

Semi-automated analysis of microplastics in complex wastewater samples

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SUPPLEMENTARY INFORMATION

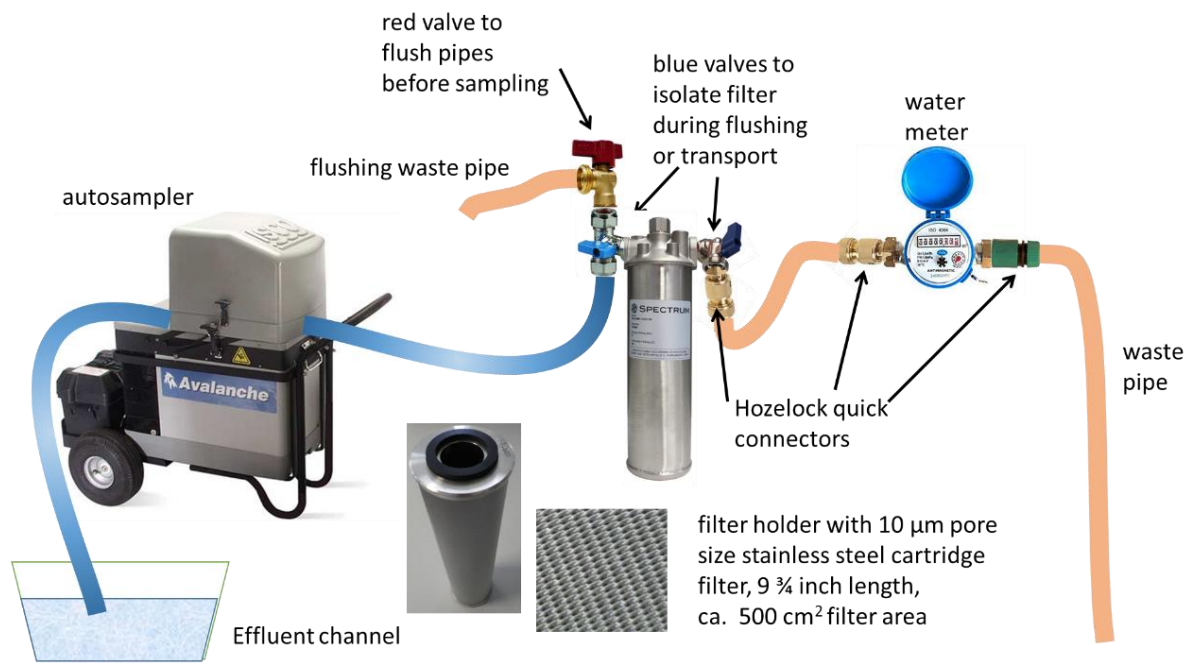


Figure S1. Schematic sampling setup for WwTW effluent

S1. Resolution and blank correction calculations

As it is not possible to detect less than one particle this is also the limit of detection (LOD) unless blank contamination results in a higher LOD (see below). The 'resolution' for each polymer is therefore calculated based one particle in the analysed area on the filter, scaled up to the volume of the original sample. This means there must therefore be equal or greater than this number of particles in the original sample, in order for one particle to be detected in the subsample on the filter. The resolution for each polymer therefore varied between samples due to the different sample volumes, especially during subsampling.

Where blank contamination occurred, the sample counts were corrected by the average of the blanks for each polymer. This was applied to the whole processed sample regardless of volume, because contamination could have occurred at any time throughout the process, not just in the final step of applying the subsample to the silver filter. The initial LOD for the blank-corrected sample was calculated as 3.3 times the standard deviation of the blanks and the LOQ as 10 times the standard deviation of the blanks as is commonly done for chemical analyses (AOAC, 2011, Table S1).

The final LOD and LOQ for blank-corrected samples can therefore be reported as being either the value of the initial LOD and LOQ as calculated from the standard deviation of the blanks, or (based on resolution) 1 or 3 particles (LOD and LOQ respectively) in the 92% visible filter area (i.e. 1.1 or 3.3 particles for the whole filter) where blank contamination was not detected, whichever is higher. The final values are converted back into values per L or per g (Tables S2-S5). In order to be reported above LOD or LOQ, the blank-corrected value must be above the LOD or LOQ value, in which case the number is reported. If the resulting number is below the LOD or LOQ, the number is reported as <LOD or <LOQ.

Designations and calculations were carried out for each polymer individually as follows:

- Number of particles on filter [A]
- Proportion of whole sample (i.e. subsample) on filter [B]
- Number of particles in original whole sample $[C] = ([A]/0.92)/[B]$
- Mean of blank particles on filter (corrected for 92%) [D]
- Blank corrected number of particles in whole sample $[G] = [C] - [D]$
- LOD [E] = 3 x standard deviation of [D], OR $1.1/[B]$, whichever is higher
- LOQ [F] = 10 x standard deviation of [D], OR $3.3/[B]$, whichever is higher
- Final value in whole sample above LOD [H] = [G] compared to [E] (only reported if higher than [E])
- Final concentration above LOD in L or g = $[H]/\text{volume or mass represented by subsample}$
- Final value in whole sample above LOQ [I] = [G] compared to [F] (only reported if higher than [F])
- Final concentration above LOD in L or g = $[I]/\text{volume or mass represented by subsample}$

Table S1. Blank contamination values for each polymer based on influent/effluent blanks (n = 8) and sludge blanks (n = 5), and subsequent LOD and LOQ values calculated for each polymer. [^]Based on one particle in the 92% filter area analysed, scaled up to the whole filter [^]Based on three particles in the 92% filter area analysed, scaled up to the whole filter

Influent/effluent				
Polymer group	Mean	SD	LOD (3.3 * SD of blank, or 1.1[^] particles, whichever is higher)	LOQ (10 * SD of blank, or 3.3[^] particles, whichever is higher)
PE	14	13	43	130
PP	11	7.8	26	78
PET	18	16	53	161
PMMA	0	0	1.1	3.3
PA	1.4	1.8	6.0	18
PS	0.8	1.0	3.2	9.6
PU	0	0	1.1	3.3
PVC	0	0	1.1	3.3
ABS	2.0	2.9	9.5	29
Sludge				
PE	9.6	9.5	31	95
PP	66	37	123	373
PET	208	324	1,068	3,236
PMMA	0	0	1.1	3.3
PA	8.8	16	52	158
PS	2.1	2.3	7.7	23
PU	0	0	1.1	3.3
PVC	0.4	0.8	2.8	8.4
ABS	0.3	0.7	2.3	7.0

Table S2. Concentrations as microplastic particles per L in WwTW influent, values <LOD are qualified by “<” and those above the LOD but below the LOQ by “≈”. Values above the LOD are coloured yellow and those above the LOQ green. LOD and LOQ values depend on the processed volumes. The suffixes a and b indicate that sludge was also collected at that site (see Table S4).

Name	Date	% original sample on final filter	Volume on final filter (ml)	resolution* MP/L	PE MP/L	PP MP/L	PET MP/L	PMMA MP/L	PA MP/L	PS MP/L	PU MP/L	PVC MP/L	ABS MP/L	Total MPs (>LOQ only)
ASTC1	26/11/18	0.7%	1.23	882	≈ 1688	5232	< 882	< 882	< 882	< 882	< 882	< 882	< 882	5232
	08/01/19	0.7%	1.38	787	3078	5457	4633	< 787	< 787	< 787	< 787	< 787	< 787	13,168
ASTC2	05/12/18	0.6%	1.24	963	≈ 2816	3796	< 963	< 963	< 963	< 963	< 963	< 963	< 963	3796
	21/01/19	0.6%	1.10	1,007	< 1007	3969	< 1007	< 1007	< 1007	< 1007	< 1007	< 1007	< 1007	3969
ASTS1	29/11/18	0.7%	1.07	875	< 875	6940	≈ 1651	< 875	< 875	< 875	< 875	< 875	< 875	6940
	10/01/19	0.6%	1.29	988	< 988	9817	< 988	< 988	< 988	< 988	< 988	< 988	< 988	9817
ASTS2	03/12/18	0.6%	1.13	1,016	≈ 1958	17,214	≈ 2954	< 1016	< 1016	< 1016	< 1016	< 1016	< 1016	17,214
	14/01/19	0.7%	1.08	845	4151	3323	< 845	< 845	< 845	< 845	< 845	< 845	< 845	7475
AS1 a	17/01/19	2.3%	1.43	258	955	≈ 457	≈ 419	< 258	< 258	< 258	< 258	< 258	< 258	955
	01/02/19	0.6%	1.19	962	8583	≈ 2830	≈ 2793	< 962	< 962	< 962	< 962	< 962	< 962	8583
AS2 b	11/02/19	0.7%	4.22	841	< 841	≈ 1624	≈ 1585	< 841	< 841	< 841	< 841	< 841	< 841	-
TFS b	17/02/19	0.6%	1.13	991	< 991	< 991	< 991	< 991	< 991	< 991	< 991	< 991	< 991	-
TFP	12/12/18	0.7%	1.29	762	2979	≈ 1471	< 762	≈ 762	< 762	< 762	< 762	< 762	< 762	2979
	23/01/19	0.6%	1.10	917	≈ 2677	≈ 1777	< 917	< 917	< 917	< 917	< 917	< 917	< 917	-
BAFF	29/01/19	0.7%	3.10	351	1371	≈ 1028	≈ 661	< 351	< 351	< 351	< 351	< 351	< 351	1371
	06/02/19	0.7%	1.33	816	≈ 1559	5656	3987	< 816	< 816	< 816	< 816	< 816	< 816	9642

* Resolution: The minimum number of particles of each polymer required in the whole processed sample to enable one particle to be quantified in the analyzed subsample. If no particles are detected in the analysed subsample, we can only deduce that the number in the whole sample was less than this (hence reporting <). Note: LOD varies between samples and polymers (see Section S1) and may be higher than resolution where blank contamination is high.

Table S3 Concentrations as microplastic particles per L in WwTW effluent, values <LOD are qualified by “<” and those above the LOD but below the LOQ by “≈”. LOD are coloured yellow and those above the LOQ green. LOD and LOQ values depend on the processed volumes. The suffixes a and b indicate that sludge was also collected at that site (see Table S4).

Name	date	% original sample on final filter	Volume on final filter (L)	resolution* MP/L	PE MP/L	PP MP/L	PET MP/L	PMMA MP/L	PA MP/L	PS MP/L	PU MP/L	PVC MP/L	ABS MP/L	Total MPs (>LOQ only)
ASTC1	26/11/18	1.9%	0.72	1.5	< 1.5	< 1.5	5.6	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5	5.6
	08/01/19	0.8%	0.69	1.6	< 1.6	≈ 3.0	≈ 4.5	< 1.6	< 1.6	< 1.6	≈ 1.6	< 1.6	< 1.6	-
ASTC2	05/12/18	1.9%	1.1	1.0	≈ 1.8	≈ 2.8	< 1.0	< 1.0	< 1.0	< 1.0	≈ 1.0	< 1.0	< 1.0	-
	21/01/19	3.7%	2.2	0.5	< 0.7	≈ 1.3	≈ 2.2	≈ 0.5	< 0.5	< 0.5	≈ 0.5	< 0.5	< 0.5	-
ASTS1	29/11/18	1.9%	1.3	0.8	< 0.8	< 0.8	≈ 1.4	< 0.8	< 0.8	< 0.8	< 0.8	< 0.8	< 0.8	-
	10/01/19	3.8%	2.8	0.4	≈ 1.3	< 0.4	< 0.7	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	-
ASTS2	03/12/18	1.5%	2.0	0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	-
	14/01/19	2.1%	1.1	1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	-
AS1 a	17/01/19	1.4%	1.1	1.0	< 1.0	4.0	4.9	< 1.0	< 1.0	≈ 2.1	< 1.0	< 1.0	< 1.0	8.9
	01/02/19	2.2%	1.7	0.7	≈ 1.8	< 0.7	2.4	< 0.7	< 0.7	< 0.7	< 0.7	≈ 0.7	< 0.7	2.4
AS2 b	11/02/19	2.2%	1.8	0.6	≈ 1.0	≈ 1.6	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6	-
	17/02/19	1.1%	1.6	0.7	≈ 1.2	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	-
TFS b	11/02/19	2.3%	2.4	0.5	2.6	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	2.6
	17/02/19	1.9%	0.15	7.3	27.4	< 7.3	26.8	< 7.3	< 7.3	< 7.3	< 7.3	< 7.3	< 7.3	54.2
TFP	12/12/18	2.4%	2.4	0.5	5.3	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	5.3
	23/01/19	4.0%	3.1	0.3	2.2	≈ 0.6	< 0.7	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	2.2
BAFF	29/01/19	4.4%	6.3	0.2	< 0.3	≈ 0.3	< 0.4	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	-
	06/02/19	4.7%	3	0.4	≈ 1.9	44.5	≈ 1.8	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	44.5

* Resolution: The number of particles of each polymer in the whole processed sample equivalent to one particle in the final analyzed subsample. If no particles are detected in the analysed subsample, we can only deduce that the number in the whole sample was less than this (hence reporting <). Note: LOD varies between samples and polymers (see Section S1) and may be higher than resolution where blank contamination is high.

Table S4. Concentrations as microplastic particles per g dry weight sludge, values <LOD are qualified by “<” and those above the LOD but below the LOQ by “≈”. LOD are coloured yellow and those above the LOQ green. LOD and LOQ values depend on the mass of sample processed by FTIR. The suffixes a and b indicate that influent and effluent were also collected at that site (see Tables S2 and S3).

Name	date	% on final filter	Mass of sample on resolution* final filter (g)	MP/g	PE MP/g	PP MP/g	PET MP/g	PMMA MP/g	PA MP/g	PS MP/g	PU MP/g	PVC MP/g	ABS MP/g	Total MPs (>LOQ only)
AAD1 a	26/10/18	0.91%	0.009	120	2267	1252	6861	< 120	≈ 351	< 120	≈ 120	≈ 359	< 120	10,380
	16/01/19	2.4%	0.024	46	673	571	3705	< 46	≈ 82	≈ 134	≈ 46	< 46	< 46	4949
	31/01/19	2.8%	0.028	38	1105	1317	6018	≈ 38	< 52	< 38	≈ 77	< 38	< 38	8440
	07/02/19	1.0%	0.010	107	526	1004	3431	< 107	< 107	< 107	< 107	< 107	< 107	4961
	21/02/19	1.4%	0.014	80	1506	1290	6732	≈ 160	≈ 151	≈ 158	< 80	< 80	< 80	9528
AAD2	10/09/18	1.2%	0.012	89	525	< 123	< 1068	< 89	< 89	< 89	< 89	< 89	< 89	525
	17/09/18	2.8%	0.028	39	< 39	≈ 166	< 1068	≈ 39	< 52	< 39	< 39	< 39	< 39	-
	24/09/18	2.0%	0.020	55	≈ 157	≈ 322	< 1068	≈ 55	< 55	< 55	< 55	< 55	< 55	-
	08/11/18	2.4%	0.024	45	< 45	525	< 1068	< 45	< 52	< 45	< 45	< 45	< 45	525
	13/12/18	1.7%	0.017	64	< 64	449	< 1068	< 64	< 64	< 64	≈ 64	< 64	< 64	449
AAD3	26/07/18**	3.2%	0.032	34	1074	509	< 1068	≈ 34	≈ 59	≈ 100	≈ 34	< 34	< 34	1584
	20/09/18	2.3%	0.024	43	1882	1828	< 1027	< 43	< 50	403	< 43	≈ 45	≈ 90	4113
	28/09/18	2.6%	0.026	41	1353	1131	< 1068	≈ 83	< 52	< 41	≈ 83	< 41	< 41	2485
	04/10/18	3.1%	0.031	35	1091	≈ 324	< 1068	≈ 35	≈ 98	< 35	≈ 35	< 35	< 35	1091
	12/10/18	2.8%	0.028	39	1877	1152	< 1068	157	227	194	118	< 39	< 39	3725
LS	17/07/18	2.5%	0.025	could not be analysed, because excess solids in the subsample obscured the microplastic particles										
	06/09/18	0.7%	0.007	could not be analysed, because excess solids in the subsample obscured the microplastic particles										
	09/10/18	0.7%	0.007	could not be analysed, because excess solids in the subsample obscured the microplastic particles										
	19/11/18	0.42%	0.004	261	1035	< 261	< 1068	< 261	< 261	< 261	≈ 261	< 261	< 261	1035
	14/12/18	0.32%	0.003	338	4047	< 338	< 1068	< 338	< 338	< 338	< 338	< 338	< 338	4047
AD b	19/10/18	2.2%	0.022	49	1021	1161	< 1068	< 49	≈ 89	≈ 145	< 49	≈ 98	< 49	2182
	24/10/18	2.4%	0.024	44	1803	524	< 1047	≈ 91	≈ 82	< 44	< 44	≈ 45	≈ 90	2327
	06/11/18	2.4%	0.024	45	757	610	< 1068	< 45	< 52	< 45	< 45	< 45	< 45	1366
	27/11/18	1.4%	0.014	78	2956	1182	< 1068	≈ 78	< 78	≈ 154	≈ 156	< 78	< 78	4138
	03/01/19	2.1%	0.021	52	301	≈ 244	< 1068	< 52	< 52	< 52	< 52	< 52	< 52	301

* Resolution: The number of particles of each polymer in the whole processed sample equivalent to one particle in the final analyzed subsample. If no particles are detected in the analysed subsample, we can only deduce that the number in the whole sample was less than this (hence reporting <). Note: LOD varies between samples and polymers (see Section S1) and may be higher than resolution where blank contamination is high.

** This subsample corresponds to rep A of the sludge replicate samples shown in table S5

Table S5. Full repeat processing and analysis of four sub-samples from the same WwTW sludge (taken from site AAD3).

Name	date	% of original sample on final filter	Mass of sample on final filter (g)	resolution* MP/g	PE MP/g	PP MP/g	PET MP/g	PMMA MP/g	PA MP/g	PS MP/g	PU MP/g	PVC MP/g	ABS MP/g	Total MPs (>LOQ only)
AAD3 (rep A)	26.7.18	3.2%	0.032	34	1074	509	< 1068	≈ 34	≈ 59	≈ 100	≈ 34	< 34	< 34	1584
AAD3 (rep B)	26.7.18	3.7%	0.037	29	697	463	< 1068	≈ 29	≈ 138	≈ 57	< 29	< 29	< 29	1160
AAD3 (rep C)	26.7.18	2.7%	0.027	40	1196	617	< 1068	< 40	≈ 112	≈ 118	≈ 40	< 40	< 40	1812
AAD3 (rep D)	26.7.18	2.2%	0.022	48	1445	661	< 1068	145	< 52	< 48	< 48	< 48	< 48	2251

* Resolution: The number of particles of each polymer in the whole processed sample equivalent to one particle in the final analyzed subsample. If no particles are detected in the analysed subsample, we can only deduce that the number in the whole sample was less than this (hence reporting <). Note: LOD varies between samples and polymers (see Section S1) and may be higher than resolution where blank contamination is high.

Table S6. Sludge spike recovery efficiency (nylon particles). Measured values reported in MP/g are extrapolated from the measured values in the subsample, as per the sludge samples.

Sample	Concentration (MP/g, based on amount spiked into sludge)	Measured MP/g	% recovery
Spike_1	33935	14122	42
Spike_2	33935	19021	56
Spike_3	33935	11989	35
Spike_4	33935	24000	71
Spike_5	33935	19832	58
Average	33935	17793	52
SD		4778.99	14.08

Table S7. Effluent spike recovery (nylon particles). Measured values reported in MP/L are extrapolated from the measured values in the subsample, as per the effluent samples.

Sample	Concentration (MP/L, based on amount spiked into sludge)	Measured MP/L	% recovery
Spike_1	33935	40083	118
Spike_2	33935	30846	91
Spike_3	33935	19592	58
Spike_4	33935	38488	113
Spike_5	33935	41708	123
Average	33935	34144	101
SD		9137.68	26.76

Table S8. Microplastic removal efficiency across WwTWs based on averages for influent (n = 2) and effluent (n = 2) per WwTW, using values > LOQ.

Site code	Influent	Effluent	% removal
ASTC1	9200.3	2.8	100.0
ASTC2	3882.4	< LOQ	100.0
ASTS1	8378.3	< LOQ	100.0
ASTS2	12344.2	< LOQ	100.0
AS1a	4769.3	5.7	99.9
AS2b	< LOQ	< LOQ	-
TFSb	< LOQ	18.9	-
TFP	1489.7	3.8	99.7
BAFF	5506.7	22.3	99.6
All WwTW	7011.0	16.0	99.8

Table S9. Microplastic removal efficiency by polymer type based on the average concentration in influent (n = 16) and effluent (n = 18) across all WwTW, using only values > LOQ. All other polymers could not be quantified >LOQ, and were regularly below LOD (Tables S2 and S3).

Polymer	Influent (average MP/L)	Effluent (average MP/L)	% removal
PE	3519.8	9.4	99.7
PET	4309.9	9.9	99.8
PP	6822.6	4.0	99.9

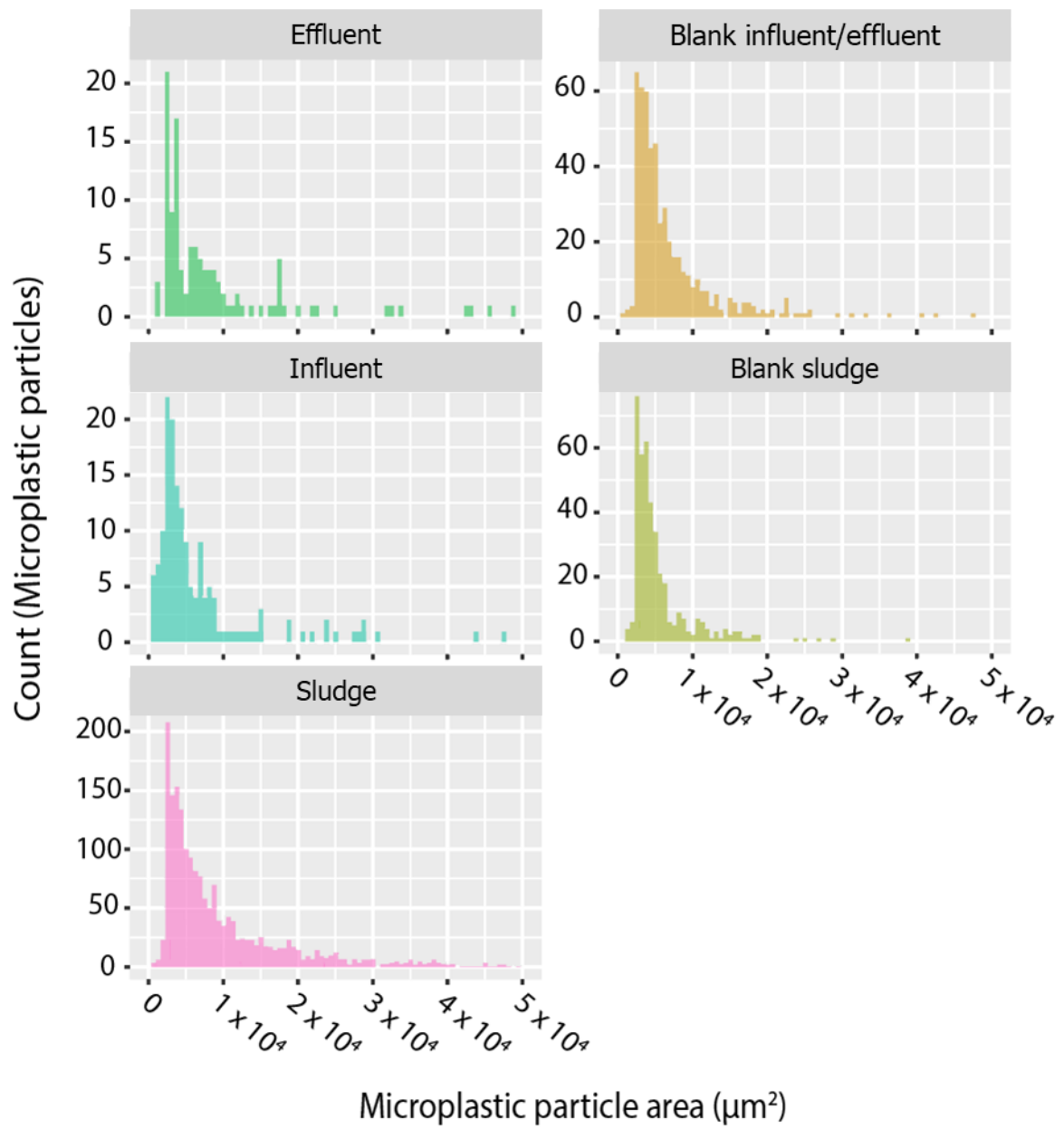


Fig. S2. Size distribution of microplastics found in the different sample types

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