Exploring the efficacy of Nile red in microplastic quantification: a co-staining approach

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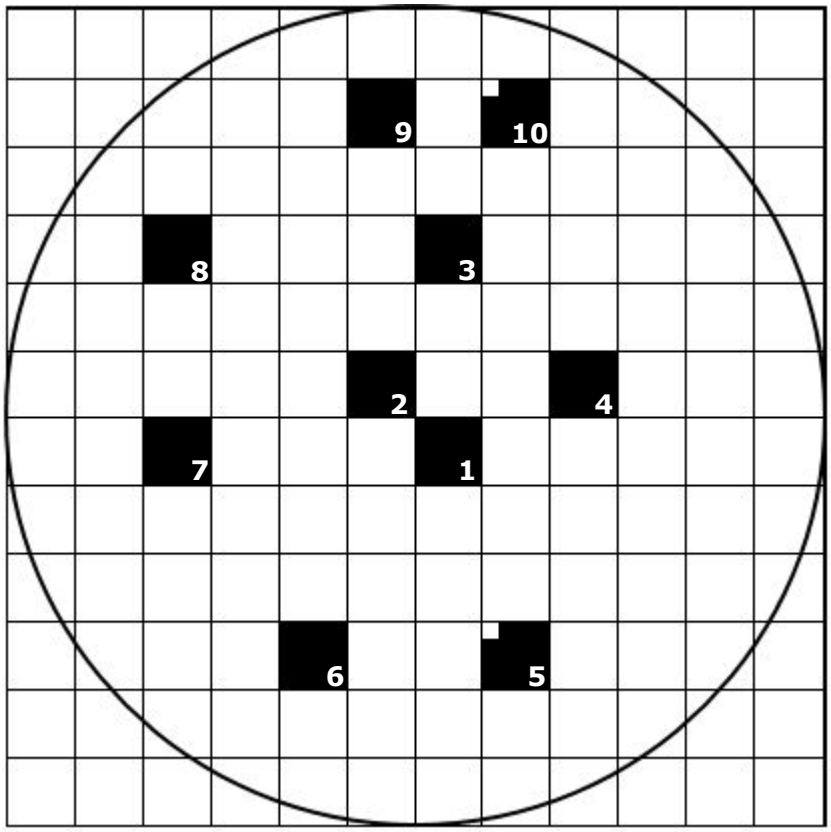


Figure S1: The cells on the gridded filter papers that were observed at 40 x magnification in each sample. The two cells with the white square within them were also observed at 100x magnification.

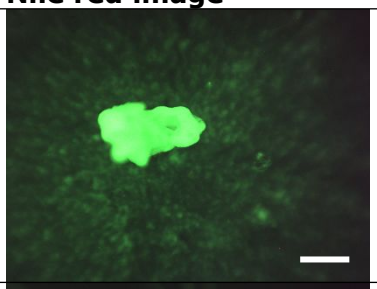
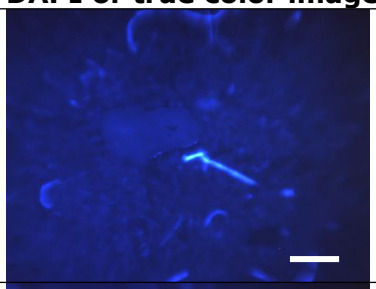
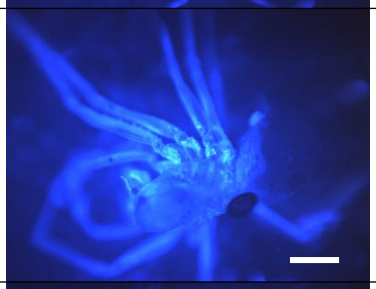
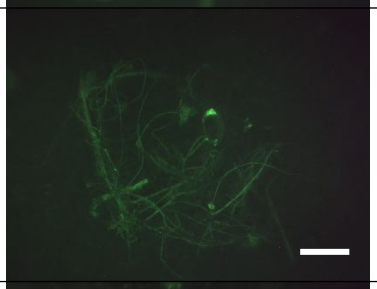
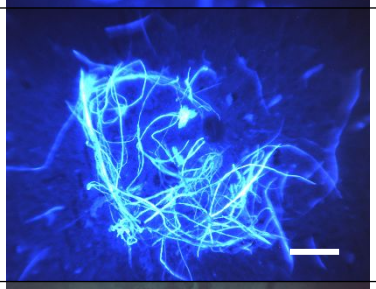
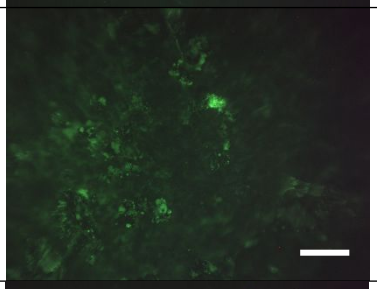
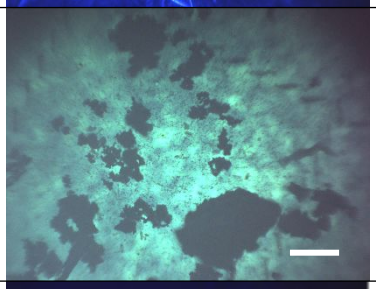
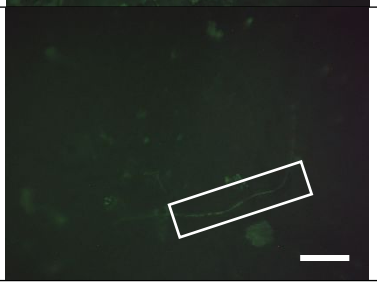
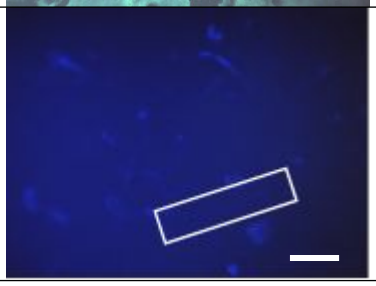
Particle	Nile red image	DAPI or true color image
Possible microplastic particle		
Invertebrate		
Bundle of fibers		
Black particles exhibiting uneven staining		
Natural textile fiber		

Figure S2: Nile Red and DAPI images of natural and possible microplastic particles stained in samples of river water. All images taken at 40x magnification and the scale bar represents 500 µm

Table S1: Table of known plastic particles and natural and synthetic fibers, detailing their color, autofluorescence at the green and blue wavelengths used to assess Nile red and DAPI staining respectively (see section 2.3), and affinity with Nile Red and DAPI fluorescent stains where autofluoresence was not observed.

Polymer	Color	Autofluorescence		Nile red fluorescence?	DAPI fluorescence?
		Green	Blue		
PP	Transparent	No	No	Yes	No
	Orange	No	No	Yes	No
	Blue	No	Yes	Yes	-
	Red	No	No	Yes	No
	Black	No	No	Yes - at edges only	No
HDPE	Translucent	No	No	Yes	No
	Brown	No	No	Yes - at edges only	No
EPS	White	No	No	Yes	No
PVC	White	Yes	No	-	No
Polyester	Black	No	No	No	No
Polyamide	Red	No	Yes	No	-
	Grey	No	Yes	Yes	-
Acrylic	Blue	No	No	No	No
	Orange	No	No	Yes	No
Cotton	White	No	No	Yes	Yes
Wool	Light blue	Yes	Yes	-	-
	Dark blue	No	No	Yes	Yes
Silk	Blue	No	No	Yes	Yes
Rayon	Blue	No	No	Yes	Yes

Table S2: Number of particles that fluoresced with Nile Red and DAPI at 40x and 100x magnification across ten and two cells of the filter area respectively (Figure S1). Significance was calculated using a paired sample t-test for counts at 40x magnification, in which the mean particle counts across these ten grid cells was compared for particles that fluoresced with Nile red, and particles that fluoresced with Nile Red but not DAPI (i.e. suspected microplastics). The paired samples T test could not be calculated at 100x magnification due to only two grid cells being quantified at this magnification.

Sample	Magnification	Particles that fluoresced with Nile Red	Particles that fluoresced with both Nile Red and DAPI	Suspected MPs (particles that only fluoresce with Nile red)	Significance (p=)
River water 1	40	33	22	11	0.003
River water 1	100	17	10	7	
River water 2	40	31	15	16	0.002
River water 2	100	11	6	5	
River water 3	40	74	8	68	0.087
River water 3	100	16	6	10	
Bottled water 1	40	14	12	2	0.005
Bottled water 1	100	14	13	1	
Bottled water 2	40	3	2	1	0.168
Bottled water 2	100	5	3	2	
Bottled water 3	40	9	9	0	0.019
Bottled water 3	100	11	8	3	
Bottled water 4	40	15	6	9	0.024
Bottled water 4	100	18	10	8	
Canned water	40	28	13	15	0.001
Canned water	100	70	57	13	
Tap water	40	24	18	6	0.001
Tap water	100	20	12	8	

Table S3: The material, color, and autofluorescence of different parts of the containers of drinking water samples. The lid of bottled water 4 had two parts.

Sample		Material	Color	Autofluorescence	
				Green	Blue
Bottled water 1	**Lid**	HDPE	Light blue	N	N
	Body	PET	Transparent	Y	Y
Bottled water 2	**Lid**	HDPE	Green	N	N
	Body	PET	Transparent	Y	Y
Bottled water 3	**Lid**	HDPE	Dark blue	N	N
	Body	PET	Transparent	Y	Y
Bottled water 4	**Lid inner**	HDPE	White	N	N
	Lid outer	PP	Dark blue	N	N
	Body	PET	Transparent	Y	Y
Canned water	**Lid**	Polypropylene	Black	N	N
	Body	Aluminium	N/A		

Table S4: Abundance of autofluorescing particles across the six drinking water samples.

Sample	Magnification	Autofluorescence	
		Green	Blue
Bottled water 1	40	0	5
Bottled water 1	100	0	1
Bottled water 2	40	0	5
Bottled water 2	100	0	0
Bottled water 3	40	0	0
Bottled water 3	100	0	0
Bottled water 4	40	0	2
Bottled water 4	100	0	2
Canned water	40	2	4
Canned water	100	1	2
Tap water	40	0	8
Tap water	100	0	3