Supplementary Information (SI) for Environmental Science: Water Research & Technology.

SUPPLEMENTARY TABLES AND FIGURES

Table S1: Characteristics of the sequenced samples

Sewage samples w collected at four sites (I-IV) in the sewer network of Lyon and Nantes with passive and grab sampling. Passive samplers were deployed for 24h.

Ct refers to the mean of three Ct values.

Coverage_30 indicates the SARS-CoV-2 genome coverage at a depth of 30.

* indicates that samples A-I and B-IV were not sequenced due to ineffective cDNA synthesis.

			SARS-CoV-2								Norovirus GII			
			Passive Sampling				Grab sampling				Passive Sampling		Grab sampling	
Sites	Date of passive sampling	Date of water sampling	Ct	cRNA/ membra-ne	Covera-ge_30 (%)	Mapped reads (%)	Ct	cRNA/10 mL	Coverage _30 (%)	Mapped reads (%)	Ct	cRNA/mem-brane	Ct	cRNA/10 mL
Lyon-I	13-Apr-22	14-Apr-22	32.3	$5.2x10^2$	90	75.6	34.8	$9.2x10^1$	86.6	64	28.5	$0.8x10^4$	31.1	$1.6.10^3$
	15-Apr-22	15-Apr-22	33.5	$2.4x10^2$	93	69.2	35.1	$7.4x10^1$	*	*	29.7	$4.0x10^3$	27.5	$1.7x10^4$
Lyon-II	13-Apr-22	14-Apr-22	32.7	$4.2x10^2$	92	90.4	35.3	$6.9x10^1$	88.3	62.9	26.0	$4.2x10^4$	28.7	$7.6x10^3$
	15-Apr-22	15-Apr-22	31.7	$7.8x10^2$	93	89.1	34.3	$1.3x10^2$	83	81.9	26.7	$2.4x10^4$	27.5	$1.7x10^4$
Lyon-III	13-Apr-22	14-Apr-22	33.2	$2.9x10^2$	90	80.1	35.6	$5.4x10^1$	87.1	90.4	28.2	$1.2x10^4$	31.2	$1.6x10^3$
	15-Apr-22	15-Apr-22	33.4	$2.5x10^2$	92	87.6	35.5	$6.0x10^1$	62.6	52.8	27.5	$1.7x10^4$	29.5	$4.5x10^3$
Lyon-IV	13-Apr-22	14-Apr-22	32.8	$3.5x10^2$	95	86.7	33.3	$2.5x10^2$	71.8	96	27.4	$1.7x10^4$	29.0	$6.2x10^3$
	15-Apr-22	15-Apr-22	31.0	$1.2x10^3$	93	84.5	33.8	$1.8x10^2$	90.5	90.3	26.9	$2.4x10^4$	26.3	$3.6x10^4$
Nantes-I	18-May-22	17-May-22	34.7	$1.1x10^2$	94	88.3	31.9	$6.4x10^2$	92.1	96.3	30.6	$2.3x10^3$	29.4	$4.9x10^3$
	20-May-22	19-May-22	34.7	$1.0x10^2$	77	84	28.6	$5.9x10^3$	95.3	96.2	29.5	$4.6x10^3$	27.8	$1.3x10^4$
Nantes-II	18-May-22	17-May-22	34.7	$1.1x10^2$	85	78.9	33.7	$1.9x10^2$	83.6	59.8	31.8	$2.0x10^3$	30.3	$2.7x10^3$
	20-May-22	19-May-22	34.8	$1.0x10^2$	80	56	33.8	1.8x10	90.2	78.1	30.4	$5.5x10^3$	31.6	$1.2x10^3$
Nantes-III	18-May-22	17-May-22	31.6	$8.4x10^2$	80.9	93.4	31.7	$7.3x10^2$	96.3	93	31.2	$1.6x10^3$	28.1	$1.1x10^4$
	20-May-22	19-May-22	34.0	$1.4x10^2$	94.4	76.1	36.0	$4.0x10^1$	92.4	94.2	29.8	$3.6x10^3$	31.3	$1.4x10^3$
Nantes-IV	18-May-22	17-May-22	35.4	0.710^2	*	*	31.7	$7.4x10^2$	95.4	94	30.0	$3.3x10^3$	27.9	$1.3x10^4$
	20-May-22	19-May-22	34.4	$1.3x10^2$	90.9	74.6	30.5	$1.7x10^3$	93.7	93	29.6	$4.3x10^3$	29.3	$5.5x10^3$

Figure S1: The frequency of mutations in the SARS-CoV-2 genome

The frequency and distribution of mutations in the genome are shown for passive and grab samples for both cities. ORF1ab: nt 266 – 13 468; S gene: nt 21 563 – 25 384.

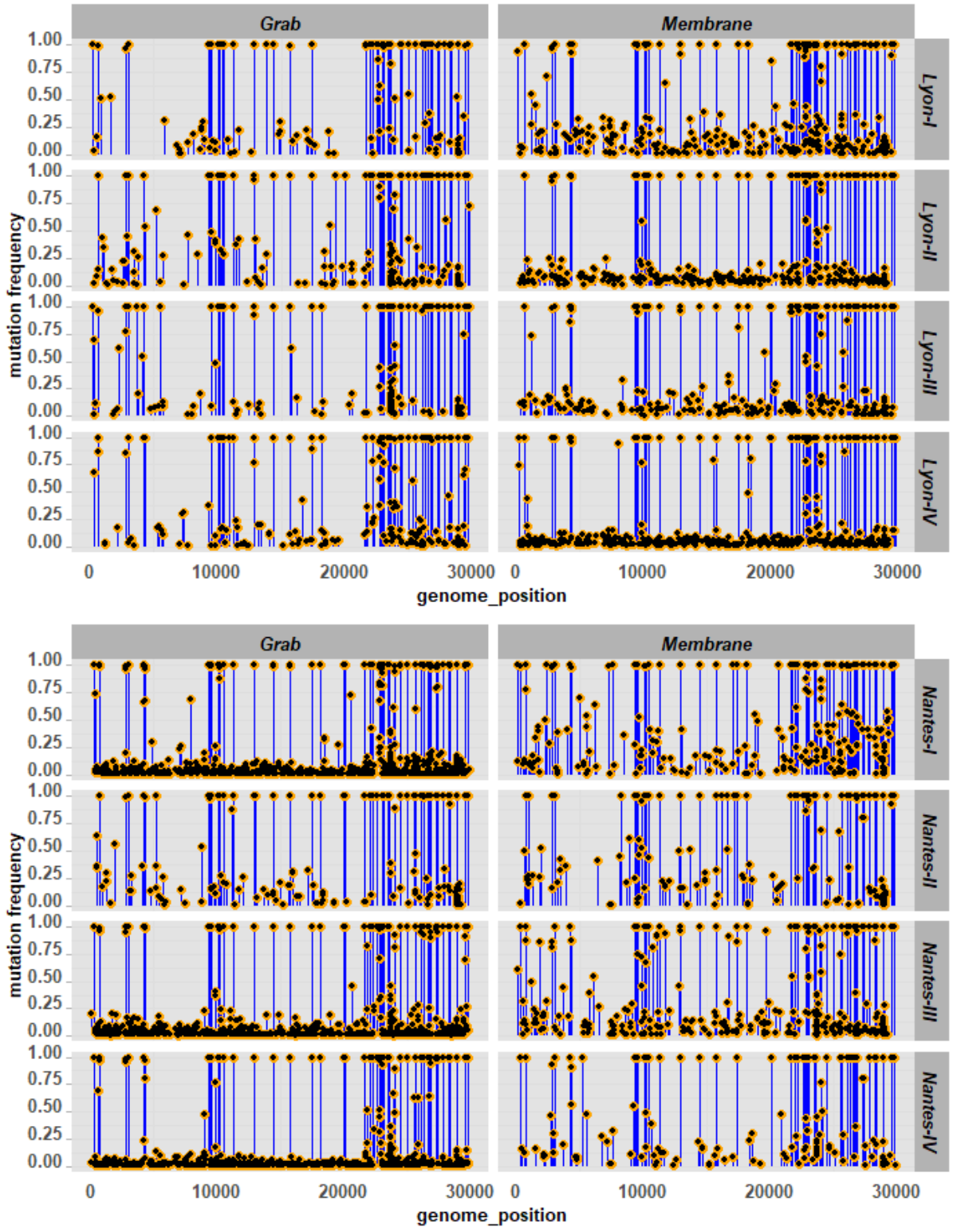

Figure S2: Heatmap of the frequencies of mutations detected in the S gene

The x-axis shows that the signature mutations of Omicron BA.2 and BA.4/5 are non-synonymous; the arrows indicate new mutations.

On the y-axis, a group of four lines corresponds to samples from the same site (sites I-IV) in the same city, including two series for membranes and grab samples (eg. Mb1 refers to: membrane series 1 and G1 refers to grab series 1). White indicates that no mutation was detected after applying quality filtering (frequency = 1%, depth = 30).

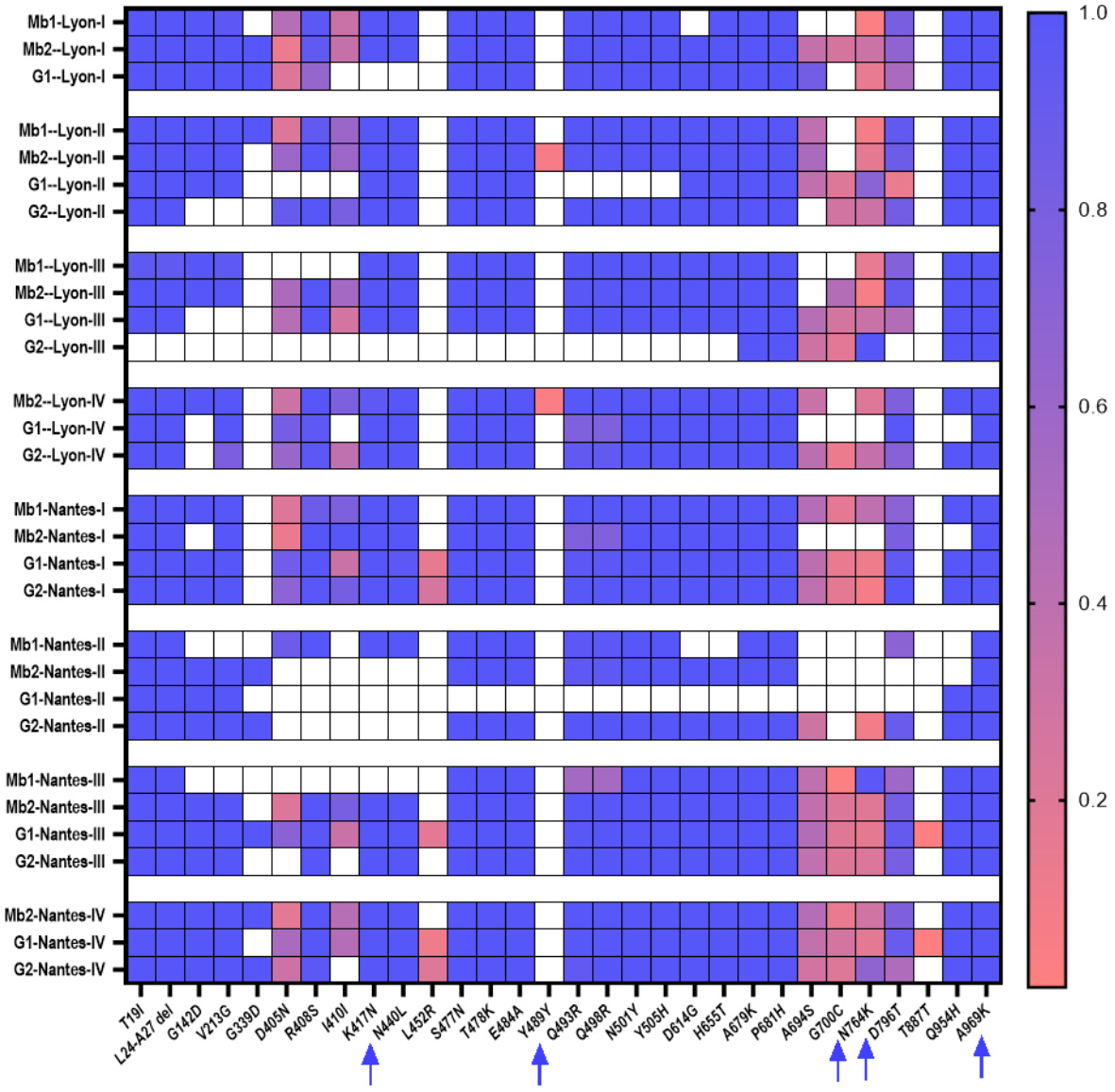

Table S2: Workflow of Environmental monitoring, quantitative RT-PCR and sequencing analysis
Assays performed for environmental monitoring of sewage, RT-qPCR analysis, storage of RNA samples and sequencing data analysis.

Assay	Description	Reference
Passive sampling device	Nylon membrane, 100 µm pore size (Ø 8.5 cm), inserted into a 3D-printed device (COSCa) designed for SARS-CoV-2 detection.	Refs. 17 & 21
Sampling design	**Membrane-based passive sampling (referenced as membrane)**: Deployment of COSCa devices for 24 h in 2 French cities (Lyon, Nantes), 4 sites per city, 2 sampling series. Transported at 4°C. **Sewage grab sampling:** Complementary to passive sampling, 1 L grab samples collected per site, transported at 4°C.	Section 2.2
Virus recovery optimisation	Comparison of methods: (i) elution with Tween 20 (Tris-HCl buffer) ± sonication (3 × 1 min) + direct lysis (bioMérieux), (ii) direct lysis (bioMérieux). Three independent experiments, 2 biological replicates each.	Section 2.3
Nucleic acid extraction from passive and grab samples	**Membrane samples:** Direct lysis using NucliSENS kit (bioMérieux). Grab samples: concentration of virus using ultracentrifugation + Nuclisens (bioMérieux). Mengovirus (MgV) spiked as process control. Treated with inhibitor removal kit (Zymo). Eluted in 100 µl nuclease-free water.	bioMérieux; Zymo; Qiagen; refs. 21, 35
Virus process control	Mengovirus (murine picornavirus) used as extraction control and PCR inhibition assessment as recommended in the ISO 15216-1.	Section 2.4; ref. 45

RT-qPCR assay	Detection of SARS-CoV-2 (IP4, polymerase gene) and NoV GII. UltraSense One-Step qRT-PCR kit (Invitrogen). Triplicates, negative amplification control included. Quantification using standard curves (CNR-Pasteur RNA transcript for SARS-CoV-2, synthetic DNA fragment for NoV GII.4).	refs. 36–38
RNA storage	Frozen at −80°C immediately after RT-qPCR and until ARTIC PCR within 6 months.	
Sequencing assay	cDNA synthesized with SuperScript IV. ARTIC PCR for amplicons sequencing V4.1 (98 amplicons, ~400 bp). Libraries prepared with NEBNext Ultra II (Illumina). Sequencing on MiSeq (2×250 cycles, 15% PhiX).	ARTIC v4.1, ref. 39
Sequence analysis	ASPICov pipeline for alignment, coverage, and variant calling. S gene mutation analysis (≥1% frequency). Aligment to SARS-CoV-2 reference (NM908947.2). VaRaPS and VirPool for deconvolution of lineages in mixed samples.	ASPICov v1.1.7, ref. 40; VaRaPS, ref. 41; VirPool, ref. 42
Statistical analysis	Comparison of recovery methods with Student's t test, Spearman correlation for grab vs membrane, ANOVA for Ct vs coverage at 30X and for lineage diversity. RStudio (4.2.2) and GraphPad Prism v10.	Section 2.8