Supplementary Information

A systems approach to understand microplastic occurrence and variability in Dutch riverine surface waters

S.M. Mintenig[1,2], M. Kooi[3], M.W. Erich[2], S. Primpke[4], P.E. Redondo- Hasselerharm[3], S.C. Dekker[1], A.A. Koelmans[3], A.P. van Wezel[1,2,5]

[1] Copernicus Institute of Sustainable Development, Utrecht University, The Netherlands.
[2] KWR Watercycle Research Institute, Nieuwegein, The Netherlands.
[3] Aquatic Ecology and Water Quality Management Group, Wageningen University, The Netherlands.
[4] Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Biologische Anstalt Helgoland, Germany.
[5] Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, The Netherlands.

Paragraph S1: Contamination mitigation and quality assurance

Before starting to handle environmental samples three working places used in literature were tested for their susceptibility to contamination and their easiness to handle which influences the recovery of MP. Tests were conducted on a normal lab bench (Dris et al. 2015, Mintenig et al. 2017), in a glove box (Torre et al. 2016) and in a laminar flow hood (Lorenz et al. 2019, Peeken et al. 2018).

Positive controls: The protocol of described sample purification (see 2.3. MP < 300 µm) was tested and the handling optimized to enable a high MP recovery during the sample purification. For this, green fluorescent PE beads (90-106 µm, Cospheric, USA) were added to 5 samples (1 L of Milli-Q water) per working place and counted before and after purification steps. The final batches of positive controls yielded in a recovery rate of 93.1 ± 1.2 % on the lab bench, of 91.1 ± 5.8 % in a laminar flow hood and of 79.0 ± 9.6 % in a glove box.

Negative controls: Prior usage all lab equipment was thoroughly rinsed, and the lab surfaces cleaned with ethanol (30 %, Carl Roth GmbH & Co. KG, Germany) and Milli-Q water. Then, the level of contamination was determined for five negative samples (1 L of Milli-Q water) per working place, also these samples underwent described sample purification (see 2.3.) Particles and fibres were counted under a stereomicroscope after the samples had been filtered onto gridded cellulose nitrate filters (Whatman, U.K.). The samples on the lab bench contained on average 9.3 (SD 1.8) fibres and 1.8 (SD 1.4) particles, in the laminar flow hood 3.7 (SD 1.2) fibres and 2.3 (SD 1.1) particles were detected, and in the glove box 5.6 (SD 3.6) fibres and 4 (SD 0.9) particles respectively.

The samples from the glove box showed a medium level of contamination, but especially a low and strongly varying recovery rate of MP (79.0 ± 9.6 %). This can be explained by the restricted freedom to operate which complicated handling and rinsing steps and resulted in the methods rejection. Samples from the laminar flow hood showed the lowest contamination levels while enabling a high recovery of MP (91.1 %). The samples on the lab bench contained the highest amount of fibres, but also had a high and continuable recovery of MP (93.1 ± 1.2 %). Ideally, all sample handling should thus be made in a laminar flow hood. However, as the access to the laminar flow hood was very limited, the recovery rates were high and reproducible on the lab bench and the levels of contamination on the lab bench were not that much higher than the ones in the laminar flow hood it was decided to conduct all work on a previously cleaned lab bench. Because of this, and because not all laboratory equipment used could be made from glass, procedural negative samples (1 L of Milli-Q water) were treated and analysed in parallel to the environmental samples which were corrected for the level of identified contamination (see below). Per sample batch (five to seven environmental samples) one negative sample was treated in parallel. At the end, environmental samples were corrected for the contamination assessed through eight negative samples. Listed are the detected MP and their respective polymer types. The samples were corrected for these MP in respective size classes.

Polymercluster	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**mean**	**SD**
Polyethylene	0	26	68	6	0	10	3	16	**16**	**23**
Polyethylene-chlorinated	0	0	26	13	3	0	0	5	**6**	**9**
Polypropylene	0	7	0	0	0	0	3	0	**1**	**2**
Polyamide	0	16	3	3	0	0	0	0	**3**	**6**
Nitrile rubber	0	0	0	0	0	3	0	0	**0**	**1**
Polyester	0	7	0	0	0	0	0	0	**1**	**2**
Acrylates/polyurethanes/varnish	0	3	0	3	3	0	0	0	**1**	**2**
Rubber type 1	0	0	3	0	0	0	0	0	**0**	**1**
Rubber type 2	0	3	0	0	0	0	0	0	**0**	**1**
Rubber type 3	7	39	334	22	0	19	0	26	**56**	**113**

To further mitigate sample contamination when working with actual environmental samples several steps were taken: During sample handling a lab coat and clothes, both made from natural fabric, were worn. Prior to any working step the lab surfaces were wiped off with ethanol (30 %, Carl Roth GmbH & Co. KG, Germany) and paper towels. Prior usage, all chemicals were filtered over 20 µm, all lab materials were rinsed with ethanol and Milli-Q water and covered with aluminium foil. Also the samples were covered immediately with aluminium foil after finishing working with them.

Table S 1: Sampling dates and locations in the Dommel (D), Meuse (M), and their tributaries/related locations (+). Riverine surface waters and WWTP effluents were sampled by filtering water over stacked sieves (mesh sizes of 20, 100 and 300 µm), sample volumes vary for the different sieves and are indicated below (m³). When sampling the WWTP effluents the tube inlet was hung directly in the effluent (15 cm below surface). The WWTPs varied in size and capacity, below their maximum hydraulic capacities are listed. When sampling riverine surface waters the tube inlet was mounted horizontally on a wooden pallet (see section 2.2) which was hung in the water to sample the upper 5cm of the water, in case of turbulent water (due to wind or waves) the tube was mounted vertically (, with specific depth indications). In general, this was done from small bridges in the Dommel, and from the riverside (~5m from shore) in the Meuse (it is indicated if done differently).*

River	Location	Latitude, Longitude	Date	Weather condition	Flow (m/s)	Start time	Sample volume (m^3)			Vertically placed inlet tube/ varying sampling location
							20µm sieve	100µm sieve	300µm sieve	
D	Berkel & Schaft	51.291384, 5.438432	18.10.2017	dry, no wind	0.29	15:30	0.213	2.151	2.183	
D	Eindhoven (WWTP, 35,000 $m^3 h^{-1}$)	51.462441, 5.504710	09.10.2017	dry, no wind	-	10:30	0.061	1.515	2.965	
D	Eindhoven DS	51.468845, 5.509891	09.10.2017	dry, no wind	0.29	17:00	0.030	1.703	1.703	
D	Eindhoven US	51.460012, 5.501451	09.10.2017	dry, no wind	0.24	13:30	0.045	1.898	1.995	
D	Het Broek	51.344994, 5.441739	18.10.2017	dry, no wind	0.28	12:00	0.198	2.084	2.160	
D	Klotputten DS	51.410085, 5.436638	26.10.2017	little wind, little rain	0.002	10:30	0.500	2.000	2.000	small boat
D	Klotputten US	51.407783, 5.435758	26.10.2017	dry, little wind	0.002	13:00	0.409	2.419	2.419	riverside
D	Nijnsel	51.554369, 5.489739	13.10.2017	dry, little wind	0.22	15:00	0.115	3.080	3.080	
D	Son	51.521215, 5.499958	13.10.2017	dry, no wind	0.23	11:30	0.102	3.370	3.370	
D	St Oedenrode (WWTP, 1,930 $m^3 h^{-1}$)	51.561801, 5.444830	10.10.2017	dry, little wind	-	09:00	0.100	2.389	3.829	
D	St Oedenrode US	51.561768, 5.446611	10.10.2017	little wind, little rain	0.28	12:00	0.100	1.751	2.650	
D	St Oedenrode DS	51.564389, 5.425714	10.10.2017	dry, little wind	0.27	15:00	0.100	3.529	2.999	
D			13.02.2018	dry, little wind	0.35	15:30	0.200	2.020	2.020	
D			06.06.2018	dry, no wind	0.17	15:00	0.351	2.000	2.000	
D			21.08.2018	dry, no wind	0.1	09:50	0.300	2.000	2.000	
D			21.08.2018	dry, no wind	0.09	11:50	0.300	2.000	2.000	
D			21.08.2018	dry, no wind	0.09	13:05	0.300	2.000	2.000	
D+	De Vleut	51.423584, 5.477953	13.11.2017	dry, little wind	0.27	11:30	0.510	2.159	2.159	
D+	Kleine Dommel	51.458808, 5.528436	13.10.2017	dry, no wind	0.265	08:30	0.150	3.000	3.000	
D+	Run	51.400646, 5.416487	18.10.2017	dry, no wind	0.23	09:10	0.300	2.565	2.565	

M	Afgedamde Maas	51.806161, 5.025366	12.10.2017	dry, little wind	-	13:00	0.105	3.525	3.700	
M	Cuijk	51.730406, 5.883969	19.10.2017	dry, no wind	-	13:00	0.995	3.000	3.000	* 10-30 cm depth, moving pond
M	Drimmelen	51.720030, 4.884695	14.11.2017	little wind, little rain	-	10:30	0.510	2.600	2.600	small boat
M			19.10.2017	dry, no wind	-	09:00	0.980	3.000	3.000	
M	Eijsden	50.779497, 5.699936	13.02.2018	dry, no wind	-	09:30	0.500	3.243	3.243	* 15 cm depth
M			06.06.2018	dry, no wind	-	10:30	0.650	2.000	2.000	
M	Haringvliet	51.822818, 4.074128	15.02.2018	dry, little wind	-	11:00	2.250	7.990	7.990	* 5- 50 cm depth, boat
M	Roermond (WWTP, 7,000 $m^3 h^{-1}$)	51.223334, 5.984140	04.10.2017	dry, little wind	-	10:00	0.200	2.825	2.825	
M	Roermond DS	51.238896, 6.006217	04.10.2017	dry, little wind	-	13:00	0.200	3.431	3.431	* 10-30 cm depth, moving pond
M	Roermond US	51.199460, 5.981864	04.10.2017	dry, little wind	-	16:30	0.400	3.603	3.603	
M+	Biesbosch1 (De Gijster)	51.725490, 4.797129	12.04.2018	dry, little wind	0	11:00	0.980	2.960	2.960	small boat
M+	Biesbosch2 (Petrusplaat)	51.754803, 4.773473	07.06.2018	dry, no wind	0	10:00	2.200	4.000	4.000	small boat
M+	Maasbommel (WWTP, 150 $m^3 h^{-1}$)	51.829682, 5.522084	06.10.2017	dry, little wind	-	09:00	n.a.	1.335	2.371	
M+	Maasbommel DS	51.828649, 5.519383	06.10.2017	dry, little wind	-	11:30	n.a.	3.058	3.058	
M+	Maasbommel US	51.830167, 5.519385	06.10.2017	dry, little wind	-	16:00	n.a.	2.033	2.033	almost no flow, wind blowing in US direction
M+	Oijen DS	51.797160, 5.512459	05.10.2017	wind, little rain	-	17:00	0.070	2.768	2.768	
M+	Oijen US	51.801022, 5.487776	05.10.2017	wind, little rain	-	14:00	0.450	3.643	3.643	
M+			05.10.2017	wind, strong rain	-	10:30	0.045	0.767	0.767	
M+	Oijen (WWTP, 12,250 $m^3 h^{-1}$)	51.810053, 5.488648	20.10.2017	little wind, little rain	-	08:50	0.647	2.000	2.000	
M+			20.10.2017	dry, little wind	-	10:25	0.500	2.050	2.050	
M+			20.10.2017	dry, little wind	-	11:40	0.520	1.995	1.995	

Table S 2: Thresholds of spectral hit quality (max. 2000) defined by manually evaluation of spectra of 3 surface water samples and 2 WWTP effluent samples based on criteria presented by Primpke et al. (2018). This was done to determine polymer specific thresholds for a safe MP identification (confidence interval of 95%). These thresholds were used during final image analysis using MPAPP (Primpke et al. 2019). (After a subsequent spectra evaluation of the sample 'Eindhoven WWTP' the threshold for this sample for polymer #12 was increased to 1050). Also indicated are polymer densities applied to calculate the MP mass (see section 2.4), these densities are the mean of reported varying densities for individual polymer types.*

no.	Polymer cluster	Hit quality threshold	Density (g cm^{-3})
1	Polyethylene	600	0.91
2	Polyethylene oxidized	600	0.91
3	Polyethylene-chlorinated	1000	0.91
4	Polypropylene	600	0.92
5	Polystyrene	600	1.04
6	Polycarbonate	800	1.21
7	Polyamide	1100	1.22
8	Polyvinylchloride	900	1.38
9	Cellulose chemical modified	900	1.20
10	Nitrile rubber	900	1.00
11	Polyester	1000	1.35
12	Acrylates/polyurethanes/varnish	850*	1.20
13	Animal fur	800	-
14	Plant fibers	800	-
15	Sand	600	-
16	Polysulfone	600	1.24
17	Polyetheretherketone	600	1.32
18	Polychloroprene	800	1.23
19	Chitin	800	-
20	Polyisoprene chlorinated	600	0.91
21	Polylactic acid	600	1.30
22	Polycaprolactone	1000	1.15
23	Ethylene-vinyl-acetate	1100	0.94
24	Polyimide	600	1.60
25	Polyoxymethylene	600	1.41
26	Polybutadiene	600	0.90
27	Acrylonitrile-butadiene	600	1.22
28	Rubber type 1	600	1.03
29	Rubber type 2	600	1.03
30	Charcoal	600	-
31	Coal	600	-
32	Rubber type 3	900	1.10

*Table S 3: MP detected in surface water and WWTP effluent samples taken in the Dommel (D), Meuse (M), or their tributaries/ related locations (+). Analysis using ATR-FTIR and µ-FTIR provided information on the individual MPs' 2 dimensional shapes and polymer types, based on this, total mass concentrations were estimated (see section 3.2). * For these samples concentrations are indicated for MP > 300 µm, the remaining locations summarize findings on MP down to a size of 20µm.*

	Location	Acr/PUR		Polyethylene		Polypropylene		Polystyrene		Rubbers		Others		Total	
		# m^{-3}	µg m^{-3}	# m^{-3}	µg m^{-3}	# m^{-3}	µg m^{-3}	# m^{-3}	µg m^{-3}	# m^{-3}	µg m^{-3}	# m^{-3}	µg m^{-3}	# m^{-3}	µg m^{-3}
D	Berkel & Schaft	33	2	129	401	31	10	17	6	75	11	24	0.01	**309**	**430**
D	Eindhoven (WWTP)	379	52	224	2677	109	289	3	13	93	7	932	29	**1741**	**3068**
D	Eindhoven DS	862	446	2180	5422	1148	982	127	50	3019	300	1114	69	**8450**	**7270**
D	Eindhoven US	71	64	95	234	826	176	146	21	781	106	760	107	**2678**	**708**
D	Het Broek	17	0	63	716	35	26	0.5	11	35	3	126	6	**277**	**761**
D	Klotputten DS	19	2	182	715	221	210	9	13	151	18	5	25	**587**	**981**
D	Klotputten US	179	205	84	173	28	20	2	10	101	16	68	12	**460**	**435**
D	Nijnsel	49	20	72	668	80	107	0	0	46	2	84	26	**331**	**824**
D	Son	2	2	129	1411	42	134	105	9	73	4	3	0	**354**	**1561**
D	St Oedenrode (WWTP)	132	126	302	1533	207	135	40	26	75	18	185	47	**941**	**1884**
D	St Oedenrode DS	53	3	182	1109	204	187	0	0	188	10	94	29	**721**	**1339**
D	St Oedenrode US	544	95	1282	834	3995	518	259	48	3726	611	1725	269	**11532**	**2375**
D	St Oedenrode 13-2	279	54	3232	2178	227	211	22	1	2906	591	193	64	**6859**	**3099**
D	St Oedenrode 6-6	191	51	293	284	396	158	41	29	247	90	691	138	**1859**	**750**
D	St Oedenrode 21-8-R1	156	8	281	136	454	78	28	1	144	4	129	11	**1192**	**237**
D	St Oedenrode 21-8-R2	372	57	326	180	388	60	32	1	192	4	327	338	**1636**	**641**
D	St Oedenrode 21-8-R3	266	28	228	250	312	39	7	1	296	8	422	35	**1531**	**360**
D+	De Vleut	35	1	79	69	36	32	8	1	182	13	21	3	**360**	**119**
D+	Kleine Dommel	26	1	19	179	23	43	1	0	34	2	57	36	**160**	**261**
D+	Run	12	0	76	12	11	5	12	19	129	3	29	12	**270**	**51**
M	Afgedamde Maas	21	5	215	348	157	119	7	17	423	68	52	12	**876**	**569**
M	Cuijk	2	0	67	131	62	59	10	25	15	2	21	5	**177**	**223**
M	Drimmelen	64	13	269	82	87	46	3	1	330	13	36	23	**789**	**178**
M	Eijsden 13-2	512	29	134	72	63	9	2	1	437	285	81	21	**1228**	**418**
M	Eijsden 19-10	96	10	230	1354	247	314	3	16	212	28	62	17	**849**	**1738**

M	Eijsden 6-6	64	18	377	169	124	98	31	56	322	281	241	92	**1160**	**713**
M	Haringvliet	4	2	116	64	36	17	20	6	224	57	67	11	**468**	**156**
M	Roermond (WWTP)	183	84	132	857	112	76	15	26	124	26	458	182	**1024**	**1250**
M	Roermond DS	66	36	277	207	215	130	1	14	175	21	207	40	**942**	**447**
M	Roermond US	110	9	431	728	309	275	13	6	200	42	317	38	**1381**	**1098**
M+	Biesbosch1 (De Gijster)	12	2	209	128	24	10	2	1	333	287	26	6	**607**	**435**
M+	Biesbosch2 (Petrusplaat)	12	3	16	37	17	12	1	0.01	16	2	6	7	**67**	**62**
M+	Maasbommel *	0	0	45	915	1	17	0	0	0	0	0	0	**46**	**932**
M+	Maasbommel-DS *	0	0	1	13	0	0	0	0	0	0	0	0	**1**	**13**
M+	Maasbommel-US *	0	0	5	110	1	30	0	0	0	0	0	0	**7**	**140**
M+	Oijen *	1	35	1468	29761	20	400	1	30	0	0	4	106	**1494**	**30332**
M+	Oijen-DS *	0	10	7	139	1	15	0	0	0	0	0	0	**8**	**164**
M+	Oijen-US *	0	0	1	22	0	0	0	0	0	0	0	0	**1**	**22**
M+	Oijen-R1 *	0	0	203	4105	7	143	0	0	0	0	1	27	**211**	**4276**
M+	Oijen-R2 *	1	26	263	5330	12	239	0	0	0	0	3	81	**279**	**5677**
M+	Oijen-R3 *	0	0	692	14033	15	308	2	46	0	0	2	50	**711**	**14437**

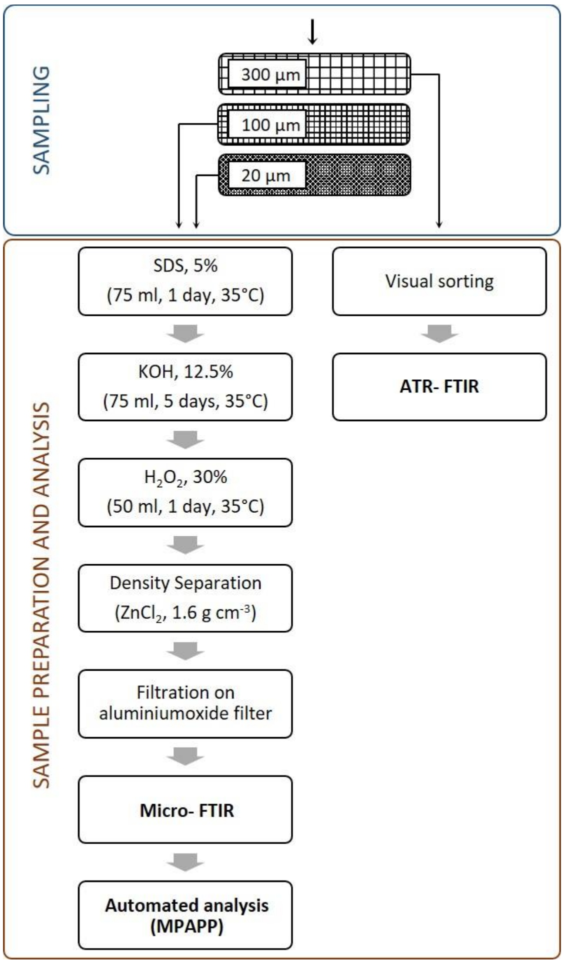

Figure S 1: Schematic flowchart of steps taken to extract and analyse MP from aqueous samples. All sample preparation to extract MP retained on the 20 and 100 µm sampling sieves was done at KWR Watercycle Research Institute (The Netherlands), the sorting of MP > 300 µm and all FTIR analyses were conducted in the laboratories of Wageningen University and Research (The Netherlands).

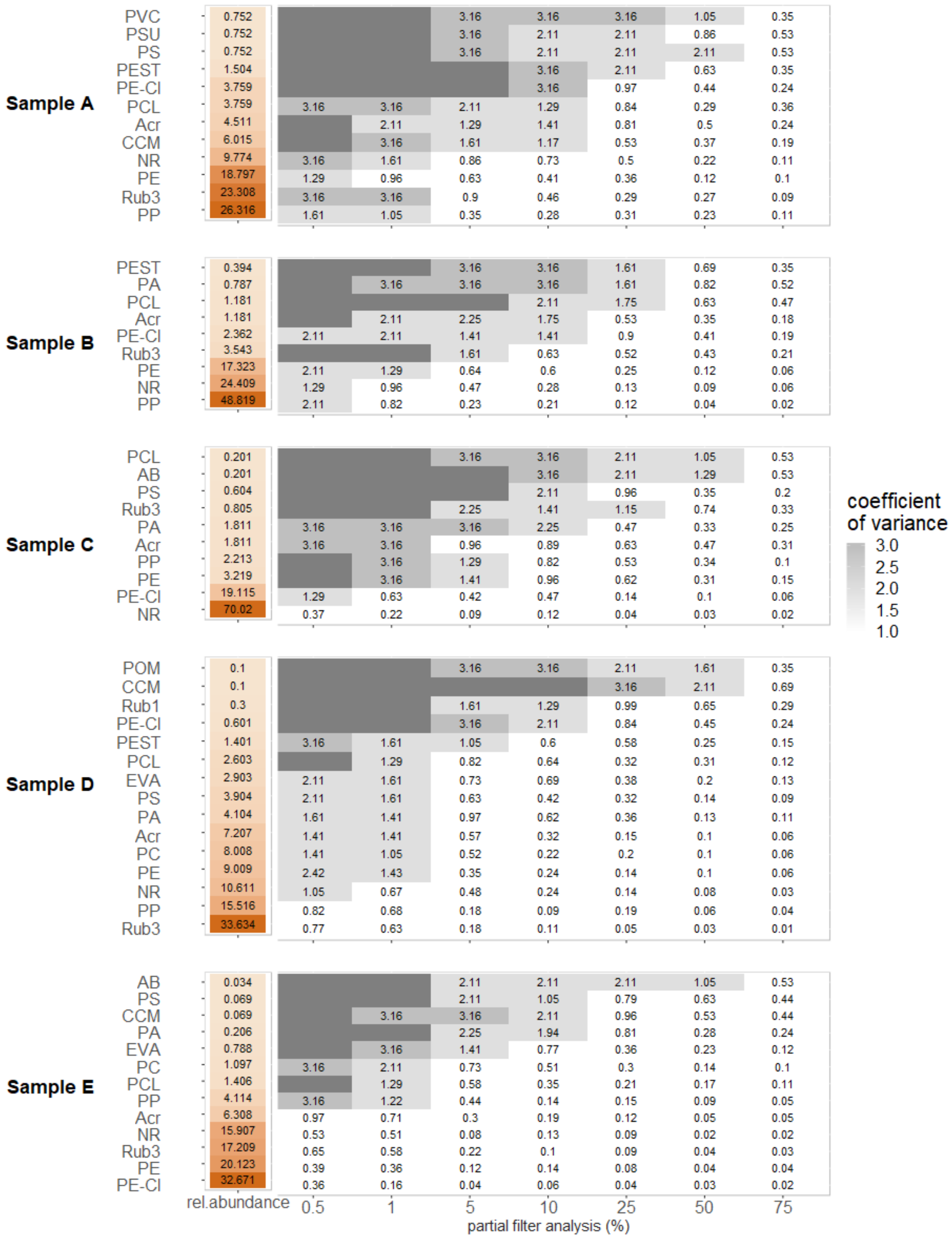

Figure S 2: Uncertainties expressed as the coefficient of variance (CV) of identified polymer types during partial filter analysis with micro-FTIR. In orange the relative abundances of polymer types are indicated, total numbers of MP were Sample A: 157; Sample B: 254; Sample C: 500; Sample D: 1039; Sample E: 2928 (Figure 1). The CV was calculated for the individual samples based on 10 randomly selected filter areas of specified size (0.5- 75%). Areas that miss a CV are coloured in dark grey indicating that this polymer type was not identified in any of the generated 10 areas.

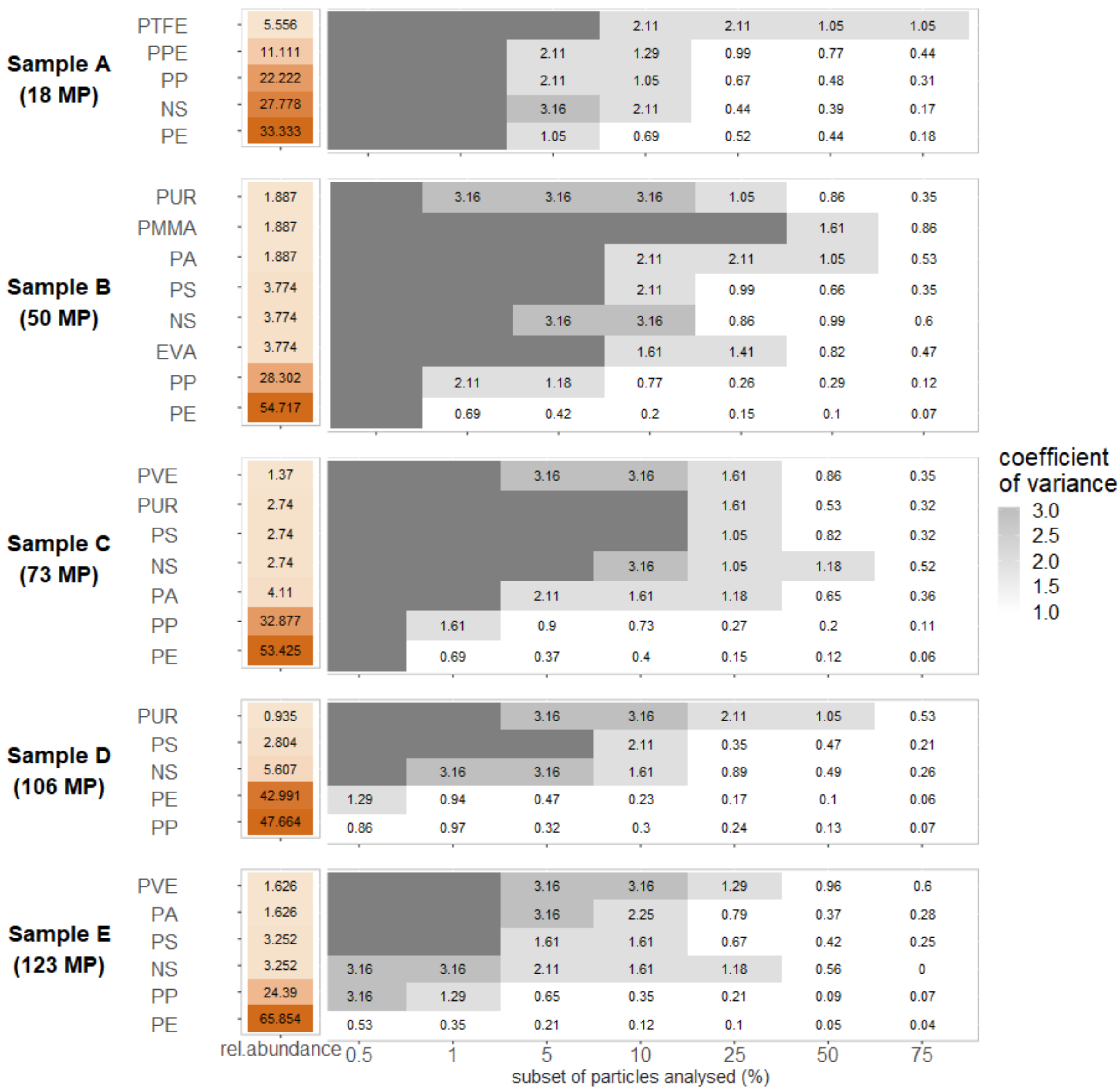

Figure S 3: Uncertainties expressed as the coefficient of variance (CV) of identified polymer types when analysing a subset of particles with ATR-FTIR. In orange the relative abundances of polymer types are indicated for each sample. The CV was calculated for the individual samples based on 10 randomly selected subsamples of specified size (0.5- 75%). Areas that miss a CV are coloured in dark grey indicating that this polymer type was not identified in any of the generated 10 subsamples.

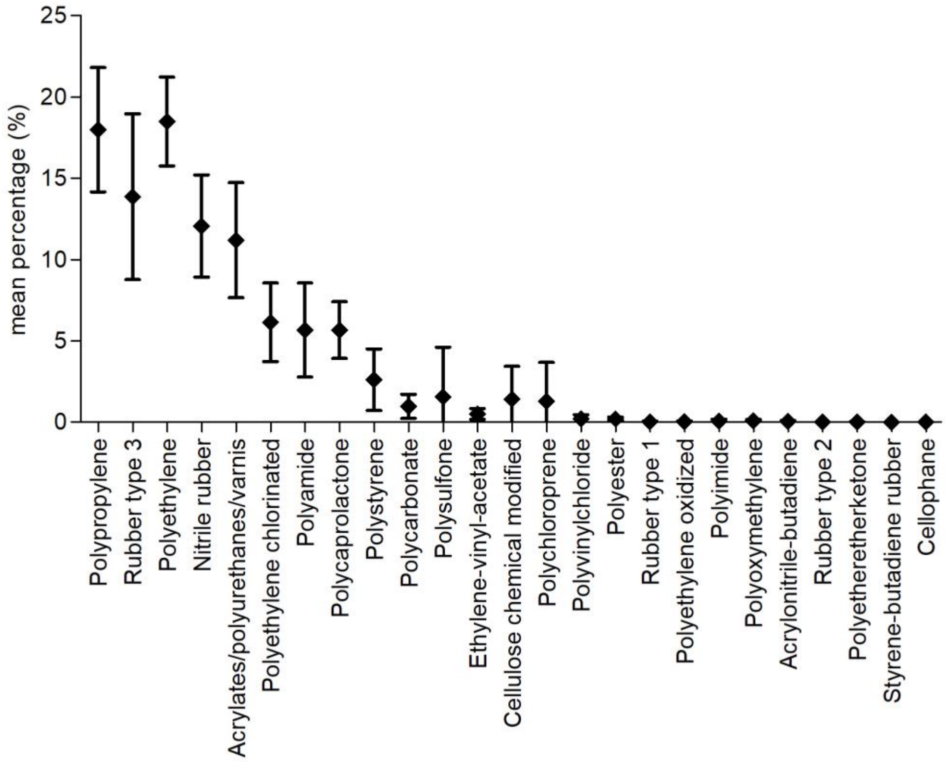

Figure S 4: Mean percentages of detected polymers in riverine surface water samples. Whiskers show the 95 % confidence interval. Polymers are ordered on the x axis based on the total amount of detected MP.

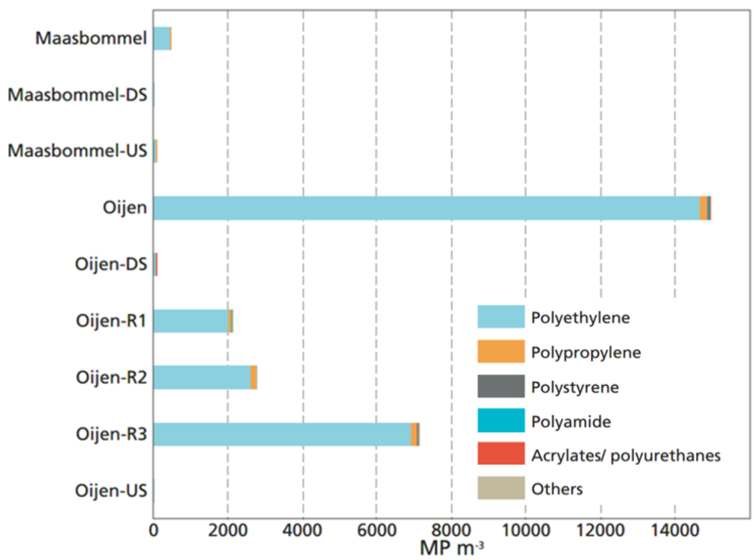

Figure S 5: MP number concentrations (MP > 300µm m^{-3}) with respect to the different polymer types identified in the effluents of the WWTPs Maasbommel and Oijen, as well as in riverine surface water upstream (US) and downstream (DS) of the discharging point.

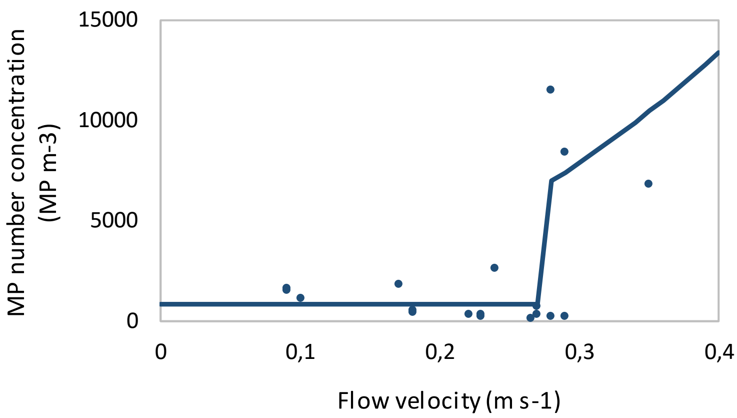

Figure S 6: MP concentrations detected in the Dommel in dependency of flow velocities. The critical shear stress equation indicated a critical flow velocity of 0.275 m/s that will result in increased MP concentrations in riverine surface water.

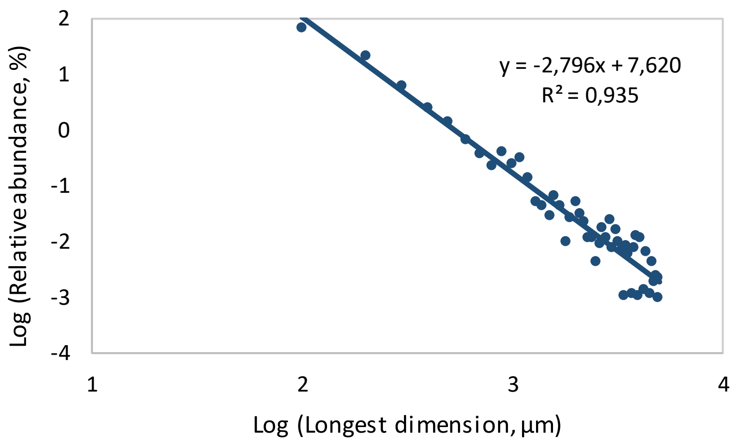

Figure S 7: Relative abundance of MP numbers in relation to MP sizes.

References

Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N. and Tassin, B. (2015) Microplastic contamination in an urban area: a case study in Greater Paris. Environmental Chemistry 12(5), 592-599.

Lorenz, C., Roscher, L., Meyer, M.S., Hildebrandt, L., Prume, J., Löder, M.G.J., Primpke, S. and Gerdts, G. (2019) Spatial distribution of microplastics in sediments and surface waters of the southern North Sea. Environmental Pollution 252, 1719-1729.

Mintenig, S.M., Int-Veen, I., Löder, M.G.J., Primpke, S. and Gerdts, G. (2017) Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. Water Research 108, 365-372.

Peeken, I., Primpke, S., Beyer, B., Gütermann, J., Katlein, C., Krumpen, T., Bergmann, M., Hehemann, L. and Gerdts, G. (2018) Arctic sea ice is an important temporal sink and means of transport for microplastic. Nature Communications 9(1), 1505.

Primpke, S., A. Dias, P. and Gerdts, G. (2019) Automated identification and quantification of microfibres and microplastics. Analytical Methods 11(16), 2138-2147.

Primpke, S., Wirth, M., Lorenz, C. and Gerdts, G. (2018) Reference database design for the automated analysis of microplastic samples based on Fourier transform infrared (FTIR) spectroscopy. Analytical and Bioanalytical Chemistry 410(21), 5131-5141.

Torre, M., Digka, N., Anastasopoulou, A., Tsangaris, C. and Mytilineou, C. (2016) Anthropogenic microfibres pollution in marine biota. A new and simple methodology to minimize airborne contamination. Mar Pollut Bull 113(1), 55-61.