Review of: "No evidence for environmental transmission risk of SARS-CoV-2 in the UK’s largest urban river system: London as a case study"

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This study investigated whether both SARS-CoV-2 RNA and/or infectious particles can be detected from both running and standing surface waters as well as sediments in the Thames catchment, including the river itself and the Hampstead Heath bathing ponds. Bivalve samples collected adjacent to major combined sewer overflows (CSOs) were also tested. The authors also compared these surveys to others conducted on the Sava and Danube rivers in Serbia, which receive large quantities of raw sewage from the Serbian capital of Belgrade. The authors indicate two objectives of the study: (i) to test a novel methodology for concentrating and detecting the RNA and infectious particles of enveloped RNA viral pathogens from high volume water samples and (ii) to evaluate whether London waterways are viable conduits for SARS-CoV-2 transmission.
In my view, this study was well conducted, the methodology is well described, and important results are reported and discussed in depth and compared with results from other relevant articles in the area. However, I will raise some issues that I think are important and could be improved or further explored in this study (or other studies), and these issues are related to what was addressed in a previous review article (https://doi.org/10.1016/j.scitotenv.2021.145721).

1. During the procedure of concentration of water samples from the River Thames, an initial volume of 10L was used and a final volume of 2 mL was reached, which was submitted to SARS-CoV-2 RNA detection and infectivity assays. This procedure was validated using the MHV as surrogate, in three replicates, obtaining recovery rates of 160x, 6.32x and 0.40x, in replicates 1, 2 and 3, respectively (see Table S2). This is a very large variability and raises doubts as to whether the sample concentration procedure should be used or not, as the concentration of viral particles in the sample can increase by up to 160x, but it can also be reduced to 0.40x.

2. SARS-CoV-2 virion is a small spherical particle with a diameter of about 100-120 nm (https://doi.org/10.1016/j.watres.2020.115899), but which can reach up to 200 nm in diameter (https://doi.org/10.1016/S0140-6736(20)30211-7). Taking this into account, a significant fraction of viral particles can be retained on the filter during the sample pre-filtration step. In addition, viral particles can adsorb on suspended solids present in the sample and be improperly removed from the sample (https://doi.org/10.1021/acs.est.6b00876).

3. It is well known that filtration through 0.22 or 0.45 μm pore size filters is a routine practice for virus analysis in samples of an environmental nature (water, wastewater), but this is a practice developed for non-enveloped viruses and the shear

stress imposed during the filtering step can break the capsid of enveloped viruses, making them non-infectious. In tighter filters, higher shear stress is expected.

4. I think that specific methodologies for analyzing the infectivity of enveloped viruses in sewage and water samples, mainly for the pretreatment and concentration stages, should be developed in future works. Some suggestions can be found in this reference: https://doi.org/10.1016/j.scitotenv.2021.145721.

5. Line 17: "The presence of SARS-CoV-2 in untreated sewage …". "SARS-CoV-2 RNA" is more accurate than "SARS-CoV-2" and the difference is very relevant in the manuscript context. This issue should be revised in all the manuscript for similar sentences. Similarly, in lines 41, 203, 216, 219, 321, …

6. Micromolar (µM) is being used to describe the pore size of the filter, which must be in micrometer (µm). This should be revised in the manuscript, lines 182, 236, 256 … and in the supplementary material.