Supplementary Materials for

CCritical Evaluation of Different Passive Sampler Materials and Approaches for the Recovery of SARS-CoV-2, Faecal-Indicator Viruses and Bacteria from Wastewater

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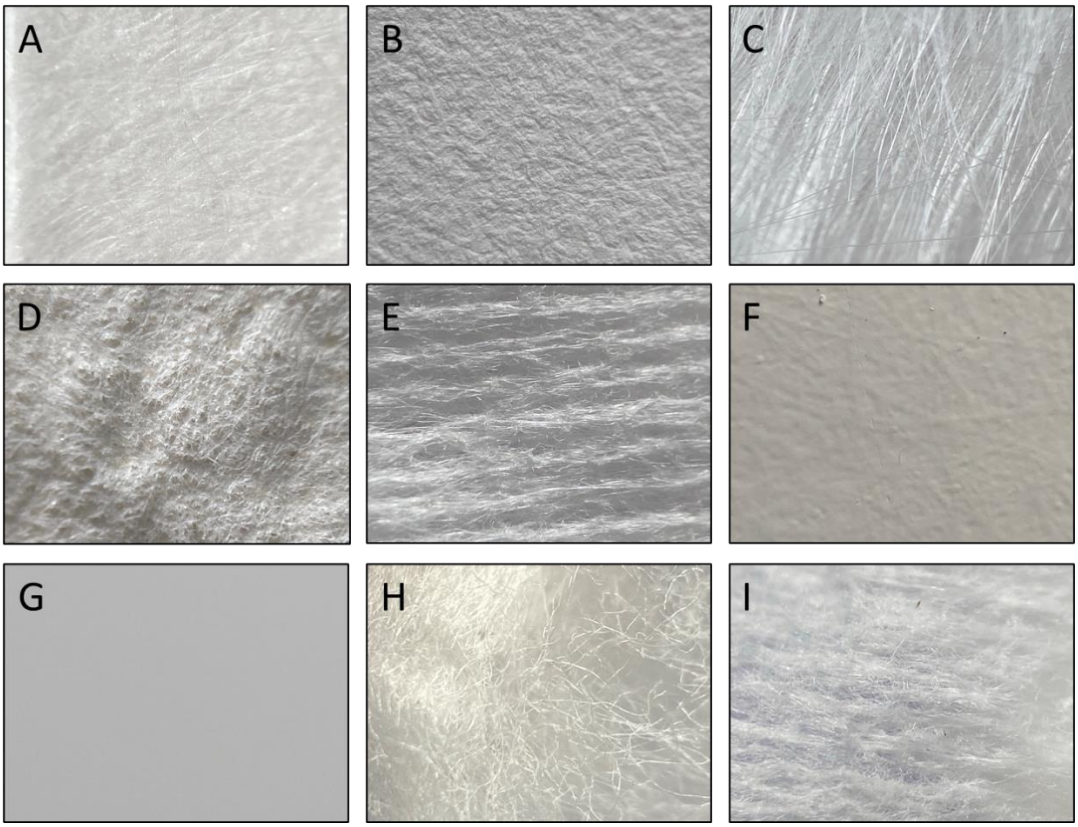


Fig. S1. Photographs of the surface structure of the different sorbent materials used in the trial. A, NanoCeram®; B, SG81 Whatman; C, GW-40 glass wool; D, ZetaPlus® 3M; E, Cotton (Moore) swab; F, Biodyne® nylon membrane; G, Cellulose acetate 11106 Sartorius; H, Inner tampon material; I, Outer tampon material. Magnification x5.

Fig. S2. Map showing the location of the Bangor city urban wastewater treatment plant used in the study and an aerial photograph illustrating its conformation. The site is operated by Dŵr Cymru-Welsh Water.

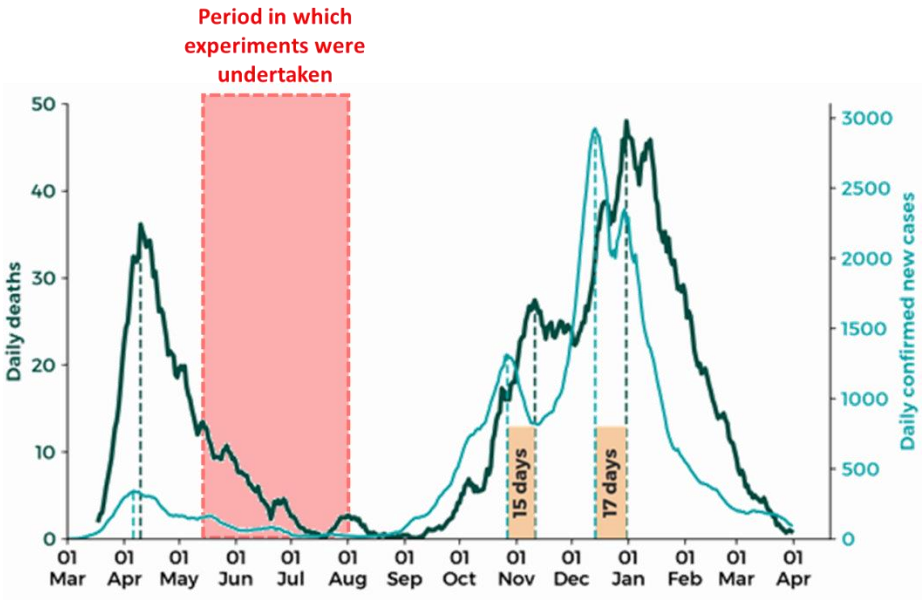


Fig. S3. Number of deaths suspected to be caused by COVID-19 in people who have tested positive in Wales (dark-green curve shows a 7-day rolling average). For comparison, the light-blue curve shows a 7-day rolling average of the daily number of new confirmed cases of COVID-19. Source: Welsh Government. The red area indicates the period in which the field experiments were performed.

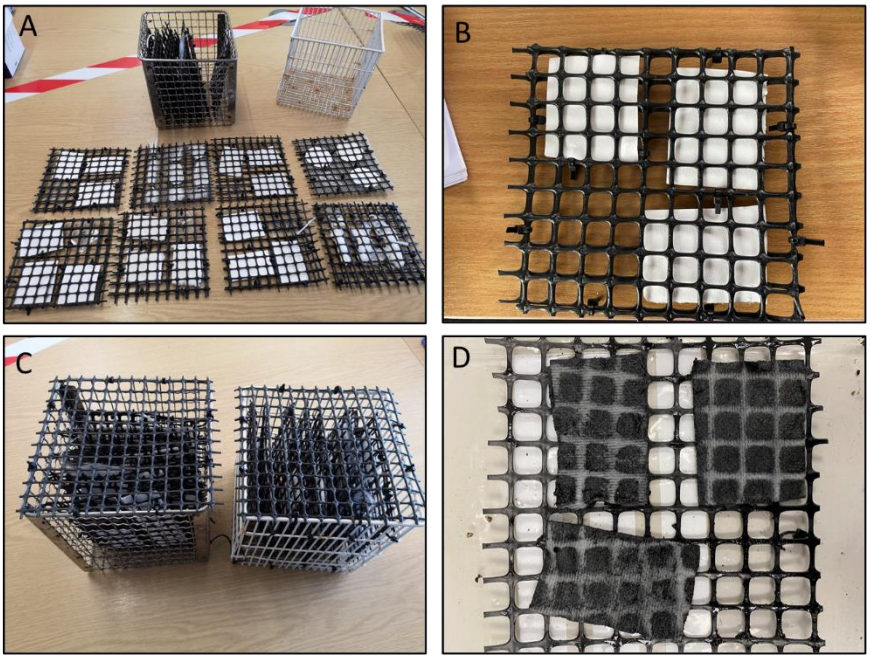


Fig. S4. Photographs of the cassettes used for passive sampler deployment in wastewater. Panel A shows the individual mesh cassettes containing three individual replicate pieces of passive sampler material. Panel B shows an individual cassette. Panel C shows a rack of cassettes in a sample holding cage. Panel D shows the material after deployment in the wastewater stream for 24 h (note the change in colour from white to black).

Fig. S5. Photographs showing (Panel A) the cotton (Moore) swab sampler, (Panel B) the tampon sampler, (C) deployment of the tampons in within the open wastewater channel, and (D) retrieval of a sampler after 24 h within the channel.

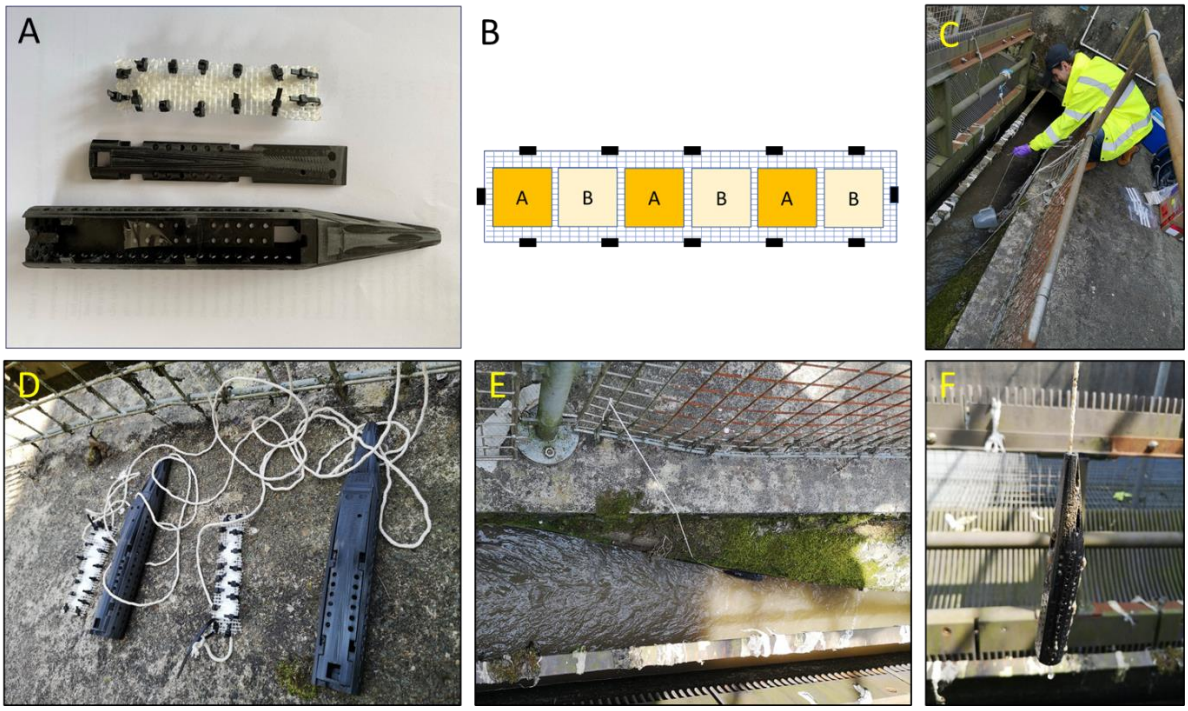
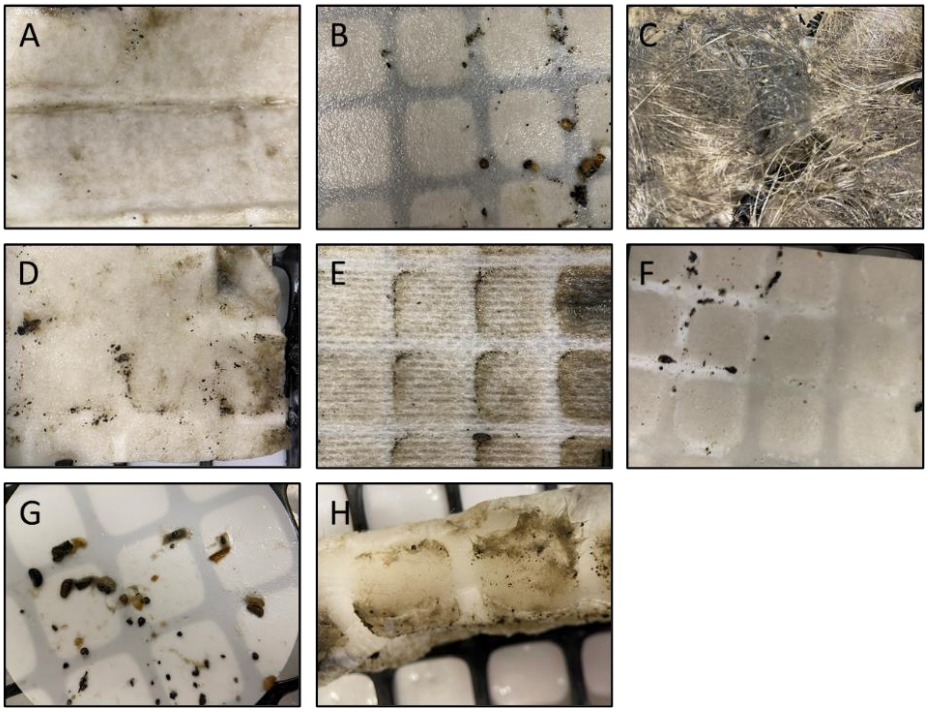


Fig. S6. Photographs showing (Panel A) the Monash torpedo sample housing, its lid and the while membrane cassette that goes inside the sampler, (Panel B) the configuration of the membrane cassette containing replicate 1 cm^2 samples of two different membrane types, (Panel C) wastewater chemistry sampling, (Panel D), replicate Monash torpedo devices ready for deployment alongside two non-housed membrane cassettes, (Panel E) the Monash torpedo device in the wastewater stream, and (Panel F) the recovery of the Monash torpedo and surface contamination after deployment in the non-screened sewer for 24 h.

1-hour deployment period



24-hour deployment period

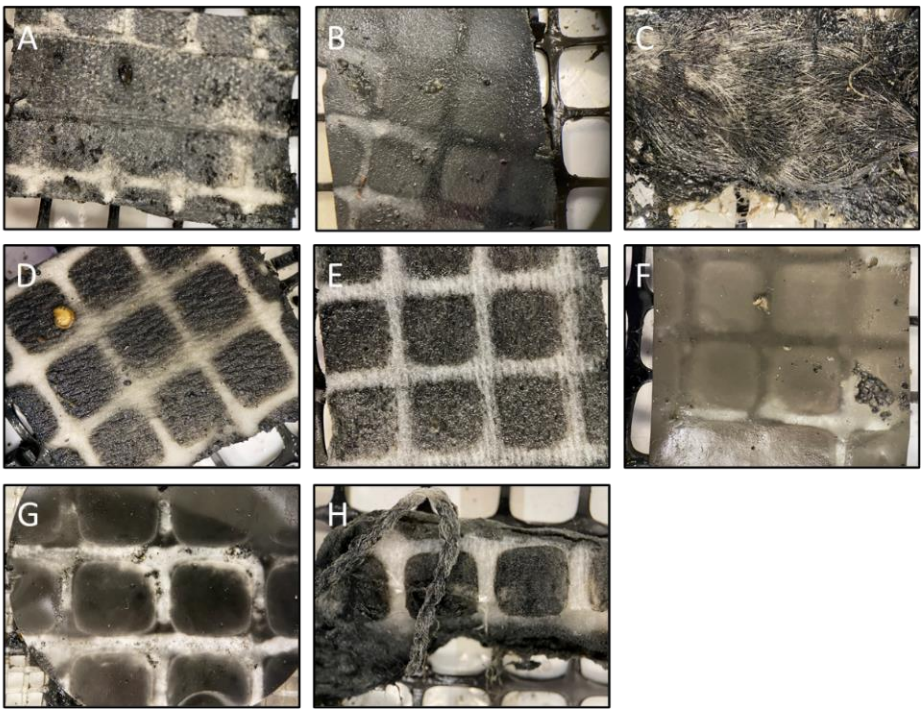


Fig. S7. Photographs of the 8 different passive sampler materials after deployment in the wastewater stream at a centralised wastewater treatment plant for either 1 h (top panel A-H) or 24 h (lower panel A-H). Note the accumulation of fine particles in some passive samplers (e.g. A, C, E, F, H) and the accumulation of larger particles in others (e.g. B, D, F, G). The alphabetical key to the passive sampler materials is presented in Table 1.

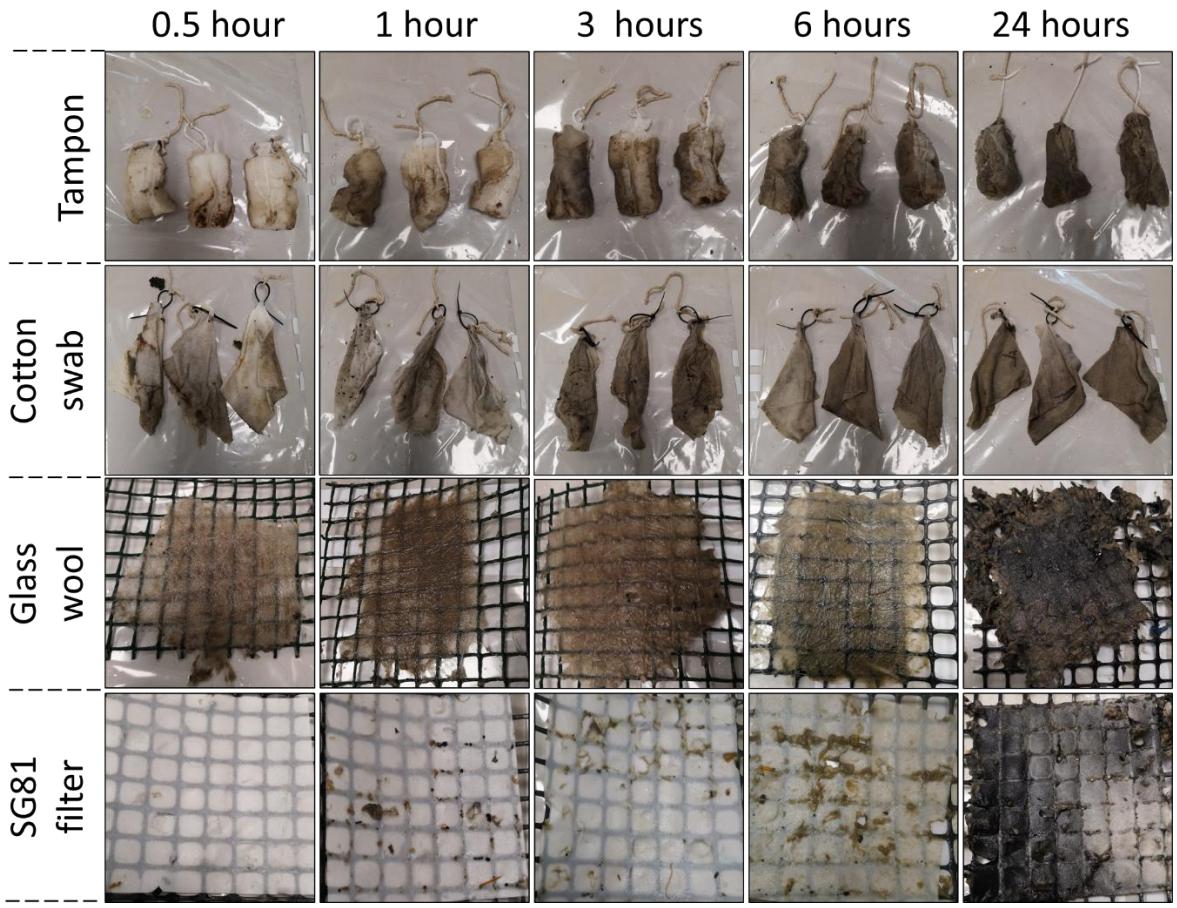


Fig. S8. Photographs showing the influence of deployment time (0.5 to 24 h) on the accumulation of particulate matter by 4 different passive sampler materials (cotton tampon, cotton swab, GW40 glass wool, SG81 Whatman filter) at a centralised wastewater treatment plant.

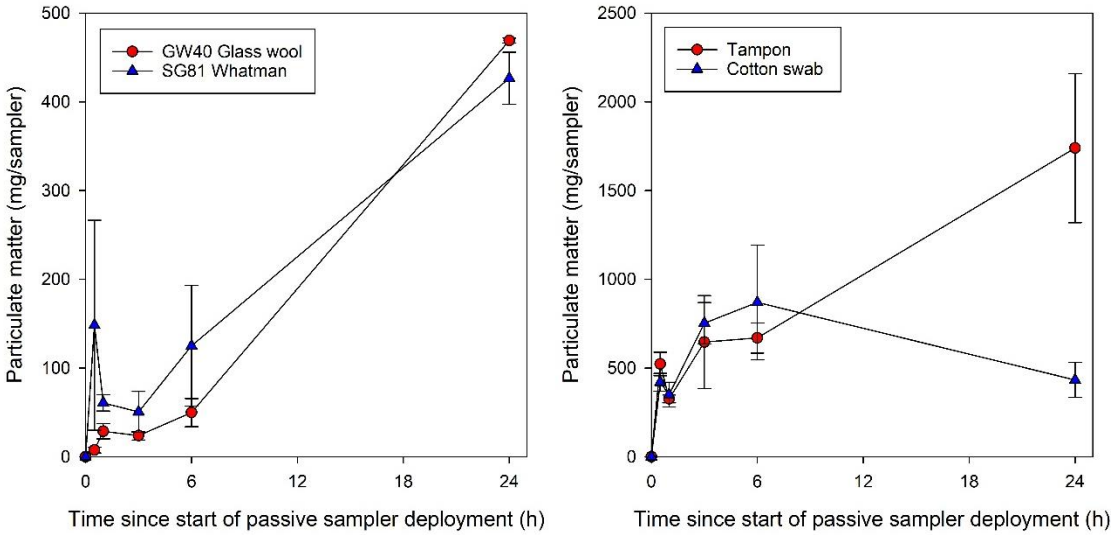


Fig. S9. Influence of deployment time (0.5 to 24 h) on the accumulation of particulate matter by 4 different passive sampler materials (tampon, cotton swab, GW40 glass wool, SG81 Whatman filter) at a centralised wastewater treatment plant. Values are expressed on a dry weight basis and represent means ± SEM (n = 3). The two graph panels represent different experimental periods and therefore cannot be directly compared.

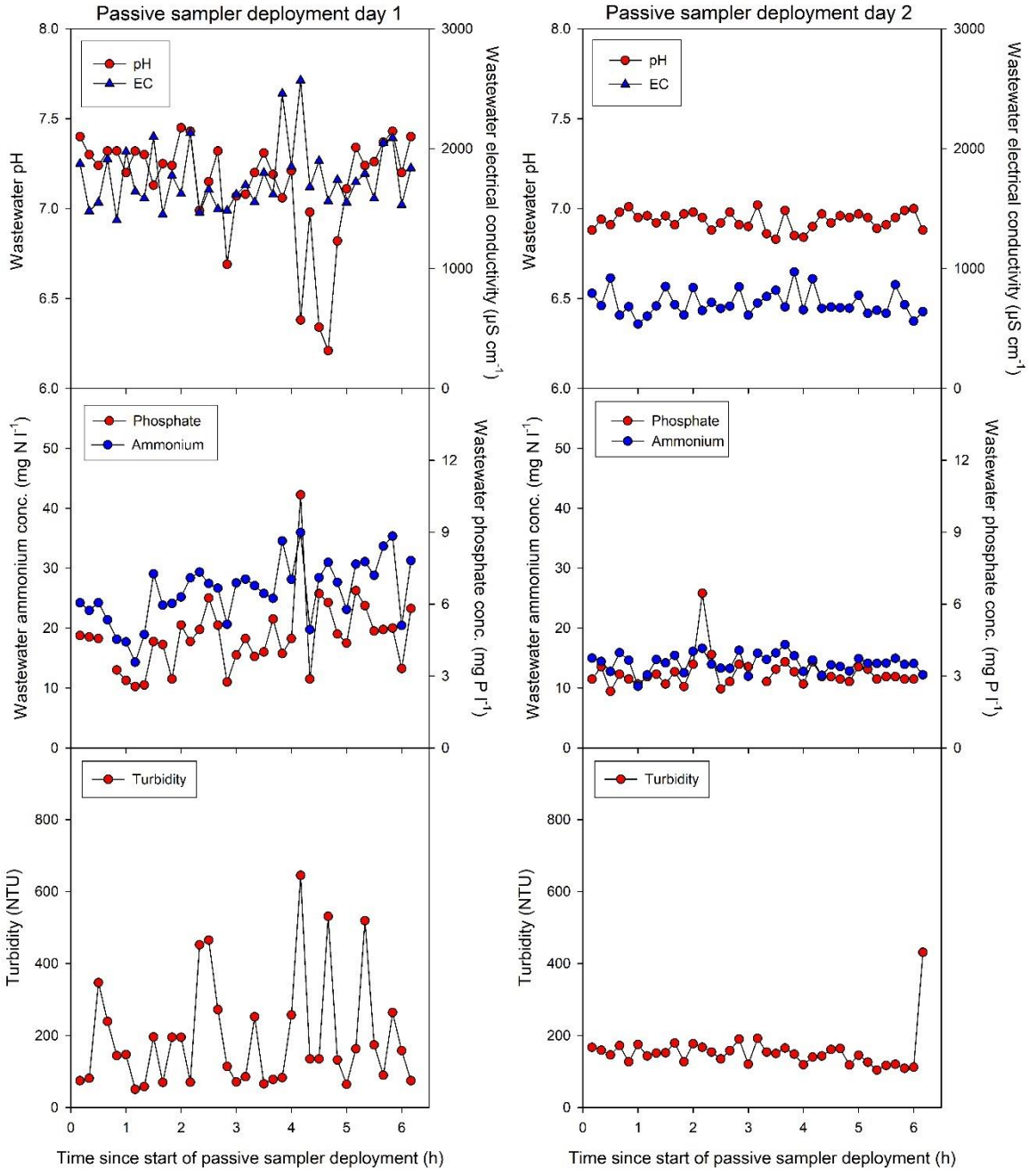


Fig. S10. Changes in wastewater chemistry and suspended solid load over the first 6 h of the passive sampler deployment over two sampling days. The tampon and cotton (Moore) swab were tested on deployment day 1 and the GW40 glass wool and SG81 Whatman filter deployed on day 2. Values represent single samples taken every 10 mins.

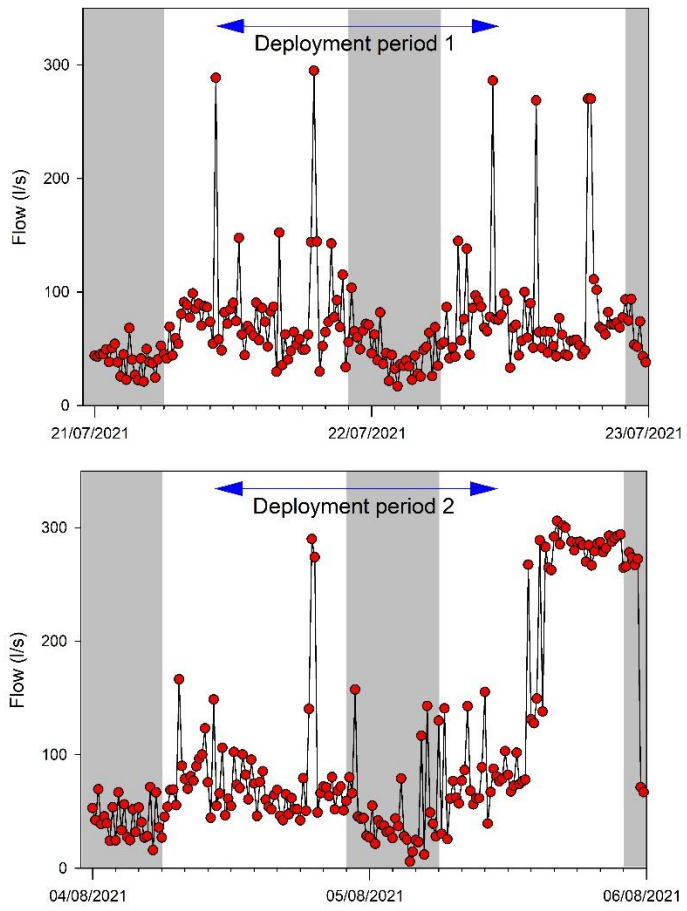


Fig. S11. Changes in wastewater flow during the time when the passive samplers were deployed over two sampling days. The tampon and cotton (Moore) swab were tested on deployment day 1 and the GW40 glass wool and SG81 Whatman filter deployed on day 2. Values represent single measurements taken every 15 mins. The dark periods represent the night-time period.

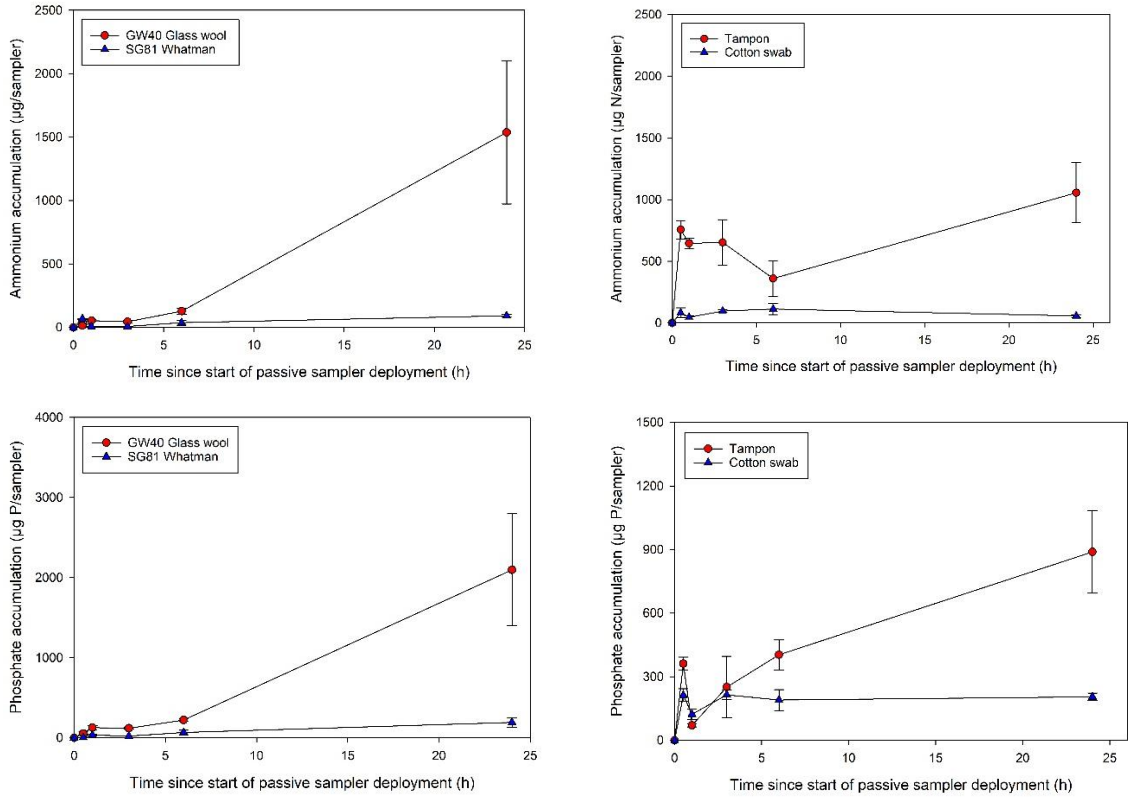


Fig. S12. Accumulation of ammonium (upper panels) and phosphate (lower panels) over time (0- 24 h) in different passive sampler materials deployed wastewater over two sampling days. The tampon and cotton (Moore) swab were tested on deployment day 1 and the GW40 glass wool and SG81 Whatman filter deployed on day 2. Values represent single samples taken every 10 mins. Values represent means ± SEM (n = 3). The two graph panels represent different experimental days and therefore cannot be directly compared.

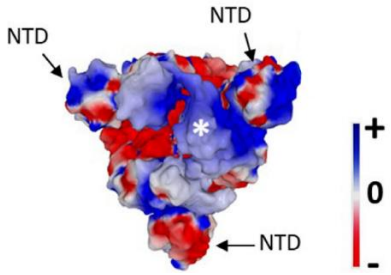


Fig. S13. Topology of the SARS-CoV-2 spike protein trimer and its electrostatic surface potential domains with the protein [64].

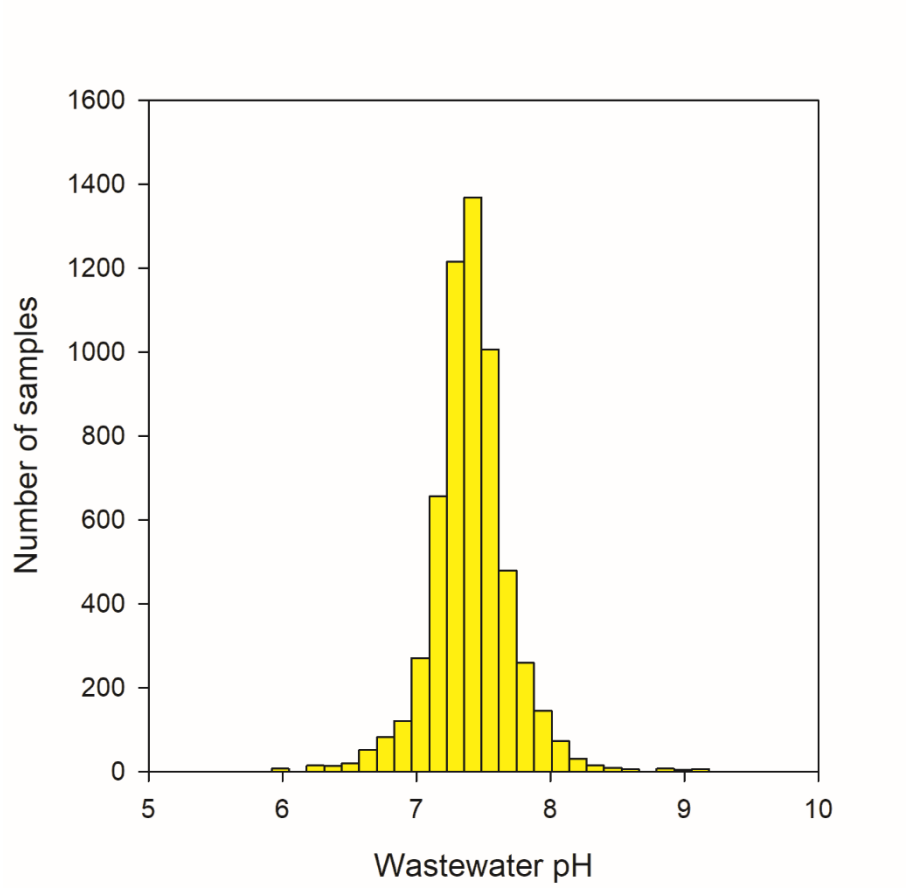


Fig. S14. Histogram of pH values in wastewater samples collected as part of the Welsh national wastewater surveillance programme between 2021-2022 across 47 individual sites (n = 5895). The mean value is 7.40, the standard deviation is 0.31, and the median value is 7.39.

Table S1. (RT-)qPCR primers and probes and standard curve quality used in this study.

Primer/Probe	Sequence (5'-3')	Reference	Standard curve		
			Slope	R^2	Efficiency %
SARS-CoV-2 (N1) Forward primer	GACCCCAAAATCAGCGAAAT	[50]	-3.500 – -3.136	0.976 – 0.998	93.052 – 108.390
SARS-CoV-2 (N1) Reverse primer	TCTGGTTACTGCCAGTTGAATCTG				
SARS-CoV-2 (N1) Probe*	[FAM]ACCCCGCATTACGTTTGGTGGACC[MGB]				
Phi6 Forward primer	TGGCGGCGGTCAAGAGC	[51]	-3.528 – -3.107	0.965 – 0.999	92.071 – 109.898
Phi6 Reverse primer	GGATGATTCTCCAGAAGCTGCTG				
Phi6 Probe*	[ABY]CGGTCGTCGCAGGTCTGACACTCGC[QSY]				
CrAssphage_Q56 Forward primer	CAGAAGTACAAACTCCTAAAAAACGTAGAG	[91]	-3.552 – -3.181	0.994 – 0.998	94.458 – 106.246
CrAssphage_Q56 Reverse primer	GATGACCAATAAACAAGCCATTAGC				
CrAssphage_Q56 Probe	[FAM]AATAACGATTTACGTGATGTAAC[TAMRA]				

*Quencher was modified to be compatible with the QuantStudio environment.

Table S2. Influence of wastewater exposure time on the amount of suspended solids trapped by two types of cotton-based passive sampler material either contained within or outside of the Monash torpedo. Suspended solids represent the dry weight of particles trapped by the sampler while turbidity represents the optical density of the particles removed from the membrane in a wash solution. Values represent means ± SEM (n = 3).

	Exposure time (h)	**Suspended solids (g m^{-2})**		**Turbidity (NTU)**	
		Tampon	**Swab**	**Tampon**	**Swab**
Torpedo	**0**	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	1	3.3 ± 1.8	4.0 ± 1.2	50 ± 14	44 ± 8
	6	13.0 ± 1.2	29.3 ± 13.4	347 ± 49	462 ± 78
Outside	**0**	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	1	8.0 ± 4.7	14.0 ± 2.1	127 ± 49	232 ± 31
	6	No sample		No sample	