Supplementary file

Unveiling Microplastics Pollution in Alaskan Waters and Snow

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Section S1. Microplastics quantification

We transferred 100 mL of sample to a 250 mL conical flask and stained with Nile Red (19123, Sigma-Aldrich) using two different methods as shown in Fig. S3. Method 1 (adopted from previous studies[39-41]) follows the protocol where Nile red was added at the last step, whereas in Method 2 (adopted from previous studies [42-44]) Nile red was added at the first stage. We faced challenges using method 1 where direct addition of Nile red on glass fiber filter led to the the staining of filters resulting in background fluorescence noise and underestimation of microplastics count during microscopic observation. Therefore, we followed method 2, where Nile red was added at the first stage to avoid the direct contact of Nile red with the glass fiber filter. In method 2, we did not observe any bleaching effect of H_2O_2 on the Nile red stained microplastics. The comparison of Nile red stained microplastics using these two methods has been shown in Fig. S4. The figure shows that the background fluorescence noise has been significantly reduced using method 2 and it provided clear visibility of the microplastic particles as compared to method 1. Previous studies adding Nile red before the oxidation steps reported minimum to no reduction of the fluorescent intensity of Nile red stained microplastics. Therefore, we followed method 2 for staining and quantifying microplastics in all the samples throughout this study.

Section S2. PCA R Code

We summarized our PCA object with the ‘summary’ function. A biplot was generated with the ‘ggbiplot’ function under the ‘ggbiplot’ package. Finally, the ‘PCAtest’ function within the PCAtest package was used to test the statistical significance of the PCA. The detailed R Studio codes for PCA analysis is provided below.

RStudio Code:

```
#Read file
PCAD= read.csv(file.choose(), header = TRUE)
head(PCAD)

#Omit n/a
PCA = na.omit(PCAD)
head(PCA)

#PCA
PCA.pca <- prcomp(PCA[,c(3:9)], center = TRUE,scale. = TRUE)
summary(PCA.pca)

#Biplot
```

```
install.packages("devtools")
library(devtools)
install_github("vqv/ggbiplot")
library(ggbiplot)

ggbiplot(PCA.pca, ellipse = TRUE, groups = PCA$Source, varname.size=7)+
  geom_point(aes(color=PCA$Source), size= 5, shape=16)+
        scale_fill_manual("Source Type")+
theme_classic()+
        theme(legend.title =  element_text(size = 16, face='bold', colour = "Black"),
                        legend.text = element_text(size = 16, face='bold', colour = "Black"),
                        legend.box.just = "right",
                        legend.margin = margin(6, 6, 6, 6),
                        panel.border = element_rect(linetype = "solid", size = 1.5, fill = NA))+
        theme(plot.title=element_text(family='', face='bold', hjust=0.05, vjust=0.5,

                        colour='Black', size=16, margin=margin(t=40,b=-30)),
                        #axis.title.y = element_blank(),
                        axis.title = element_text(color="Black", size=18, face="bold"),
                        #axis.text.y=element_blank(),
                        axis.text=element_text(color="Black", size=16),
                        axis.ticks= element_line(size = 1.3),
                        axis.ticks.length = unit(-0.2, "cm"))

#PCAtest

devtools::install_github("arleyc/PCAtest")
library(PCAtest)
result <- PCAtest(PCA[,c(3:9)], 100, 100, 0.05, varcorr=FALSE, counter=FALSE, plot=TRUE)
```

Tables and Figures

Table S1: Sample metadata showing detailed sample information, including source, coordinates, and MP count. Deionized water (with 18.2 MΩ-cm conductivity) was used as a control blank and stored, processed, and visualized for MPs following an identical protocol used for the field samples. The microplastics data presented in this table were blank subtracted and left censored values were substituted (<LOD with LOD/2 and <LOQ with LOQ/2)

Ser no.	Sampling Date	Location Name	Sample Source	Latitude	Longitude	Region	Microplastics Count (L^{-1})	Size (µm)
1	July, 2020	Lowe River	River	61.0669444	-146.04983	Southcentral	259[a]	67.3
2	July, 2020	Blueberry Recreation Lake	Lake	61.1211435	-145.68814	Southcentral	259[a]	509.0
3	July, 2020	Blueberry Recreation Lake	Lake	61.147999	-145.72611	Southcentral	259[a]	71.9
4	July, 2020	Worthington Glacier River	River	61.1678964	-145.71523	Southcentral	634	32.5
5	July, 2020	Squirrel Creek	Creek	61.665815	-145.16787	Southcentral	259[a]	64.8
6	July, 2020	Chitina Town Lake	Lake	61.5147414	-144.43775	Southcentral	86[b]	629.4
7	July, 2020	Lake near Chitina	Lake	61.5656074	-144.44466	Southcentral	571	47.0
8	July, 2020	Klutina River	River	61.9547204	-145.32487	Southcentral	259[a]	56.0
9	July, 2020	Meiers Lake	Lake	62.8184931	-145.49737	Southcentral	737	132.9
10	July, 2020	Summit Lake	Lake	63.1184385	-145.49501	Southcentral	841	59.9
11	July, 2020	Castner Creek	Creek	63.4032702	-145.7352	Interior	86[b]	813.9
12	March, 2021	Haman St.	Snow	64.828918	-147.91443	Interior	821	69.4
13	March, 2021	Fox Den Road	Snow	64.958054	-147.61833	Interior	614	83.5
14	March, 2021	University of Alaska Fairbanks	Snow	64.8575	-147.82194	Interior	1011	NA
15	March, 2021	Tanana River	Snow	65.1605556	-151.96028	Interior	681	NA
16	March, 2021	North Slope Haul Road 1	Snow	67.811351	-149.82265	Interior	259[a]	62.0
17	March, 2021	North Slope Haul Road 2	Snow	67.810548	-149.82179	Interior	681	96.7
18	March, 2021	Yukon-Koyukuk School District 1	Snow	64.622432	-149.55242	Interior	701	61.6
19	March, 2021	Yukon-Koyukuk School District 2	Snow	64.67733	-149.81173	Interior	259[a]	102.2
20	March, 2021	Yukon-Koyukuk School District 3	Snow	64.916559	-149.43073	Interior	867	90.8

21	March, 2021	Yukon-Koyukuk Census Area	Snow	64.756909	-149.13132	Interior	1206	NA
22	March, 2021	Adjacent to Alyeska Pipeline (APL) valve house	Snow	67.7986533	-149.81979	Interior	995	92.6
23	March, 2021	Adjacent to Dietrich River and Met Station (ADS1)	Snow	67.6448217	-149.73775	Interior	848	103.6
24	March, 2021	Located between TAPS and Dalton highway	Snow	67.7359717	-149.75863	Interior	810	93.0
25	March, 2021	Upper Sag River valley 1	Snow	68.44598	-148.70284	Far North	864	58.8
26	March, 2021	Upper Sag River valley 2	Snow	68.4135083	-148.95772	Far North	814	56.6
27	March, 2021	Arctic National Wildlife Refuge Steep Ridge	Snow	68.4119267	-148.13664	Far North	781	57.0
28	March, 2021	Arctic National Wildlife Refuge Platue	Snow	68.64327	-147.35129	Far North	1031	95.2
29	March, 2021	East of Dalton Hoghway Remote Location 1	Snow	69.102205	-146.82428	Far North	681	95.9
30	March, 2021	North Slope Borough School District	Snow	69.229	-147.6184	Far North	1010	130.2
31	March, 2021	East of Dalton Hoghway Remote Location 2	Snow	68.9766883	-147.23306	Far North	661	95.4
32	March, 2021	East of Dalton Hoghway Remote Location 3	Snow	68.6820283	-148.04123	Far North	698	59.6
33	March, 2021	East of Dalton Hoghway Remote Location 4	Snow	68.617605	-148.15362	Far North	259 [a]	119.7
34	March, 2021	East of Dalton Hoghway Remote Location 5	Snow	68.484505	-147.83512	Far North	895	125.0
35	April, 2021	Remote Location 1 at the West of Arctic National Wildlife Refuge	Snow	68.6932283	-147.47897	Far North	259 [a]	NA
36	April, 2021	Remote Location 2 at the West of Arctic National Wildlife Refuge	Snow	68.7719867	-147.43274	Far North	552	100.6
37	April, 2021	North Slope Borough School District 1	Snow	69.349025	-148.0163	Far North	781	82.8
38	April, 2021	Yukon Flats School District 2	Snow	67.6690733	-149.63816	Interior	259 [a]	69.7
39	April, 2021	North Slope Borough School District 2	Snow	68.0791167	-149.77842	Interior	259 [a]	52.6
40	April, 2021	North Slope Borough School District 3	Snow	68.0859433	-149.87353	Interior	259 [a]	72.5
41	April, 2021	Yukon Flats School District 3	Snow	67.85767	-149.67829	Interior	756	80.1
42	April, 2021	Yukon Flats School District 4	Snow	67.668775	-149.64668	Interior	719	95.9
43	July, 2021	Turnagain pass	Creek	60.801799	-149.18376	Southcentral	881	73.0
44	July, 2021	East fork Chulitna River	River	63.23264	-149.20288	Interior	86 [b]	58.2
45	July, 2021	Trail Lakes	Lake	60.500248	-149.37407	Southcentral	86 [b]	571.9
46	July, 2021	Bird Creek	Creek	60.973461	-149.46657	Southcentral	259 [a]	86.6

47	July, 2021	Bear Creek	Creek	64.13449	-149.25352	Interior	86 [b]	29.9
48	July, 2021	Sheep Creek	Creek	61.994241	-150.05883	Southcentral	748	85.1
49	July, 2021	Lower Troublesome Creek	Creek	62.600828	-150.23361	Southcentral	86 [b]	866.1
50	July, 2021	Nenana	River	63.517396	-148.80512	Interior	259 [a]	1239.9
51	July, 2021	East fork six mile creek	Creek	60.75547	-149.38585	Southcentral	937	113.7
52	April, 2021	Top of the ridge next to radiation tower 1	Snow	68.61584	-149.3027	Far North	1001	148.5
53	April, 2021	Top of the ridge next to radiation tower 2	Snow	68.61584	-149.3027	Far North	595	125.6
54	April, 2021	Top of the ridge next to radiation tower 3	Snow	68.61584	-149.3027	Far North	781	104.3
55	April, 2021	Top of the ridge next to radiation tower 4	Snow	68.61584	-149.3027	Far North	259 [a]	112.7
56	April, 2021	Top of the ridge next to radiation tower 5	Snow	68.61584	-149.3027	Far North	581	105.1
57	August, 2021	Yukon River	River	65.8785556	-149.72119	Interior	86 [b]	53.5
58	August, 2021	Yukon-Koyukuk School District 5	River	67.0178889	-150.28753	Interior	86 [b]	1034.7
59	August, 2021	Marion Creek	Creek	67.3008889	-150.14819	Interior	86 [b]	1315.9
60	August, 2021	North Fork	River	66.6762222	-150.64333	Interior	86 [b]	1865.9
61	August, 2021	Carlo Creek	Creek	63.566363	-148.8135	Interior	259 [a]	1267.2
62	August, 2021	Savage River	River	63.740558	-149.29186	Interior	711	32.3
63	August, 2021	Deep Creek North	Creek	60.029995	-151.68245	Southcentral	259 [a]	56.4
64	August, 2021	Beluga Lake	Lake	59.6419	-151.5146	Southcentral	259 [a]	108.6
65	August, 2021	Kenai River	River	60.4919	-149.81038	Southcentral	259 [a]	NA
66	August, 2021	Anchor Point	Creek	59.752436	-151.76747	Southcentral	259 [a]	117.7
67	August, 2021	Kasilof River	River	60.3168592	-151.25864	Southcentral	259 [a]	113.4

68	August, 2021	Summit lake	Lake	60.640898	-149.49892	Southcentral	631	87.8
69	August, 2021	Centennial Park	River	60.475886	-151.08436	Southcentral	1331	NA
70	August, 2021	Quartz Creek	Creek	60.478706	-149.72417	Southcentral	695	86.2
71	August, 2021	Sisvalik Lake 1	Lake	67.0744722	-163.29353	Interior	259 [a]	88.6
72	August, 2021	Sisvalik Lake 2	Lake	67.0744722	-163.29353	Interior	86[b]	90.0
73	August, 2021	Paxon Lake	Lake	62.8946	-145.5318	Southcentral	259 [a]	50.2

[a]Microplastics count below LOQ, [b]Microplastics count below LOD

Table S2. LOD and LOQ of MPs analysis method

Control	MP Count (L^{-1})	Mean	SD	LOD	LOQ
Deionized water	444	519.31	51.83	171.04	518.30
Deionized water	560				
Deionized water	580				
Deionized water	530				
Deionized water	510				
Deionized water	550				
Deionized water	440				
Deionized water	540				

Table S3. Analyzing the distribution of microplastics data for MPs count, size, and morphology using various statistical methods. P values with bold font indicate a non-normal distribution.

Statistical method	Data type	Source		P Value
Shapiro-Wilk test	MPs count (Particles/L)	River		**8×10^{-4}**
		Lake		**3×10^{-2}**
		Creek		**6×10^{-3}**
		Snow		**5×10^{-3}**
Kolmogorov-Smirnov test	MPs size (µm)	River		**2.2×10^{-16}**
		Lake		**2.2×10^{-16}**
		Creek		**2.2×10^{-16}**
		Snow		**2.2×10^{-16}**
Shapiro-Wilk test	MPs count (Particles/L)	Southcentral		**5×10^{-4}**
		Interior		**1×10^{-3}**
		Far North		0.09
Shapiro-Wilk test	MPs percentage (%)	Lake	Fiber	**1.9×10^{-2}**
			Fragment	**1.7×10^{-3}**
			Pellet	**2.3×10^{-3}**
			Filament	**1.5×10^{-3}**
		River	Fiber	**4.6×10^{-2}**
			Fragment	**1.5×10^{-2}**
			Pellet	**5.1×10^{-4}**
			Filament	**1.2×10^{-2}**
		Creek	Fiber	**8.4×10^{-3}**
			Fragment	**1.8×10^{-2}**
			Pellet	**1.5×10^{-3}**
			Filament	**4.9×10^{-3}**
		Snow	Fiber	**3.1×10^{-4}**
			Fragment	**5.8×10^{-3}**
			Pellet	**4.2×10^{-4}**
			Filament	**6.1×10^{-2}**

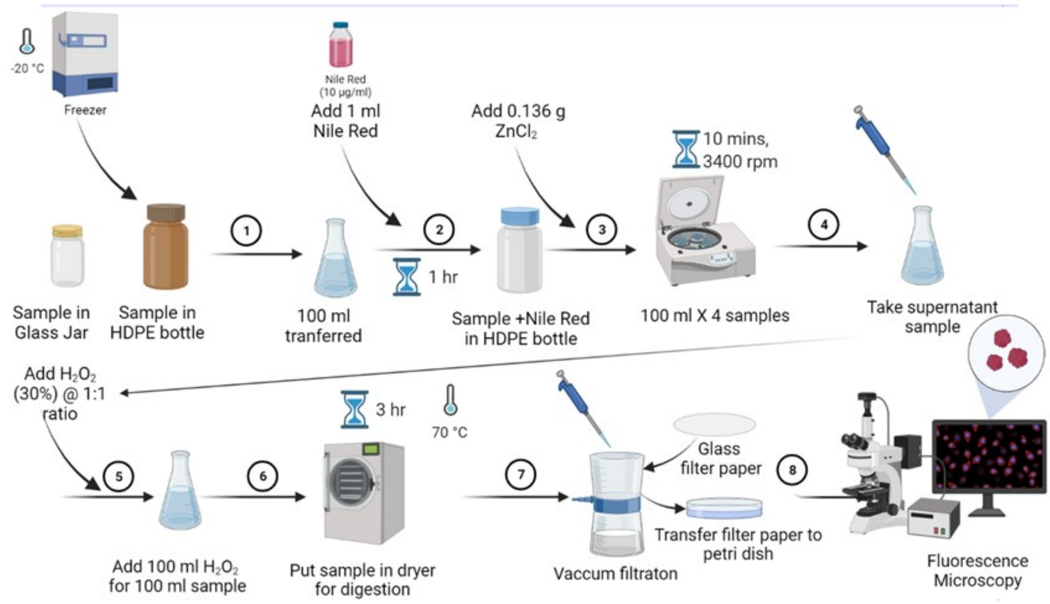

Figure S1. Methods developed for analyzing microplastics from collected samples

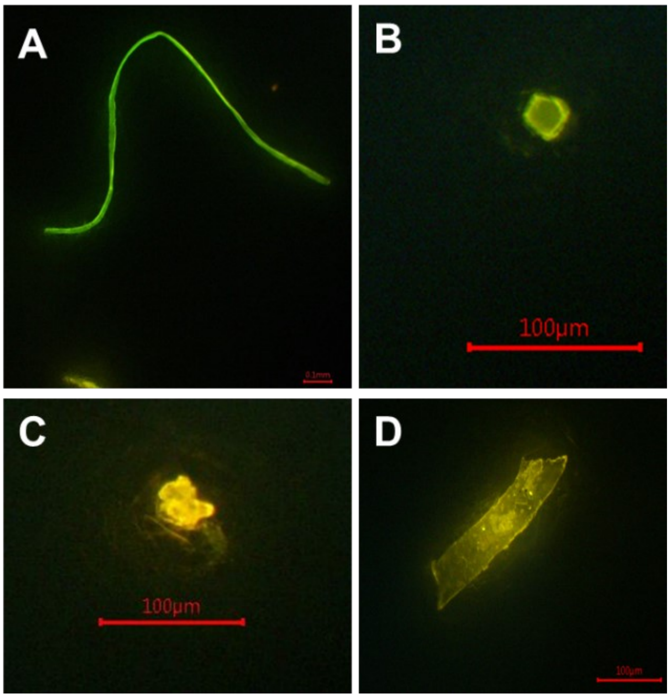

Figure S2. Microplastics types based on morphology observed under fluorescent microscope at 400X magnification; fiber (A), fragments (B), pellet (C), film (D).

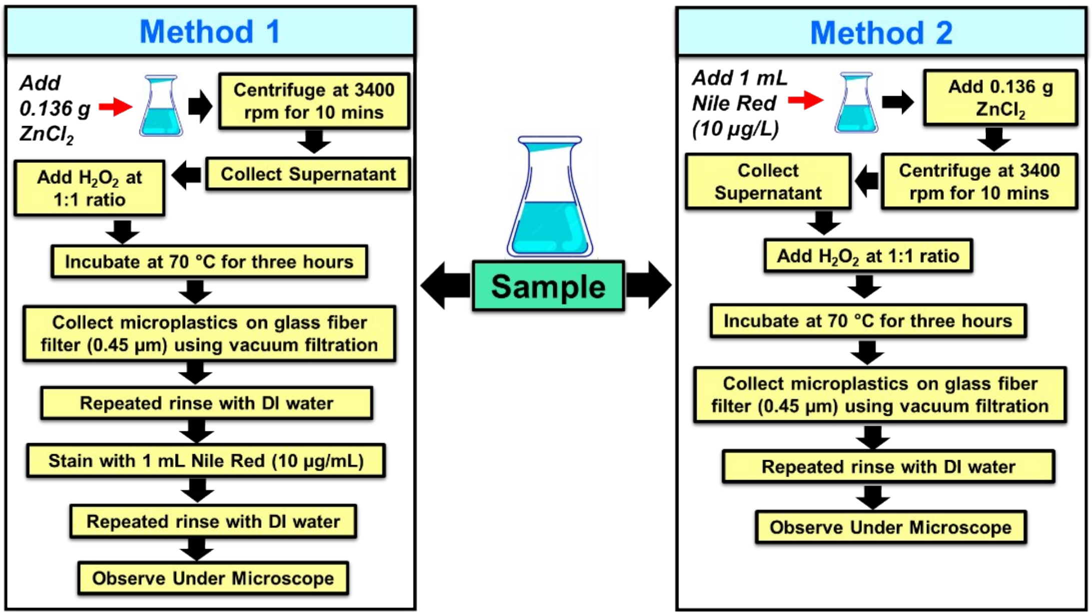

Figure S3. Schematic presentation of the two different methods compared in this study for staining microplastics with Nile Red.

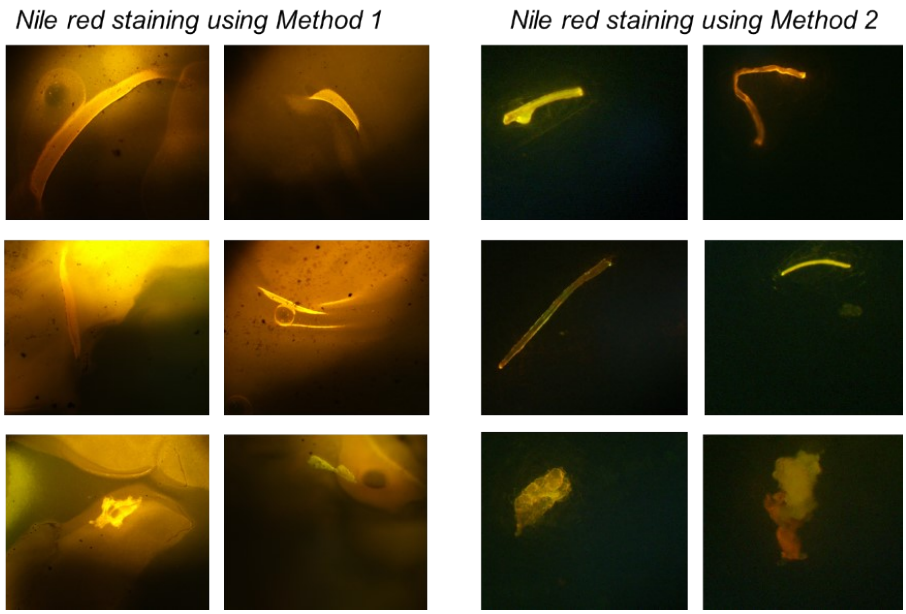

Figure S4. Nile Red stained microplastics observed under fluorescent microscope at 400X magnification. The figure shows that method 2 enabled significant reduction of background fluorescence noise and much better visibility of microplastics as compared to method 1.

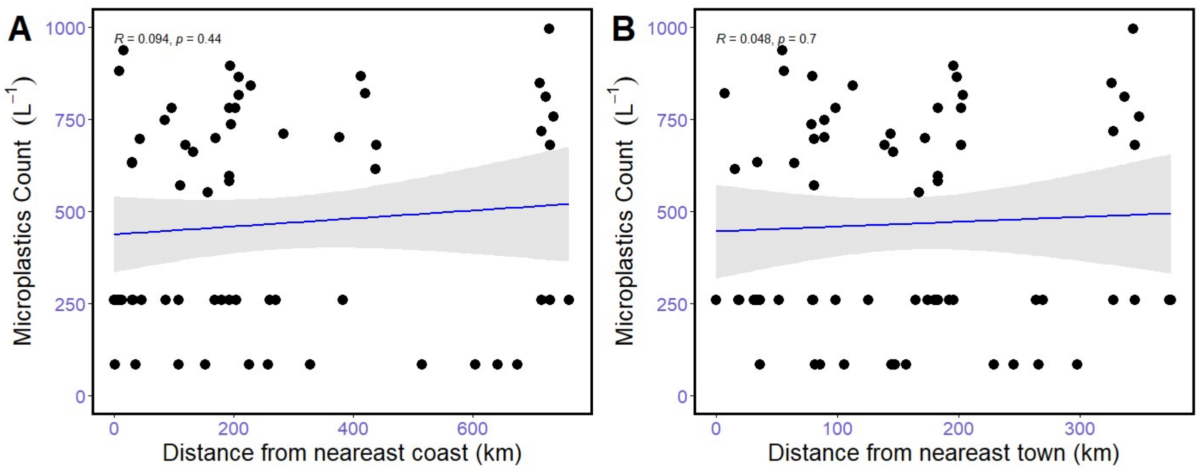

Figure S5. Correlations of overall MP counts from all sources in this study with respect to distance from nearest coast (A) and nearest town (B).