Supporting Information: Miscounting microplastics as a result of matrix molecules: Optimizing identification and quantification of microplastics in natural freshwater systems

Authors and Affiliations

Joseph Marchand[a] ; ORCID ID 0009-0000-3607-5790

Ariyah Thomas[a]; ORCID ID 0009-0007-8568-7399

Elizabeth C. Minor[a,b*] ; ORCID ID 0000-0001-6930-9338

Amber Ruthenbeck[a] ; ORCID ID 0009-0005-7891-4624

Alexa Nielsen[a]

Melissa A. Maurer-Jones[a] ; ORCID ID 0000-0003-1517-6183

a Department of Chemistry and Biochemistry, University of Minnesota Duluth, 1038 University Dr, Duluth, Minnesota 55812, United States

b Large Lakes Observatory, University of Minnesota Duluth, 2205 East 5th St, Duluth, MN 55812. E-mail: eminor@d.umn.edu

* Corresponding author

Table S1. Sample name, and coordinates of surface water matrices.

Sample #	**Latitude (DMS)**	**Longitude (DMS)**	**Name**
1	-94°47'37" W	48°54'23" N	Lake of the Woods Transect 1
2	-92°49'53" W	45°22'50" N	South Center Lake
3	-96°21'19" W	44°24'26" N	Shaokatan

Table S2. When to use method quick guide to help identify when to use each method. Full description of the method and each step can be found in section 2.

Method	**Method 1**	**Method 2**	**Method 3**	**Method 4**
Step 1	Filter onto Anodisc	Dry down	Dry Down	Dry Down
Step 2	FTIR Analysis	Fenton Oxidation	Fenton Oxidation	Fenton Oxidation
Step 3	-	5M NaCl	5M NaCl	Ethanol
Step 4	-	Filter onto Anodisc	50 um Filter	50 um Filter
Step 5	-	FTIR Analysis	Sonicate, and rinse	Sonicate, and rinse
Step 6	-	-	Filter onto Anodisc	5M NaCl
Step 7	-	-	FTIR Analysis	Filter onto Anodisc
Step 8	-	-	-	FTIR Analysis

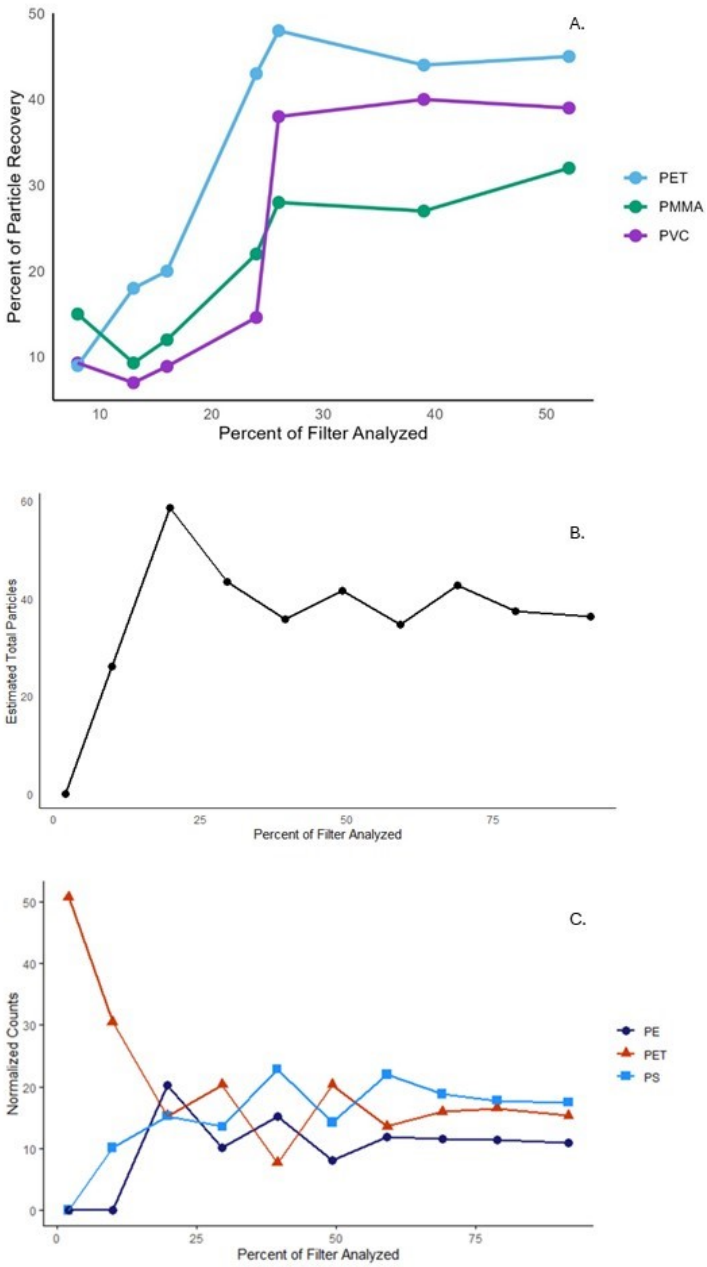

Figure S1. Evaluation of plastic standards to determine ideal percentage of Anodisc to analyze via μFTIR. A). In-house developed microplastic standards, PET, PMMA, and PVC were analyzed by scanning various percentages of the filter and extrapolating to determine recovery percentages. Recoveries increased until about 30% and then plateaued. B and C). A MicroPrefs Tablet (PE, PET, PS, 50-300 μm, from Chiron Microplastics) was dissolved in MilliQ water, filtered onto an Anodisc and scanned over 91.6% of the filter area. Random fields of view summing to different proportions of the filter were counted to determine how much of a filter needed to be scanned for consistent results. Total counts are shown in B and the individual polymer counts in C. Based upon the data in A, B, and C, approximately 30% of the filter appears to be a reasonable compromise between scanning time and estimation of microplastic total counts and composition.

Table S3. Drinking water facility sample concentrations and respective total particles and volume.

Sample	Number of Microplastics (With 3.1 area multiplier)	Volume (Liters)	Microplastics per Liter (MPL)
DWS Raw 100 µm – Before Resuspension	6.2 (n = 2)	776.596	0.008
DWS Raw 100 µm – After Resuspension	18.6 (n = 6)	776.596	0.024
DWS Finished 100 µm	68.2 (n = 22)	996.21	0.068

Table S4. Drinking water facility samples individual particle descriptions of composition, length and width in microns, visual morphology, and visual color.

Sample ID	Number	Composition	Length (µm)	Width (µm)	Morphology	Color
DWS Raw 100 µm – Before Resuspension	1	PP	51.97	29.11	Unknown	Unknown
	2	PP	262.6	58.17	Fragment	Unknown
DWS Raw 100 µm – After Resuspension	1	PET	261.87	28.5	Fiber	Translucent
	2	PU	196.97	132.72	Fragment	Gray
	3	PU	124.53	79.05	Fragment	Gray
	4	PET	475.63	66.44	Fiber	Translucent
	5	PET	734.67	511.2	Fragment	Translucent
	6	PA	193.67	81.84	Fragment	Translucent
DWS Finished 100 µm	1	PE	64.6	43	Fragment	Translucent
	2	PA	140.5	119.5	Fragment	Translucent
	3	PA	62	50.5	Fragment	Translucent
	4	PMMA	326.8	219.7	Fragment	Translucent
	5	PE	93.5	48.5	Fragment	Translucent
	6	PA	68.4	50.6	Fragment	Translucent
	7	PE	72	56.1	Fragment	Translucent
	8	PA	98.2	42.4	Fragment	Translucent
	9	PMMA	134	112.9	Fragment	Brown
	10	PA	90.1	70	Fragment	Brown
	11	PA	80.3	57.5	Fragment	Brown
	12	PA	89.6	51.3	Fragment	Translucent
	13	PP	80.9	45.4	Fragment	Translucent
	14	PP	139.9	132.2	Fragment	Translucent
	15	POM	212.5	72.8	Fragment	Translucent
	16	PU	289.4	175.8	Fragment	Brown
	17	PU	1112.2	718.3	Fragment	Brown

	18	PP	441.6	58.8	Fragment	Brown
	19	PP	324.3	91.8	Fragment	Brown
	20	PP	169.4	45.2	Fragment	Translucent
	21	PET	690	31.7	Fiber	Translucent
	22	PET	1926.3	64.8	Fiber	Translucent

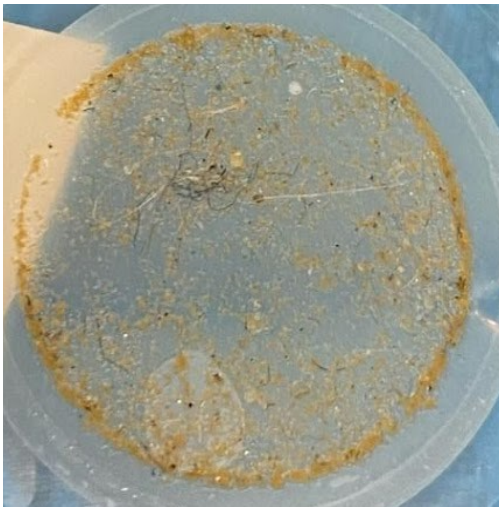

Figure S2. Sample 2, after a 1:1 addition of denatured ethanol to the sample volume (Method 4), was filtered onto an Anodisc analysis filter.

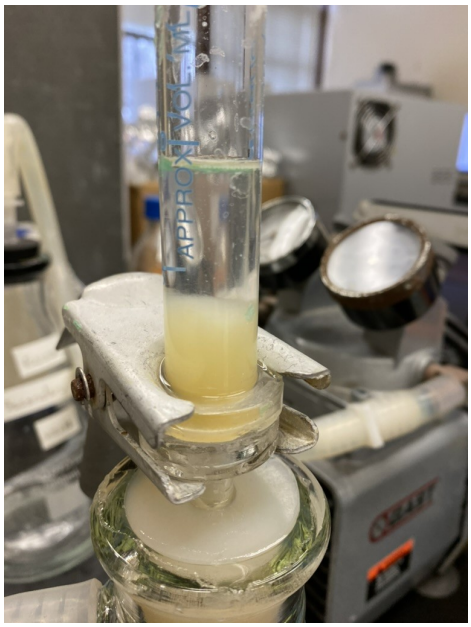

Figure S3. Filtration apparatus following the addition of 5M NaCl to the Fenton/Alcojet solution. The introduction of salt induced the formation of a soap-like principate, leading to the sample clogging the filtration apparatus.

Table S5. Particle size information for positive control standards processed by Method 1 vs Method 4. 25 random particles from Method 1 vs Method 4 were sized using the visual microscopy sizing tool in the Bruker LUMOS II software.

Method 1			**Method 4**		
Particle Number	*Length (um)*	*Width (um)*	*Particle Number*	*Length (um)*	*Width (um)*
1	883	773	*1*	1075	489
2	1041	697	*2*	1146	848
3	1021	710	*3*	1309	800
4	867	547	*4*	1417	1183
5	992	702	*5*	2248	1199
6	1076	719	*6*	851	749
7	1216	959	*7*	1619	600
8	1235	639	*8*	1042	647
9	1421	1051	*9*	1455	1109
10	1289	938	*10*	764	581
11	1107	803	*11*	1503	885
12	1048	658	*12*	1882	1107
13	791	609	*13*	1147	776
14	1471	858	*14*	1249	466
15	959	681	*15*	1779	946
16	866	764	*16*	1630	1384
17	852	741	*17*	1706	1029
18	1072	727	*18*	1137	738
19	1156	789	*19*	2070	1244
20	976	607	*20*	1122	872
21	1108	615	*21*	1057	805
22	939	520	*22*	1254	567
23	791	571	*23*	690	728
24	872	603	*24*	1859	1142
25	918	542	*25*	1236	778
Average	**1039**	**713**	***Average***	**1370**	**867**
Median	**1021**	**702**	***Median***	**1254**	**805**
St dev	**182**	**135**	***St dev***	**401**	**251**
Quartile 1	**883**	**609**	***Quartile 1***	**1122**	**728**
Quartile 3	**1108**	**773**	***Quartile 3***	**1630**	**1107**

Table S6. Comparison of the hotel-restaurant complex finished drinking water sample as initially processed via Method 1 and after resuspension from the Anodisc filter and reprocessing via Method 4.

Raw counts	*Method 1*	*Method 4*
PP	21	9
PET	11	3
PA	12	2
Average length (µm)	202	256

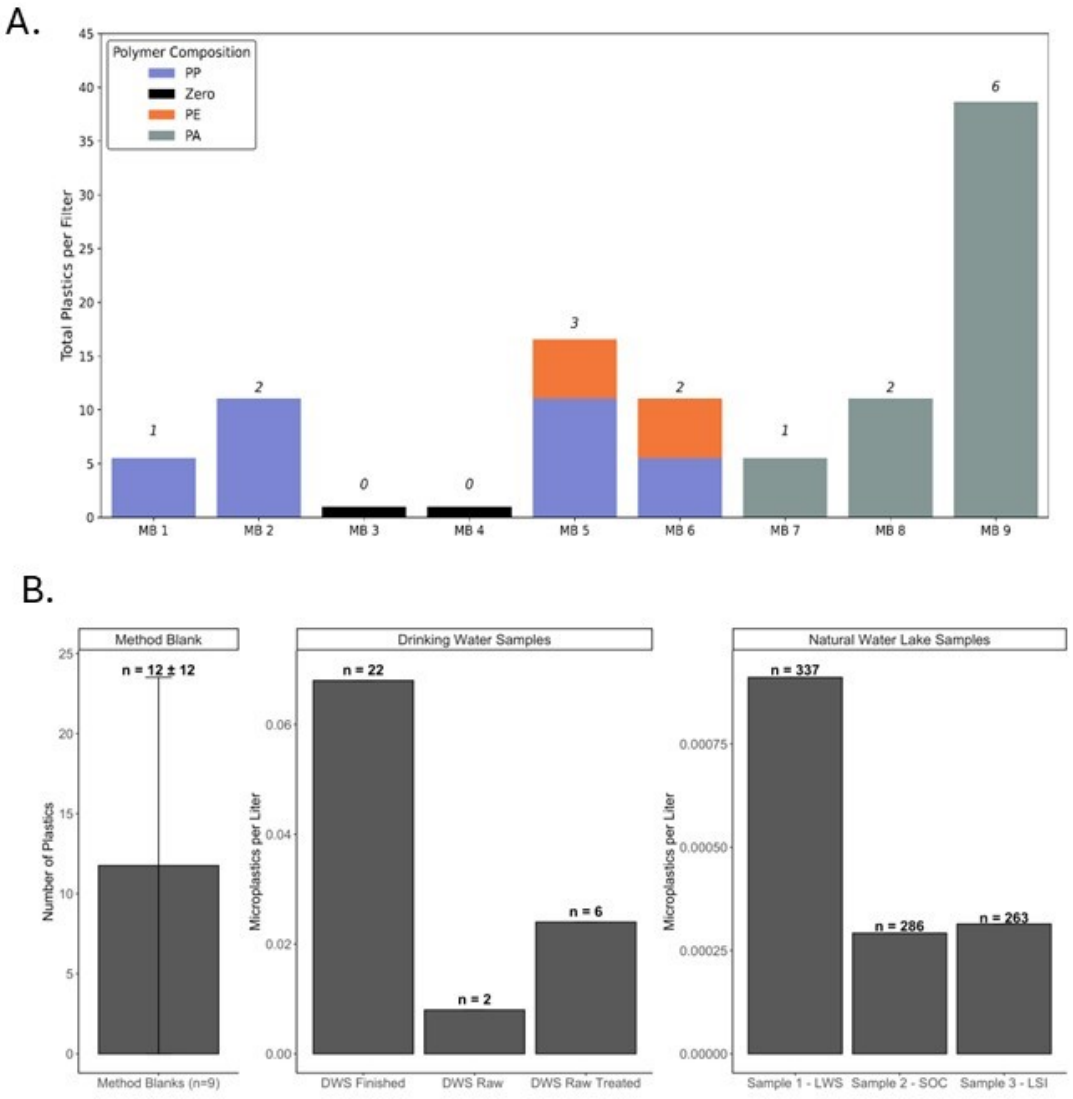

Figure S4. Overview of plastics counts in the methods blanks vs actual samples discussed in the manuscript. A. Data and polymer composition for each of the nine methods blanks performed during this project; 17% of a filter is scanned for each of these. B. Average method blank vs sample counts and concentrations for samples described in the manuscript. Note that DWS Raw are the counts before removal of clay while DWS Raw Treated is after the clay removal step (see manuscript, Fig. 1). The numbers above the bars are the raw counts from the portion of the filter scanned, with the blank counts (from 17% of the filter as shown in A) adjusted to match the sample filter percentage of ~32%. Thus, if blank correction were performed, 12 particles would need to be subtracted from each sample before conversion to the microplastics per liter value.

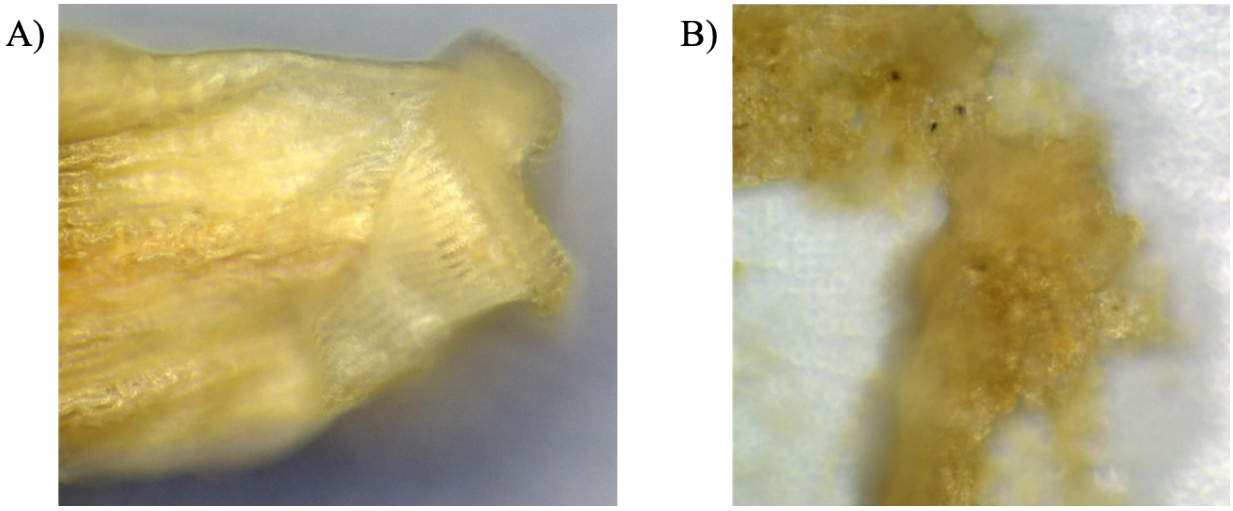

Figure S5 Microscopic images of the wax end of a spruce needle (A) and the residue removed post-Fenton oxidation (B), both analyzed in this study.

Table S7. Specific identification peaks and corresponding normalized intensities for EVAc standards and two leaf waxes. Intensities are normalized to the highest intensity peak in each spectrum.

EVAc Standard		**Spruce Needle Wax**		**Basswood Leaf Wax**	
Wavenumber (cm^{-1})	Normalized Intensity	Wavenumber (cm^{-1})	Normalized Intensity	Wavenumber (cm^{-1})	Normalized Intensity
1464	0.232	1464	0.375	1463	0.216
1738	0.515	1732	1.000	1734	0.790
2851	0.674	2851	0.764	2852	0.611
2922	1.000	2925	0.968	2927	1.000

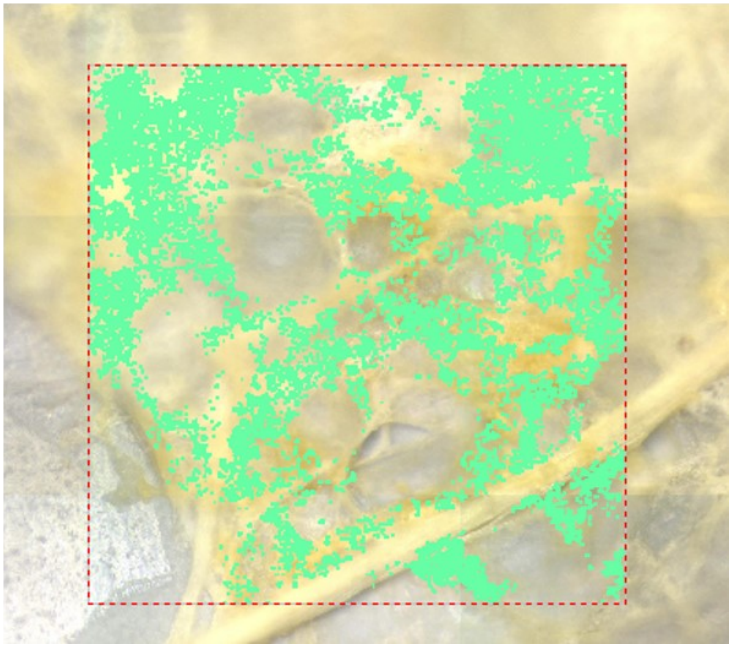

Figure S6 Purency Microplastic Finder (PMF) polymer identification map of Fenton-oxidized basswood leaf wax. The majority of the scanned area is identified as ethylene-vinyl acetate copolymer (EVAc), represented by light green pixels.

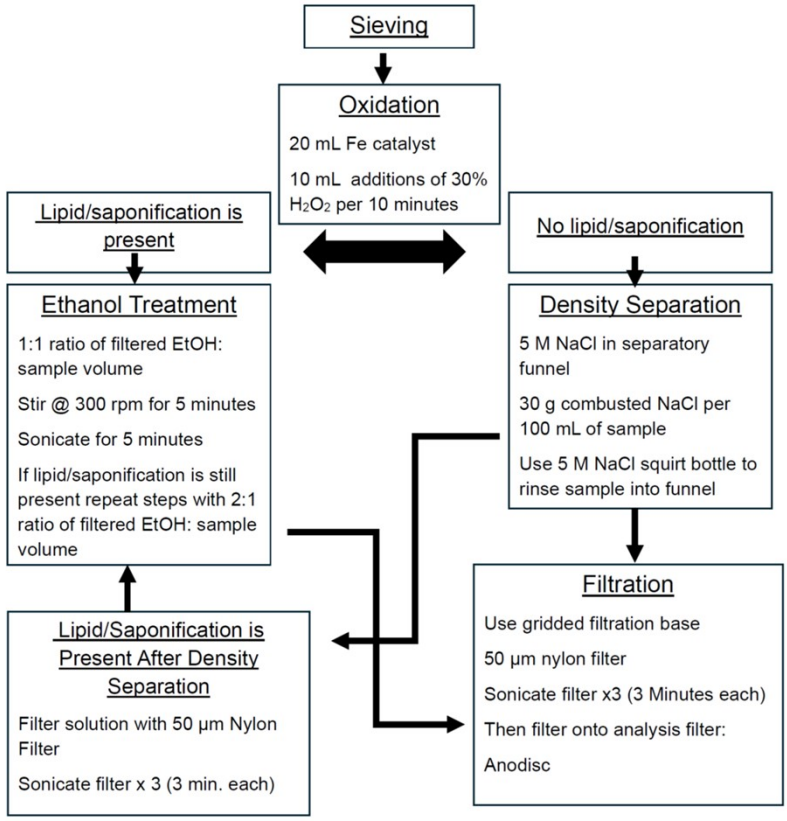

Figure S7 Flowchart of the newly proposed method for in-lab use.