

Supplementary information

Wastewater treatment plants performance for reuse: evaluation for bacterial and viral risks

*Ileana Federigi**, *Roberto Salvadori***, *Giulia Lauretani**, *Anna Leone***, *Simone Lippi***, *Francesca Marvulli***, *Alessandra Pagani**, *Marco Verani**, *Annalaura Carducci**

** Laboratory of Hygiene and Environmental Virology, Department of Biology, University of Pisa, Via S. Zeno 35/39, 56127 Pisa, Italy*

*** Acque S.p.A., Via A. Bellatalla 1, 56121 Pisa, Italy*

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Table S1. Oligonucleotide primers and probes used for the viral detection by (RT)qPCR. Target regions are also reported for each viral parameter. References are reported in the footnotes.

Primer or probe names	Concentration (μM)	Sequence (5'-3')	Thermal cycle	Reference
Human adenovirus (hexon gene)				
Primer AdF	0.9	CWTACATGCACATCKCSG G	50°C: 2 min, 95°C: 10 min, 45 cycles (95°C: 15s; 60°C: 1 min)	Hernroth et al., 2002
Primer AdR	0.9	CRCGGGCRAAYTGCACCA G		
Probe AdP	0.225	FAM-CCGGGCTCAGGTACTCCGA GGCGTCCT-TAMRA		
Norovirus genogroup II (RdR Pol gene)				
Primer JJV2F	1	CAAGAGTCAATGTTTAGGTGGATGAG	48°C: 30 min, 95°C: 10 min, 45 cycles (95°C: 15s; 60°C: 1 min)	Skraber et al., 2009
Primer COG2R	1	TCGACGCCATCTTCATTCACA		
Probe RING2-TP	0.1	FAM-TGGGAGGGCGATCGCAA TCT-BHQ		
Enterovirus (5' UTR region)				
Primer EVF	0.6	GGCCCCTGAATGCGGCTAAT	48°C: 30 min, 95°C: 10 min, 45 cycles (95°C: 15s; 60°C: 1 min)	Donaldson et al., 2002
Primer EVR	0.6	CACCGGATGGCCAATCCAA		
Probe EV	0.25	FAM-CGGACACCCAAAGTAGT CGGTTCCG-TAMRA		
SARS-CoV-2 (ORF1ab)				
2297-CoV-2-F	0.5	ACA TGG CTT TGA GTT GAC ATC T	50°C: 30 min, 95 °C: 5 min, 45 cycles (95 °C: 15s; 60°C: 30s)	La Rosa et al., 2021
2298-CoV-2-R	0.9	AGC AGT GGA AAA GCA TGT GG		
2299-CoV-2-P	0.25	FAM-CAT AGA CAA CAG GTG CGC TC-MGBEQ		

Hernroth et al. (2022) Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, *Mytilus edulis*: the first Scandinavian report. doi: 10.1128/AEM.68.9.4523-4533.2002; Skrabber et al. (2009) Occurrence and persistence of enteroviruses, noroviruses and F-specific RNA phages in natural wastewater biofilms. doi: 10.1016/j.watres.2009.05.020; Donaldson et al. (2002) Detection, quantitation and identification of enteroviruses from surface waters and sponge tissue from the Florida Keys using real-time RT-PCR. doi: 10.1016/s0043-1354(01)00479-1; La Rosa et al. (2021) SARS-CoV-2 has been circulating in northern Italy since december 2019: evidence from environmental monitoring. doi: 10.1016/j.scitotenv.2020.141711

Table S2. Effect of two independent variables, water treatment and weather condition, on *E. coli* levels in the exit samples (dependent variable), separately for each WWTP. *E. coli* concentrations are reported as geometric mean \pm standard deviation

Weather condition	Water treatment	n° obs	<i>E. coli</i> levels Log ₁₀ (MPN/100ml)	2-way ANOVA (p-value)
WWTP1				
Dry	Chlorinated	52	3.8 \pm 0.9	< 0.0001 weather condition; < 0.01 water treatment
	Not chlorinated	9	4.5 \pm 0.6	
Wet	Chlorinated	14	4.4 \pm 0.5	
	Not chlorinated	2	5.2 \pm 0.1	
WWTP2				
Dry	Chlorinated	51	1.3 \pm 1.2	< 0.01 weather condition; < 0.0001 water treatment
	Not chlorinated	55	4.19 \pm 0.9	
Wet	Chlorinated	5	1.8 \pm 1.5	
	Not chlorinated	12	4.46 \pm 0.6	

Table S3. Bacterial and viral indicator concentrations at different stages of the treatment process. Results are reported as median and interquartile range (first and third quartiles) and refer to eight sampling dates.

Microbial parameter	WWTP type	Sewage entrance	Secondary effluents	Tertiary effluents
<i>E. coli</i> (MPN/L)	WWTP1	6.68 $\times 10^7$ (5.47 $\times 10^7$ - 6.87 $\times 10^7$)	1.62 $\times 10^5$ (9.55 $\times 10^4$ - 2.02 $\times 10^5$)	2.44 $\times 10^4$ (9.45 $\times 10^3$ - 2.30 $\times 10^5$)
	WWTP2	1.43 $\times 10^8$ (1.12 $\times 10^8$ - 1.73 $\times 10^8$)	1.80 $\times 10^5$ (9.35 $\times 10^4$ - 1.05 $\times 10^6$)	2.14 $\times 10^4$ (8.65 $\times 10^3$ - 1.17 $\times 10^5$)
Intestinal enterococci (MPN/L)	WWTP1	1.16 $\times 10^7$ (8.17 $\times 10^6$ - 1.31 $\times 10^7$)	3.22 $\times 10^4$ (1.48 $\times 10^4$ - 3.99 $\times 10^4$)	3.65 $\times 10^3$ (2.03 $\times 10^3$ - 1.72 $\times 10^4$)
	WWTP2	1.53 $\times 10^7$ (1.38 $\times 10^7$ - 1.77 $\times 10^7$)	4.81 $\times 10^4$ (2.25 $\times 10^4$ - 7.07 $\times 10^4$)	6.65 $\times 10^3$ (8.00 $\times 10^2$ - 2.92 $\times 10^4$)
Somatic coliphages (PFU/L)	WWTP1	9.00 $\times 10^6$ (5.37 $\times 10^6$ - 1.60 $\times 10^7$)	2.95 $\times 10^4$ (1.93 $\times 10^4$ - 6.80 $\times 10^4$)	4.90 $\times 10^3$ (2.15 $\times 10^3$ - 1.62 $\times 10^4$)
	WWTP2	1.05 $\times 10^7$ (7.00 $\times 10^6$ - 1.35 $\times 10^7$)	6.50 $\times 10^4$ (3.38 $\times 10^4$ - 9.60 $\times 10^4$)	4.90 $\times 10^3$ (2.20 $\times 10^3$ - 5.35 $\times 10^4$)
Clostridia spores (CFU/L)	WWTP1	1.10 $\times 10^5$ (7.00 $\times 10^4$ - 1.80 $\times 10^5$)	3.50 $\times 10^2$ (2.30 $\times 10^2$ - 9.20 $\times 10^2$)	5.50 $\times 10^1$ (4.75 $\times 10^1$ - 9.00 $\times 10^1$)
	WWTP2	9.25 $\times 10^4$ (4.55 $\times 10^4$ - 1.24 $\times 10^5$)	9.00 $\times 10^3$ (6.25 $\times 10^3$ - 1.35 $\times 10^4$)	3.50 $\times 10^3$ (2.75 $\times 10^3$ - 7.00 $\times 10^3$)

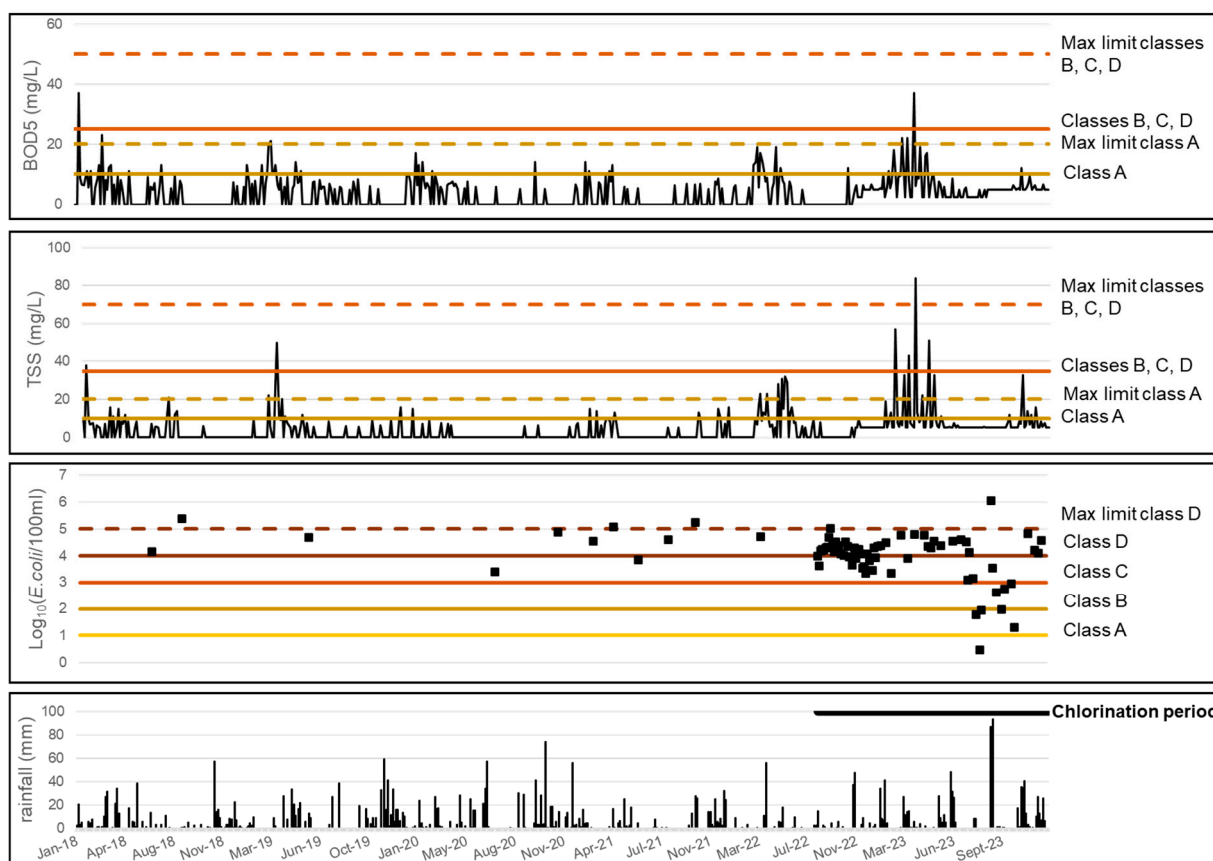


Figure S1. Compliance of WWTP1 with EU minimum requirements for BOD₅, TSS and *E. coli* (data of *E. coli* have been Log₁₀-transformed). Information on rainfall and chlorination are also reported for the entire monitoring period in bottom box. Precipitation corresponds to accumulated rainfall (mm) in the 24 prior to the sampling dates, according to Copernicus definition of wet days (<https://climate.copernicus.eu/ESOTC>; accessed on 12 January 2024).

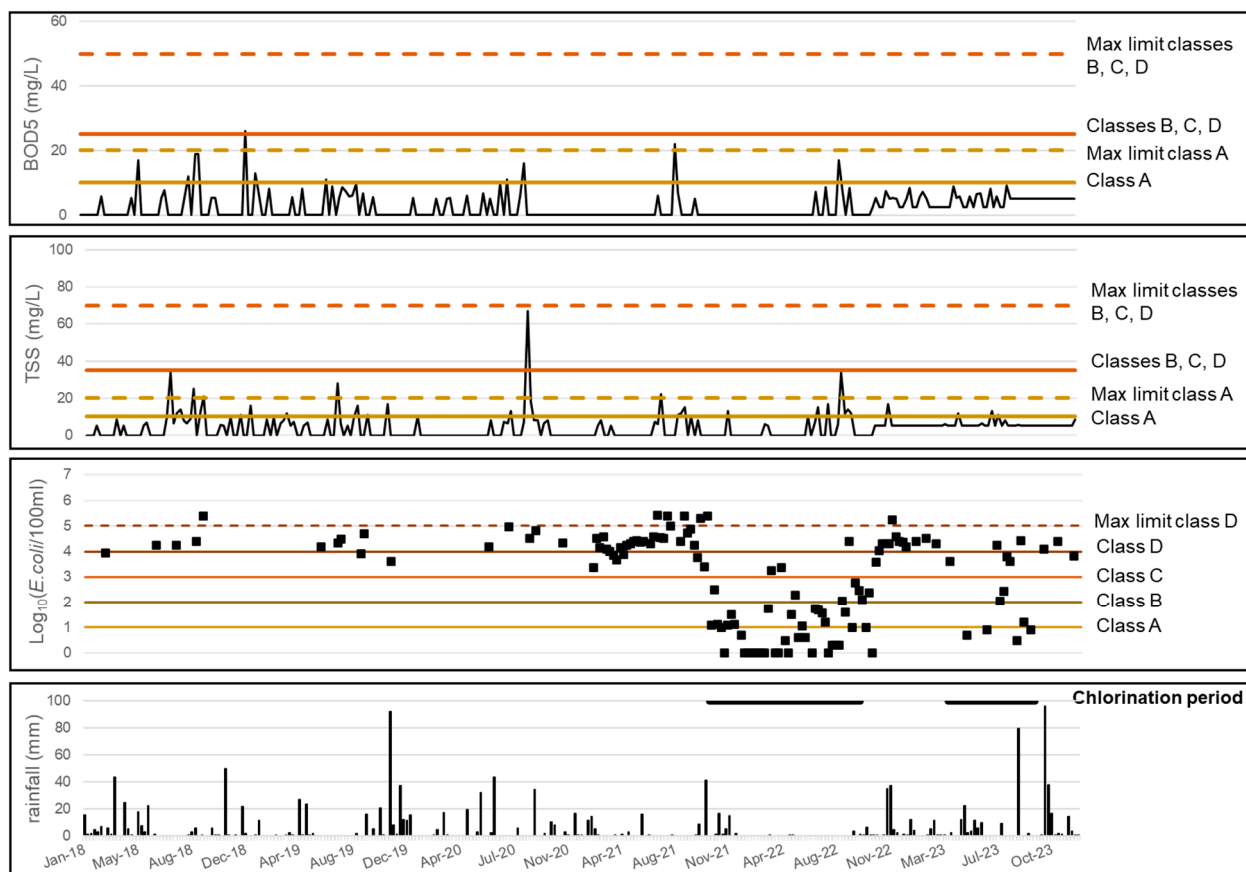


Figure S2. Compliance of WWTP2 with EU minimum requirements for BOD₅, TSS and *E. coli* (data of *E. coli* have been Log₁₀-transformed). Information on rainfall and chlorination are also reported for the entire monitoring period in the bottom box. Precipitation corresponds to accumulated rainfall (mm) in the 24 prior to the sampling dates, according to Copernicus definition of wet days (<https://climate.copernicus.eu/ESOTC>; accessed on 12 January 2024).