pa	59	61	FRAGMENT
Pa	41	74	FRAGMENT
Pa	56	64	FRAGMENT
Pa	60	60	FRAGMENT
Pa	48	61	FRAGMENT
Pa	42	71	FRAGMENT
Pet	46	76	FIBER
Pet	50	66	FIBER
Pet	64	62	FRAGMENT
Pet	68	63	FIBER
Pet	42	69	FRAGMENT

24-0918-Takhini			
Polymer	**Size (µm)**	**Library Match %**	**Morphology**
pe	89	82	FRAGMENT
pe	237	69	FRAGMENT
pe	70	72	FRAGMENT
pe	77	85	FRAGMENT
pp	55	67	FRAGMENT
pp	57	61	FRAGMENT
pp	72	72	FRAGMENT
pp	84	67	FRAGMENT
pet	99	78	FIBER
pet	110	77	FRAGMENT
pet	83	91	FRAGMENT
abs/ps	120	74	FRAGMENT

Figure 10. Microplastic Extraction - Density Separation with Sodium Iodide (NaI_{aq})

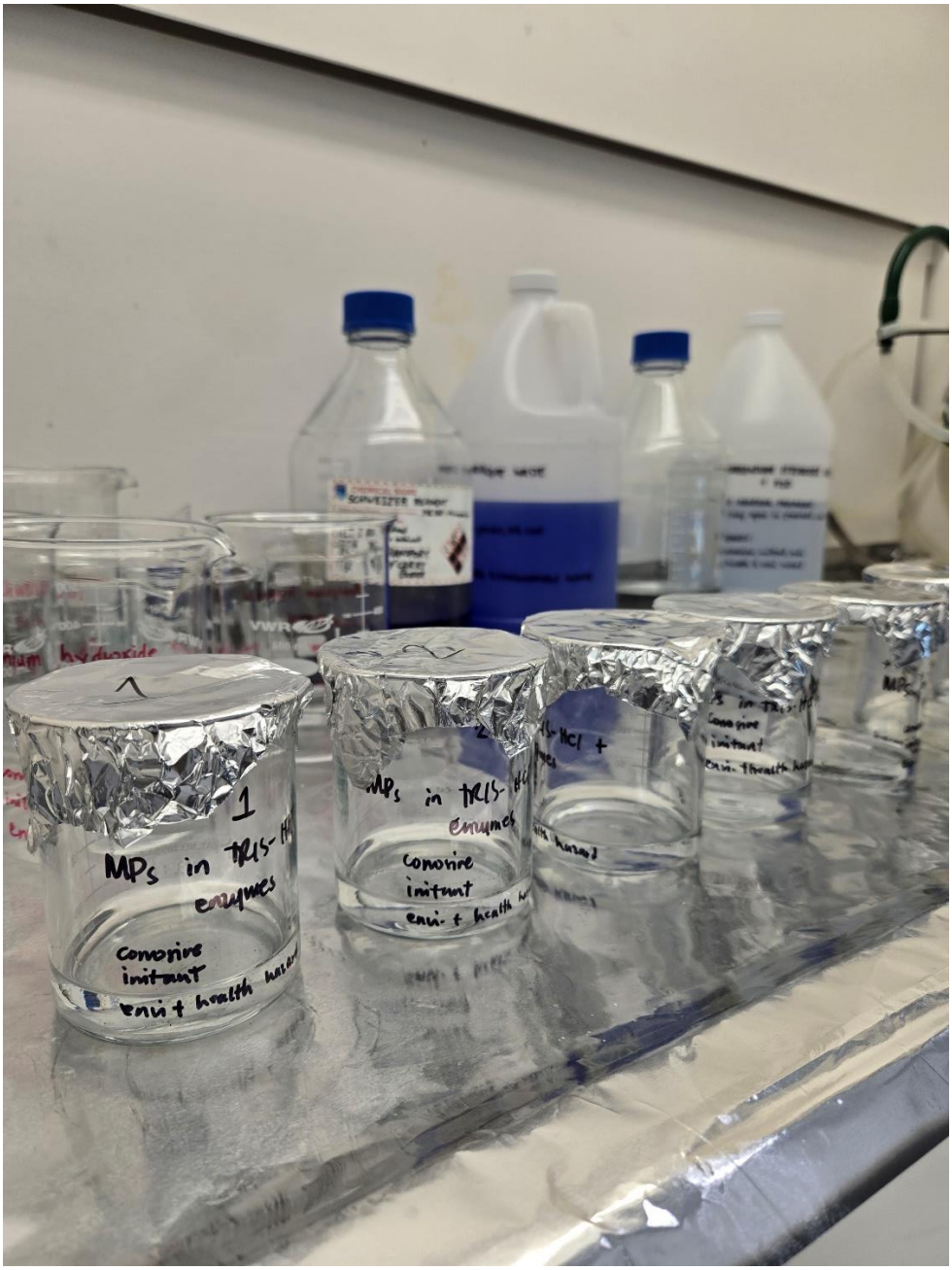

Figure 11. Microplastic Extraction -Enzymatic Digestion

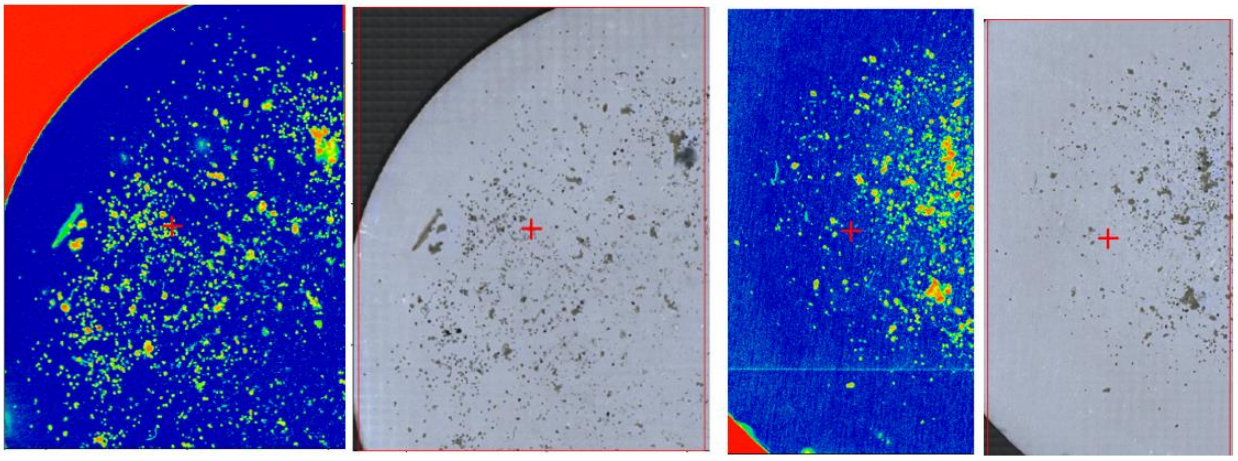

Figure 12. Hyperspectral and Microscope Image. 24-0912-MARSHDAM (left) 24-0911-Livinston (right)

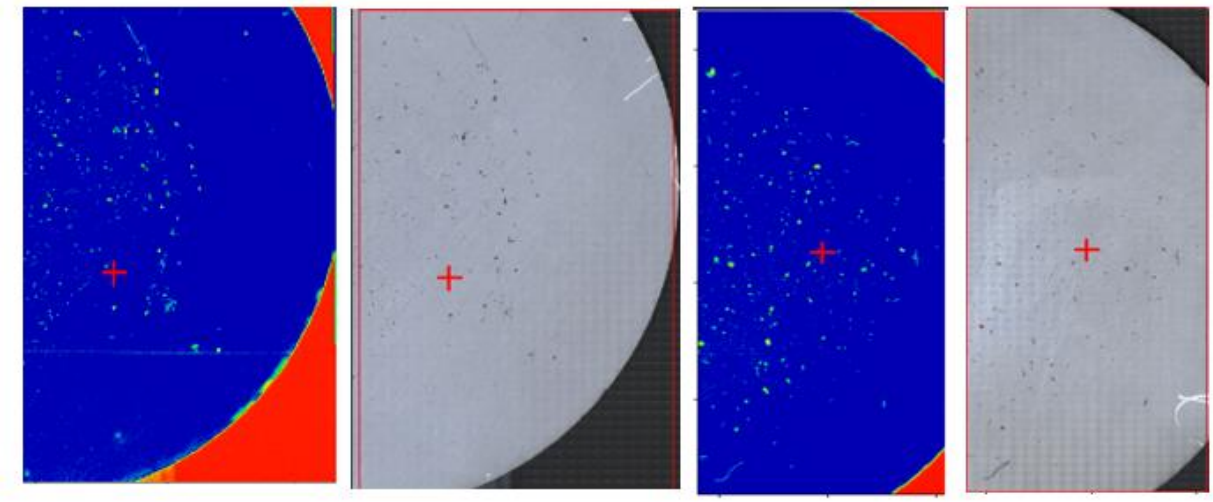

Figure 13. Hyperspectral and Microscope Image. 24-0913-Burma Field blanks (left) Lab Blank (right).

Note. For spectral interpretation using microscopic FTIR, it's important to note that the signal intensity is lower compared to classic or ATR FTIR due to the small size of the particles being analyzed. Microscopic particles produce weaker signals than bulk samples. In transflectance mode, the signal will be reduced because the smaller surface area of the particles, which limits the interaction of IR light with the sample.

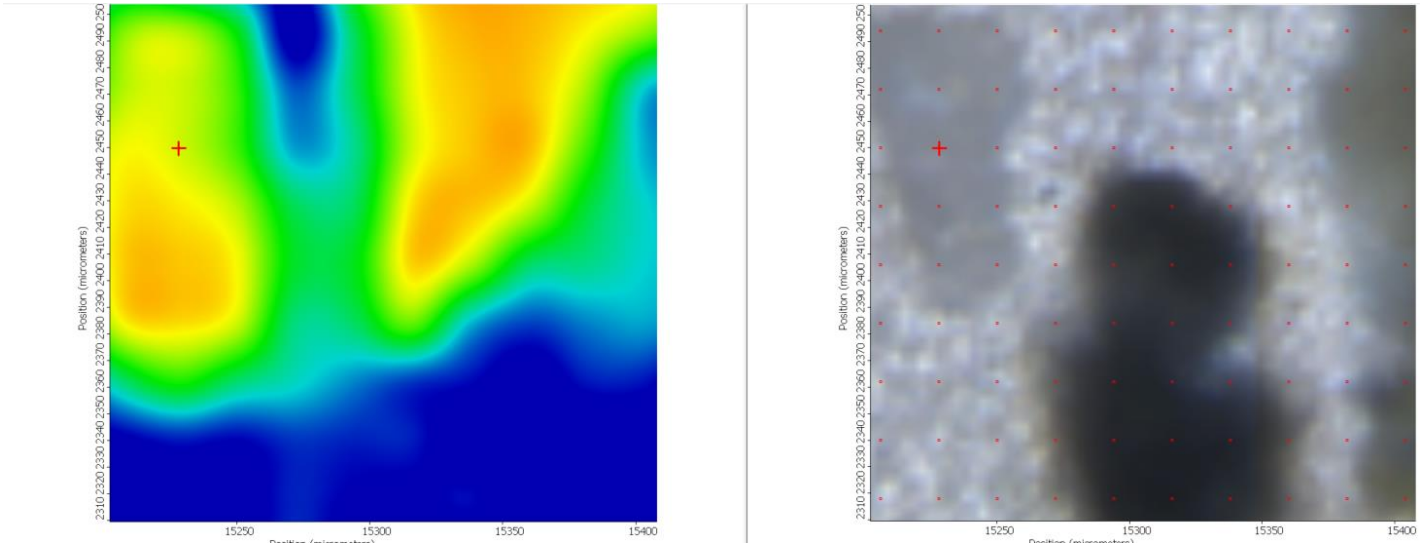

Figure 14. Hyperspectral and Microscope Image. PE.

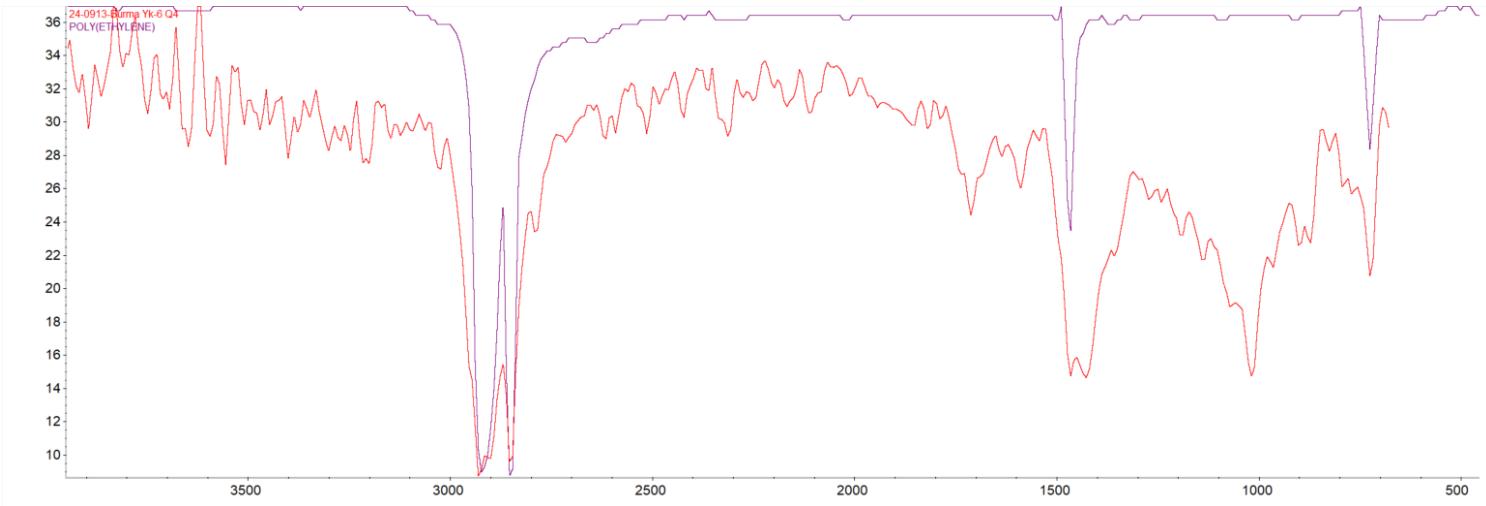

Figure 15. Spectral Overlay. PE.

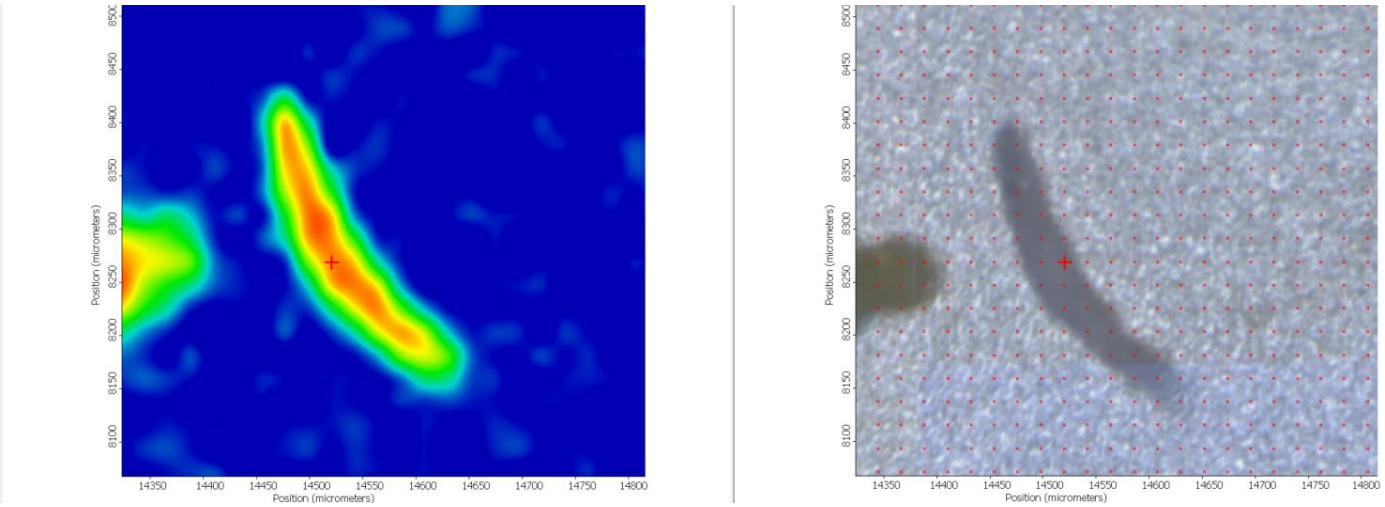

Figure 16. Hyperspectral and Microscope Image. PP.

Figure 17. Spectral Overlay. PP.

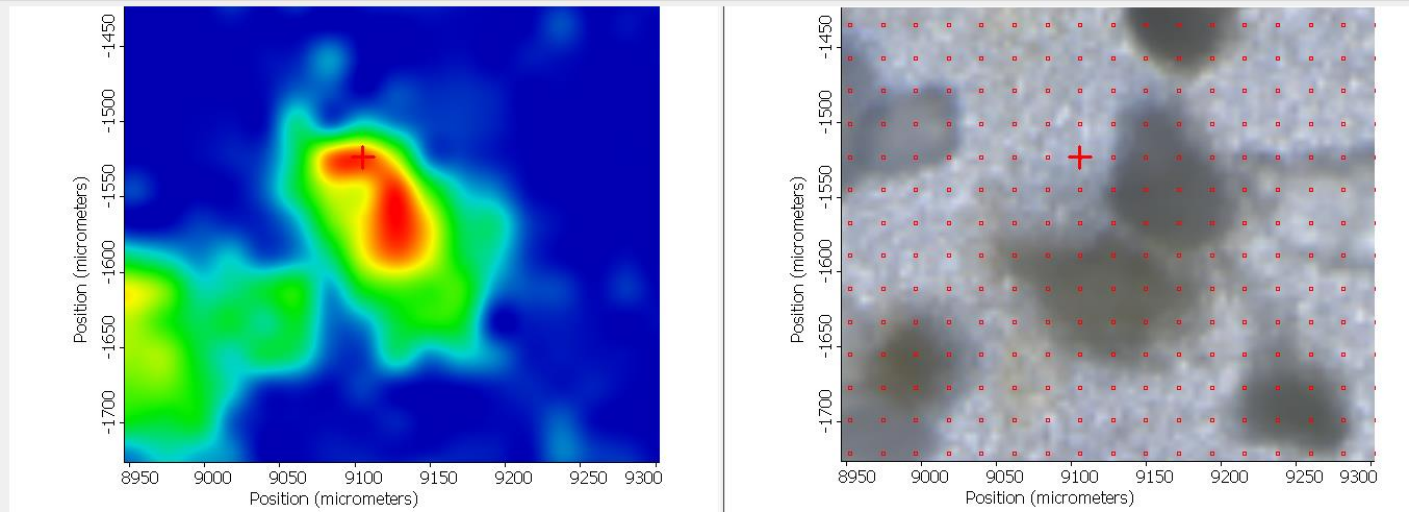

Figure 18. Hyperspectral and Microscope Image. PS.

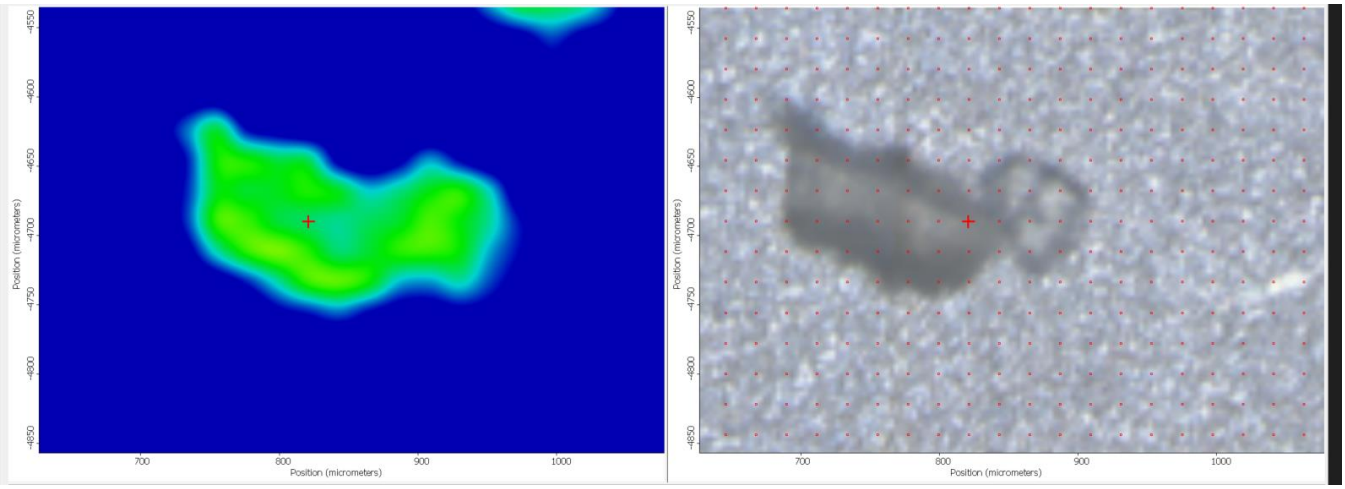

Figure 19. Hyperspectral and Microscope Image. PS.

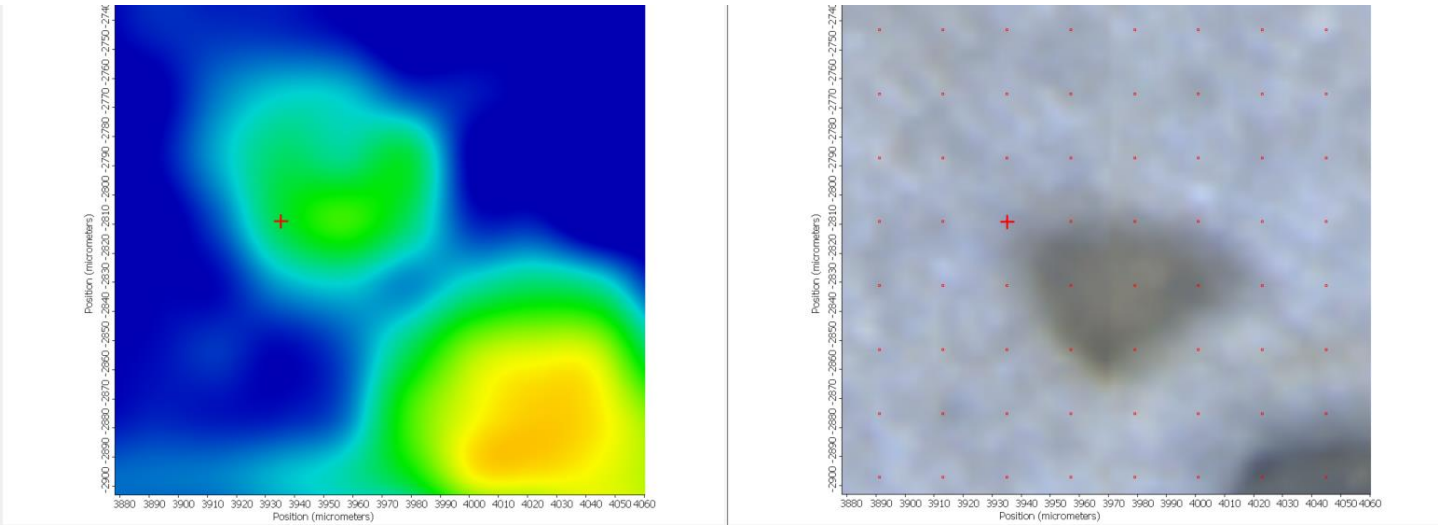

Figure 20. Hyperspectral and Microscope Image. PS.

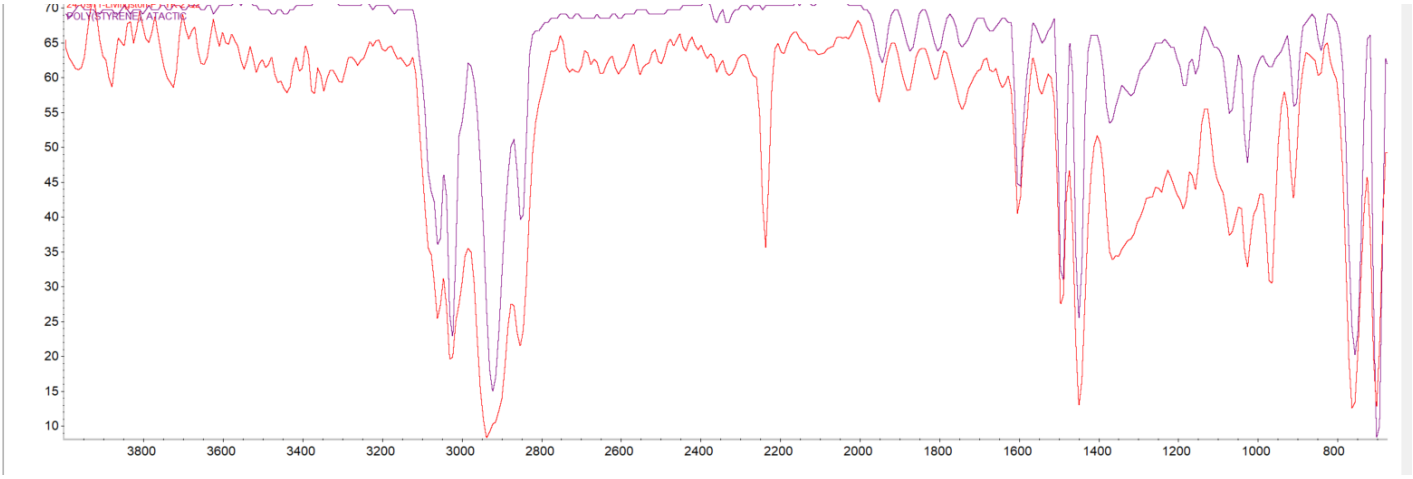

Figure 21. Spectral Overlay. PS.

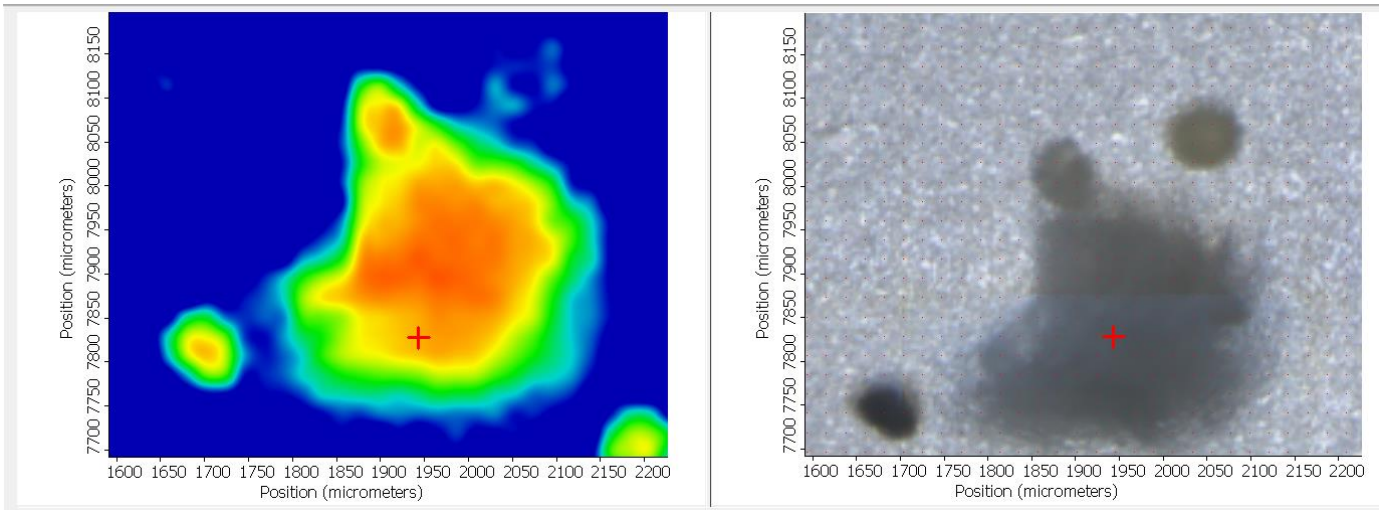

Figure 22. Hyperspectral and Microscope Image. PMMA.

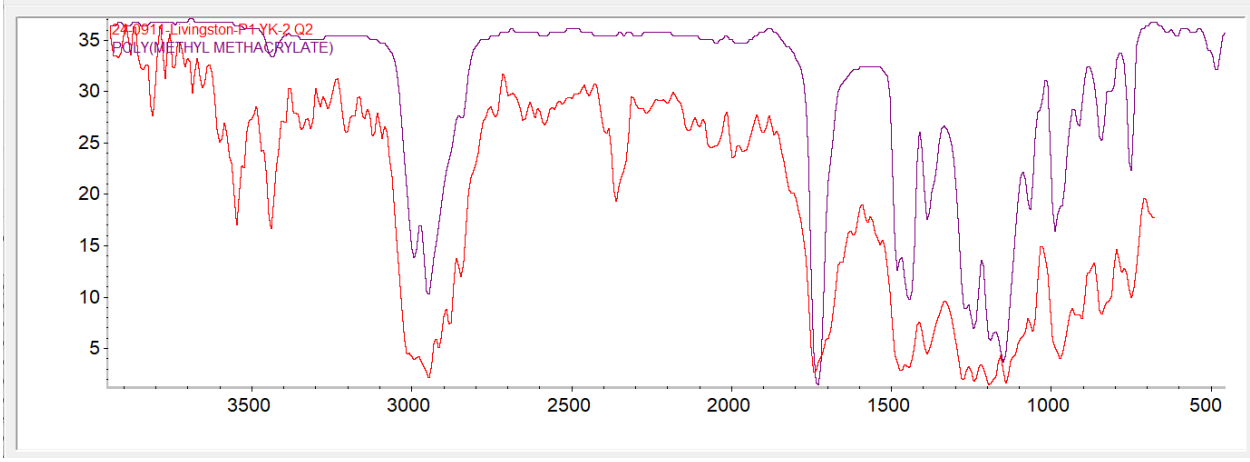

Figure 23. Spectral Overlay. PMMA.

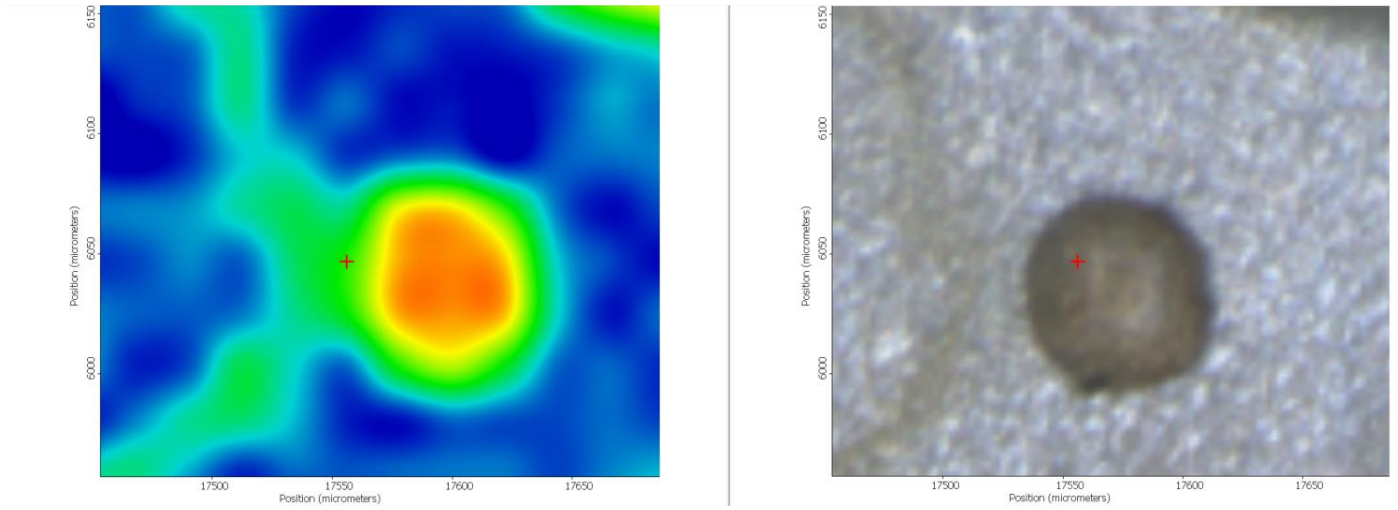

Figure 24. Hyperspectral and Microscope Image. PET.

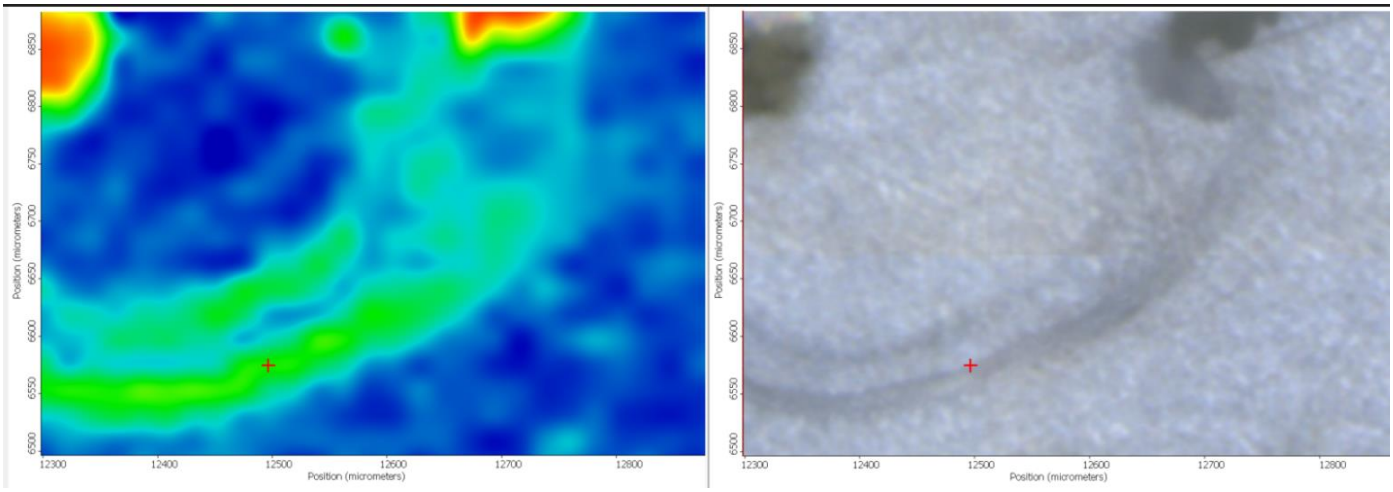

Figure 25. Hyperspectral and Microscope Image. PET.

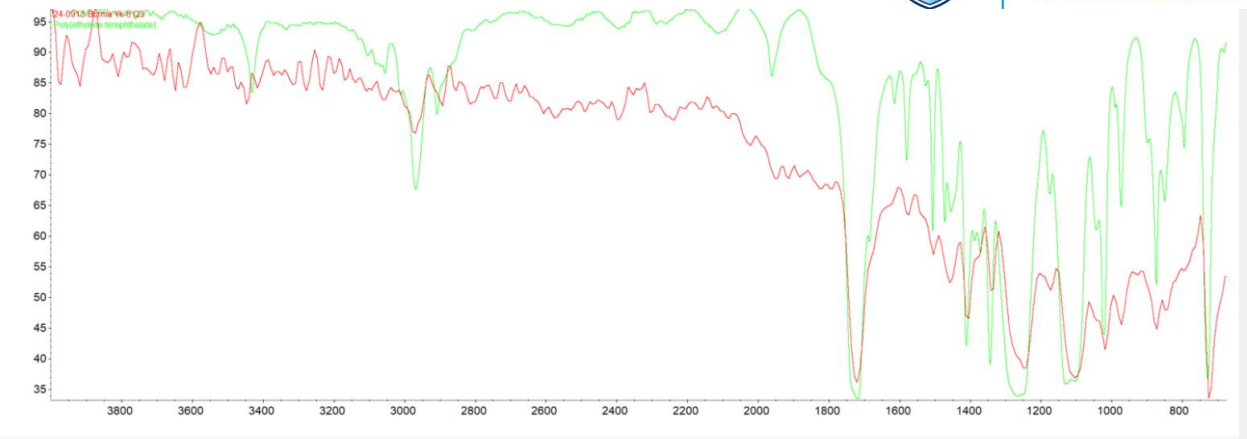

Figure 26. Spectral Overlay. PMMA.

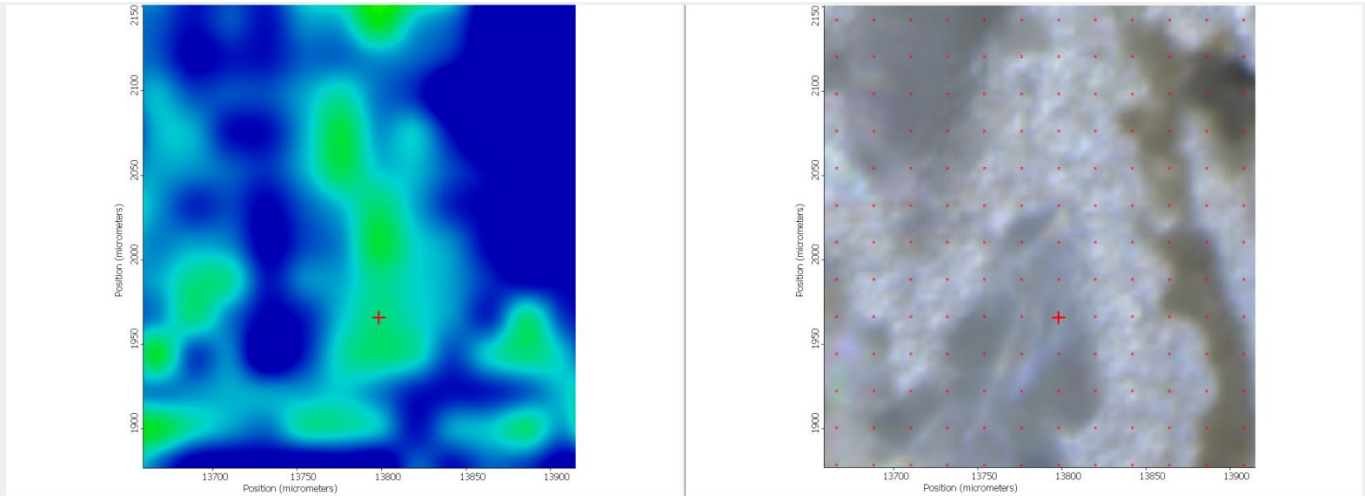

Figure 27. Hyperspectral and Microscope Image. PA.

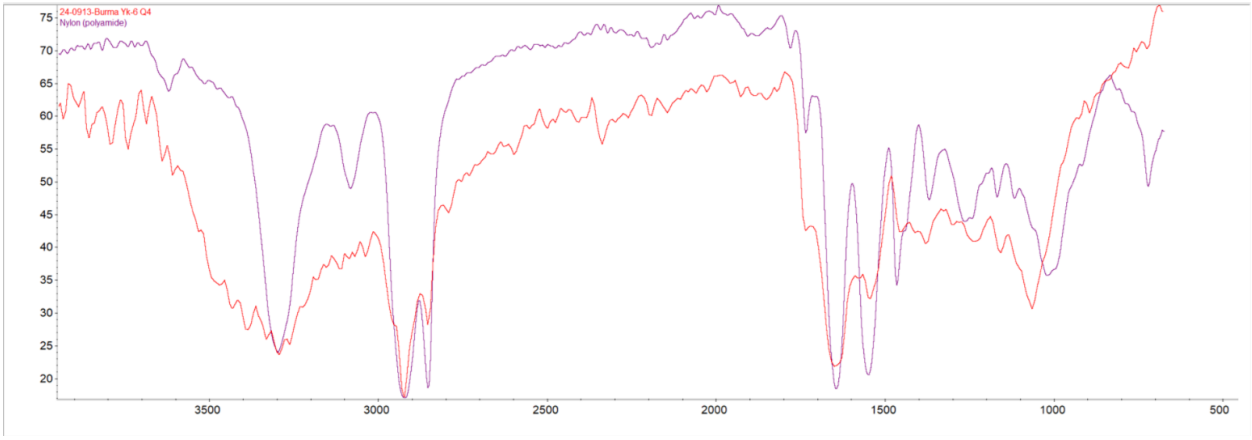

Figure 28. Spectral Overlay. PA.