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Potential discharge, attenuation and exposure risk of SARS-CoV-2 in natural water bodies receiving treated wastewater

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Recently reported detection of SARS-CoV-2 in wastewater around the world has led to emerging concerns on potential risk in water bodies receiving treated wastewater effluent. This review aims to provide an up-to-date state of key knowledge on the impact of SARS-CoV-2 in natural water bodies receiving treated wastewater. In this review, SARS-CoV-2 concentrations in wastewater, expected removal in WWTPs, and possible dilution and decay in water bodies are reviewed based on past studies on SARS-CoV-2 and related enveloped viruses. We suggest a quantitative microbial risk assessment (QMRA) framework to estimate the potential risk of SARS-CoV-2 in natural water bodies through various water activities. Dose–response model of SARS-CoV and Poisson's distribution is employed to estimate possible viral ingestion and the annual chance of infection through several water activities in natural water bodies. Finally, future perspectives and research needs have been addressed to overcome the limitations and uncertainty in the risk assessment of SARS-CoV-2 in natural water bodies.

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INTRODUCTION

A number of studies have reported the detection of SARS-CoV-2 in wastewater in the context of wastewater-based epidemiology for early warning of COVID-19 outbreak^{1–7}. Increased concern has recently emerged, pertaining to the occurrence of SARS-CoV-2 in water environments during the current COVID-19 pandemic^{8,9}. The awareness of the potential risk of SARS-CoV-2 from wastewater has increased since RNA detection of SARS-CoV-2 in wastewater reached public domains^{3,10–12}. Recently, possible transmission of COVID-19 from sewage was reported by a cohort study in Guanzhou, China¹³. Although the occurrence of fecal–oral route transmission and infectivity of SARS-CoV-2 in wastewater is still controversial, there are growing concerns about the exposure risk of SARS-CoV-2 in natural water bodies that receive treated effluent from wastewater treatment plants (WWTPs), among citizens, administrative sectors, and policymakers. Because of the limited prior knowledge, the fate of SARS-CoV-2 from wastewater treatment to the water environment is still being scholarly speculated in a qualitative manner. For quantitative discussion on the potential risk of SARS-CoV-2 in water bodies, the following knowledge base is necessary: i) possible ranges of SARS-CoV-2 concentrations in wastewater, (ii) reduction of SARS-CoV-2 in wastewater treatment, (iii) decay of SARS-CoV-2 in ambient water bodies, and iv) dose–response model of SARS-CoV-2. While these key knowledges are not readily available or limited yet, revisiting the previous studies on viruses in water and wastewater brings valuable knowledge for quantitative discussion on the potential risk of SARS-CoV-2 in wastewater. There have been a number of studies on behaviors of the related coronaviruses and enveloped viruses, e.g., SARS-CoV, murine hepatitis virus (MHV), $\phi 6$, and nonenveloped enteric viruses in wastewater treatment and water bodies. Behaviors of these related viruses (e.g., disinfection efficiency, their stability, and decay in aerosol and on

environmental surfaces) enable to provide more probable assumptions on the fate of SARS-CoV-2 in wastewater.

This review aims to provide an up-to-date state of key knowledge to understand the potential risk of SARS-CoV-2 in natural water bodies receiving treated wastewater in water activities. For this purpose, we present recent findings on the prevalence of SARS-CoV-2 in untreated and treated wastewater reported around the globe, revisited past studies on reduction of SARS-CoV-2-related enveloped viruses and nonenveloped enteric viruses in various wastewater treatment and disinfection processes, and dilution and decay of SARS-CoV-2 and related viruses under environmental stresses in natural water bodies. Based on this knowledge, a framework of quantitative microbial risk assessment (QMRA) for SARS-CoV-2 in natural water bodies receiving treated wastewater is suggested, along with the intensive collection of quantitative data for readily calculations of potential risks of SARS-CoV-2 based on water activities (Fig. 1). The potential risk of the various water activities was estimated based on several example scenarios of exposure by using dose–response and probability models. Finally, perspectives are addressed on limitations and applicability of the suggested QMRA framework, and possible impacts of untreated wastewater discharge like combined sewer overflows on the occurrence of SARS-CoV-2 in water bodies. This study would be beneficial to understand the potential impacts of SARS-CoV-2 in wastewater on natural water bodies and to implement QMRA-based risk management during the COVID-19 pandemic.

CONCENTRATIONS OF SARS-COV-2 IN WASTEWATER

Detection of SARS-CoV-2 RNA in feces of symptomatic and asymptomatic patients of COVID-19 has directed toward the presence of SARS-CoV-2 RNA in the wastewaters^{10,14–16}. Moreover, SARS-CoV-2 RNA in feces can be detected for a longer duration

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Fig. 1 Framework of QMRA of SARS-CoV-2 in natural water bodies receiving treated wastewater.

(22 days) followed by respiratory samples (18 days) and serum samples (16 days)¹⁷. Due to such clinical observations, it became the need of time to conduct wastewater surveillance for assessing the presence of the actual number of COVID-19 patients. To date, 11 countries have reported the presence of molecular SARS-CoV-2 RNA in wastewater samples, including untreated wastewater, treated wastewater, and sludge (Table 1). According to most of these studies, SARS-CoV-2 RNA in wastewater was detected when the number of confirmed cases reached 1–100 per million population^{1,2,7,11,18–21}.

Table 1 summarizes the reported detection of SARS-CoV-2 RNA in treated and untreated wastewaters and sludge samples. The detection frequency was the highest in the untreated wastewater with maximum concentration ranging from 10^2 to $10^{6.5}$ copies/L^{1–7,11,18–23}. Though many studies reported no detection of SARS-CoV-2 RNA in treated wastewater^{5–7,23}, some cases reported detection with maximum concentration ranging from 10^3 to 10^5 copies/L^{3,11,24}. Interestingly, during the study conducted on Wuchang Fangcang Hospital wastewater samples, SARS-CoV-2 RNA was not detected in the untreated wastewater but reported in treated wastewater. This indicates accumulation during the treatment as well as low efficacy of current disinfection practices on hospital effluents of China²⁴. There is a report of the detection of SARS-CoV-2 RNA in treated wastewater before chlorination, which was attributed to the low limit of detection and high filtration volume of the wastewater sample³. Previously, the sludge settled in the wastewater treatment plants has shown to contain a broad variety of human viruses including the coronaviruses²⁵. With this knowledge, two cases, in the United States²⁶ and Spain⁶ reported successful detection of SARS-CoV-2 RNA in sludge with maximum concentration ranging from 10^5 to 10^7 copies/L.

The detected concentrations of SARS-CoV-2 RNA copies in wastewater samples were consistent with possible viral shedding through excretion²⁷. Tschärke et al.²⁸ estimated the average quantity of wastewater produced per person, as 250 L/person/day, although it is bound to vary with geographical locations, water availability, and lifestyle. The feces quantity was modeled as a normal distribution with a mean of 2.11 log g/person and a standard deviation of 0.25 log obtained from high-income countries^{1,29}. During the period of heaviest shedding among the mild cases of COVID-19, the shedding rate of SARS-CoV-2 RNA in feces was reported in the range 2–8 log copies/g^{15,17}.

REDUCTION OF SARS-COV-2 IN WASTEWATER TREATMENT

According to the environmental surveillance reports so far, SARS-CoV-2 RNA in treated wastewater is mostly under detection limit ($\sim 10^4$ copies/L) (Table 1), while the typical range of the detected SARS-CoV-2 RNA in untreated wastewater has been 10^4 – 10^6 copies/L. Fate and removal of SARS-CoV-2 in wastewater treatment possibly differ from those of well-studied enteric viruses. SARS-CoV-2 is an enveloped virus, which is encased within an outer lipid bilayer membrane called as “envelope”, while most human enteric viruses detected in wastewater, such as norovirus, rotavirus, enterovirus, and others are nonenveloped viruses, which do not possess envelope³⁰. This difference in surface chemical property is likely to affect virus removal efficiency in wastewater treatment, which typically ranges between 2 and 3 log₁₀ from untreated wastewater to the final effluent of WWTP for most of the known enteric viruses (Fig. 2).

In general, LRV for conventional activated sludge (CAS) processes exhibits 1–3 log; membrane bioreactor (MBR) processes often have 1 or 2 log higher LRVs than CAS processes. Anaerobic–anoxic–oxic (A2O) processes reportedly have lower LRVs (ranging 1–2 log) than CAS (Table 2). Major removal mechanisms of viruses in wastewater are partitioning to wastewater solids in primary treatment and adsorption on sludge flocs in secondary treatment^{31,32}. However, there is still limited knowledge of the removal of enveloped viruses in wastewater. Ye et al. investigated partitioning behaviors of two enveloped viruses, i.e., murine hepatitis virus (MHV) and $\phi 6$ in wastewater and found that enveloped viruses exhibit four times larger adsorption than the nonenveloped virus of MS2 and T3³³. It is known that the adsorption of virus particles is governed by extended Derjaguin–Landau–Verwey–Overbeek (XDLVO) forces, i.e., attractive Lewis van der Waals (LW), acid–base (AB) forces, and repulsive electrostatic (EL) force^{34,35}. Since apolar or hydrophobic substances generally have larger AB force and exhibit more adhesiveness³⁶, an enveloped virus with a relatively hydrophobic lipid bilayer membrane is also likely to be more adhesive to wastewater solids and sludge flocs than a nonenveloped enteric virus.

Disinfection is often applied to the secondary treatment effluent for inactivation of bacteria and viruses before discharge. Although a number of studies reported on disinfection of nonenveloped human enteric viruses^{37–39}, only a limited number of studies reported disinfection efficiency of enveloped viruses in wastewater. Wang et al. investigated inactivation of SARS-CoV in untreated wastewater by chlorination and reported 100% reduction by a dose of free chlorine at 1 mg Cl L^{−1} (free residue chlorine 0.40 mg Cl/L) for 10 min⁴⁰. Inactivation efficiencies of F2 phage and *E. coli* under the same condition were 28 and 0%. Under all tested conditions of dose and contact time, SARS-CoV was inactivated more easily than F2 phage and *E. coli*. Also, in treated wastewater, disinfection efficiency by chlorination could be better because it contains less amount of interfering substances that can consume free chlorine. To our knowledge, no prior studies on the efficiency of ozonation for inactivation of enveloped viruses in water are available in the public domain. Since the viral envelope is vulnerable to chemical oxidation, inactivation of SARS-CoV-2 by ozonation could be also more efficient than enteric viruses as expected in chlorination. Ultraviolet irradiation is also probably effective for the inactivation of SARS-CoV-2 in wastewater. Inagaki et al. reported SARS-CoV-2 in water could be inactivated to 3 log by UV at 250–300 nm with exposure of 3.7 mW/cm² for 10 s ($=37$ mJ/cm²)⁴¹. Inactivation efficiency by UV depends on viral genome types. Generally, the single-stranded RNA (ssRNA) genome is reported to be more vulnerable than the double-stranded RNA (dsRNA) genome, and the double-stranded DNA (dsDNA) genome exhibits the highest tolerance⁴². Typical LRV of enteric viruses by UV disinfection process is 0.5–2 (Table 2). Walker

Table 1. Reported detection of SARS-CoV-2 in untreated and treated wastewaters along with virus concentration methods and the maximum copies.

Country	Locality	Water matrix	Positive detection rate	Maximum concentration (copies/L)	Virus concentration method	Targeted gene by RT-(q)PCR	Reference
Australia	Brisbane, Queensland	Untreated wastewater	2/9 (22%)	1.2×10^2	Electronegative membrane-direct RNA extraction; ultrafiltration	N gene	2
China	Wuchang Fangcang Hospital	Untreated wastewater	N. Sarbeco: 1/9 NIID_2019-nCoV_N: 0/9	1.9	Centricon (Merck) ultrafiltration of centrifuged supernatant	ORF1ab gene N gene	24
France	Paris	Treated wastewater	7/9	$0.05-1.87 \times 10^4$	PEG precipitation of centrifuged supernatant	E gene	11
		Untreated wastewater	23/23 (100%)	$>10^{6.5}$	Ultracentrifugation	E gene	11
		Treated wastewater	6/8 (75%)	$\sim 10^5$		E gene RdRp gene	11
India	Ahmedabad	Untreated wastewater	NA (100%)	10^3-10^6		ORF1ab gene S gene N gene	7
		Untreated wastewater	8 May: 100% 27 May: 100%	$0.78 \times 10^2-8.05 \times 10^2$	PEG precipitation of centrifuged supernatant	E gene	18
		Treated wastewater	8 May: 0% 27 May: 0%	0			
Israel	Various locations	Untreated wastewater	10/26	NA	Primary: PEG or Alum precipitation of centrifuged supernatant. Secondary: Amicon ultrafiltration	E gene	18
Italy	Milan and Rome	Untreated wastewater	12/12 (100%)	NA (qualitative PCR detection)	PEG/dextran precipitation of centrifuged supernatant	ORF1ab gene S gene	4
Japan	-	Untreated wastewater	0/5	0	Electronegative membrane-vortex; membrane adsorption-direct RNA extraction	ORF1ab gene S gene N gene	3
		Treated wastewater	1/5 (20%)	2.4×10^3		N gene	21
	Ishikawa and Toyama	Untreated wastewater	7/27	4.4×10^4	PEG precipitation of centrifuged supernatant	N gene	6
Spain	Murcia	Untreated wastewater	N1: 21/42 N2: 23/42 N3: 27/42	N1: 1.4×10^4 N2: 3.4×10^4 N3: 3.1×10^4	Aluminum flocculation-beef extract precipitation	N gene	23
	Ourense	Untreated wastewater	Secondary: 2/18 Tertiary: 0/12 Influent: 5/5	$<2.5 \times 10^4$		N gene E gene RdRp gene	23
		Treated wastewater	Primary effluent: 1/4 Final effluent: 0/5	NA	Amicon ultrafiltration of centrifuged supernatant		
		Sludge	14/34		Glycine/beef extract elution-centrifugation-filtration-PEG precipitation	N gene	6
	Valencia	Untreated wastewater	12/15	10^4-10^5	Aluminum flocculation-beef extract precipitation	N gene	6
		Treated wastewater	0/9	0			
The Netherlands		Untreated wastewater	14/24 (58%) N1: 14/24	NA	Centricon (Merck) ultrafiltration of centrifuged supernatant	N gene E gene	1

Table 1 continued

Country	Locality	Water matrix	Positive detection rate	Maximum concentration (copies/L)	Virus concentration method	Targeted gene by RT-(q)PCR	Reference
Turkey	Amsterdam, The Hague, Utrecht, Apeldoorn, Tilburg, Amersfoort, Schiphol, Istanbul	Untreated wastewater	N2: 0/24 N3: 8/24 E: 5/24 7/9	10^2 – 10^5	Amicon ultrafiltration OR PEG precipitation of centrifuged supernatant	RdRp gene	22
USA	Bozeman, Montana	Untreated wastewater	7/7 (100%) N1: 7/7 N2: 7/7	$> 3 \times 10^4$ N1: 10^2 – 10^4 N2: 10^2 – 3×10^4	Corning Spin-X ultrafiltration of filtered sample	N gene	19
	Louisiana	Untreated wastewater	2/7	$10^{3.2}$	Centricon (Merck) ultrafiltration of centrifuged supernatant;	N gene	5
		Treated wastewater	0/4	0	Adsorption–elution method using an electronegative membrane		
	Massachusetts	Untreated wastewater	10/14 (71%) N1: 10/14 N2: 10/14 N3: 10/14	$> 2 \times 10^5$ N1: 2.3×10^5 N2: 1.4×10^5 N3: 1.7×10^5	PEG precipitation of filtered sample	N gene	20
	New Haven, Connecticut	Primary sludge	44/44	1.7×10^5 – 4.6×10^7	Direct RNA extraction	N gene	26

and Ko reported that MHV coronavirus, a ssRNA virus-like SARS-CoV-2, had much lower survival than MS2, another ssRNA virus, and dsDNA adenovirus had even lower survival, under a dose of UV 254 nm⁴³. Briefly, SARS-CoV-2 is expected to have higher (or at least equivalent) LRV than other nonenveloped enteric viruses for any disinfection processes applied in wastewater treatment.

DILUTION AND DECAY OF SARS-COV-2 IN RECEIVING WATER BODIES

Viruses present in treated wastewater are typically released into environmental waters (i.e., rivers, lakes, and oceans) where virus concentrations are affected by a number of environmental processes categorized into two major processes of dilution and decay. While dilution is driven by seasonal dynamics of water flows, it is the primary process affecting concentration levels of any contaminant that vary significantly in time and space depending on various climatic, geological, hydrological, and anthropogenic conditions^{44–46}. The decay of viruses in receiving waters involves processes like adsorption, aggregation, sedimentation, resuspension, inactivation by temperature and solar radiation, and predation. It is governed by environmental factors such as heat, light, dryness, pH, salinity, ammonia, organic matter, microbial activity, and biofilms^{47–49}.

Keller et al. evaluated dilution factors of effluents from WWTPs in rivers around the world based on spatial modeling in 0.5° resolution (with an average cell size of 2250 km²)⁵⁰. The authors found that annual median dilution factors differ very largely among countries, for example, Canada (33,500), Indonesia (4240), New Zealand (1070), Vietnam (508), Switzerland (260), China (145), United States (120), Bangladesh (87), Japan (58), Korea (40), United Kingdom (37), India (36), Spain (26), Oman (17), Belgium (8.7), and Morocco (5). Large variations in dilution factors also exist within a country, e.g., annual dilution factors in different locations in the United States illustrated a variation of two orders of magnitude (from 33 to 3600) difference between 25th and 75th percentiles, and four orders of magnitude (from 4.5 to 89,000) difference between 5th and 95th percentiles. Spatial variation usually becomes larger for a higher spatial resolution (i.e., more local scale)^{51–53}. Temporal/seasonal variations also cause up to seven orders of magnitude variations in some tropical countries⁵⁰. Similarly, lakes, ponds, and coastal waters have a diverse range of dilution factors for wastewater-derived contaminants, depending on the size of water bodies, local hydrology (e.g., inflow/outflow, vertical/horizontal transport, and exchange with the outer sea), and wastewater discharge volumes. In engineering practice, a ratio of river to wastewater flow (a dilution factor) of 40 is recommended to prevent risk⁵⁴. Overall, it is pertinent to note that in stagnant and (semi) closed water systems, contaminants that are not readily attenuated (e.g., by sedimentation and degradation) can persist in the waters and pose infection risks for a long time.

The dilution of wastewater in the receiving waters can be determined by using specific chemicals or microbes as indicators/tracers. Various pharmaceuticals, personal care products, and artificial sweeteners have been suggested as chemical tracers of domestic wastewater in surface and marine waters^{55–58}. Microbial indicators or tracers of wastewater include a diverse range of specific bacteria and viruses having various source specificity and environmental stability, which have been summarized as microbial source tracking tools^{59–61}. Recent studies have employed both chemical and microbial indicators of wastewater for evaluating fecal pollution^{62,63}. Migration of wastewater (fecal sources) to environmental waters can be sensitively detected by using these indicators/tracers, for example, raw wastewater with up to a dilution factor of 19,000 ($4.3 \log_{10}$) can be detected by using PMMoV and human adenovirus (AdV)⁶³.

Fig. 2 Typical log removal values (LRVs) of viruses in the wastewater treatment process. CAS conventional activated sludge process, MBR membrane bioreactor process, A2O anaerobic–anoxic–oxic process, UV ultraviolet disinfection, MF microfiltration, UF ultrafiltration.

Among the factors affecting the decay of viruses, the temperature has been most studied and often regarded as the most influential factor on virus survival in environmental waters^{49,64}. Other important factors include virus association with solids, exposure to UV, and the presence of microbial flora⁴⁷. In general, enveloped viruses have less stability against environmental stresses than nonenveloped viruses owing to the easy disruption of proteins and lipids of the envelope⁶⁵. The reported stability of various coronavirus strains varies significantly with temperature^{40,66,67}. In water at 25 °C, the time for virus titer to decrease by 2 log₁₀ (T₉₉), was 22 days for transmissible gastroenteritis virus (TGEV) and 17 days for mouse hepatitis virus (MHV). On the other hand, in the water at 4 °C, both viruses did not exhibit decay over 6 weeks⁶⁷. Gundy et al. showed that two coronavirus strains, human coronavirus 229E (HCoV) and feline infectious peritonitis virus (FIPV), were less stable than non-enveloped poliovirus 1 LSc-2ab (PV-1) in water and wastewater in a dark condition⁶⁶. It was found that T₉₉ in secondary effluent at 23 °C was 1.85 days for HCoV, 1.62 days for FIPV, and 3.83 days for PV-1. The difference in the stability between the two enveloped viruses and the nonenveloped virus was even larger at 23 °C (T₉₉: 8.09 days for HCoV, 8.32 days for FIPV, and 47.5 days for PV-1). The study also reported shorter viral survival in the case of secondary effluent, encountering aggregation and adsorption to solid phases, removal by sedimentation, and envelop disruptions by solvents and detergents⁶⁶.

The occurrence of enteric viruses in environmental waters has been extensively studied^{47,68}. Interestingly, surface water and seawater can contain virus concentrations more than treated wastewater by a few orders of magnitude, particularly in the case of combined sewer overflow (CSO) events, where untreated wastewater is discharged into water bodies^{69,70}. A recent survey in Tokyo Bay showed that both *E. coli* and viruses had an increase in abundance for few days after a heavy rain involving CSOs (highest concentration: 9.8×10^4 CFU/L of *E. coli*, 6.8×10^6 copies/L of PMMoV, 2.9×10^4 copies/L of Aichi virus, 5.6×10^3 copies/L of norovirus genogroup I, and 1.2×10^4 copies/L of norovirus genogroup II)⁷¹. However, while the increase and subsequent

decrease in *E. coli* level were large (e.g., three orders of magnitude), virus concentrations had small fluctuations. It can probably be due to the survival of viruses derived from the previous CSOs, low removal of the viruses at WWTPs, and high inhibition in molecular detection of the viruses by environmental matrices. This highlights the stability of viruses in coastal waters, where various recreational activities can take place, as well as the importance of CSOs as a source of viruses in such waters. It is also important to use appropriate process controls and/or additional treatment of virus concentrates^{21,68} in analyzing viruses in environmental waters, which have various degree of inhibitory substances that reduce the efficacy of RNA extraction and RT-PCR⁷².

ESTIMATED RISK OF EXPOSURE TO SARS-COV-2 IN RECEIVING WATER BODIES

COVID-19 is likely to have similar transmission potential with SARS, MERS, and 1918 pandemic flu A (H1N1). The transmission potential of disease is represented by the basic reproduction number (R_0), which is the number of secondary infections resulting from a single primary infection into an otherwise susceptible population. The basic reproduction number (R_0) of COVID-19 reportedly ranged 2.2–3.6^{73–75}, which is in the range of the reported R_0 values for SARS, MERS, and 1918 pandemic flu A (H1N1)^{74,76}. Since the basic reproduction number depends on not only the infectivity of the virus but also its infectious period, the similarity of the transmission potential does not directly mean the similarity of viral infectivity indicated as a dose–response relationship. Although sufficient data are not available for modeling the dose–response relations of SARS-CoV-2 yet, reported viral doses in some animal experiments to indicate that SARS-CoV and SARS-CoV-2 are more infectious than influenza virus⁷⁷.

Infection of SARS-CoV-2 by ingestion is still controversial but potentially occurs⁹. SARS-CoV-2 is the phylogenetically related species of SARS-CoV, which caused the SARS outbreaks in 2003. SARS-CoV and SARS-CoV-2 have the same infection mechanisms, in which the virus enters human cells by binding their spike (S)

Table 2. Summary of virus concentration and log removal values in various (A) wastewater treatment, and (B) disinfection processes.

Virus	Process of WWTPs	Concentration in influent (copies/L)	Concentration in final effluent (copies/L)	LRV (log reduction)	References
<i>(A) Wastewater Treatment Systems</i>					
GI-Norovirus	CAS	4×10^4 – 8.2×10^9	1.4×10^2 – 2.5×10^4	0.50–2.87	99–101
	MBR	10^6 – 10^9	1×10^3 – 1×10^4	2.40–4.30	102–105
	A2O	1×10^1 – 1×10^3	1×10^2	1–2	106–108
	Trickling filter	1.5×10^1 – 1×10^5	1×10^{-1} – 1.5×10^3	1.5–3.5	100,108
	WSP	1×10^1 – 1×10^9	1.4×10^2 – 2.5×10^7	0.5–2	103,108
GII-Norovirus	CAS	4×10^2 – 8.2×10^9	1.4×10^{-1} – 2.5×10^3	1.5–3	99–101
	MBR	10^5 – 10^8	1×10^2 – 1×10^3	1.1–5.3	102–105
	A2O	1×10^1 – 1×10^3	1×10^2	1–2	106–108
	Trickling filter	1.5×10^1 – 1×10^5	1×10^{-1} – 1.87×10^4	2.5–3.5	100,108
	WSP	1.5×10^2 – 1×10^7	1×10^{-1} – 1×10^6	0.5–1.5	103,108
GIV-Norovirus-	CAS	1×10^4 – 1.5×10^5	1.4×10^{-1} – 2.5×10^1	4–5	100
	Trickling filter	1.5×10^3 – 1×10^5	1×10^{-1} – 1.87×10^3	2–4	100,108
Murine Norovirus	CAS	–	–	2–3	106
	MBR	–	–	1–3	106
	A2O	–	–	0–1	106
PMMoV	CAS	1×10^6 – 1×10^{10}	1×10^5 – 2.5×10^9	2–3	100,109
	MBR	1×10^5 – 1×10^6	1×10^3 – 1×10^4	0.70–2	110
	Trickling filter	1×10^5 – 1×10^6	1×10^5 – 2.5×10^5	0.5–1	100
Adenovirus	CAS	1×10^2 – 1×10^6	1×10^0 – 2.5×10^1	2–3	105,111
	MBR	1×10^3 – 1×10^6	1×10^1 – 1×10^3	3.7–5.6	104,111,112
	A2O	1×10^5 – 1×10^6	1×10^4 – 1×10^5	0.4–1.6	104
	Trickling filter	1×10^5 – 1×10^6	1×10^4 – 1×10^5	0.5–2	100
	WSP	1×10^1 – 1×10^2	1×10^0 – 1×10^1	0.7–1	108
Enterovirus	CAS	1×10^4 – 1×10^6	1×10^0 – 2.5×10^1	0.5–2.5	100,112
	MBR	1×10^3 – 1×10^5	1×10^2 – 1×10^3	1.52–3.89	99,105,106,112,113
	A2O	1×10^2 – 1×10^5	1×10^3 – $1 \times 10^{4.5}$	0.5–1	106
	Trickling filter	1×10^5 – 1×10^6	1×10^2 – 1×10^3	2.5–3	100
Sapovirus	CAS	1×10^5 – 1×10^6	1×10^1 – 2.5×10^2	1.77–3.96	30,114
	MBR	1×10^5 – 1×10^6	1×10^3 – 1.5×10^4	3	102
	A2O	1×10^2 – 1×10^4	1×10^3 – 1×10^4	1–2	106
	Trickling filter	1×10^4 – 1×10^6	1×10^2 – 1×10^3	2–3	100
Rotavirus	CAS	1×10^1 – 1×10^6	1×10^2 – 1×10^5	0.20–1.53	30,100,114
	MBR	1×10^5 – 1×10^6	1×10^3 – 1.5×10^4	0.52–2	99,102
	A2O	1×10^1 – 1×10^3	1×10^{-1} – 1×10^0	1–2	114
	Trickling filter	1×10^1 – 1×10^6	1×10^1 – 1×10^4	1–2	100
<i>(B) Disinfection processes</i>					
UV	GI-Norovirus	1×10^2 – 1×10^4	1.4×10^0 – 2.5×10^2	0.5–2	108
	GII-Norovirus	1×10^0 – 1.5×10^2	1.4×10^0 – 2.5×10^3	0.5–1	108
	GIV-Norovirus	1×10^5 – 1×10^6	1×10^3 – 1.5×10^4	1–1.5	30
	PMMoV	1×10^5 – 1×10^6	1×10^{-1} – 1×10^0	1.4–3.4	110
	Adenovirus	1.4×10^2 – 1×10^4	1×10^0 – 2.5×10^3	0.5–1	105,108
	Enterovirus	1×10^1 – 2.9×10^2	1×10^0 – 1.6×10^2	0.7–1	105,108
	Sapovirus	1×10^5 – 1×10^7	1×10^4 – 1×10^5	1–2	30
	Rotavirus	2.4×10^4 – 1×10^7	1×10^3 – 1×10^6	0.5–1.1	30
	JC polyomavirus	1×10^2 – 1×10^4	1×10^1 – 1×10^2	1.7–2.8	30
Chlorination	GI-Norovirus	1×10^3 – 1×10^5	1.4×10^2 – 1.5×10^4	0.3–1.1	30,72
	GII-Norovirus	1×10^5 – 1×10^6	1×10^3 – 1.5×10^4	0–1	72
	GIV-Norovirus	1×10^4 – 1×10^5	1×10^3 – 1.5×10^3	0.2–0.5	72
	PMMoV	1×10^5 – 1×10^7	1×10^4 – 1×10^5	0.7–1.9	115
	Adenovirus	1.4×10^2 – 4.5×10^4	1.1×10^1 – 2.9×10^4	1–2	72,105
	Enterovirus	1×10^2 – 1×10^3	1×10^0 – 1.6×10^2	1–2	72,105

Table 2 continued

Virus	Process of WWTPs	Concentration in influent (copies/L)	Concentration in final effluent (copies/L)	LRV (log reduction)	References
Ozonation	Sapovirus	2.9×10^3	1×10^3	1.2–1.3	30,72
	Rotavirus	2.1×10^3	1×10^3	0–0.5	30
	JC polyomavirus	2.4×10^2 – 1×10^3	1×10^2 – 1×10^3	0–1	30,72
	GI-Norovirus	1×10^2 – 1×10^6	1.4×10^0 – 2.5×10^3	2–3	116
	GII-Norovirus	1×10^2 – 1.5×10^4	1.4×10^2 – 1×10^3	1–2	116
	PMMoV	1×10^5 – 1×10^6	1×10^{-1} – 1×10^1	2.5–3.2	110
	Adenovirus	1×10^4 – 1×10^6	1.5×10^{-1} – 1×10^4	2–3	117
	JC polyomavirus	1×10^2 – 1.5×10^5	1.4×10^{-1} – 1×10^3	1–2	116

proteins to ACE2 receptors of human cells⁸. Since the ACE2 receptor is abundant in small intestine cells⁷⁸, SARS-CoV-2 infection possibly occurs in the gastrointestinal tract when the virus survives in gastric acid and reaches small intestines⁷⁹. Survival of SARS-CoV-2 was observed at a high titer under acidic conditions (pH 2.2), which mimics the gastric environment⁸⁰. Moreover, gastric pH could be around 4.0–5.0 by medication of acid-reducing drugs and gastrointestinal feeding⁸¹. In such cases, SARS-CoV-2 is more likely to survive in gastric acid and cause infection, as reported with SARS-CoV by Darnell et al.⁸².

In QMRA, the chances of infection by a virus are calculated by a dose–response model, which describes relations of viral exposure dose and the probability of infection. Currently, the dose–response model of SARS-CoV-2 is not available yet; however, a dose–response model of SARS-CoV through intranasal infection has been developed by Watanabe et al.⁸³. This model could be applied to complement the lack of a dose–response model of SARS-CoV-2 with careful consideration of its uncertainty⁸⁴. Watanabe's model suggested an exponential model with the following equation:

$$p(r|d) = 1 - \exp\left(-\frac{d}{k}\right) \quad (1)$$

where $p(r|d)$: the chance of infection at the viral dose of d , d : dose of virus (PFU, plaque-forming unit), k : 4.2×10^2 (PFU). According to this model, 1 PFU of viral dose causes 0.24% of infection and 2 PFU causes 0.49%.

The expected dose of the virus is estimated from the volume of water ingestion and viral concentration in the water. SARS-CoV-2 concentration in treated wastewater expectedly ranges between 1 and 100 copies/L, assuming the presence of 10^4 – 10^5 copies/L of SARS-CoV-2 in untreated wastewater and 3 log of removal (equivalent to that of nonenveloped enteric viruses) in wastewater treatment. In water bodies receiving the treated wastewater, SARS-CoV-2 concentrations are expected to be even lower by dilution and decay. As discussed above, factors of dilution and decay are variable to be five times to 4–5 log, depending on the locations of discharge. Hence, SARS-CoV-2 RNA in receiving water bodies does not probably exceed <100 copies/L, except in the worst case like in an urban river that consists of a large proportion of treated wastewater from highly infected areas. Moreover, the viable count of the virus as PFU is probably smaller than viral RNA concentration since viral RNA concentration possibly includes viral RNA from a virus that has lost its infectivity.

The expected volume of water ingestion depends on the activity to expose the water body. For example, the median volume of water ingestion per event is reported as 2.0 mL in fishing, 6.0 mL in swimming⁸⁵. When the water body contained 1 PFU/L of viral concentration, the expected viral dose will be 0.002 PFU/event in fishing and 0.006 PFU/event in swimming. When the expected viral dose is smaller than 1 PFU, the probability where virus particles are ingested should also be considered. Assuming

Fig. 3 Chance of infection to SARS-CoV under the expected dose of virus per exposure event. Bold line shows the probability of infection as a function of the expected viral dose. Arrows point to the probability of infection and expected viral dose in various water activities under different scenarios of viral concentration in water.

that viruses are randomly distributed in the water, the viral dose from a certain volume of water follows the Poisson distribution:

$$p(d|\lambda) = \frac{\lambda^d}{d!} \exp(-\lambda) \quad (2)$$

where $p(r|d)$: the probability of the event where the viral dose of d occurs under the expected dose of λ , λ : the expected dose per event.

Therefore, the chance of infection under the expected dose of λ , $P(r|\lambda)$, can be calculated as

$$P(r|\lambda) = \sum_{d=1,2,\dots,\infty} p(r|d) \cdot p(d|\lambda) = \sum_{d=1,2,\dots,\infty} \left(1 - \exp\left(-\frac{d}{k}\right)\right) \left(\frac{\lambda^d}{d!} \exp(-\lambda)\right) \quad (3)$$

The potential chance of infection of SARS-CoV from a given expected viral dose in water activities is derived as in Fig. 3 by assuming all the ingestion dose reaches the nasal cavity. For example, when the water body contained 1 PFU/L of virus concentration, the expected viral dose will be 0.002 PFU/event in fishing and 0.006 PFU/event in swimming. The estimated chance of infection per event is derived from equation (3) as 4.9×10^{-6} in fishing and 1.5×10^{-5} in swimming (Table 3).

SARS-CoV-2 is phylogenetically related to SARS-CoV with 79% (in ~30,000 bp) of genomic similarity to SARS-CoV⁸⁶. Both species have the spike (S) protein to enter the host cells by interacting with angiotensin-converting enzyme 2 (ACE2). The structure of the receptor-binding domain of SARS-CoV-2 has been evolved from SARS-CoV, and possibly causes higher infectivity^{86,87}. Viral doses in some animal model experiments suggest that SARS-CoV-2 is

Table 3. Expected dose and probability of infection to SARS-CoV-2 for various water activities under various viral concentration scenarios.

Activity	Virus concentration in water body (PFU/L) (assumption)	Volume of water ingestion* (mL/event)	Expected viral dose, λ (log PFU/event)	Chance of infection per event, $P(r \lambda)$ (log)	No. of events per year (assumption)	Annual chance of infection (log)
Swimming	0.01	6.0	-4.2	-6.84	10	-5.84
	0.1		-3.2	-5.84		-4.84
	1		-2.2	-4.84		-3.84
	10		-1.2	-3.84		-2.84
Fishing	0.01	2.0	-4.7	-7.31	50	-5.61
	0.1		-3.7	-6.31		-4.61
	1		-2.7	-5.31		-3.61
	10		-1.7	-4.31		-2.61
Canoeing/ rowing	0.01	2.3	-4.6	-7.25	20	-5.95
	0.1		-3.6	-6.25		-4.95
	1		-2.6	-5.25		-3.95
	10		-1.6	-4.25		-2.95

*Medians of water ingestion estimated by Dorevitch et al.⁸⁵.

potentially more infectious than SARS-CoV⁷⁷. Hence, the estimated chance of infection for COVID-19 in this study could be underestimated and has large uncertainty.

PERSPECTIVES

We presented a QMRA framework to estimate the risk of SARS-CoV-2 in treated wastewater and receiving water bodies by employing the best of the currently available related knowledge. However, the estimated risk values obtained potentially have large uncertainty due to various assumptions and limitations. The major limitations that possibly cause uncertainties in the estimation are (i) employment of the dose–response model for SARS-CoV, and (ii) unknown ratio of SARS-CoV-2 in wastewater as RNA copies to infectious viable count as PFU. The dose–response model for SARS-CoV used in this study needs to be replaced with yet-to-be-developed actual dose–response model of SARS-CoV-2. The dose–response model of SARS-CoV was developed from data sets of challenges in mice with intranasal inoculation of murine hepatitis virus 1 (MHV-1) as a surrogate for SARS-CoV. There must be large uncertainties regarding differences of SARS-CoV-2 from MHV-1, gastrointestinal from intranasal infection including a possible reduction in gastric acid, and human from mice. Although SARS-CoV-2 and SARS-CoV have a similar infectious mechanism, there could be a significant difference in gastrointestinal dose–response relations of SARS-CoV-2 from the dose–response model for SARS-CoV employed in this study. Moreover, viral RNA copies are assumed to be the maximum viable count as PFU because virus quantity as RNA copies possibly includes the remaining RNA from viruses, which has decayed or lost their infectivity. There are limitations pertaining to the use of virus quantity by molecular methods, e.g., RNA copies, for QMRA as addressed by Haas et al.⁸⁸. The actual infectivity of SARS-CoV-2 in wastewater quantified as RNA copies is still uncertain. While the persistence of SARS-CoV-2 is being studied under different environmental circumstances, Remoldi et al. reported insignificant infectivity of SARS-CoV-2 in untreated and treated wastewater from the observation of no cytopathic effects on Vero E6 cells following the inoculation of 48 h and 72 h⁸⁹. It seems that the most SARS-CoV-2 in wastewater possibly already lost its infectivity or inherently inactive owing to its vulnerability to detergents and alcohols, as well as exposure to detergents in sewer lines for hours^{90–92}. Hence, the estimated risk in this study could be overestimated from this assumption, because viable count as PFU

can be smaller than virus quantity as RNA copies. However, even with such large uncertainty, decision-makers are required to put the best efforts to manage the possible risk by SARS-CoV-2 in treated wastewater, because WWTPs need to continue their operation even under COVID-19 outbreaks. The use of QMRA could be helpful for the management of the potential risk of SARS-CoV-2 in water bodies rather than without any quantitative idea on possible risk.

Although this study focused on the impacts of SARS-CoV-2 in treated wastewater on water bodies, many natural water bodies are often affected by untreated wastewater, as the decay of SARS-CoV-2 is still a debated issue⁹³. Discharge of untreated wastewater from combined sewer overflows (CSOs) is highly significant and very common in many countries, including Central Europe (around 70% systems are combined sewer systems) and in the United States (serving more than 40 million individuals)^{94,95}. The quantity of untreated CSOs reached 20% of the total annual discharge of combined sewage in the United States⁹⁵. CSOs possess a higher concentration of bacteria, viruses, and other pathogens than the WWTP effluents, as it contains untreated sewage and hence causes pathogenic contamination in the ambient waters^{62,96–98}. Loads of fecal indicator bacteria from CSOs into natural water bodies can be 4-log larger than those from treated effluent when 23% of combined sewage was discharged as untreated CSOs⁹⁶. The detection of SARS-CoV-2 RNA is already reported in rivers in Italy and Ecuador, indicating contaminated streams of untreated wastewater^{12,89}. It should be noted that the occurrence of CSO events during COVID-19 outbreaks possibly causes a substantial increase in the infection risk of SARS-CoV-2 by exposure to receiving water bodies. Thus, more careful risk assessment and management are necessary for water bodies that possibly receive untreated wastewater including CSOs.

Overall, the motivation of this paper was to provide information on potential risk in treated wastewater from the best of the current knowledge for such decision-making. It should be carefully noted that the estimated risk of SARS-CoV-2 presented in our study should be considered as the bottom line of calculating possible risk under the QMRA framework for SARS-CoV-2 in treated wastewater and water environment. It must further be updated with the development of dose–response model of SARS-CoV-2, the proportion of viable SARS-CoV-2 to RNA copies in wastewater and water environment, for better confidence and reliability.

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AUTHOR CONTRIBUTIONS

M.K.: writing, reviewing, and editing all parts of the paper; M.A.: literature review and writing in the section “Reduction of SARS-CoV-2 in wastewater treatment”; K.K.: literature review and writing in the section “Dilution and decay of SARS-CoV-2 in receiving water bodies”; K.D.: literature review and writing in the section “Concentrations of SARS-CoV-2 in wastewater”; A.H.: review and editing all parts of the paper; H.Y.: formal analysis, reviewing, and editing in the section “Estimated risk of exposure to SARS-CoV-2 in receiving water bodies”; R.H.: conceptualization, formal analysis, writing the sections “Estimated risk of exposure to SARS-CoV-2 in receiving water bodies” and “Perspectives”, reviewing, and editing all parts of the paper.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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