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Presence and persistence of SARS-CoV-2 in aquatic environments: A mini-review
Jürgen Mahlknecht

Abstract
The introduction of SARS-CoV-2 into water bodies via sewage raises public health concerns. For the assessment of public health risks, it is necessary to know the presence and persistence of infectious SARS-CoV-2 in water and wastewater. The present mini-review documents the occurrence and decay rates of viable infectious SARS-CoV-2 and SARS-CoV-2 RNA in different water matrices including wastewater, river water, groundwater, tap water, and seawater. Persistence of viable SARS-CoV-2 is mainly temperature dependent. A rapid inactivation of infectious SARS-CoV-2 is found in river water, sea water, and wastewater compared to tap water. SARS-CoV-2 RNA was found to be considerably more stable than infectious SARS-CoV-2, indicating that the environmental detection of RNA alone does not prove risk of infection. Persistence assays need to consider physicochemical and biological water composition as well as the effect of detergents, enzymes, and filtering particulate matter.

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Introduction
SARS-CoV-2, a positive-strand RNA coronavirus responsible for the respiratory disease COVID-19 has a low mortality rate of 1.2% compared to other coronavirus (SARS-CoV and MERS-CoV) but is highly contagious due to the spread via droplets (from respiration), direct contact, and fomites [1]. Viable virus particles have been detected in feces and to a lesser extent in urine of infected persons [2,3], as well as viral RNA has been identified early in untreated wastewater, for example, the studies by Ahmed et al., Medema et al., Randazzo et al., and La Rosa et al. [4–7], suggesting that other routes of infection may exist. These findings have raised concerns about global health, especially about the possibility that SARS-CoV-2 passes from wastewater treatment plants (WWTPs) to the receiving environment, affecting downstream human activities such as agriculture, recreation, and public health in general [7,8].

This has led to the extensive use of wastewater surveillance for containing and mitigating outbreaks [9,10]. Wastewater-based epidemiology has been used to predict community outbreaks in urban centers, universities, hospitals, and airplanes [11]. While wastewater surveillance has been impactful, it is relevant to know the presence and persistence of infectious SARS-CoV-2 in water and wastewater for the assessment of public health risks, that is, for describing the exposure risks associated with waters and wastewater to the public. Besides, water bodies may act as natural reservoirs or repositories for the virus [8,12,13].

Due to safety challenges in performing laboratory work with highly infectious human coronaviruses, researchers have preferred to evaluate closely related viral surrogates to assess the persistence of SARS-CoV-2 in water [14]. However, the suitability of enveloped virus surrogates varies depending upon the environmental conditions, which could lead to obvious wrong estimates. Therefore, researchers started to perform confirmatory experiments in high biological safety level laboratories analyzing spiked viable SARS-CoV-2 or viral strains in different water matrices [9,15].

The objective of this mini-review is to compile and update quantitative information on the presence and stability of SARS-CoV-2 in different water matrices based on experiments using viable virus or its genetical strains. Further, equations for calculating decay rates and corresponding statistics are presented. This
contribution is thought to serve as reference and practical guide for future work in this field.

**Detection in aquatic systems**

Studies on aquatic environments reported a wide range of SARS-CoV-2 concentration [13] (Figure 1). In wastewater, the viral load of reported studies varied between \(1.9 \times 10^1\) and \(7.0 \times 10^6\) copies/L \([4,9,6,16–23]\), in secondary treated wastewater between \(1.6 \times 10^2\) and \(2.51 \times 10^5\) copies/L \([6,24,25]\), in tertiary treated effluent between \(1.3 \times 10^3\) and \(4.6 \times 10^5\) copies/L \([28,29]\), in river water between \(2.0 \times 10^2\) and \(3.19 \times 10^6\) copies/L \([16,22,25,30–32]\), and in groundwater between \(2.6 \times 10^3\) and \(3.8 \times 10^5\) copies/L \([8]\). As expected, primary sludge has in general higher viral loads than wastewater, because it contains a broad diversity of human viruses, including commonly circulating coronavirus strains. On the other hand, it is notable that the tertiary effluents and influents have almost similar data trends.

**Estimation of decay rates and statistics**

The decay rates typically are obtained with log—linear Eq. (1). For determination of the decay rate constants of the first-order kinetics, the virus titer, and corresponding time points are used together. The \(T_{90}\) value (time required to reach a 90% reduction) is calculated using Eq. (2).

\[
\ln \left( \frac{C_t}{C_0} \right) = -k \cdot t
\]

\[
T_{90} = \frac{-\ln(0.1)}{k}
\]

where, \(k\) is the constant first-order decay rate, \(C_0\) and \(C_t\) are the corresponding initial viral loads and viral loads at time \(t\) of the assays. A linear regression analysis may be used to calculate the associated 95% confidence intervals of the decay rate constants. Reported errors and model fit values are usually reported as \(R^2\) and/or root-mean-square error (RMSE). As alternative to the log-linear model, nonlinear models may also be applied to describe the decay, including exponential-nonlinear least square, exponential biphasic, and Weibull models \([33]\).

To select the best model, all models may be fit to the observed data and compared using an extra sum-of-squares F test. In most cases, the log-linear model may fit best. The \(k\) values may be \(\log_{10}\)-transformed and linear regression used to characterize the relationship between temperature and decay rate constant within each matrix. The fit of the regression is assessed using \(R^2\) values and RMSE. All plotting, regressions, and statistical analyses can be performed in scientific graphing and statistics programs, for example, open-source Rstudio (stats package) and commercial GraphPad PRISM.

**Persistence and decay in different water matrices**

Data from previous studies suggest that the coronavirus seems to have a low persistence in the environment and is very subtle to oxidants, such as chlorine; it also demonstrates that its inactivation is significantly quicker in water compared to non-enveloped human enteric viruses with known waterborne transmission \([7,10,34]\). Like with SARS-CoV, genetic material of the SARS-CoV-2 virus was found in wastewater, whose decay is altered by various factors, including virus physiology, time outside the host, wastewater composition, temperature, and pH \([35]\). In addition, it is known that enveloped viruses exhibit shorter lifespans when outside the host \([36]\). Finally, the effect of proteolytic enzymes and detergents on the external lipid envelope of the virus may impose a shorter survival time for enveloped viruses \([12,35]\).

Several researchers have investigated the persistence in different water matrices using viable SARS-CoV-2 and SARS-COV-2 RNA (Table 1). For security reasons, most
| Article | Virus                  | Matrix                        | Temperature (°C) | Best fit model | k (d⁻¹) | R² of k | RMSE  | T₉₀ (days) | Data location in the mentioned article |
|---------|------------------------|-------------------------------|-----------------|----------------|---------|---------|-------|------------|--------------------------------------|
| [13]    | SARS-CoV-2 RNA         | Raw wastewater                | 4               | First order    | −0.13   | 0.74    | –     | 17.17      | Table 2                              |
|         | Raw wastewater         | 26                            |                 | First order    | −0.27   | 0.99    | –     | 7.68       | Table 2                              |
| [36]    | SARS-CoV-2             | River water (filter sterilized)| 4               | First order    | 0.61    | 0.93    | 0.57  | 3.8         | Table 2                              |
|         | River water (filter sterilized) | 20                           | First order    | 1.01           | 0.92    | 0.66    | 2.3   | Table 2                              |
|         | Seawater (filter sterilized) | 4                            | First order    | 1.07           | 0.91    | 0.73    | 2.2   | Table 2                              |
|         | Seawater (filter sterilized) | 20                           | First order    | 2.02           | 0.99    | 0.28    | 1.1   | Table 2                              |
|         | SARS-CoV-2 RNA         | River water (filter sterilized) | 20              | First order    | 0.14    | 0.80    | 0.3   | 16.6        | Tables 4 and 5                        |
|         | Seawater (non-filter sterilized) | 20                           | First order    | 0.26           | 0.46    | 1.08    | 8.9   | Tables 4 and 5                        |
| [33]    | SARS-CoV-2             | River water                   | 24              | First order/Weibull | −0.37   | 0.65    | 0.049 | 1.9         | Table 4                              |
|         | River water            | 4                             | First order/Weibull | −0.16         | 0.76    | 0.064  | 7.7   | Table 4                              |
|         | River water (filtered) | 24                            | First order/Weibull | −0.32         | 0.82    | 0.067  | 3.3   | Table 4                              |
|         | Wastewater             | 24                            | First order/Weibull | −0.83         | 0.79    | 0.022  | 1.2   | Table 4                              |
|         | Wastewater             | 4                             | First order/Weibull | −0.19         | 0.75    | 0.062  | 5.5   | Table 4                              |
|         | Wastewater (filtered)  | 24                            | First order/Weibull | −0.80         | 0.80    | 0.052  | 1.5   | Table 4                              |
| [9]     | SARS-CoV-2 RNA         | Raw wastewater                | 37              | First order    | 0.29    | 0.74    | 1.10  | 8.04        | Table 3                              |
|         | Raw wastewater         | 25                            | First order    | 0.18           | 0.87    | 0.67   | 12.6  | Table 3                              |
|         | Raw wastewater         | 15                            | First order    | 0.11           | 0.71    | 0.59   | 20.4  | Table 3                              |
|         | Raw wastewater         | 4                             | First order    | 0.08           | 0.79    | 0.37   | 27.8  | Table 3                              |
|         | Autoclaved wastewater  | 37                            | First order    | 0.41           | 0.94    | 0.59   | 5.71  | Table 3                              |
|         | Autoclaved wastewater  | 25                            | First order    | 0.17           | 0.93    | 0.48   | 13.5  | Table 3                              |
|         | Autoclaved wastewater  | 15                            | First order    | 0.08           | 0.85    | 0.32   | 29.9  | Table 3                              |
|         | Autoclaved wastewater  | 4                             | First order    | 0.05           | 0.95    | 0.14   | 43.2  | Table 3                              |
|         | Tap water              | 37                            | First order    | 0.04           | 0.88    | 0.86   | 9.4   | Table 3                              |
|         | Tap water              | 25                            | First order    | 0.05           | 0.78    | 0.68   | 15.2  | Table 3                              |
|         | Tap water              | 15                            | First order    | 0.15           | 0.28    | 0.33   | 51.2  | Table 3                              |
|         | Tap water              | 4                             | First order    | 0.25           | 0.83    | 0.17   | 58.6  | Table 3                              |
| [15]    | SARS-CoV-2             | Wastewater (low titer)        | 20              | First order    | 1.4     | 0.71    | 1.8   | 1.6         | Table 1                              |
|         | Wastewater (high titer)| 20                            | First order    | 1.1            | 0.54    | 1.2    | 2.1   | Table 1                              |
|         | Tap water (high titer) | 20                            | First order    | 1.2            | 0.88    | 1.2    | 1.7   | Table 1                              |
|         | Wastewater             | 50                            | First order    | 0.15 min⁻¹     | 0.88    | 1.4    | 15 min | Table 1                              |
|         | Wastewater             | 70                            | First order    | 1.0 min⁻¹      | 0.88    | 1.9    | 2.2 min | Table 1                              |
studies have been carried out using RNA rather than isolated infectious virions in water matrices. In general, it can be anticipated that SARS-CoV-2 RNA is considerably more persistent than infectious SARS-CoV-2, implying that the environmental detection of RNA alone does not validate risk of infection [15,36].

**Wastewaters**

As expected from other human coronaviruses, the persistence and decay of SARS-CoV-2 in municipal wastewater are strongly dependent on the environmental temperature [37,38]. At high ambient temperatures (37°C) the decay rates are higher than at low temperatures (4°C). Also, there exists a faster degradation of low titer viruses in untreated wastewater than in sewage spiked with high titer exogenous viruses due to the incomplete viral structure in the wastewater that makes viral RNA easier to degrade [9]. Wurtzer et al. [39] detected that SARS-CoV-2 RNA could persist in wastewater for 19 days after the last confirmed case of infection. The reason might be that SARS-CoV-2 RNA may be shed by possible asymptomatic infected individuals for a long time, rather than the persistence of the virus in the wastewater [13]. Bivins et al. [15] reported that the T90 of viable SARS-CoV-2 in wastewater at room temperature was 1.6–2.1 days (Table 1). However, the virus remained infectious for 7 days during high-titer experiments and for three days at the low-titer experiments.

Extended SARS-CoV-2 survival times were experimented in filtered samples compared to unfiltered samples [33]. They obtained a T90 of 1.5 and 1.2 days, respectively, for viable SARS-CoV-2 in filtered and unfiltered wastewater at 24°C. Ahmed et al. [9] determined that the average T90 for SARS-CoV-2 RNA ranged from 8.0 (37°C) to 27.8 days (4°C) and from 5.7 (37°C) to 43.2 days (4°C) in untreated and autoclaved wastewater, respectively. Yang et al. [13] obtained a slightly differing result, demonstrating that the T90 value of SARS-CoV-2 RNA in raw wastewater was between 7.7 (26°C) and 17.2 days (4°C). Regarding the different treatment steps in a WWTP, the two main mechanisms that control the attenuation/decay of human coronavirus in wastewater are inactivation and viral adsorption [35]. Finally, SARS-CoV-2 viral RNA is appreciably less persistent compared to other microbiological contaminants, such as E. coli [12].

**River water**

SARS-CoV-2 decay in river water is less sensitive to temperature than wastewater, and the SARS-CoV-2 survival times are longer in river water than in wastewater. A 90% reduction of viable SARS-CoV-2 in river water has been reported ranging from 1.9 (24°C) to 7.7 days (4°C) [33] (Table 1). Also, dissolved solids and pH may alter SARS-CoV-2 persistence. For example, the lower pH of river water may stimulate higher electrostatic interactions and viral adsorption to the solids, which presents a more mineral composition (lower volatile solids/total solids ratio) compared to the solids present in wastewater samples (higher volatile solids/total solids ratio) [33]. This agrees with previous literature [40], in which a faster virus inactivation was observed in complex rather than in simpler matrices.

According to Scheller et al. [41], pH and organic/inorganic solids may play important roles in the formation of pH-dependent electrically charged surfaces by producing significant alterations in the virus structure proteins due to changes in its isoelectric point. Likewise with wastewater, lengthier SARS-CoV-2 survival times were reported in filtered samples compared to unfiltered samples [33]. A T90 value of 3.3 days for filtered river water against 1.9 days for raw river water at 24°C was observed. The faster inactivation in unfiltered compared to filtered river water samples is probably due to the presence of inorganic clays, given their highly adsorptive properties, which could potentially act as SARS-CoV-2 sink [42].

**Drinking water (tap water)**

All in all, in surface water and drinking water, the identification of genetic material was less successful compared to wastewater [12]. This was expected with drinking water, since this water was chlorinated, which completely inactivates enveloped viruses such as the SARS-CoV-2. Ahmed et al. [9] investigated SARS-CoV-2 RNA in untreated wastewater, autoclaved wastewater, and dechlorinated tap water. They reported an average T90 of 9.4 (37°C), 15.2 (25°C), 51.2 (15°C), and 58.6 days, respectively, in tap water (Table 1) and found that tap water was more stable than wastewater. Bilvins et al. [15] determined the persistence of SARS-CoV-2 in water and wastewater; they observed a T90 of viable SARS-CoV-2 in tap water at room temperature 2.0 days, being slightly higher than wastewater.

**Seawater**

The study of Lee et al. [43] reported that SARS-CoV-2 quickly lost activity as soon as it is introduced in seawater. This trend was also observed when examining viral RNA levels. In contrast, SARS-CoV-2 in filtered seawater was significantly more stable than that in unfiltered. The authors concluded that the presence of predatory microbes could inactivate viruses through protease and nuclease activity. However, even after SARS-CoV-2 particles were inactivated, viral RNA remained still detectable (up to 5 or 6 days). This was expected, because similar as in other media (sputum, nasal mucus) the nucleic acid remained after the virus was inactivated [44].

Sala-Comolera et al. [36] used filter sterilized seawater spiked with infectious SARS-CoV-2 incubated at two
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Concluding remarks

The emerging analytical methods have shown that environmental waters could contain viable SAR-CoV-2 with a relatively high level of persistence. However, no study has demonstrated or confirmed the transmission of SARS-CoV-2 via water or other environmental compartments, nor the fecal–oral transmission could be confirmed so far.

Nevertheless, the decay rate data is extremely important for the quantitative microbial risk assessment associated to exposure to SARS-CoV-2 contaminated water. Thus, there is still a need to further investigate and gather data to unravel the complete mechanism for the SARS-CoV-2, especially regarding the different pathways of transmission. This makes it imperative to use infectious SARS-CoV-2 virus rather than strains or RNA, although this would involve a high level of safety to conduct. So far, only a very limited number of studies has detected and isolated infectious virions in water matrices.

Temperature has an important effect on SARS-CoV-2 persistence in water and wastewater. Nevertheless, the understanding of other factors like water composition, the presence of oxidants, potentially competing microorganisms, pH, and detergents, as well as the impact of filtration of particulate matter needs to be advanced. In this sense, standard protocols must be defined for persistence assays.

Conventional virus detection techniques show several shortcomings, such as a highly tedious and skill-dependent virus detection. Therefore, biosensing techniques were proposed recently as alternative, including the combination of various detection techniques (optical, electrochemical, PCR), with functional nanomaterials, LAMP, and low-cost microfluidics. These techniques need further development.

Declaration of competing interest

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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