Supplementary Materials

Wastewater Surveillance of SARS-CoV-2 and Chemical Markers in Campus Dormitories in an Evolving Pandemic COVID -19

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Table S1. Campus wastewater sampling sites comprisisng 14 residential facilities, 28 final discharge chambers servicing 9090 students from 89 blocks.

Site (n=7)	Final discharge ID (n=28)	Number of block (n=89)	Estimated total population (n=9090)
A	A1	2	450
	A2	2	420
	A3	2	470
	A4	2	460
	A5_n	3	450
	A5_s	3	510
	A6_a	1	300
	A6_b	1	
	A6_c	1	
	A6_d	1	
	A6_e	1	
	A6_f	1	
	A6_g	1	
	A6_h	1	
B	B1	6	485
C	C1	5	650
D	D1	6	490
	D2	6	470
E	E1	6	510
	E2	6	520
F	F1	8	400
G	G1_a	4	350
	G1_b	4	555
	G1_c	1	90
	G1_d	2	210
	G1_e	3	300
	G1_f	5	470
	G1_g	5	530

Table S2. Primer/Probe sets and PCR conditions used in this study.

Primer/Probe	Sequence	Number of cycles	Annealing temperature (°C)	Reference
SARS-CoV-2_N1-Forward	5'-GACCCCAAAATCAGCGAAAT-3'	45	55	1,2,3
SARS-CoV-2_N1-Reverse	5'-TCTGGTTACTGCCAGTTGAATCTG-3'			
SARS-CoV-2_N1_Probe	5'-ACCCCGCATTACGTTTGGTGGACC-FAM-3'			
PMMoV FP1 - Forward	5'-GAGTGGTTTGACCTTAACGTTGA-3'	45	55	4
PMMoV-RP1 - Reverse	5'-TTGTCGGTTGCAATGCAAGT-3'			
PMMoV-Probe	5'-CCTACCGAAGCAAATG-FAM-3'			

References

1. Hirotsu, Y., Mochizuki, H., Omata, M., 2020. Double-Quencher Probes Improved the Detection Sensitivity of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by One-Step RTPCR. medRxiv 2020.03.17.20037903.
2. Jung, Y.J., Park, G.-S., Moon, J.H., Ku, K., Beak, S.-H., Kim, S., Park, E.C., Park, D., Lee, J.-H., Byeon, C.W., Lee, J.J., Maeng, J.-S., Kim, S.J., Kim, S.I., Kim, B.-T., Lee, M.J., Kim, H.G., 2020. Comparative analysis of primer-probe sets for the laboratory confirmation of SARS-CoV-2. bioRxiv 2020.02.25.964775. https://doi.org/10.1101/2020.02.25.964775
3. https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html
4. Zhang T, Breitbart M, Lee WH, Rum JQ, Wei CL, Soh SW, Hibberd ML, Liu ET, Rohwer F, Ruan Y. 2006. RNA viral community in human feces: prevalence of plant pathogenic viruses. PLoS Biol. 4:e3.10.1371/journal.pbio.0040003

Table S3. List of target emerging contaminants, MS transition and surrogate isotope-labelled standards.

Name	Abbreviation	Transition	Surrogate isotope-labelled standards
Acetaminophen	ACT	152.1 -> 110.1	ACT-D4
Azithromycin	AZT	749.5 -> 158.0	AZT-D3
Benzethonium Cl	BCL	413.3 -> 91.0	Nevirapine-d3
Benzyldimethyldodecylammonium chloride	BAC-12	305.0 -> 58.1	CF-D9
Caffeine	CF	195.1 -> 138.1	CF-D9
Clarithromycin	CLAR	748.5 -> 158.2	CLAR-D3
Clindamycin	CLI	425.2 -> 126.0	CLAR-D3
Chlortetracycline	CTC	479.1 -> 444.1	TET-D6
Didecyldimethylammonium chloride	DADMAC-10	327.4 -> 43.2	TMP-D3
Doxycycline	DXC	446.2 -> 429.1	ACT-D4
Erythromycin-H_2O	ERY-H20	716.5 -> 158.0	ERY-D6
Lincomycin	LIN	407.2 -> 126.2	LIN-D3
Oxybenzone	OXB	229.1 -> 151.1	DEET-D10
Oxytetracycline	OXY	461.1 -> 426.1	TET-D6
Sulpiride	SUL	342.2 -> 112.2	CBZ-D8
Triclocarban	TCC	313.0 -> 160.0	TCC-13C6
Triclosan	TCS	286.9 -> 35.0	TCS-D3
Tetracycline	TET	445.2 -> 410.3	TET-D6
Tylosin	TYL	916.5 -> 174.0	AZT-D3

Table S4 Quality control data of the targets.

Target analytes	MDL (ng/L)	MQL (ng/L)	RR±SD (%)
ACT	2	9	101.7±4.4
AZT	0.15	0.5	107.3±8.9
BCL	2	9	85±19
BAC-12	2	9	78±12
CF	2.5	8	100.9±4.7
CLAR	0.15	0.5	97.6±5
CLI	0.1	0.3	101±4.8
CTC	2.5	7.5	101.1±2.5
DADMAC-10	2	9	79±8.9
DXC	2	5	105.8±4.8
ERY-H_20	0.2	0.6	94.2±10.5
LIN	0.1	0.3	99±5.5
OXB	0.08	0.25	96.9±1.6
OXY	23	75	103.6±9.4
SUL	0.06	0.2	94.1±4.4
TCC	1.4	4.5	102.9±4.4

TCS	3	10	98.9±6.7
TET	15	50	104.1±1
TYL	0.5	1.5	87.7±3.4

RR: Relative recovery (RR) of individual analytes were performed on the wastewater samples (200 mL) spiked with 50 ng/L target analytes before and after the solid phase extraction (SPE), and then calculated using relative response with a corresponding labeled internal standard.

$$\%RR = \frac{C_{Pre-S} - C_N}{C_{Sp-Sol}} \times 100$$

Where C_{Pre-S} is the measured concentration of the analyte in the sample extract and the samples were spiked with the analytes and surrogate standards prior to the extraction, C_N is the measured concentration of the analyte in the corresponding sample extracts without spiking, C_{Sp-Sol} is the measured concentration of the analyte in the spiking solution.

MDL and MQL: Method detection limit (MDL) and method quantification limit (MQL) were defined to be the lowest observable concentration of analytes in spiked extracts of the samples giving a signal-to- noise (S/N) of 3 and 10, respectively.

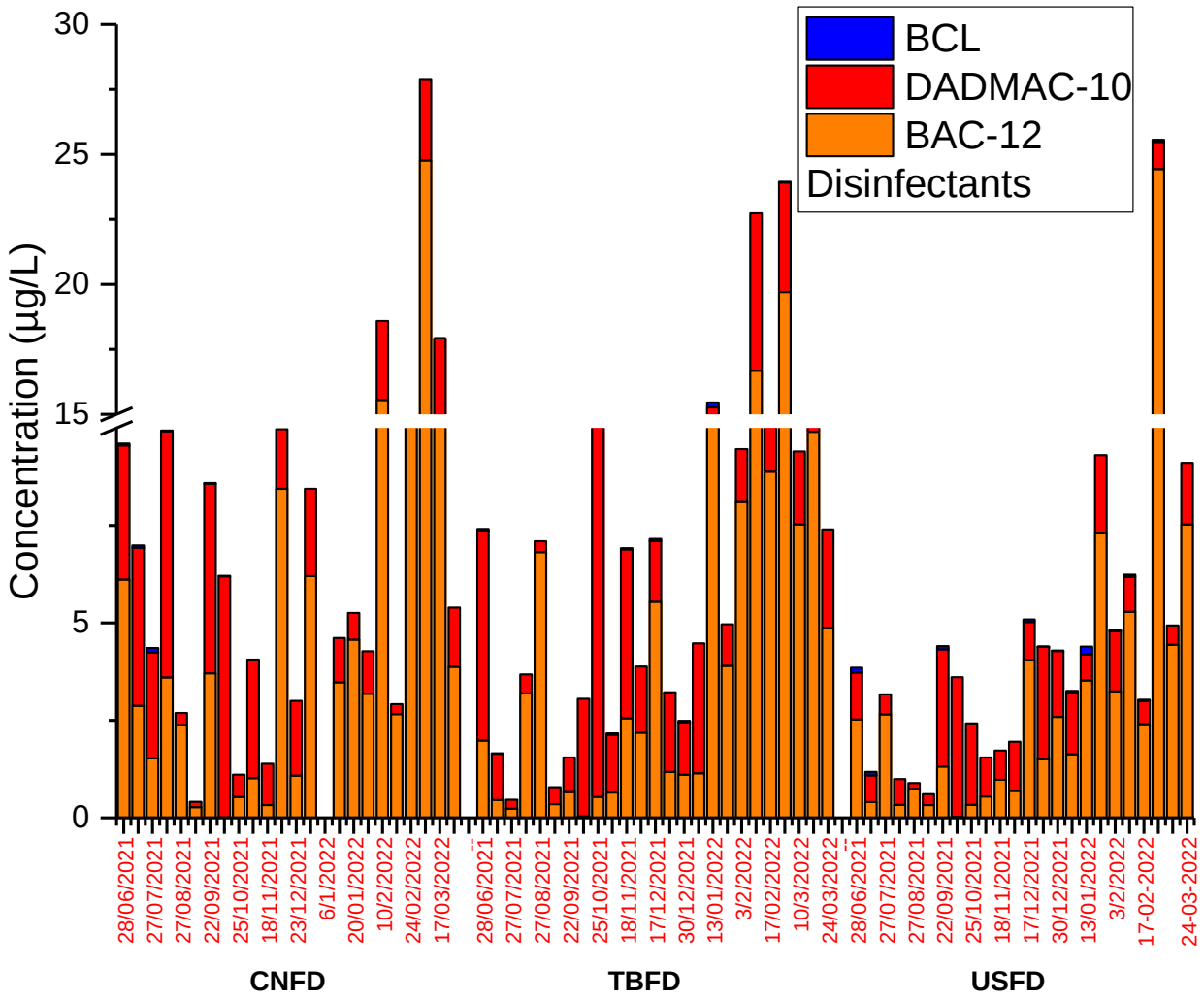

Figure S1. Variations in the concentrations of disinfectants

CNFD, TBFD and USFD can be read as A2, A4 and A5, respectively.

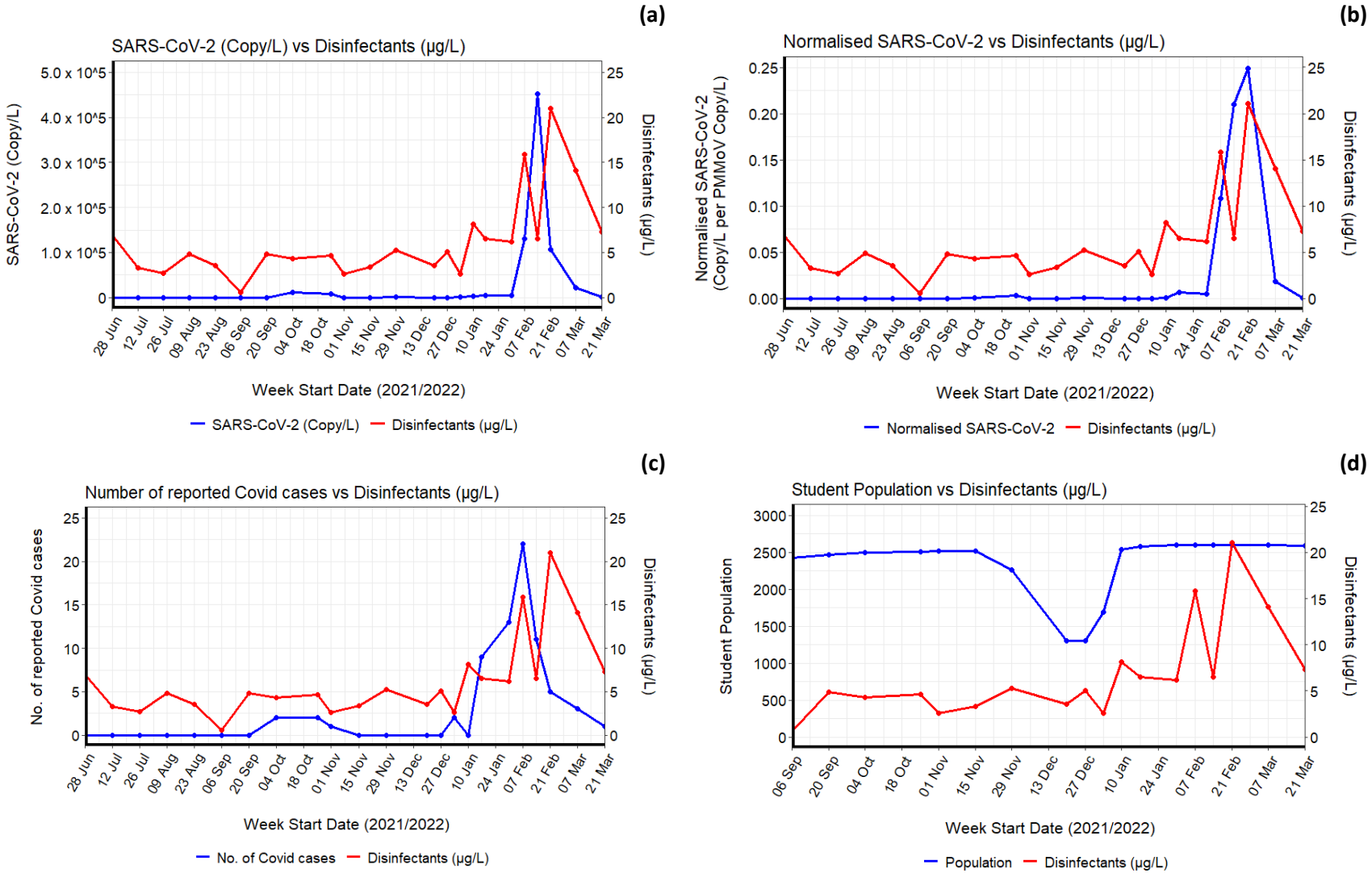

Figure S2. Correlation analysis between disinfectants and (a) SARS-CoV-2 (copy/L), (b) normalized SARS-CoV-2 (Copy/L per PMMoV Copy/L), (c) number of reported COVID-19 cases, (d) student population

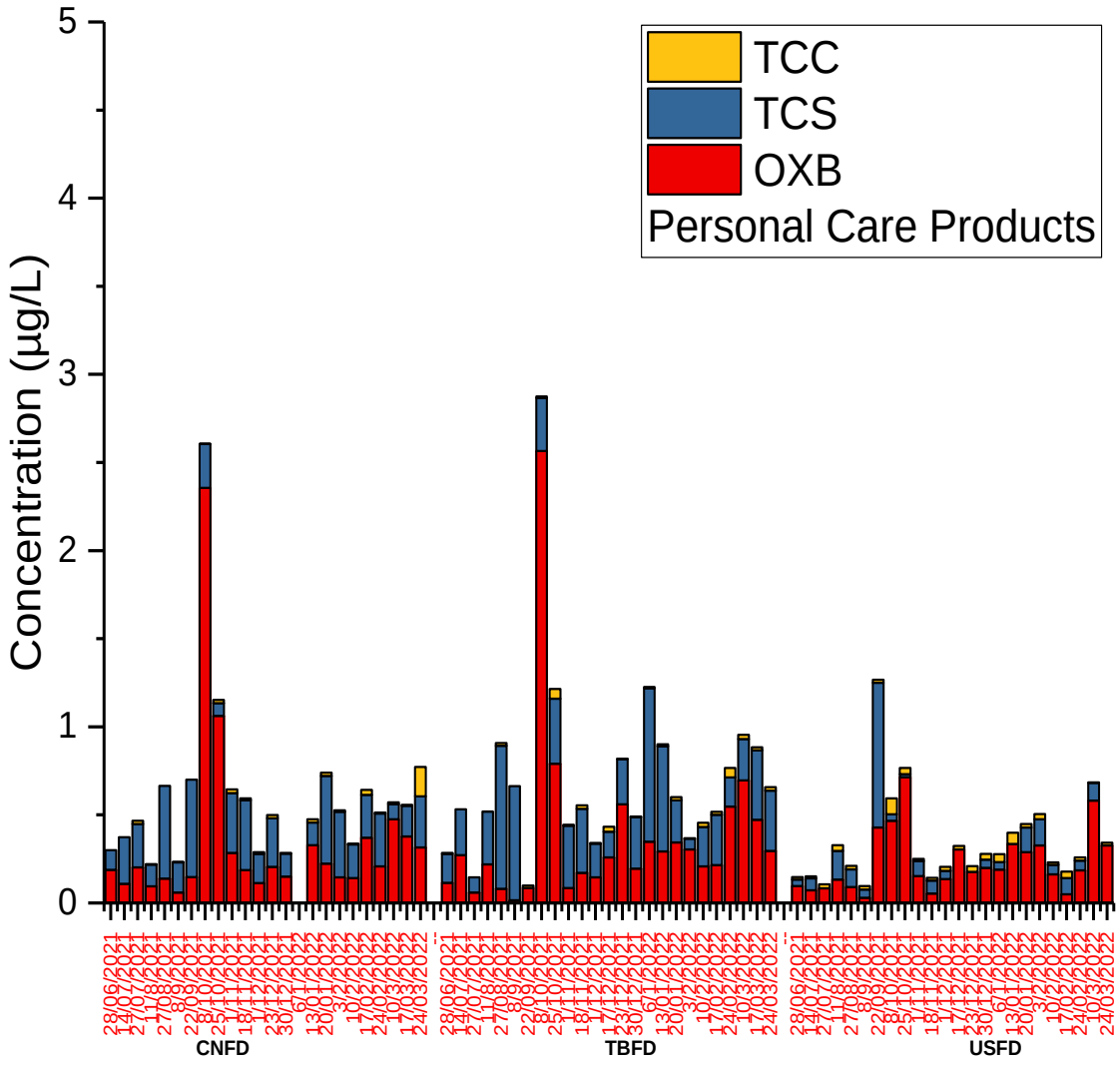

Figure S3. Variations in the concentrations of personal care products

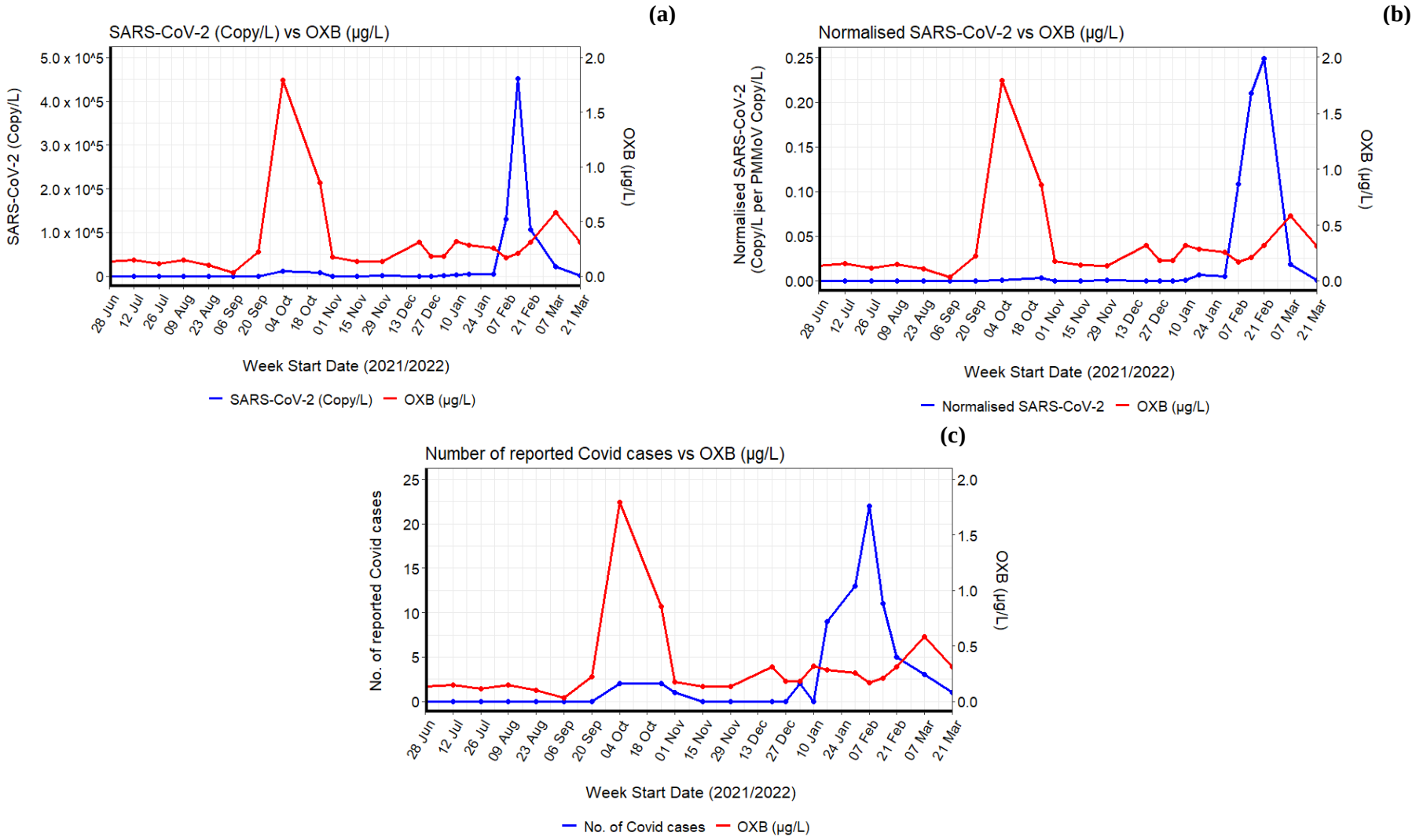

Fig. S4. Correlation analysis between OXB and (a) SARS-CoV-2 (copy/L), (b) normalized SARS-CoV-2 (Copy/L per PMMoV Copy/L), (c) number of reported COVID-19 cases

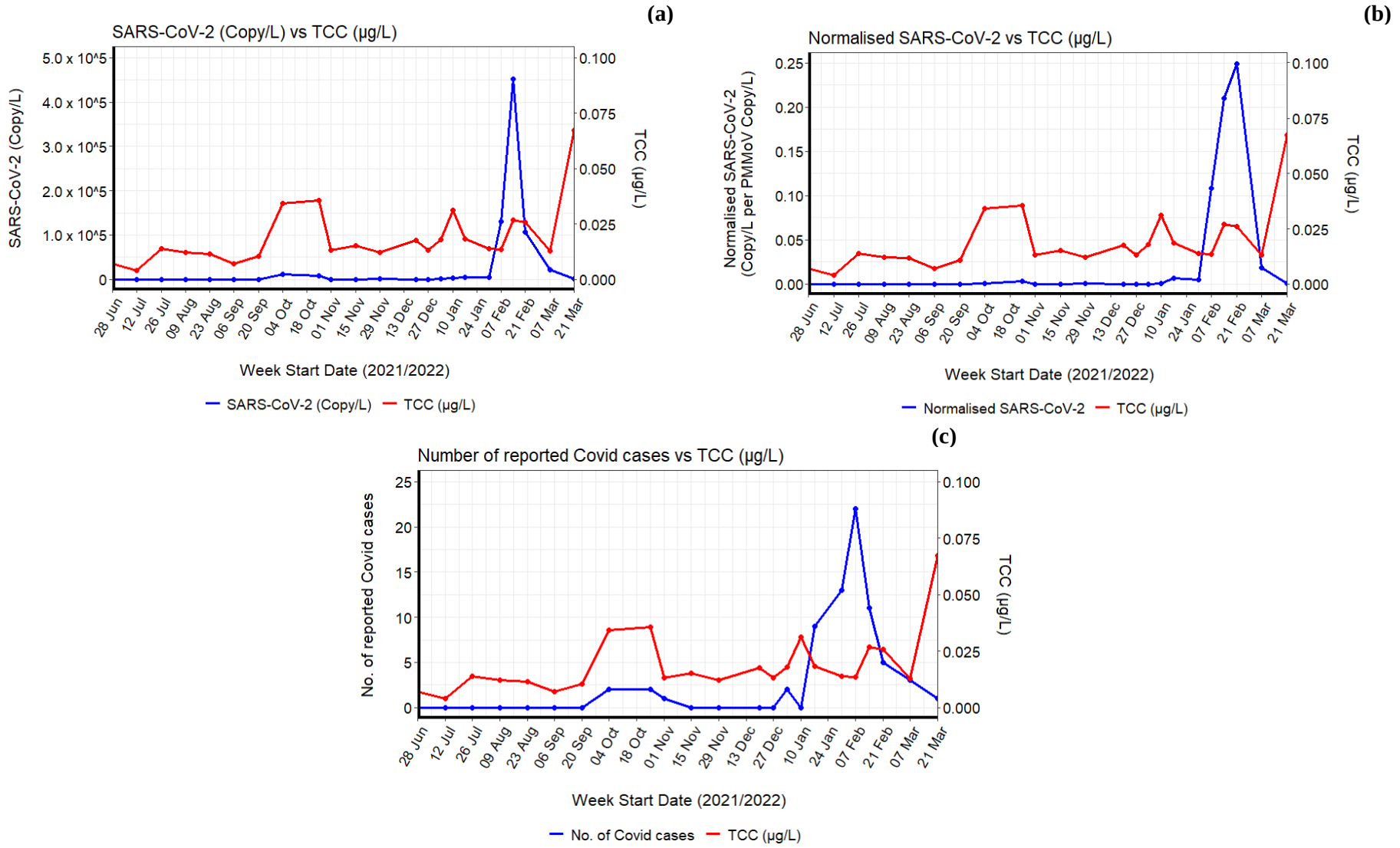

Fig. S5. Correlation analysis between TCC and (a) SARS-CoV-2 (copy/L), (b) normalized SARS-CoV-2 (Copy/L per PMMoV Copy/L), (c) number of reported COVID-19 cases

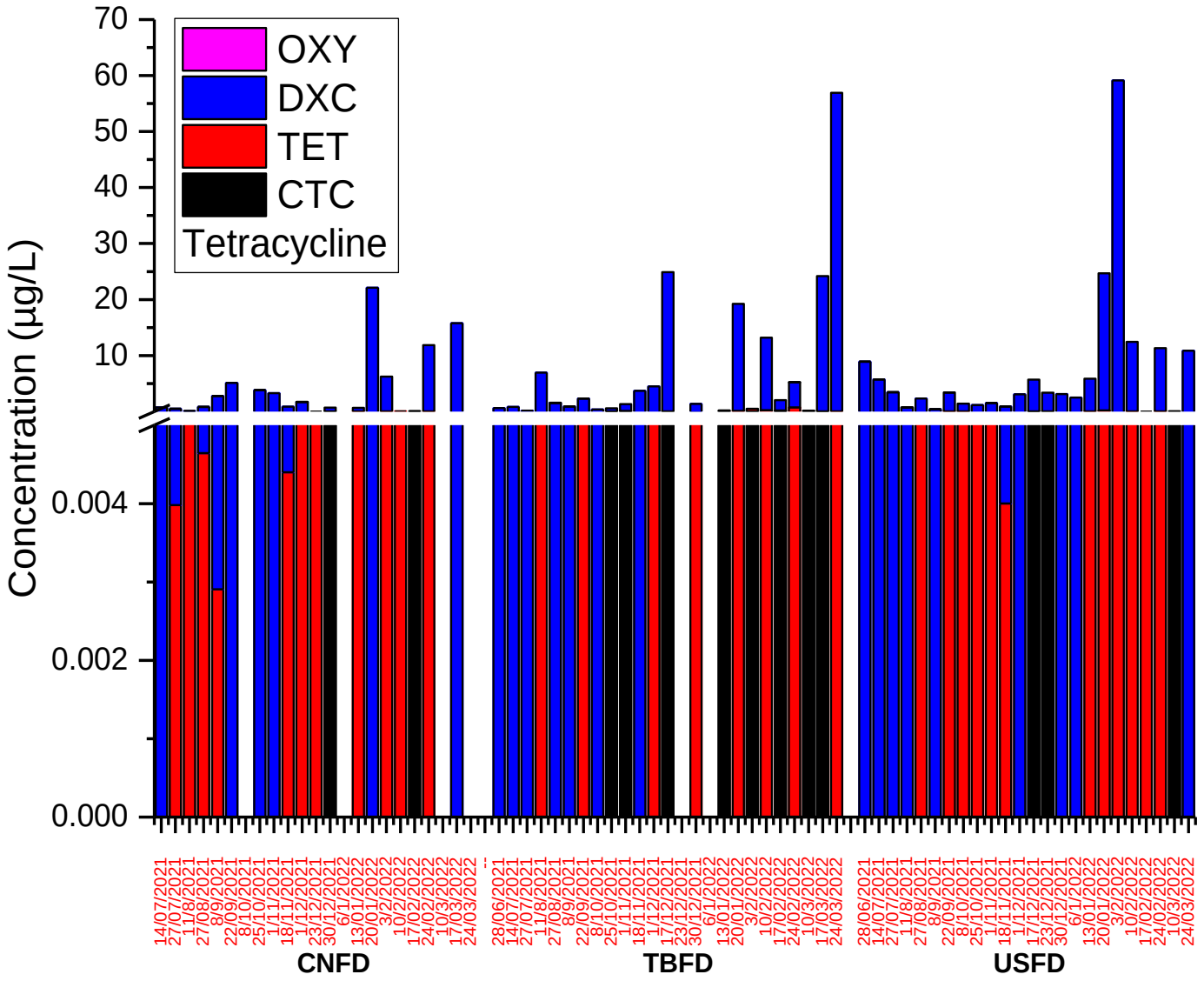

Figure S6. Variations in the concentrations of antibiotics- tetracycline

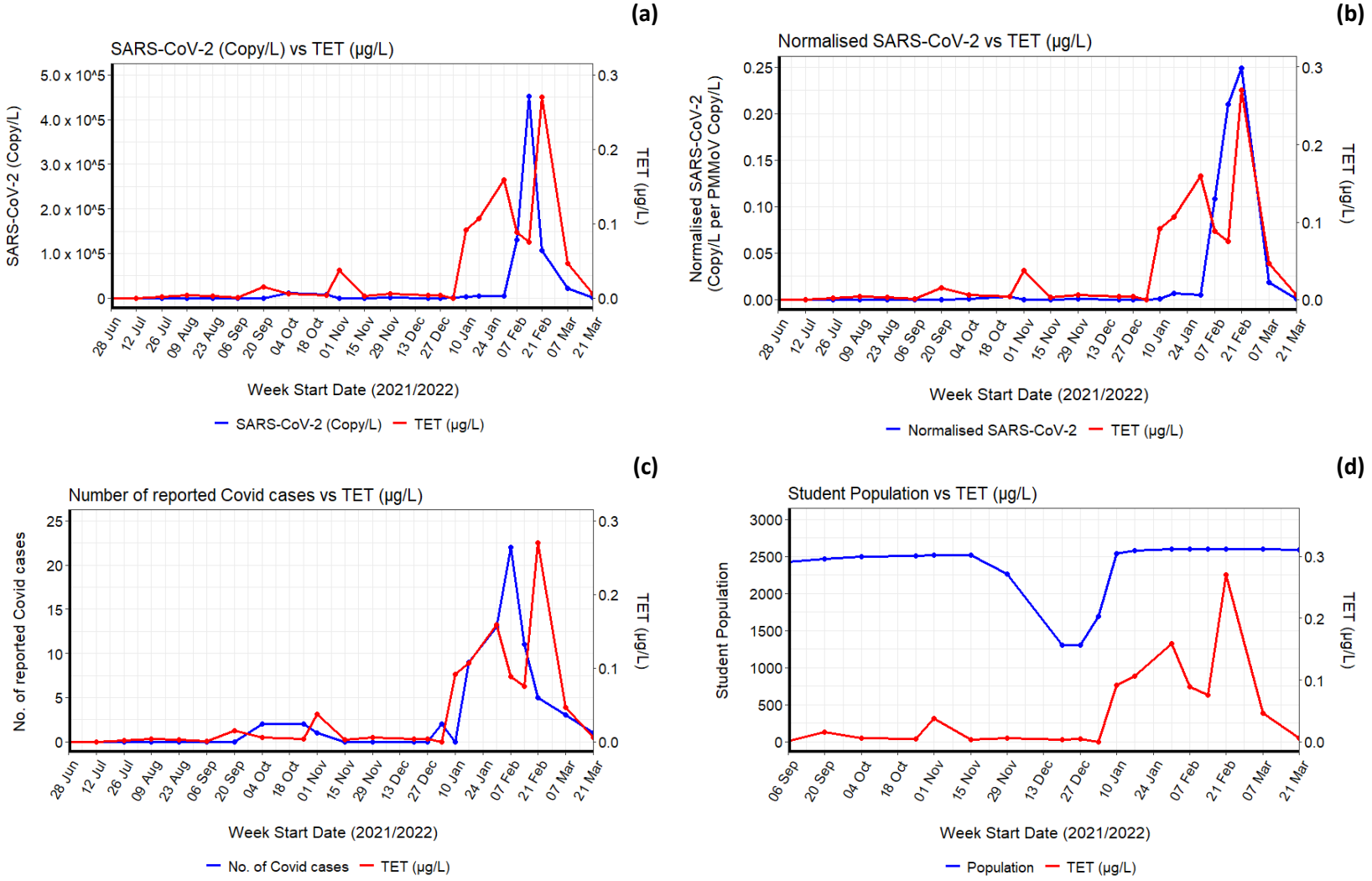

Figure S7. Correlation analysis between tetracyclines and (a) SARS-CoV-2 (copy/L), (b) normalized SARS-CoV-2 (Copy/L per PMMoV Copy/L), (c) number of reported COVID-19 cases, and (d) student population

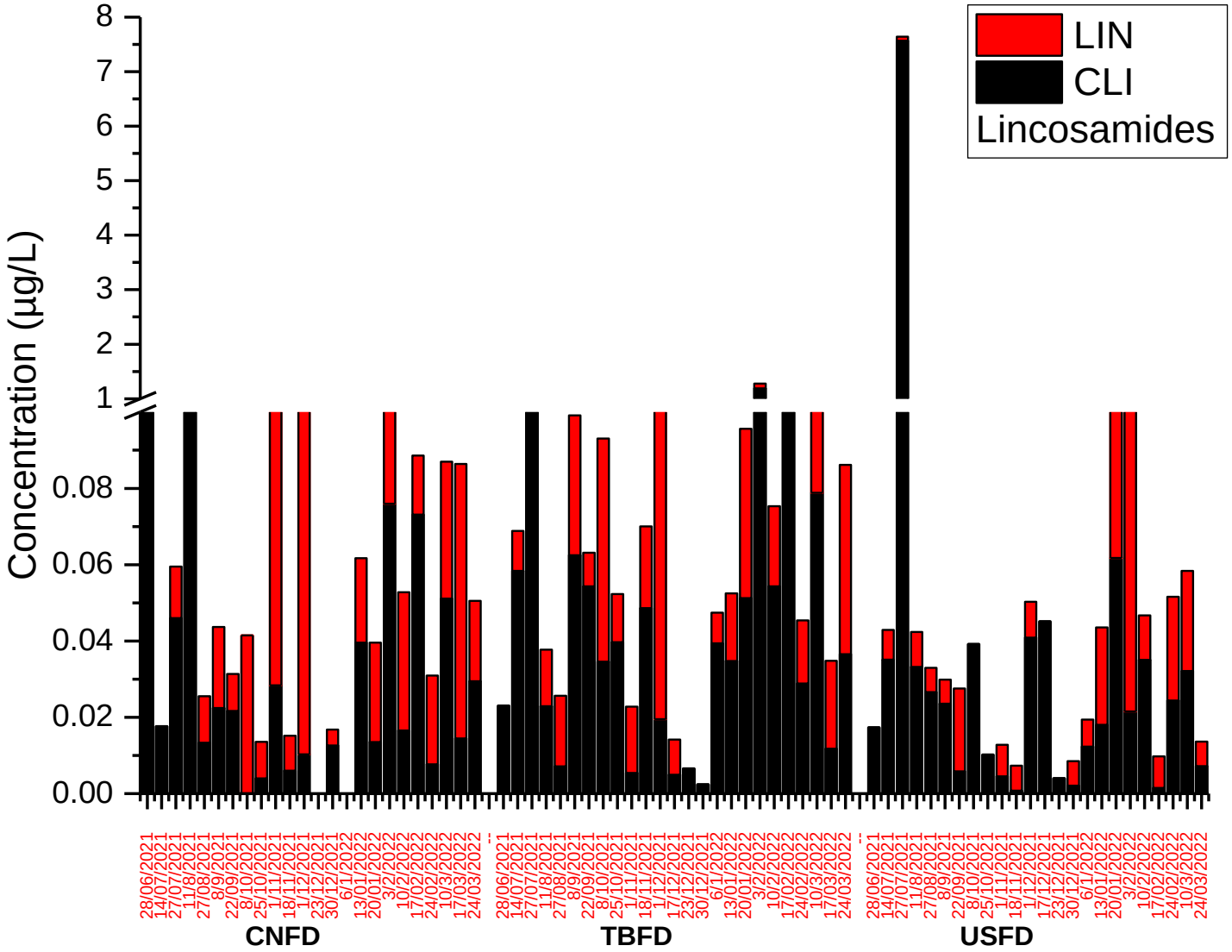

Figure S8. Variations in the concentrations of antibiotics-lincosamides

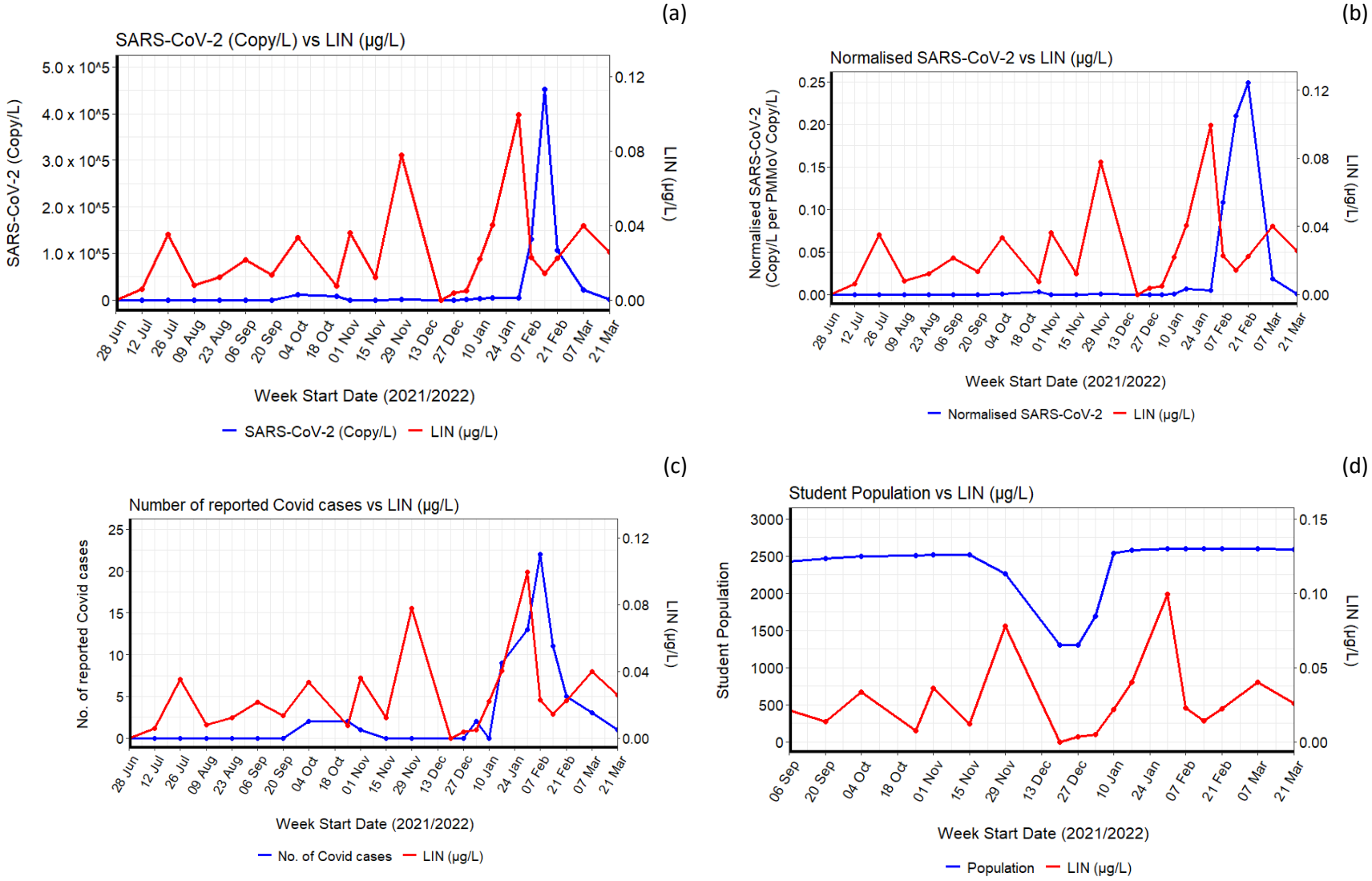

Figure S9. Correlation analysis between lincosamycin and (a) SARS-CoV-2 (copy/L), (b) normalized SARS-CoV-2 (Copy/L per PMMoV Copy/L), (c) number of reported COVID-19 cases, and (d) student population

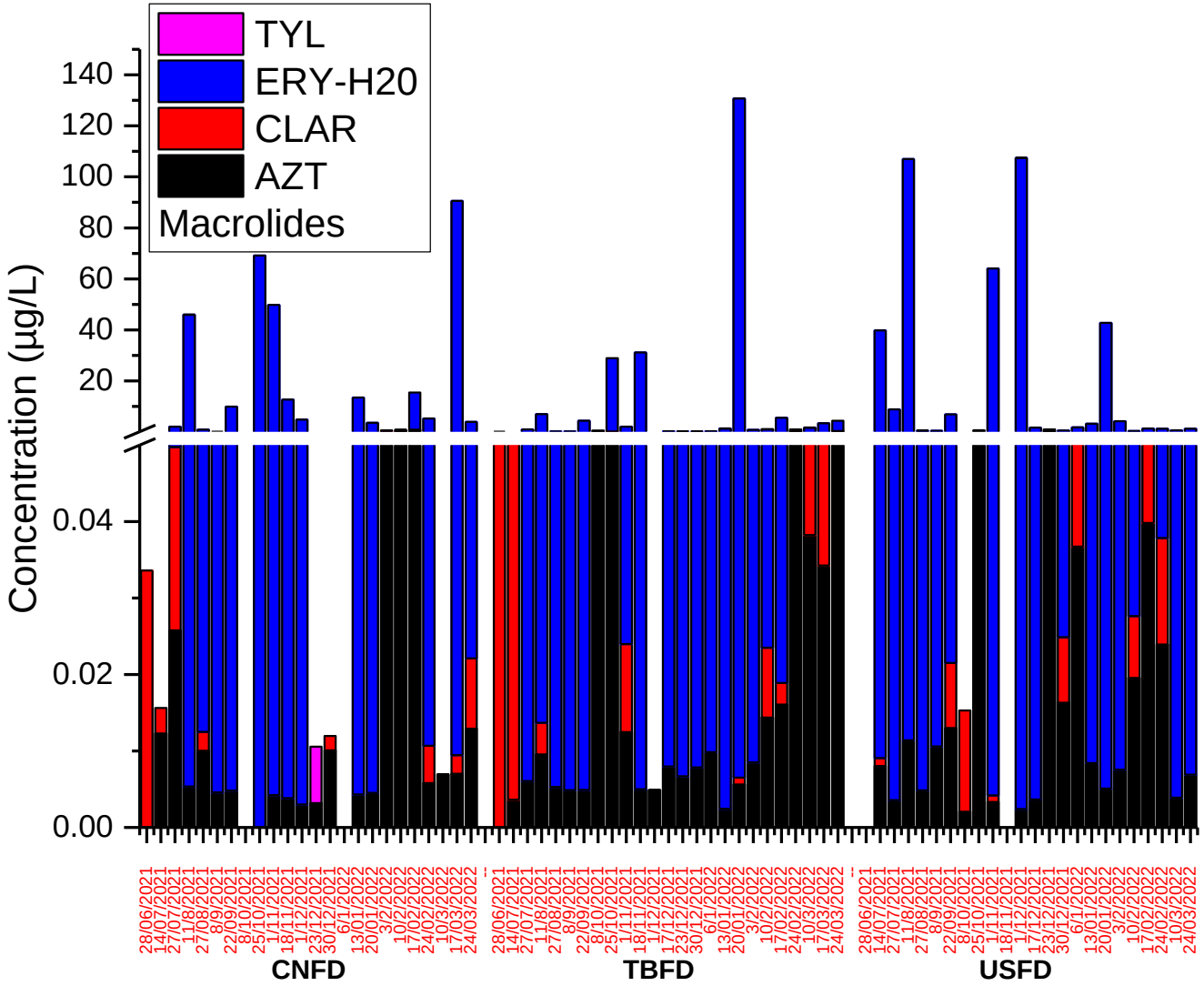

Figure S10. Variations in the concentrations of antibiotics-macrolides

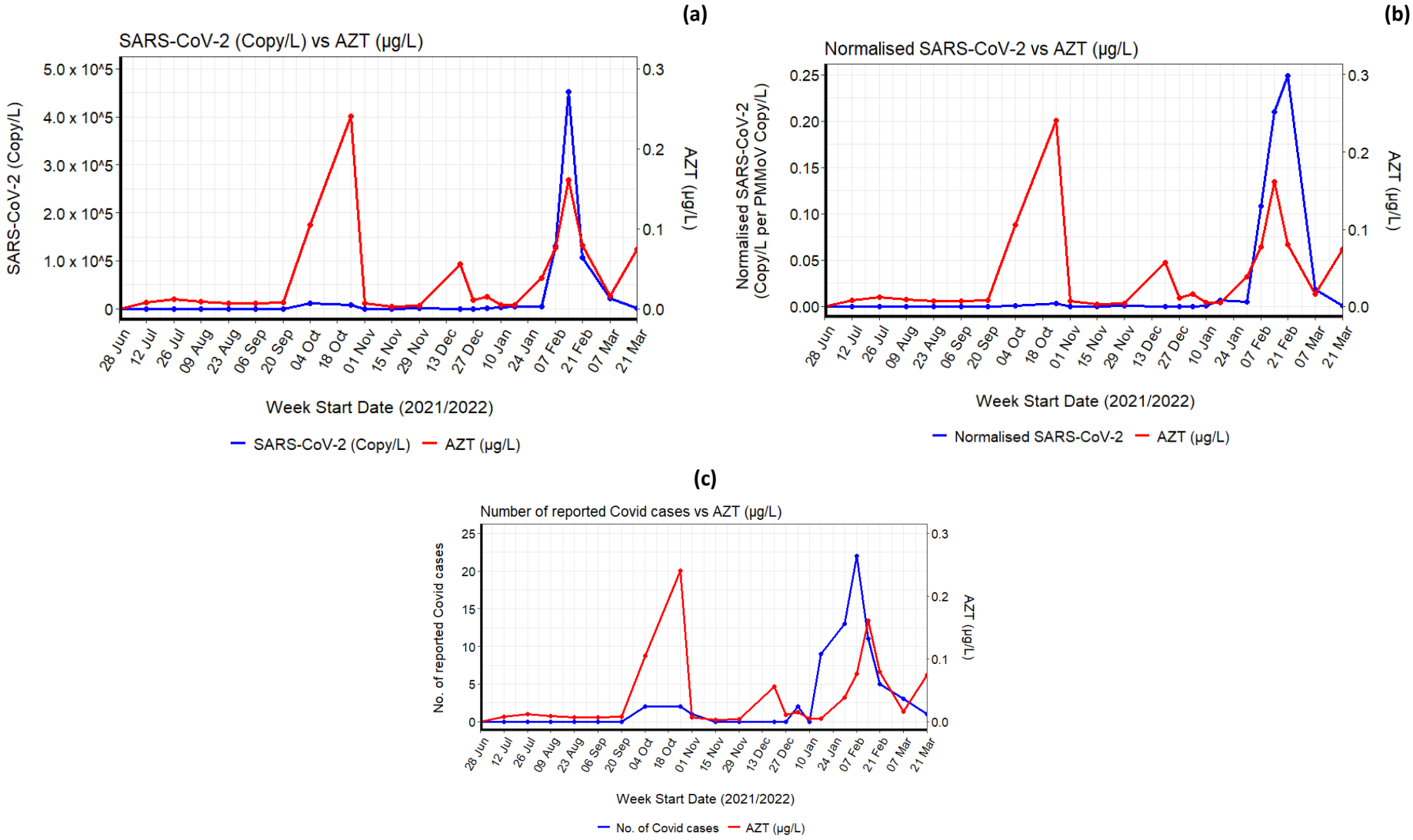

Figure S11. Correlation analysis between azithromycin and (a) SARS-CoV-2 (copy/L), (b) normalized SARS-CoV-2 (Copy/L per PMMoV Copy/L), and (c) number of reported COVID-19 cases

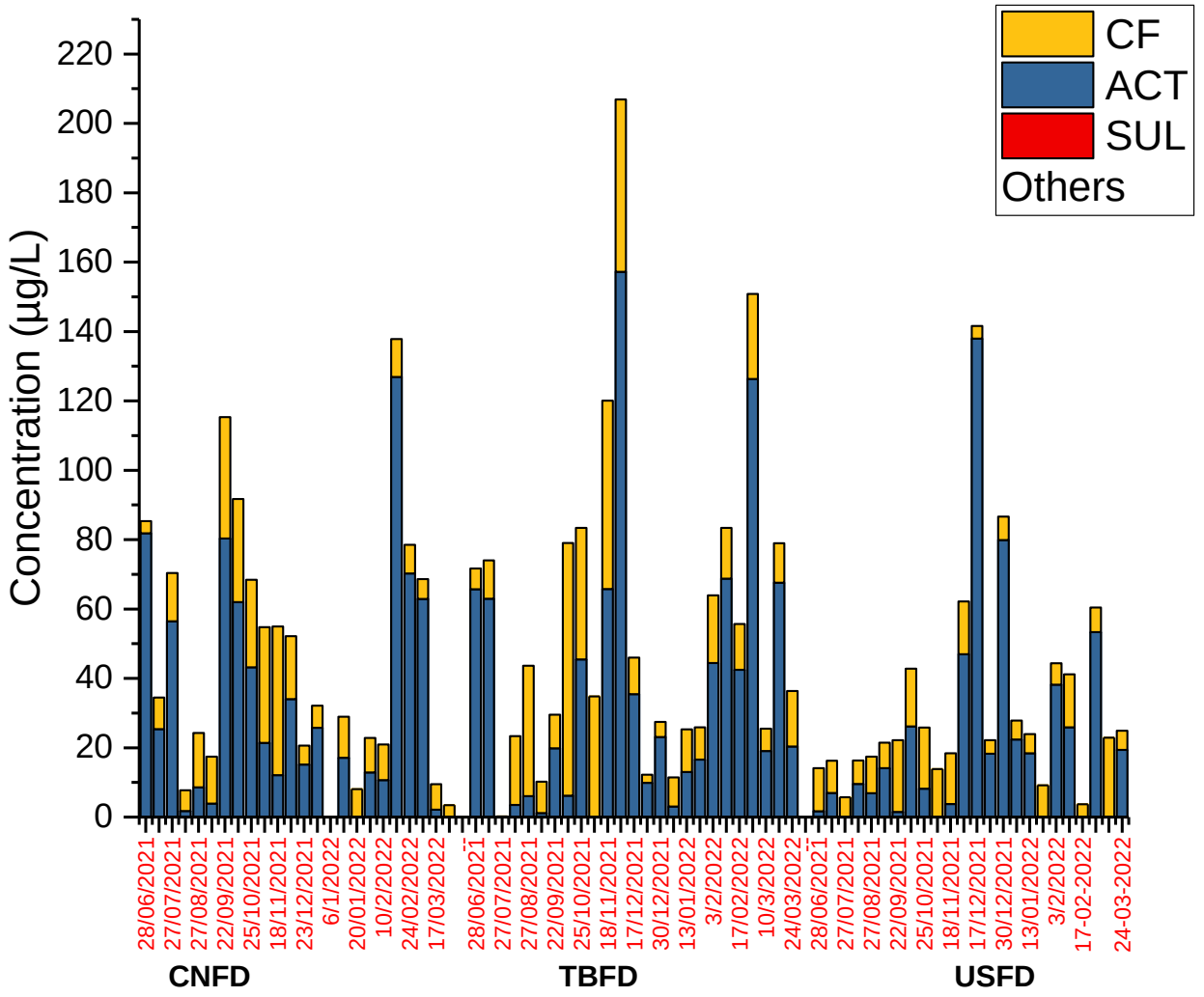

Figure S12. Variations in the concentrations of other pharmaceuticals

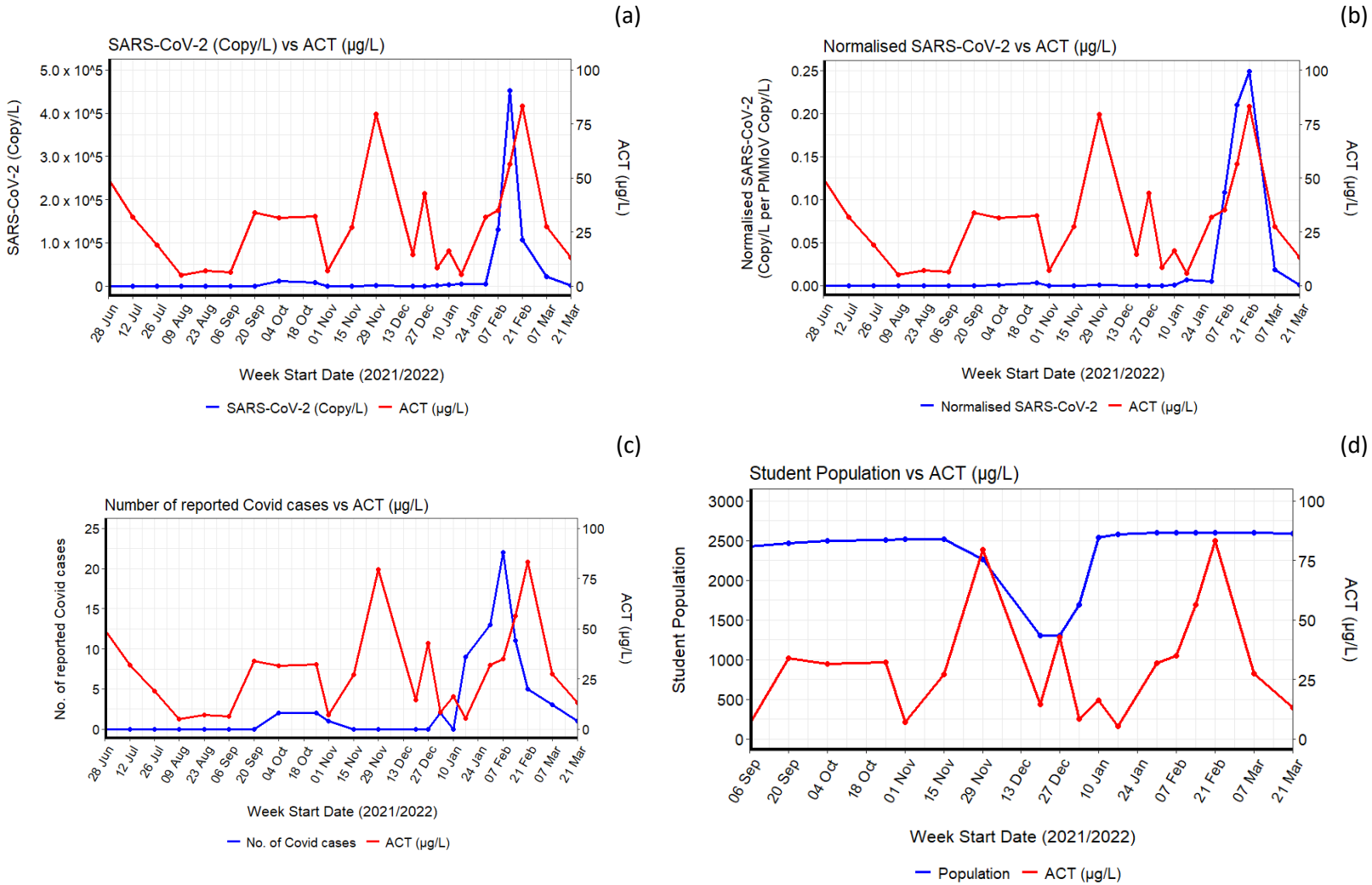

Figure S13. Correlation analysis between acetaminophen and (a) SARS-CoV-2 (copy/L), (b) normalized SARS-CoV-2 (Copy/L per PMMoV Copy/L), (c) number of reported COVID-19 cases, and (d) student population

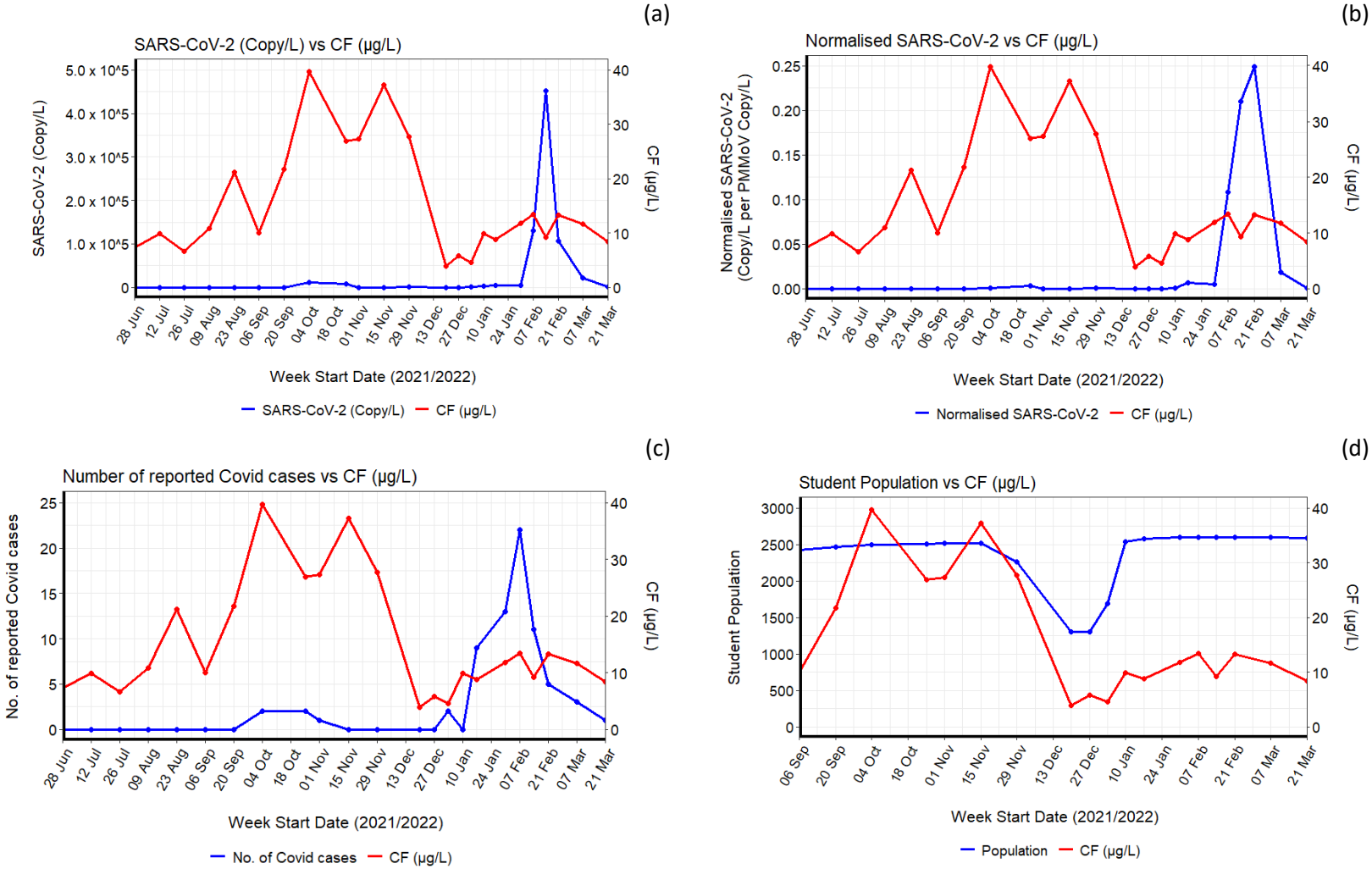

Figure S14. Correlation analysis between caffeine and (a) SARS-CoV-2 (copy/L), (b) normalized SARS-CoV-2 (Copy/L per PMMoV Copy/L), (c) number of reported COVID-19 cases, and (d) student population

Correlation Analysis

The column "Variable" refers to the other variable against which the Antiviral concentration was correlated against

Table S5: Acetaminophen correlation analysis for various conditions

Variable	SpearmanCoeff	p_valueRho
SARS-CoV-2 (Copy/L)	0.41	0.053
Normalised_SARS-CoV-2	0.38	0.077
No. of Covid cases	0.19	0.385
Covid cases per Population	0.17	0.435
Population	0.14	0.586

No statistically significant correlation

Table S6: Caffeine correlation analysis for various conditions

Variable	SpearmanCoeff	p_valueRho
SARS-CoV-2 (Copy/L)	0.22	0.32
Normalised_SARS-CoV-2	0.15	0.49
No. of Covid cases	0.12	0.59
Covid cases per Population	0.1	0.66
Population	0.15	0.54

No statistically significant correlation