

Longitudinal study on the multifactorial public health risks associated with sewage reclamation.

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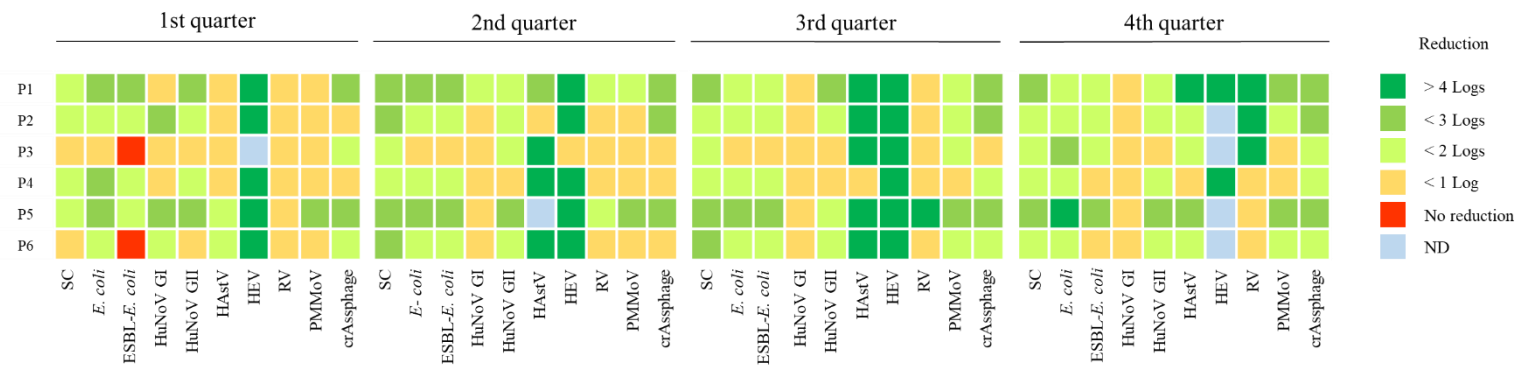
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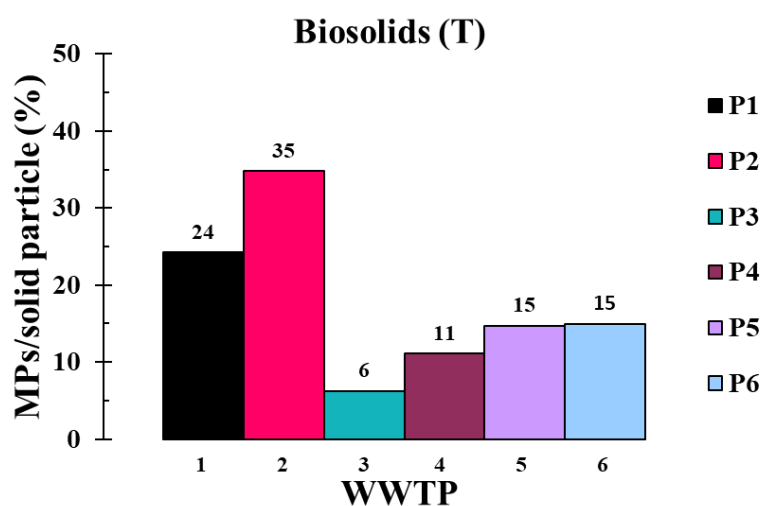
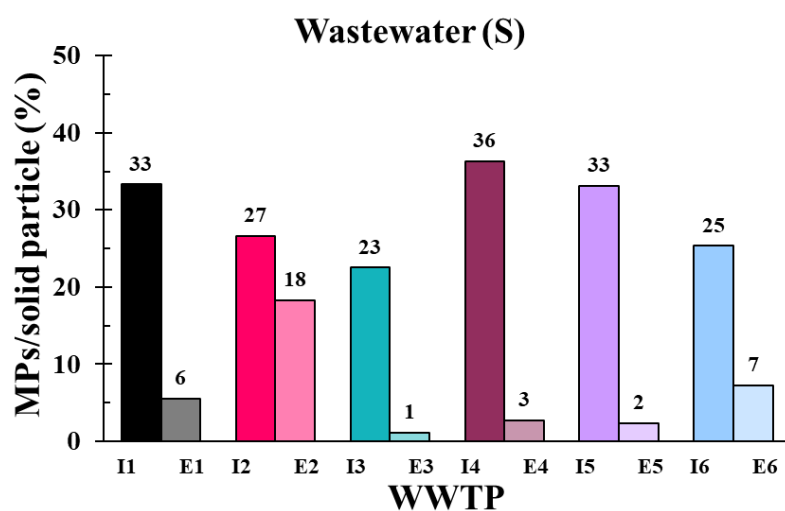
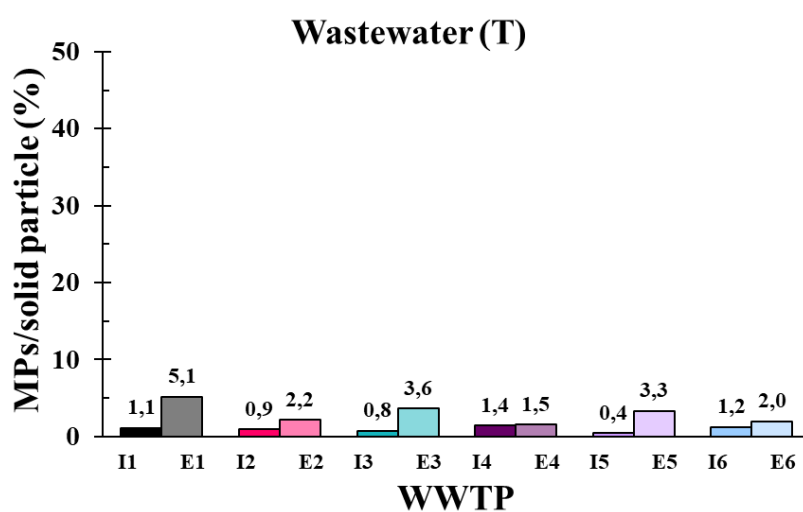
Supplementary material



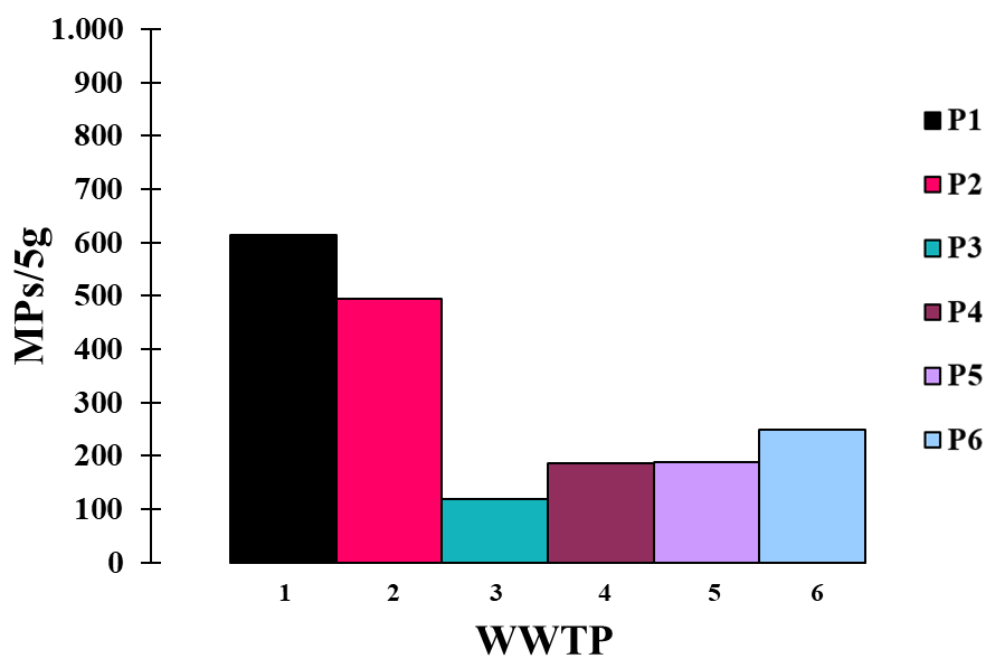
Supplementary Figure 1. Reduction in the levels of human pathogenic viruses, viral faecal contamination indicators and *Escherichia coli* during the water reclamation process in samples collected from six different WWTPs (P1-P6). SC: Somatic coliphages; ND: Not detected.

Virus	Primers + probe	Sequence (5' --> 3')	5' Dye	Quencher	PCR conditions	LoQ/L	LoD/L	Reference			
CrAssphage	Forward (064F1)	TGTATAGATGCTGCTGCAACTGTACTC			Inactivation: 95 °C, 30 sec	1.64 x 10 ⁵	4.94 x 10 ⁴	(Stachler et al., 2017)			
	Reverse (064R1)	CGTTGTTTTTCATCTTTATCTTGCCAT			RT: 45 °C, 15 min						
	Probe (064P)	CTGAAATTGTTCTATAAGCAA	6-FAM	NFQMGB	[Denaturation: 95 °C, 05 sec + Annealing: 60 °C, 30 sec] x45 cycles						
PMMoV	COMERCIAL KIT (PMMoV Fecal Indicator RT-qPCR Kit)				Inactivation: 95 °C, 2 min	1.94 x 10 ³	1.41 x 10 ³	COMERCIAL KIT (PMMoV Fecal Indicator RT-qPCR Kit)			
					[Denaturation: 95 °C, 15 sec + Annealing: 60 °C, 60 sec] x45 cycles						
HuNoV GI	Forward (QNIF4)	CGCTGGATGCGNTTCCAT			RT: 55 °C, 20 min	8.77 x 10 ³	2.77 x 10 ³	(“ISO - ISO 15216-1:2017 – Microbiology of the food chain)			
	Reverse (NVILCR)	CCTTAGACGCCATCATCATTAC			Inactivation: 95 °C, 2 min						
	Probe (TM9)	TGGACAGGAGATCGC	6-FAM	NFQMGB	[Denaturation: 95 °C, 10 sec + Annealing: 60 °C, 30 sec] x45 cycles						
HuNoV GII	Forward (QNIF2)	ATGTTCAGRTGGATGAGRTTCTCWGA			RT: 55 °C, 20 min	2.12 x 10 ⁴	1.60 x 10 ⁴	(“ISO - ISO 15216-1:2017 – Microbiology of the food chain)			
	Reverse (COG2R)	TCGACGCCATCTTCATTACACA			Inactivation: 95 °C, 2 min						
	Probe (QNIFs)	AGCACGTGGGAGGGCGATCG	6-FAM	3HBQ_1	[Denaturation: 95 °C, 10 sec + Annealing: 60 °C, 30 sec] x45 cycles						
HAV	Forward (HAV68)	TCACCGCGTTTGCTTAG			RT: 55 °C, 20 min	2.58 x 10 ⁴	1.26 x 10 ⁴	(“ISO - ISO 15216-1:2017 – Microbiology of the food chain)			
	Reverse (HAV240)	GGAGAGCCCTGGAAGAAA			Inactivation: 95 °C, 2 min						
	Probe (HAV150)	CCTGAACCTGCAGGAATTAA	6-FAM	NFQMGB	[Denaturation: 95 °C, 10 sec + Annealing: 60 °C, 30 sec] x45 cycles						
HEV	Forward (JHRVF)	GGTGGTTTCTGGGGTGAC			RT: 50 °C, 30 min	2.99 x 10 ⁴	1.20 x 10 ⁴	(“ISO - ISO 15216-1:2017 – Microbiology of the food chain)			
	Reverse (JHEVR)	AGGGGTTGGTTGGATGAA			Inactivation: 95 °C, 15 min						
	Probe (JHEVPmod)	TGATTCTCAGCCCTTCGC	6-FAM	NFQMGB	[Denaturation: 95 °C, 10 sec + Annealing: 55 °C, 20 sec + Elongation: 72 °C, 15 sec] x45 cycles						
HAstV	Forward (AstVor1b+)	AAGCAGCTTCGTGACTCTGG			RT: 55 °C, 20 min	1.73 x 10 ⁵	1.07 x 10 ⁵	(“ISO - ISO 15216-1:2017 – Microbiology of the food chain)			
	Reverse (AstVor1b-)	AGCCATCACACTTCTTTGGTC			Inactivation: 95 °C, 2 min						
	Probe (AstVor1BP)	AGAGCAACTCCATCGCATTT	6-FAM	3BHQ	[Denaturation: 95 °C, 10 sec + Annealing: 60 °C, 30 sec] x45 cycles						
RV	Forward (JVKF)	CAGTGGTTGATGCTCAAGATGGA			RT: 55 °C, 20 min	4.01 x 10 ⁵	3.57 x 10 ⁵	(Jothikumar et al., 2009)			
	Reverse (JVKR)	TCATTGTAATCATATTGAATACCCA			Inactivation: 95 °C, 2 min						
	Probe (JVKP)	ACAACTGCAGCTTCAAAAGAAGWGT	6-FAM	3BHQ	[Denaturation: 94 °C, 10 sec + Annealing: 55 °C, 30 sec] x45 cycles						
PEDV	Forward	CAGGACACATTCTTGGTGGTCTT			RT: 42 °C, 15 min	9.87 x 10 ⁴	1.01 x 10 ⁴	(Zhou et al., 2017)			
	Reverse	CAAGCAATGTACCACTAAGGAGTGTT			Inactivation: 95 °C, 2 min						
	Probe	ACGCGCTTCTCACTAC	6-FAM	NFQMGB	[Denaturation: 95 °C, 15 sec + Annealing: 60 °C, 60 sec] x45 cycles						
MgV	Forward	GCGGGTCTGCCGAAAGT			RT: 42 °C, 15 min	2.07 x 10 ⁴	1.46 x 10 ⁴	(“ISO - ISO 15216-1:2017 – Microbiology of the food chain)			
	Reverse	GAAGTAACATATAGACAGACGCACAC			Inactivation: 95 °C, 2 min						
	Probe	ATCACATTACTGGCCGAAGC	6-FAM	NFQMGB	[Denaturation: 95 °C, 15 sec + Annealing: 60 °C, 60 sec] x45 cycles						
SARS-CoV-2 (N1)	COMERCIAL KIT (2019-nCoV_N1 Combined Primer/Probe Mix, CDC)				RT: 50 °C, 15 min	7.69 x 10 ⁴	4.45 x 10 ⁴	(CDC, 2020)			
					[Denaturation: 95 °C, 03 sec + Annealing: 60 °C, 30 sec] x45 cycles						
RSV	Forward	CACWGAAGATGCWAATCATAAATTCA			RT: 50 °C, 20 min	1.71 x 10 ⁴	4.06 x 10 ³	(Sanghavi et al., 2011)			
	Reverse	GTATYTTTATRGTTGCTTCYCTTCCTAACC			Inactivation: 95 °C, 15 min						
	Probe	TAATAGGTA/ZEN/TGTTATATGCKATGTC	FAM	3IABkFQ	[Denaturation: 94 °C, 45 sec + Annealing: 56 °C, 75 sec] x45 cycles						
IAV	Forward 1	CAAGACCAATCYTGTCACTCTGAC			RT: 50 °C, 30 min	1.92 x 10 ⁶	1.69 x 10 ⁶	(CDC, 2009)			
	Forward 2	CAAGACCAATYCTGTCACTTYTGAC			Inactivation: 95 °C, 2 min						
	Reverse 1	GCATTYTGACAAAACVGTCTACG			[Denaturation: 95 °C, 15 sec + Annealing: 55 °C, 30 sec] x45 cycles						
	Reverse 2	GCATTTTGGATAAAGCGTCTACG									
	Probe	TGCAGTCCT/ZEN/CGCTCACTGGGCACG	FAM	3IABkFQ							

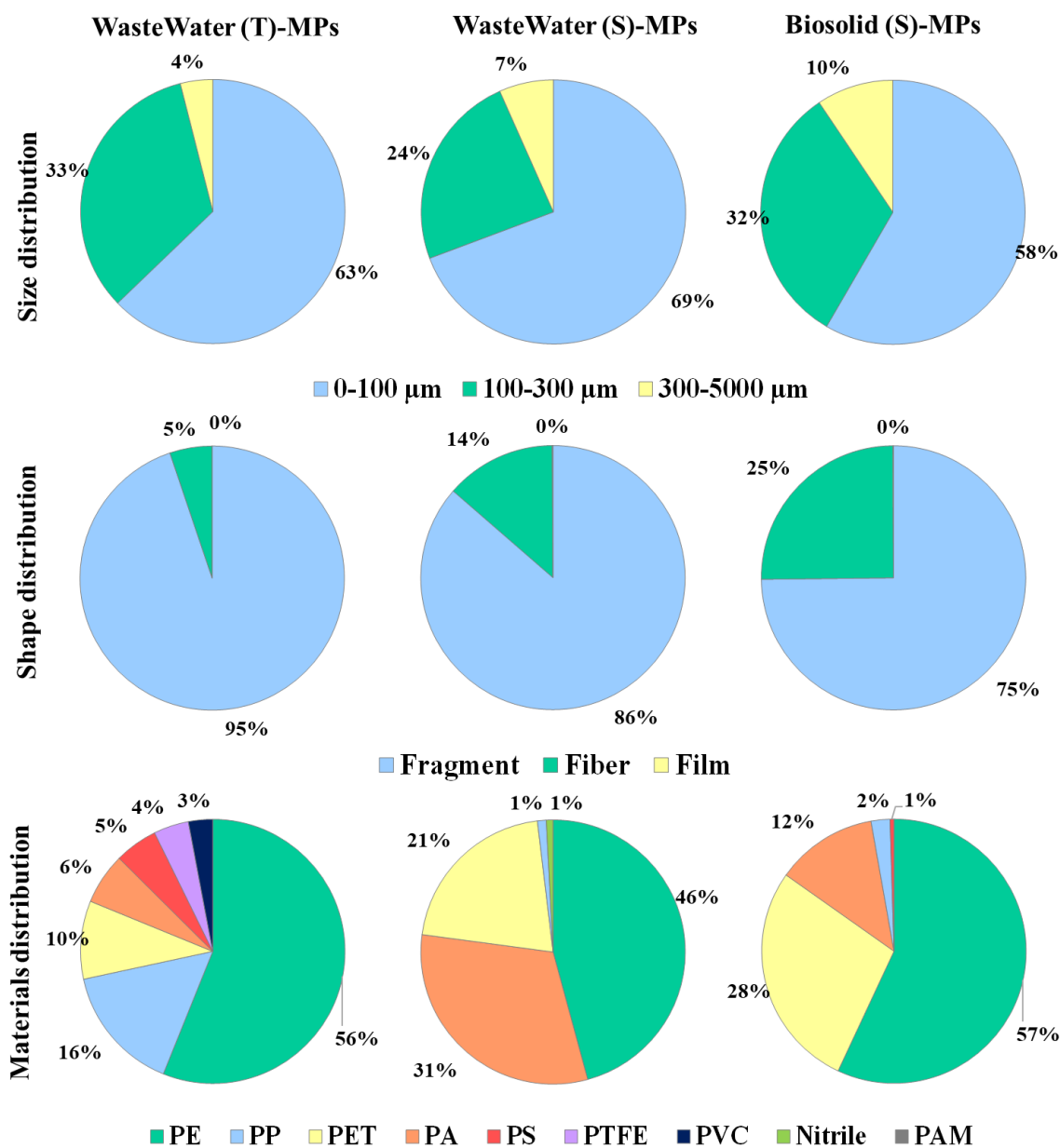
Supplementary Table 1. Primers, probes, PCR conditions, limit of quantification (LOQ/L), and limit of detection (LOD/L) for all targeted viruses in this work.



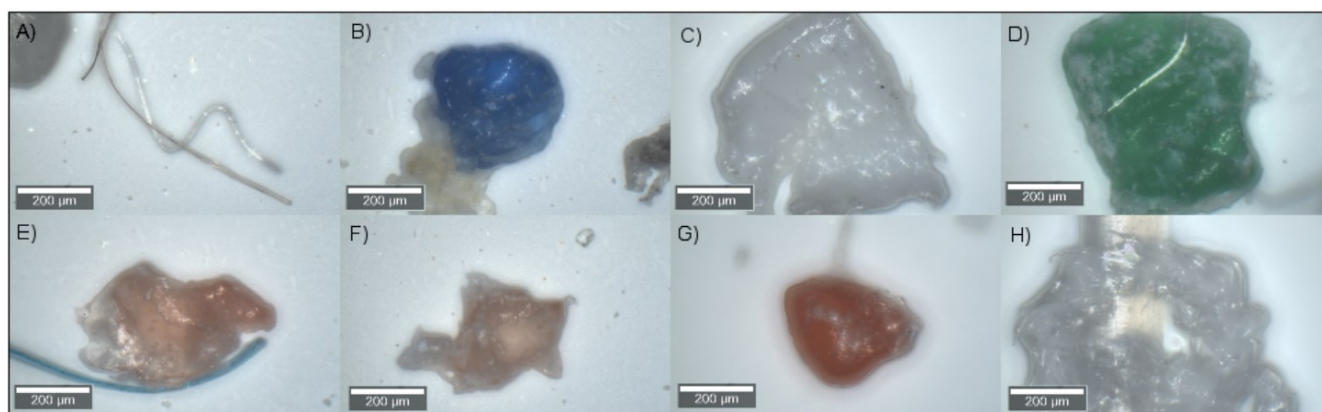
Supplementary Figure 2. Annual average percentage of MPs respect to all solid particles in influent (I) and effluent (E) wastewater samples and in biosolids samples in six different WWTPs (P1-P6).



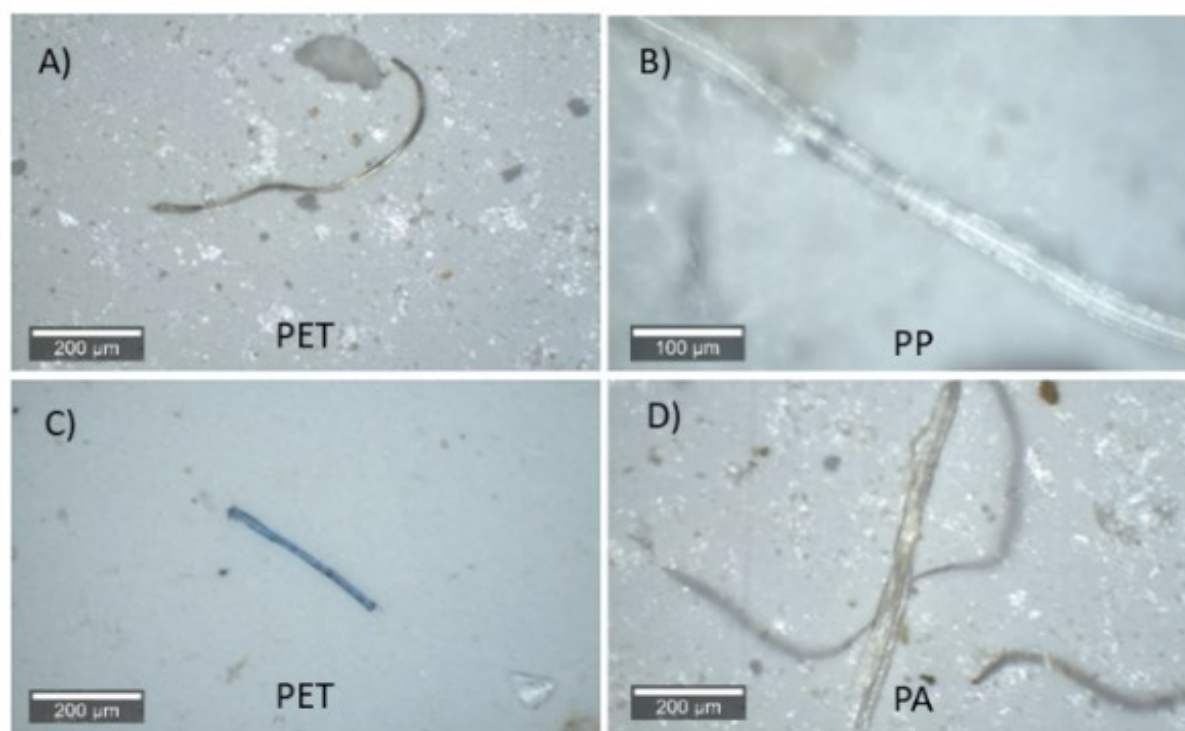
Supplementary Figure 3. Annual average concentration of (S)-MPs (larger than 300 µm) in biosolids collected from six different WWTPs (P1-P6).



Supplementary Figure 4. Morphological distribution of type, size, and shape of all identified MPs from six different WWTPs (P1-P6) in influent and effluent wastewaters and biosolids. PE: polyethylene; PET: polyethylene terephthalate; PA: polyamide; PP: polypropylene; PS: polystyrene; PVC: polyvinyl chloride; PTFE: polytetrafluoroethylene; PAM: polyacrylamide.



Supplementary Figure 5. Examples of identified microplastics. A) PET; B-C-D) PP; E-F-G-H) PE. PE: polyethylene; PET: polyethylene terephthalate; PP: polypropylene.



Supplementary Figure 6. Optical microscopy images of microplastic fibers identified in the wastewater and biosolid samples