

SARS-CoV-2 Wastewater Monitoring in Thuringia, Germany: Analytical Aspects and Normalization of Results

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Supplement

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a) Boxplots

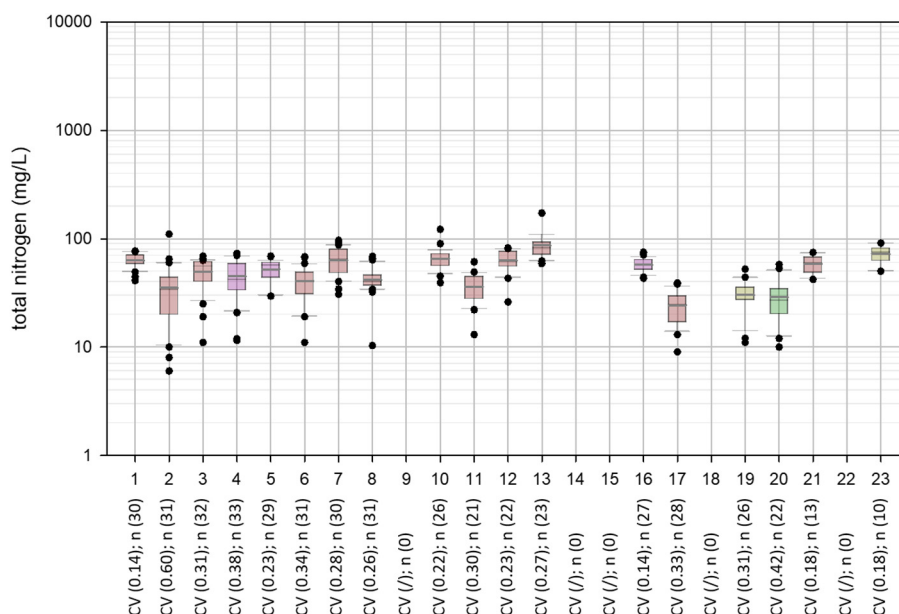


Figure S1: Temporal variation of N_{tot} concentration [mg/L] for the 23 different sampling sites with size classes (GK) (violet = GK 5; red = GK 4; yellow = GK 3; green = GK 2). boxplots: median (black line), mean (gray line), 10% and 90% quantiles as whiskers and all possible outliers.

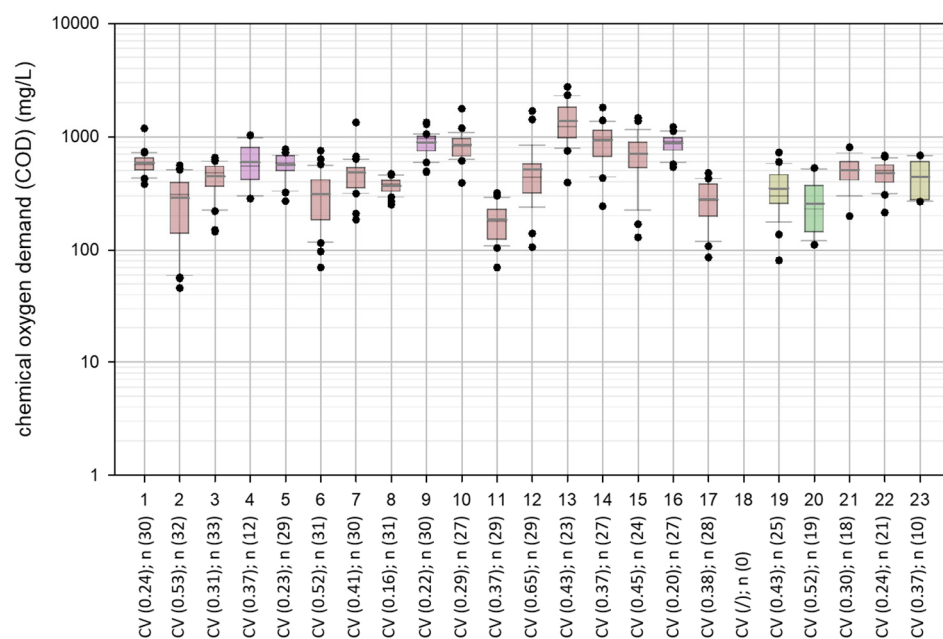


Figure S2: Temporal variation of COD concentration [mg/L] for the 23 different sampling sites with size classes (GK) (violet = GK 5; red = GK 4; yellow = GK 3; green = GK 2). boxplots: median (black line), mean (gray line), 10% and 90% quantiles as whiskers and all possible outliers.

b) Time course of cumulative average concentration of PMMoV RNA and COD and N_{tot} for federal state

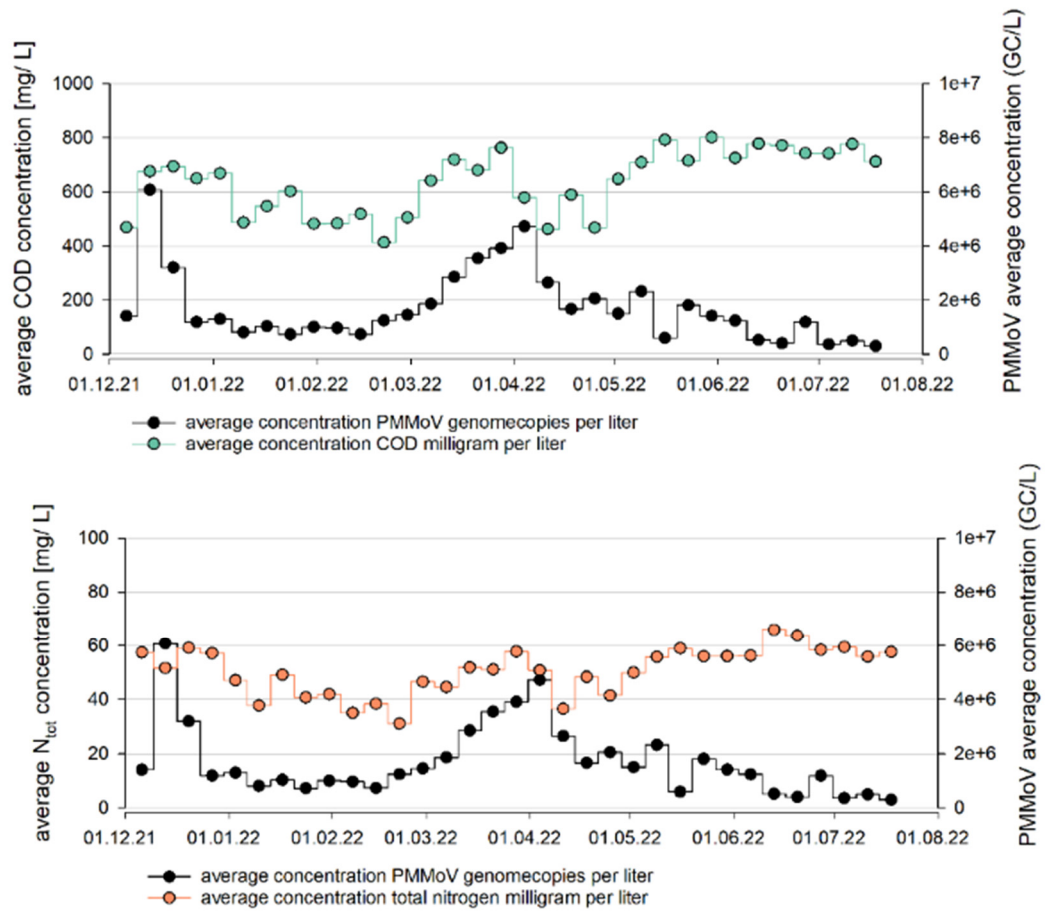


Figure S3: Investigation of seasonal variation. Cumulative mean concentrations (flow-normalized) for total pooled data set of average COD and N_{tot} concentration [mg/L] and PMMoV genome copies [GC/L] in comparison.

Table S1: Spearman Correlation between cumulative average concentration of PMMoV, NH₄-N, COD and N_{tot}.
P-value *<0.05; **>0.05

Spearman Correlation	Cumulative concentration NH ₄ -N [mg/L]	Cumulative concentration COD [mg/L]	Cumulative concentration N _{tot} [mg/L]	Cumulative concentration PMMoV [GC/L]
Cumulative concentration NH ₄ -N [mg/L]	1	0.815*	0.888*	-0.168**
Cumulative concentration COD [mg/L]	0.815*	1	0.807*	-0.194**
Cumulative concentration N _{tot} [mg/L]	0.888*	0.807*	1	-0.166**
Cumulative concentration PMMoV [GC/L]	-0.168 **	-0.194**	-0.166**	1

c) Time course

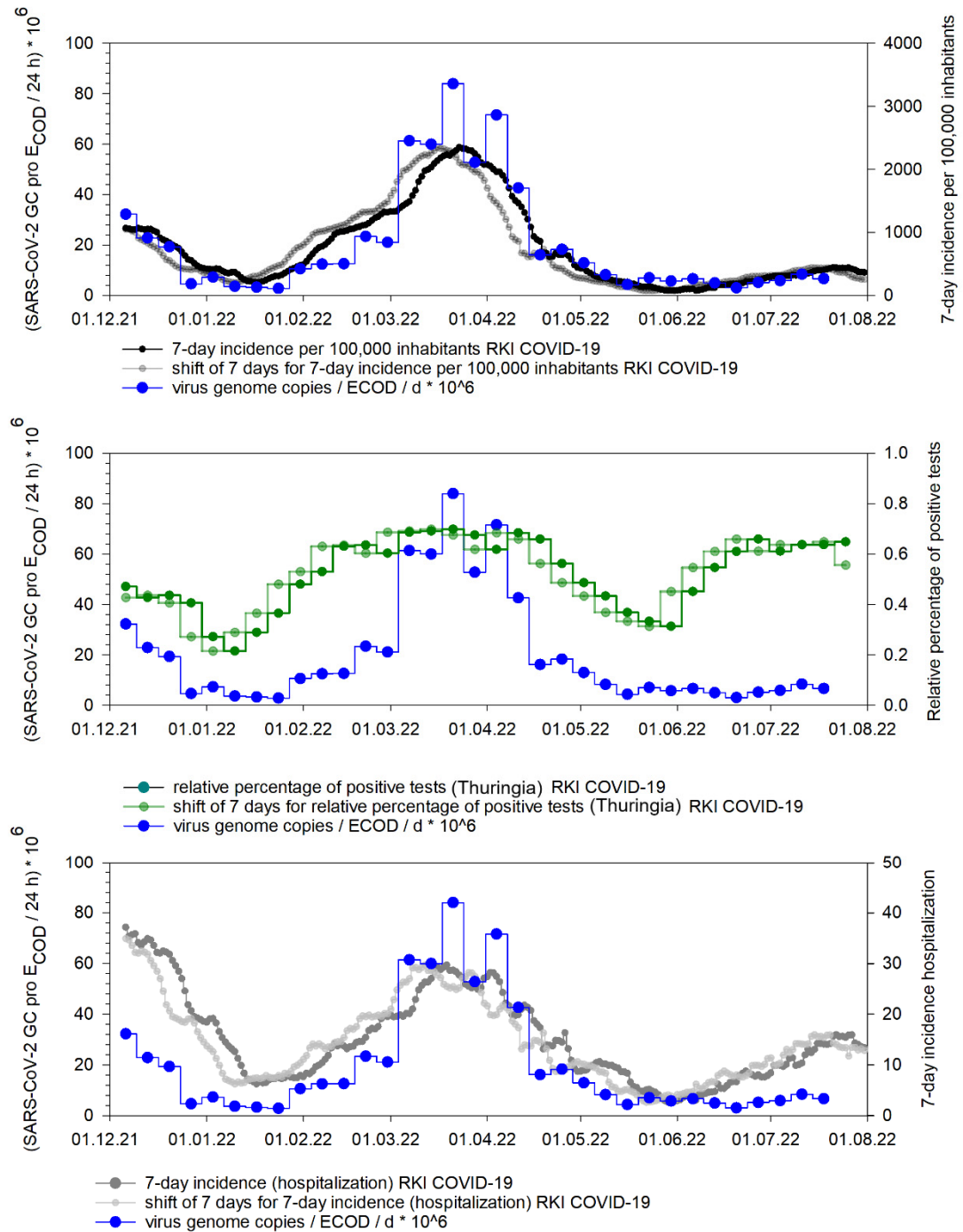


Figure S4: Timeline of SARS-CoV-2 genome copies per inhabitant_{COD} / 24 h of all sampled WWTPs (see table 1) and different epidemiological metrics for the Free State of Thuringia - without time shift of epidemiological metrics and a with a time shift.

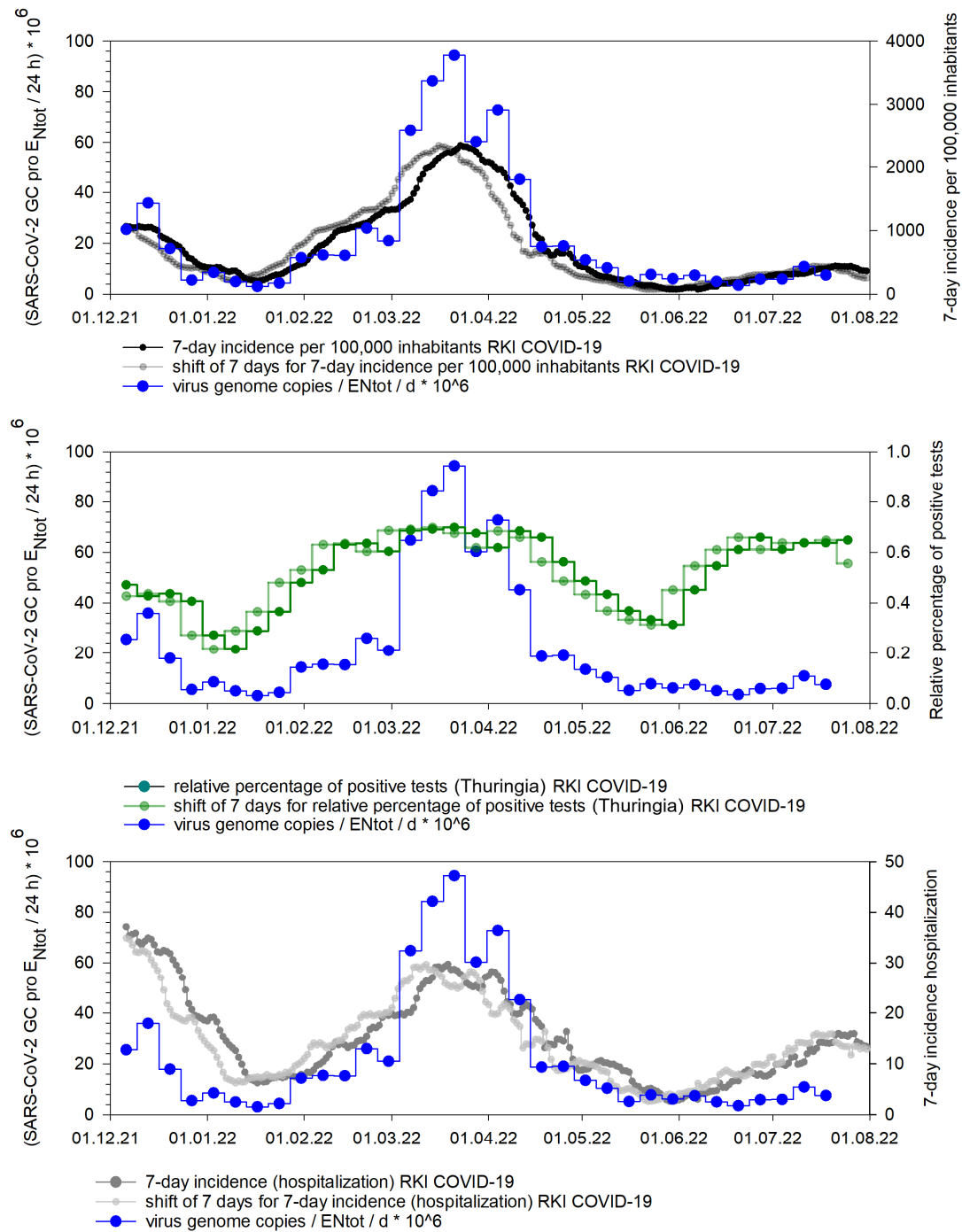


Figure S5: Timeline of SARS-CoV-2 genome copies per inhabitant $N_{tot} / 24 \text{ h}$ of all sampled WWTPs (see table 1) and different epidemiological metrics for the Free State of Thuringia - without time shift of epidemiological metrics and a with a time shift.

d) Quality Control

Table S2: Minimum and maximum coefficients of variation (CVs) of biological and technical replicates for SARS-CoV-2 (N1 assay) and PMMoV.

	CVs for triplicate biological extracts	CVs for triplicate technical replicates
SARS-CoV-2 (N1 assay)	MIN: 0.002 MAX: 0.76	MIN: 0.0001 MAX: 0.079
PMMoV	MIN: 0.015 MAX: 1.19	MIN: 0.0003 MAX: 0.035

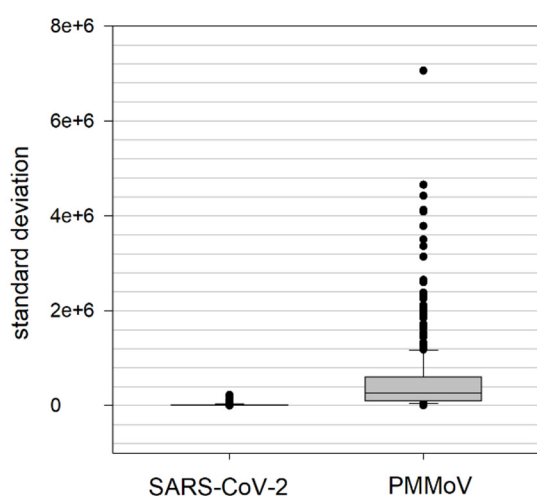


Figure S6: Standard deviation of biological replicates for the concentration [GC/L] of SARS-CoV-2 and PMMoV. boxplots: median (black line), 10% and 90% quantiles as whiskers and all possible outliers.

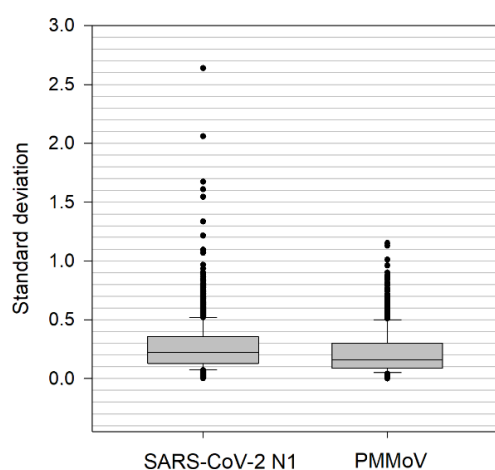


Figure S7: Standard deviation of CT-values from the technical replicates for the SARS-CoV-2 N1 and PMMoV assay in RT-qPCR. boxplots: median (black line), 10% and 90% quantiles as whiskers and all possible outliers.

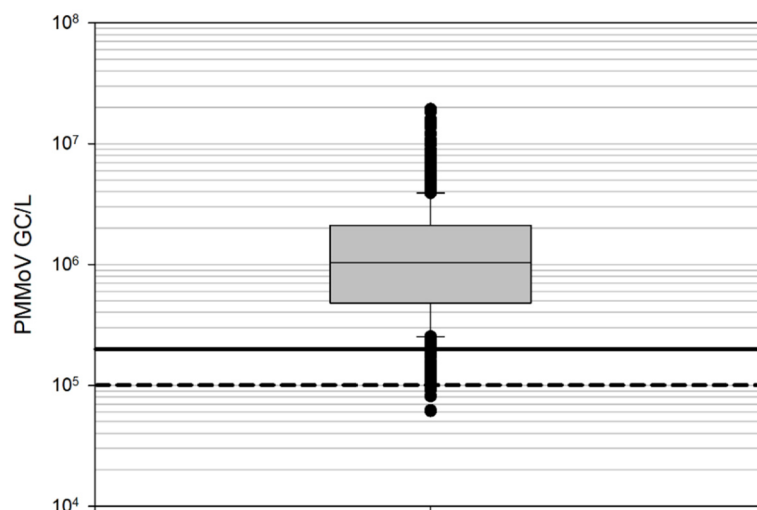


Figure S8: PMMoV as internal extraction control with threshold of 10^5 PMMoV GC/L (lower values indicated poor quality; dashed line) and 2×10^5 PMMoV GC/L (higher values indicated acceptable quality; line). Threshold values were used in accordance with Kantor et al. (52) . boxplot: median (black line), 10% and 90% quantiles as whiskers and all possible outliers.

RT-qPCR Performance Characteristics

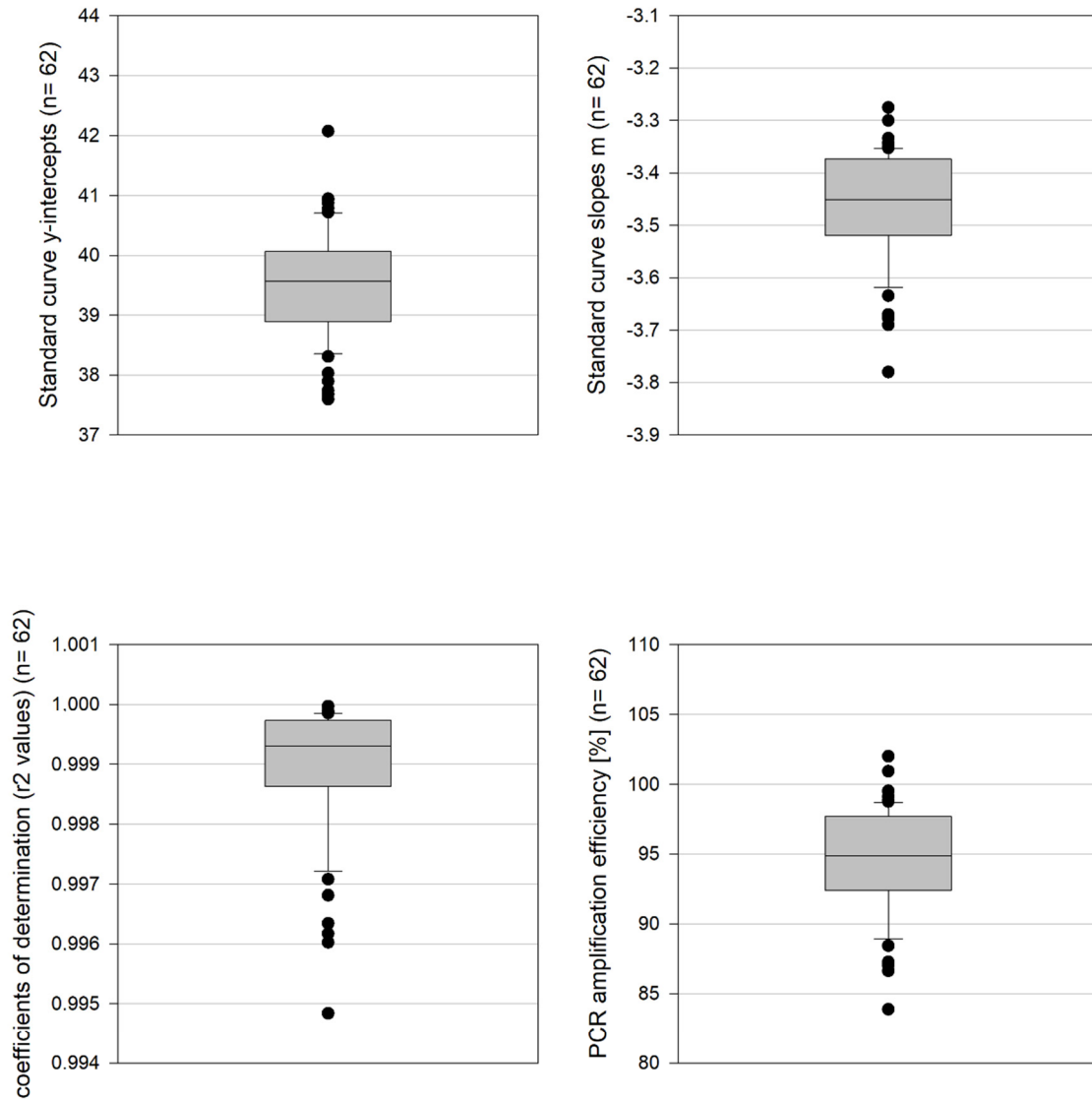


Figure S9: RT-qPCR Performance Characteristics SARS-CoV-2. boxplots: median (black line), 10% and 90% quantiles as whiskers and all possible outliers.

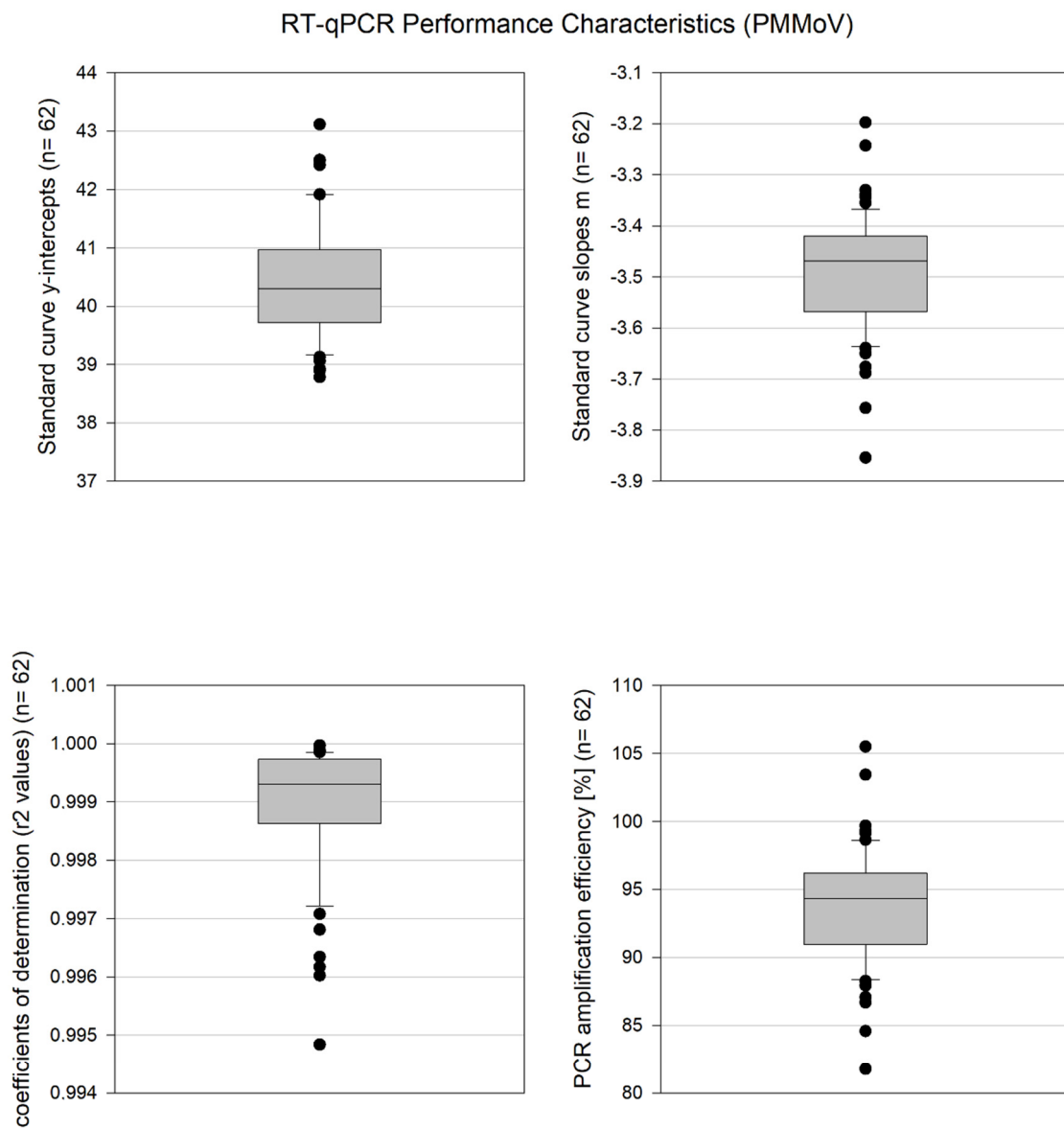


Figure S10: RT-qPCR Performance Characteristics PMMoV. boxplots: median (black line), 10% and 90% quantiles as whiskers and all possible outliers.

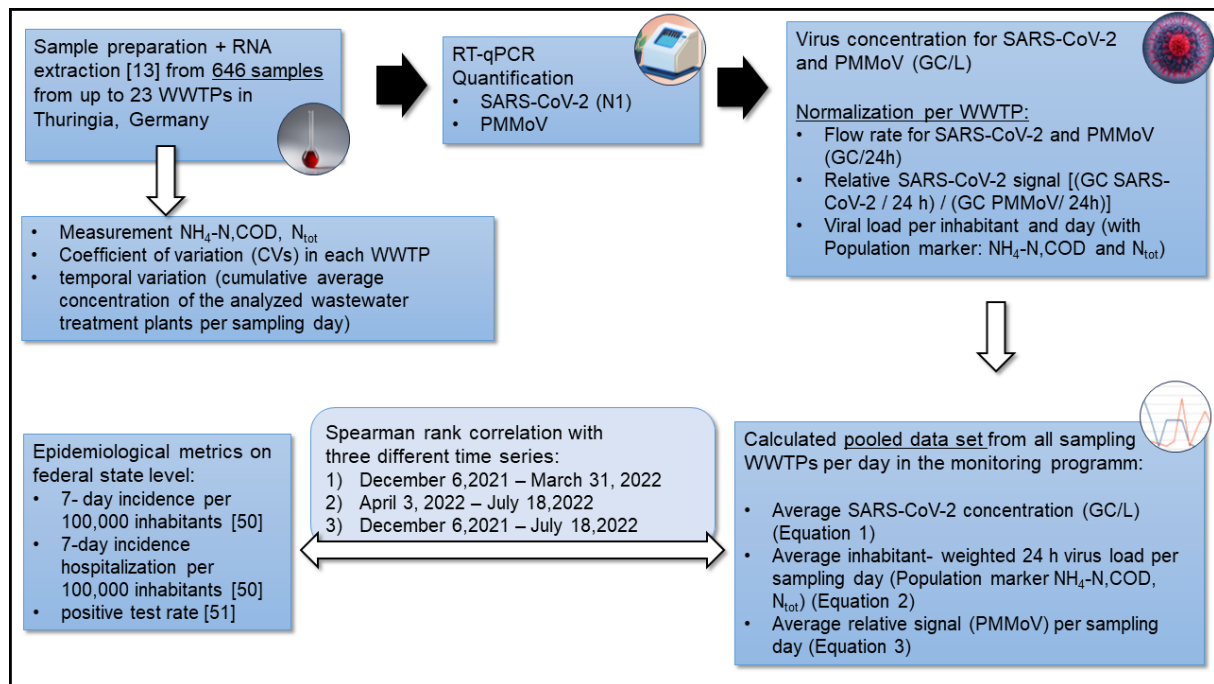


Figure S11: Figure providing an overview of the methods and data analyses performed.

e) Cost per biological replicate

Table S3: Cost per biological sample replicate for RNA extraction (consumables and reagents) within the comparative analyses in the wastewater monitoring laboratory at Hamm Lippstadt University of Applied Sciences.

Items	Metric	Amount used per wastewater sample biological replicate	Cost per sample replicate [€]
<u>Lysis</u>			<u>0.37</u>
NaCl (≥99 %, Ph. Eur., USP, Carl Roth GmbH + Co. KG, Karlsruhe, Germany)	kg	0.01	
50 mL conical tubes (Eppendorf SE, Hamburg, Germany)	tube	1	
Tris (reagent grade) (Carl Roth GmbH + Co. KG, Karlsruhe, Germany)	kg	0.00007	
EDTA, Disodium ACS Reagent Grade (Carl Roth GmbH + Co. KG, Karlsruhe, Germany)	g	0.012	
1000uL pipette tips with filter (Socorex Isba SA, Ecublens, Switzerland)	tips	1	
<u>Prefiltration</u>			<u>3.08</u>
PVDF flat membrane filters; 5 µm; 47mm (Durapore®, Merck KGaA, Darmstadt, Germany)	filters	1	
50 mL syringe (sterile) (Carl Roth GmbH + Co. KG, Karlsruhe, Germany)	syringes	1	
<u>RNA extraction</u>			<u>10.62</u>
Ethanol (70%) from Ethanol 99.8% p.a	L	0.04	
Ethanol (99.8% p.a) (Carl Roth GmbH + Co. KG, Karlsruhe, Germany)	L	0.009	
Wash Buffer 1	L	0.005	
Wash Buffer 2	L	0.01	
ZymoPURE Elution Buffer (ZYMO Research Europe GmbH, Freiburg, Germany).	mL	0.2	
Zymo IIP columns with reservoir (ZYMO Research Europe GmbH, Freiburg, Germany).	columns and reservoirs	1	
200uL pipette tips with filter (Socorex Isba SA, Ecublens, Switzerland)	tips	3	
120 ml sterile cups (Carl Roth GmbH + Co. KG, Karlsruhe, Germany)	cup	1	
2,0 mL tubes (Eppendorf SE, Hamburg, Germany)	tubes	1	
1.5 mL tubes (Eppendorf SE, Hamburg, Germany)	tubes	2	
<u>Cost per biological sample replicate (Sample preparation and RNA extraction)</u>			<u>14.07</u>
<u>*One-time cost</u>			
EZ-Vac Vacuum Manifold (ZYMO Research Europe GmbH, Freiburg, Germany).			<u>226</u>