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REVIEW ARTICLE

# The impact of coronavirus SARS-CoV-2 (COVID-19) in water: potential risks

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**Abstract**
This review summarizes research data on SARS-CoV-2 in water environments. A literature survey was conducted using the electronic databases Science Direct, Scopus, and Springer. This complete research included and discussed relevant studies that involve the (1) introduction, (2) definition and features of coronavirus, (2.1) structure and classification, (3) effects on public health, (4) transmission, (5) detection methods, (6) impact of COVID-19 on the water sector (drinking water, cycle water, surface water, wastewater), (6.5) wastewater treatment, and (7) future trends. The results show contamination of clean water sources, and community drinking water is vulnerable. Additionally, there is evidence that sputum, feces, and urine contain SARS-CoV-2, which can maintain its viability in sewage and the urban-rural water cycle to move towards seawater or freshwater; thus, the risk associated with contracting COVID-19 from contact with untreated water or inadequately treated wastewater is high. Moreover, viral loads have been detected in surface water, although the risk is lower for countries that efficiently treat their wastewater. Further investigation is immediately required to determine the persistence and mobility of SARS-CoV-2 in polluted water and sewage as well as the possible potential of disease transmission via drinking water. Conventional wastewater treatment systems have been shown to be effective in removing the virus, which plays an important role in pandemic control. Monitoring of this virus in water is extremely important as it can provide information on the prevalence and distribution of the COVID-19 pandemic in different communities as well as possible infection dynamics to prevent future outbreaks.

**Keywords** SARS-CoV-2 · Drinking water · Cycle water · Surface water · Wastewater treatment · COVID-19

## Introduction

The new outbreak of COVID-19 has recently been a serious threat to the health of people around the world. COVID-19 is produced by SARS-CoV-2, which is a single-stranded, positive-sense RNA virus that causes infection and respiratory failure and has led to thousands of deaths (Eslami and Jalili 2020). The current coronavirus pandemic has resulted in huge impacts worldwide. Coronavirus is estimated to infect up to 70% of the world's population and kill millions of people. The main public health strategy for limiting mortality is to reduce exposure to the virus via physical distancing, which carries tremendous economic costs (Quinete and Hauser-Davis 2021; Stookey et al. 2020).

This abrupt epidemic outbreak of coronavirus disease has currently caused enormous global concerns within the scientific and healthcare community and the general population alike due to the unavailability of human coronavirus vaccines and different virus variants or genetic mutations. Infection with the COVID-19 strain has since spread from Wuhan, China, to approximately 216 countries and territories around the world and has been established as a global pandemic health emergency (Matterne et al. 2020; Kouhsari et al. 2020; Tran et al. 2021). This outbreak is estimated to cause more than 184 324 026confirmed cases of COVID-19,

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including 3,992,680 deaths (WHO 2021). As of 7th July 2021, a total of 3,032,056,355 vaccine doses were administered.

Faced with this problem, the water is one of the most effective ways to contain the fast-spreading novel coronavirus (COVID-19), however, increased potable water demand and the needs of billions of people living in developing countries that lack access to safe drinking water, facilitate the spread of the virus (Zvobgo and Do 2020). Additionally, the wastewater generated represents a considerable portion of the water consumed, its discharge can contain complex pharmaceuticals, antibiotics, narcotics, radioactive elements, hazardous compounds, and pathogens (Anayah et al. 2021). Wastewater is an ecologically rich environment that contains a plethora of pathogens such as bacteria, fungi, protozoans, toxins, and viruses. Viruses are emerging pathogens and are able to adapt by mutation, recombination, and reassortment and can thus become able to infect new hosts and adjust to new environments. Enteric viruses are among the commonest and most hazardous waterborne pathogens, causing both sporadic and outbreak-related illnesses (Bouseettine et al. 2019). Waterborne enteric viruses, primarily transmitted via fecal-oral route either via person-to-person or interaction or the consumption of contaminated water or food, can pose a serious health hazard to personnel working at wastewater treatment facilities as well as the surrounding community. They find their way into wastewater streams from waste, vomiting, and urine of infected humans as well as animals, hence enter the wastewater stream via infiltration and inflow. Waterborne enteric viruses that are commonly found in wastewater can cause several sporadic cases of diseases and outbreaks because of water or food contamination. Their detection, quantification, and effective removal from wastewater are of great importance because their related diseases result in the death of millions of people across the world, making them a matter of great concern. Adenoviruses (AdVs), coxsackievirus, polioviruses, hepatitis (A and E) viruses, rotaviruses, reoviruses, noroviruses (NoVs), and coronaviruses (including SARS-CoV-2) are examples of some viruses that can be detected in wastewater (Ibrahim et al. 2021; Pandey et al. 2021). The ability of some viruses to travel a much greater distance than bacteria in the soil and eventually to groundwater sources due to their sizes and persistence for a considerable period makes their removal difficult and high risk (Adelodun et al. 2021). Waterborne pathogens, including SARS-CoV-2, can be released into the urban-rural water cycle through domestic sewage, urban runoff, agricultural runoff, and wastewater discharges. For this reason, efficient management of the urban-rural water cycle should be considered, as it is essential to understand the transmission of SARS-CoV-2 through different routes before reaching wastewater effluents or surface water such as oceans, rivers, or lakes.

For this reason, a treatments system for drinking water and wastewater consisting of different physical and chemical treatment units can provide multiple barriers to the alleviation of viruses in water (L. Chen et al. 2021). Abatement of waterborne viruses at different treatment processes is reviewed below (coagulation, sedimentation, filtration, and disinfection) for prevention of sewage discharge into freshwater and saltwater are essential to reduce human exposure to the virus (Naddeo and Liu 2020; Sharifi and Khavarian-Garmsir 2020).

The epidemics and pandemics should be included among the natural disasters to which drinking water and wastewater systems are vulnerable. The services provided by drinking water and wastewater utilities are critical to protecting public health, daily life, and economic well-being (Spearing et al. 2020; States 2020; Anayah et al. 2021).

The present review will focus on (a) analyze the characteristics of SARS-CoV-2 and its effects on public health, (b) identify the analytical method for SARS-CoV-2 detection and quantification in water, (c) evaluate the presence of SARS-CoV-2 in drinking water, natural water, and wastewater; (d) compare its environmental impact through the key findings reported by several authors; (e) it will also provide an overview of the potential transmission risks of this novel coronavirus and how COVID-19 could spread in water resources; (f) water and wastewater treatment polluted with SARS-CoV-2. Monitoring effluents from wastewater treatment plants is important to preventing both environmental contamination and the spread of disease and (g) identify the main trends in this field that help future research.

## Definition and features of coronavirus

### SARS-CoV-2 structure and classification

SARS-CoV-2 is a member of an enveloped positive-sense single-stranded ribonucleic acid (RNA) virus family named *Coronaviridae* belonging to the *Nidovirales* order. It is spherical, has a diameter of approximately 65-125 nm, and has crown-like spikes on the outer surface (Astuti and Ysrafil 2020). Enveloped proteins are involved in several aspects of the virus life cycle, such as assembly, envelope formation, and pathogenesis. Inside the envelope is the helical capsid containing nucleoprotein and the RNA genome (up to 33.5 kilobases (kb) genomes) (La Rosa et al., 2020c; Fehr and Perlman 2015). *Coronaviridae* is divided into two subfamilies: *Coronavirinae* and *Torovirinae*. *Coronavirinae* is then further classified into alpha, beta, gamma, and delta coronavirus (Ullah et al. 2020). Respiratory infection in humans is usually caused by alpha and beta coronaviruses, which cause mild to severe lower respiratory tract disease. Several human coronaviruses. Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus (SARS-CoV), and acute respiratory distress syndrome (ARDS)) have been identified, and they can be transmitted by humans via droplets and contact (Chen et al. 2020; Li et al. 2020). Occasionally, new variants of coronaviruses emerge due to their genetic diversity, rapid mutation, high prevalence, and wide distribution (Ullah et al. 2020; Mandal et al. 2020).

### Infectious structures of SARS-CoV-2

Coronaviruses usually have a spike (S), envelope (E), membrane (M), and nucleocapsid (N) as structural proteins. Through the S protein, this virus enters the host cell, and it is cut up by the host protease into two functional subunits, S1 and S2, which oversee host cell binding and viral-cellular membrane fusion, respectively. Several CoVs recognize different proteases and entry receptors, where SARS-CoV and SARS-CoV-2 process their S protein by employing the cellular serine called protease TMPRSS2 and subsequent interaction with angiotensin-converting enzyme two (ACE2) cellular receptors (Zhou et al. 2021; Scagnolari et al. 2021). These viral components can be used for drug therapy against COVID-19. The pathophysiology of SARS-CoV-2 is not well understood, but similar to SARS-CoV, viral replication leads to aggressive inflammation and causes acute lung injury (Scagnolari et al. 2021).

This virus infects humans and animals, causing hepatic, gastrointestinal, neurologic, and respiratory illnesses (Ullah et al. 2020).

## The effects of SARS-CoV-2 on public health

Global coronavirus disease 2019 (COVID-19) affects the economy, environment, people’s livelihoods, and mainly their health (Rume and Islam 2020). For this reason, several studies have reported its effects on the physical and mental health of children, teenagers, young adults, and elderly adults (Di Santo et al. 2020).

Regarding physical health, COVID-19 is a viral respiratory infection that is easy to spread due to the rapid transmission via the respiratory tract from person to person (Naser et al. 2020). Concerning mental health, people with pre-existing mental illness and substance use disorders will be at increased risk of infection with COVID-19 (Cullen et al. 2020). From 2020 to 2021, the percentage of total studies on the effects of COVID-19 was 44% for mental health and 34% for physical health, while 22% of publications covered both ($n$ = 32 (Banerjee et al. 2020). Table 1 summarizes the effects of COVID-19 on public health linked to age group for comparison.

## SARS-CoV-2 transmission

The main route of transmission of SARS-CoV-2 is either by direct contact with an infected subject or indirect contact through a hand-mediated transfer of the virus from contaminated fomites to the mouth, nose, or eyes or via respiratory droplets generated by breathing, sneezing, coughing, etc., (La Rosa et al., 2020a), but knowledge about other potential modes of transmission, e.g., fomite-based, vertical, and fecal-oral transmission, remains sparse (Amirian 2020). The wastewater plumbing system is believed to have acted as a potential route of transmission and caused the superspreading occurrence in Hong Kong due to the transportation of “virus-laden droplets.” The presence of SARS-CoV-2 nucleic acids has been reported in raw wastewater, sewage samples collected from hospitals, and wastewater samples after secondary treatment (Mandal et al. 2020; Sepúlveda-Loyola et al. 2020; Amirian 2020; Lapolla et al. 2020; Ahmed et al. 2020a; Cuevas-Ferrando et al. 2021).

### Initial transmission

The bat is the largest natural host of the α- and β-coronaviruses due to its adapted immune system (Kitajima et al. 2020; Street et al. 2020). COVID-19 clinical case-isolated coronavirus has a taxonomic homology > 95% to bat coronavirus (Parthasarathy and Vivekanandan 2021). There is evidence that human coronaviruses have a zoonotic origin (Drexler et al. 2014) and that at some point in their evolution, these viruses became able to infect people. Figure 1 shows the likely mechanism for starting virus transmission.

It was thought that the transmission of SARS-CoV-2 was made possible by the consumption of bats in the food market of the city of Wuhan, and thus transmission to humans was possible (Shereen et al. 2020; Adelodun et al. 2020). Some viruses are easily denatured by the increase in temperature during the food cooking process between 56 and 60 °C (Chida et al. 2021), and SARS-CoV-2 has shown a low affinity for the ACE2 receptor, a protein responsible for initiating the infectious process in human cells, above 40 °C (Shereen et al. 2020; Chida et al. 2021). Thus, consuming this processed food could not be the cause of the initial transmission.

The virus developed the ability to use ACE2 as a receptor in host cells (Street et al. 2020; Wan et al. 2020). This capability may have been the result of the natural evolution of the virus or a modification acquired in another intermediate zoonotic host (Wan et al. 2020). The chances of coming into contact with humans are higher and transmission is more probable (Platto et al. 2020) when you have daily human contact with the animal host for a long time.

### Primary transmission mechanism

Primary transmission occurs from contact with droplets that are dispersed by the coughing or sneezing of an infected or asymptomatic individual (Rothan and Byrareddy 2020), and transmission of the virus occurs by person-person contact,

**Table 1** Effect of COVID-19 on mental and physical health

| Age group | Mental health | Physical health | Author, year |
|---|---|---|---|
| Children (0–12 years) | Acute stress disorder, adjustment disorder, post-traumatic stress disorder, worry, anxiety, and fear. | - The economic crisis increases stress, violence, and parental abuse against children. | (Kontoangelos et al. 2020) |
| Adolescents (13–17 years) | Post-traumatic stress symptoms, depression, low mood, irritability, insomnia, and anger. Emotional exhaustion due to reorganization of family life, fear of death of relatives, massive stress, and anxiety for the economic crisis. | - Increase in physical and sexualized violence against adolescents, as well as multiple cases of self-injurious and suicidal behavior.<br>- Drug and alcohol abuse has increased during this pandemic. | (Banerjee et al. 2020; Kontoangelos et al. 2020) |
| Young adults (18–35 years) | Depression, stress, and reduced sleep quality. Indeed, delays in university activities due to COVID-19 have been correlated with anxiety. Also, higher panic and fear levels because of information through social media. | - Symptoms are fever, cough, shortness of breath or difficulty breathing, chills, fatigue, muscle pain, headache sore throat, loss of smell or taste, runny nose, nausea, or diarrhea.<br>- Most infections are asymptomatic or do not require hospitalization/treatment. However, a study shows that young people who contracted COVID-19 and require hospitalization ended up in intensive care/were placed on a breathing machine/died. Patients with multiple risk factors (morbid obesity, hypertension, and diabetes) faced severe cases. | (WHO 2020; Fegert et al. 2020; Clay and Parker 2020) |
| Middle-aged adults (36–55 years) | Negative changes in physical activity, sleep, smoking, and alcohol consumption. Increased depression, anxiety, distress, irritability, fearfulness, insomnia, oppositional behaviors, and somatic complaints. Some levels of panic, mental health issues, psychotic symptoms, and even suicide, were reported during the early severe acute respiratory syndrome outbreak. | - COVID-19 symptoms include fever, dry cough, nasal congestion, shortness of breath, fatigue, diarrhea, and vomiting.<br>- Some patients, gradually deteriorate, with the involvement of internal organs such as the lungs, kidneys, and heart.<br>- Complications after admission include secondary infection, acute heart injury, and acute liver and kidney injury. | (Lekamwasam and Lekamwasam 2020; Clay and Parker 2020; Cunningham et al. 2020; Tarighi et al. 2021; |
| Older adults (> 55 years) | Anxiety, depression, sleep disturbances, high levels of psychological stress, and loneliness were observed during the lockdown by a coronavirus. | - Clinical symptoms include cough, sputum, chest tightness, difficulty breathing, fever, fatigue, nasal congestion, runny nose sick, vomit, and pneumonia severity index. Complication after hospitalization: acute respiratory distress syndrome, acute heart injury, secondary infections, shock, and death.<br>- Elderly patients are prone to multi-system organ dysfunction and even failure, including gastrointestinal bleeding, renal failure, DIC, or deep vein thrombosis. | (Lekamwasam and Lekamwasam 2020; Feroz et al. 2020) |

which is favored at a distance < 1.5 m (Fig. 1) (Drexler et al. 2014; Manigandan et al. 2020b). Human and social proximity increases the transmission of the virus (Manigandan et al. 2020b).

Primary transmission epithelial cells of the host lung are infected by the virus through recognition of the membrane protein ACE2 by the glycoprotein S of SARS-CoV-2 (Rothan and Byrareddy 2020). The ACE2 protein is expressed primarily in type II alveolar cells, airway epithelial cells, fibroblasts, endothelial cells, and various immune cells (Belete 2020).

## Secondary transmission mechanisms

Surfaces contaminated with drops of body fluids from symptomatic or asymptomatic patients, such as door handles, elevator buttons, tables, and glasses, become secondary mechanisms of transmission (Fig. 1) (Shang et al. 2021). Touching surfaces and tools contaminated with the virus increase the risk of contagion (Manigandan et al. 2020b). Transmission is possible through the ocular surface, nose, and mouth (Lu et al. 2020). Medical procedures such as endoscopies or dental treatments can also be a transmission mechanism (Manigandan et al. 2020b). In these transmission mechanisms, there is no direct contact with an infected person; therefore, all different means can be considered secondary mechanisms.

Airborne transmission of SARS-CoV-2 is critical, and this mechanism has been confirmed. hospital room has been isolated (Fig. 1) (Noorimotlagh et al. 2021).

The SARS-CoV-2 virus has been isolated and identified in hospital wastewater (Fig. 1) (Gonçalves et al. 2021). The virus has been identified in fecal excretions, and wastewater from hospitals and households has a high viral concentration of $10^4$ genomic copies/L (GC/L) (Gholipour et al. 2021).

Contact with SARS-CoV-2 wastewater aerosols may be a secondary mechanism of transmission (Fig. 1), and these wastewater aerosols can come into contact with workers in wastewater treatment plants (Gholipour et al. 2021). Hospitalized patients or those isolated at home can spread the virus through wastewater. In countries where wastewater is not treated, the risk of transmission is greater (Adelodun et al. 2020).

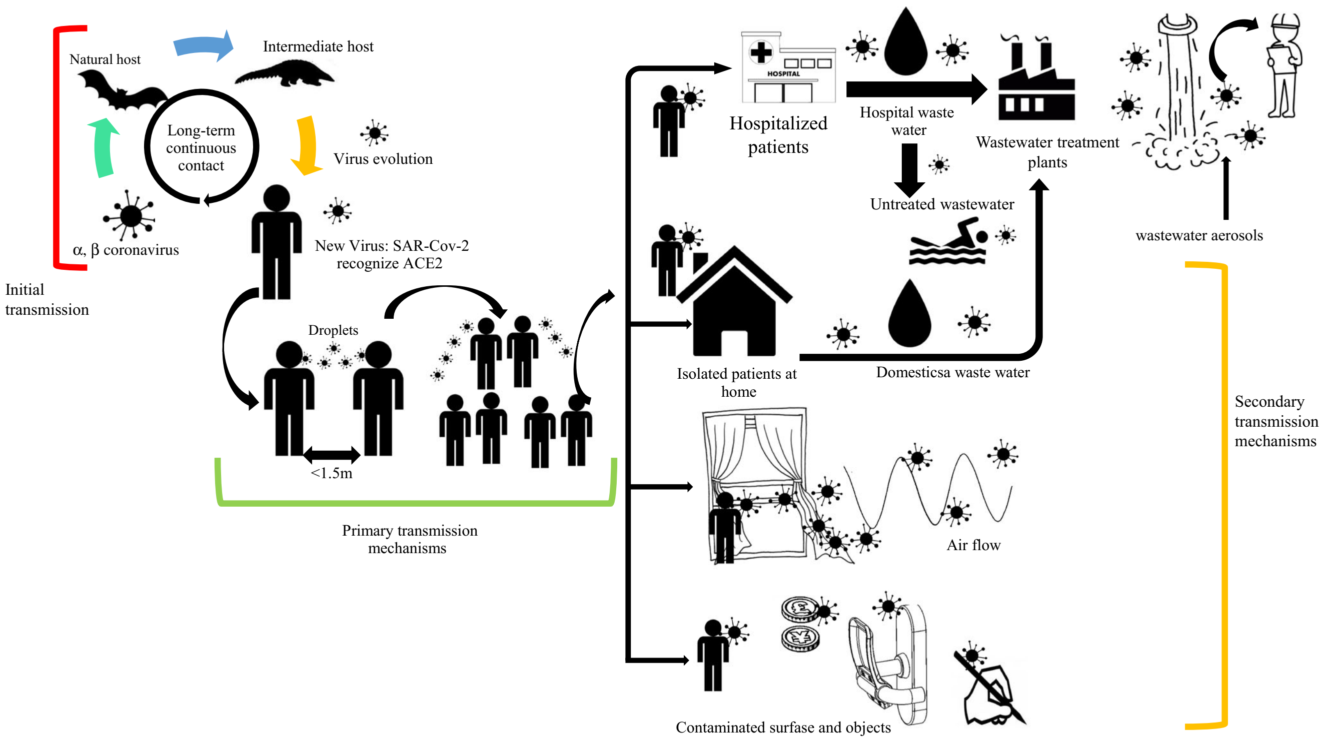


**Fig. 1** Transmission mechanisms virus SARS-CoV-2

Secondary transmission mechanisms represent an important means of contagion, and prevention measures should consider containment strategies to combat SARS-CoV-2 disease by controlling these mechanisms.

## Analytical methods for SARS-CoV-2 detection

The recent spread of SARS-CoV-2, exemplifies the critical need for accurate and rapid diagnostic assays. Since the WHO provided the diagnostic protocol on January 13, 2020, clinical and research health laboratories have quickly developed a series of diagnostic kits for COVID-19 (Vogels et al. 2020; Corman et al. 2020).

The most widely used assay is real-time PCR (RT-PCR) (Celis et al. 2021). Which is used for the molecular diagnosis of SARS-CoV-2. Several protocols in laboratories include the RNA extraction and purification process before RT-PCR as a necessary step for the measurement of viral RNA loads, as it isolates genomic RNA from the viral capsid and removes PCR inhibitors from the original material (Deiana et al. 2020).

RT-PCR proceeds with laboratory conversion of viral genomic RNA into DNA by RNA-dependent DNA polymerase (reverse transcriptase), as shown in Fig. 2. This reaction hangs on small DNA sequence primers designed to recognize complementary sequences on the RNA viral genome and the reverse transcriptase to generate a short complementary DNA copy (cDNA) of the viral RNA.

SARS-CoV-2 detection and COVID-19 diagnosis depend on RT-qPCR tests, and results are usually reported as positive or negative. However, the test can also provide a measure of the viral load in the sample, called a cycle threshold value (Tu and O'Leary 2020).

The RT-PCR test provides real-time quantification by reverse transcription of SARS-CoV-2 RNA into DNA, and after performing PCR, the fluorescence signal increases proportionally to the amount of nucleic acid amplified, which allows accurate quantification of RNA in the sample. If the fluorescence reaches a specified threshold within a certain number of PCR cycles (Ct value), the sample is considered positive (Manigandan et al. 2020a). $Ct < 40$ is considered positive, allowing the detection of very few starting RNA molecules. The cycle threshold (Ct) value from the RT–PCR is inversely proportional (on a logarithmic scale) to viral load; hence, lower Ct values correspond to higher viral loads (Trang et al. 2015).

### SARS-CoV-2 detection in environmental water samples

One common method is to quantify the amount of viral RNA in an environmental water sample via RT-qPCR. This method can measure the number of viral RNA copies or genomic copies in water samples such as surface water or wastewater (Rodríguez et al. 2009; Bar-On et al. 2020). However, virus concentration steps will likely be necessary

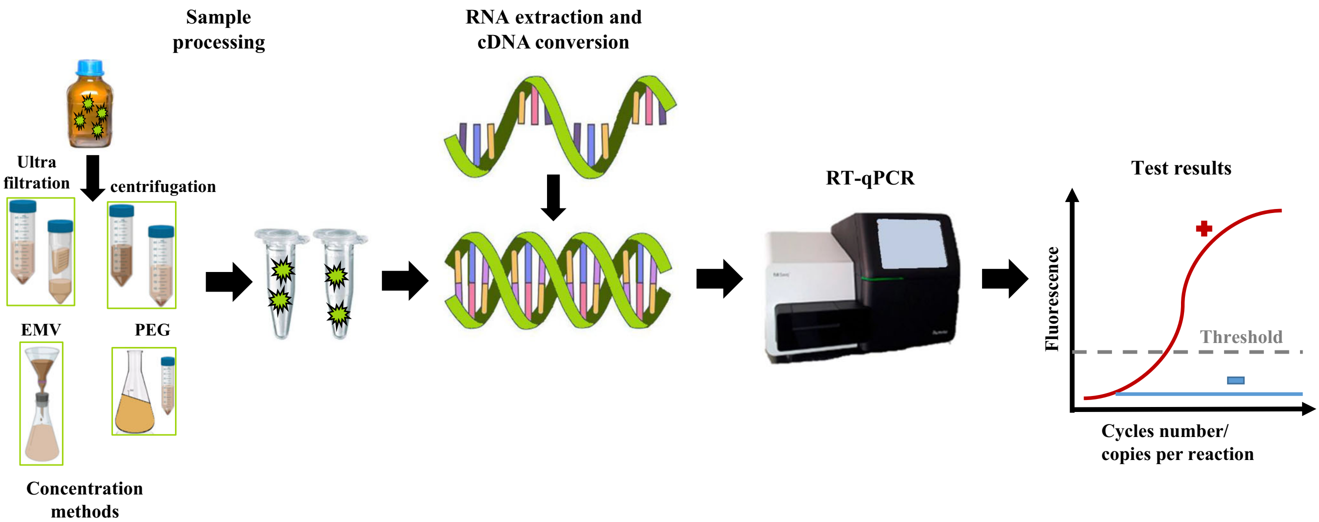


**Fig. 2** Real-time RT-PCR analysis of samples to detect SARS-CoV-2 in water

before subsequent detection of SARS-CoV-2 in samples, as shown in Fig. 2 (Nemudryi et al. 2020; Wurtzer et al. 2020a; Medema et al. 2020).

Several methods have been developed for concentrating viruses in water samples, such as electropositive or electronegative membranes. Another membrane-based method used for concentrating viruses in environmental water samples is ultrafiltration, which is based on size exclusion. Other methods, including polyethylene glycol (PEG), ultracentrifugation, and skimmed milk flocculation, have also been used for concentrating viruses from water samples (Lapolla et al. 2020; Haramoto et al. 2020; Sherchan et al. 2020).

Concentration volumes of water are important factors that should be considered in the results of detection of viruses; usually, concentrating < 100 mL of untreated water samples is sufficient to detect enteric viruses (Haramoto et al. 2018; Medema et al. 2020). Detection of SARS-CoV-2 is principally based on RT-qPCR, and their results are usually reported in RNA copies per reaction or genomic copies within a concentration volume, such as liters, milliliters, or cubic meters (Corman et al. 2020; Shirato et al. 2020).

Absolute or relative quantification can be used when calculating the results of the RT-qPCR assay: the first is used to quantify unknown samples by interpolating their quantity from a standard curve, and the second is used to analyze changes in gene expression in a given sample relative to another aqueous reference sample. The calculation methods usually used for relative quantitation are the standard curve method and comparative cycle threshold method, as shown in Fig. 2 (Boulter et al. 2016; Vogels et al. 2020; Arnaout et al. 2020).

## Environmental impact associated with SARS-CoV-2 in water

### Drinking water

Drinking water is derived from either surface waters, comprising rivers, streams, wetlands, and lakes, or groundwater. However, several contaminants of public health importance naturally occur in these water bodies. For example, metals may originate from natural geochemical lithosphere conditions, and geological processes, in addition to anthropogenic activities, have led to extensive contamination by thousands of chemicals. In general, urbanization and industrialization processes and agriculture and forestry activities are considered the two major contaminant inputs for these freshwater water bodies (Quinete and Hauser-Davis 2021). Currently, clean water sources and community drinking water are vulnerable to stool contamination, and there is evidence that sputum, feces, and urine contain SARS-CoV-2 (Purnama and Susanna 2020; Bilal et al. 2020).

SARS-CoV-2 virus has recently been reported in wastewater. It is possible that patients diagnosed with coronavirus disease are the main route of coronavirus transmission to water and sewage (Tran et al. 2021). Detection of SARS-CoV-2 RNA in wastewater is of interest as it can be used as an early warning method to detect the contact risk associated with untreated water or inadequately treated wastewater (García-Ávila et al. 2020).

Waterborne viruses are different regarding their genome contents and capsid proteins, but such viruses have some common attributes, making them of specific interest concerning the risk of disease endemics related to drinking water infections. According to Hoseinzadeh et al. 2020, water-spread viral pathogens, categorized in terms of moderate to high health importance, consist of *adenovirus*, *astrovirus*, *hepatitis A* and *E viruses*, *rotavirus*, *norovirus*, and other *caliciviruses*, as well as *enteroviruses,* including *coxsackieviruses* and *polioviruses*. Additionally, urine urinary excretion viruses (e.g., *polyomaviruses and cytomegalovirus*) have the potential to be disseminated via water. Other viruses (e.g., influenza and coronaviruses) have been considered organisms that are transmittable by drinking water, but the evidence is indecisive. Table 2 shows examples of viruses found in samples of drinking water, and some treatments are reported to remove or reduce these pathogens. SARS-CoV-2 has not yet been found in any drinking water facilities, and per current evidence, it is safe to comment that the risk to drinking water supplies is low if proper precautions are being taken (Bhowmick et al. 2020).

The SARS-CoV-2 virus has been detected in fecal samples and consequently in untreated wastewater. The fecal-oral route of transmission has possible subpathways: contaminated drinking water, contaminated raw and undercooked aquatic, marine, aquacultural, and sewage-irrigated food, and vector-mediated transmission. Contamination of drinking water sources can occur through seepage from sanitation systems (pit latrines and septic tanks), landfills leachates without geomembrane protection towards the shallow groundwater systems. One study reported 99.9% mortality after 10 days in tap water at 23 °C and over 100 days at 4 °C in other types of coronaviruses. This finding also suggests a longer survival time of coronaviruses in tap water than in wastewater (Gwenzi 2021).

Water for human consumption is treated by conventional methods with correct chlorine-based disinfection and ensuring a residual chlorine level of 0.5 mg/L. Chlorine has also been shown to be more effective in inactivating SARS-CoV-2 than chlorine dioxide and other chemical products (García-Ávila et al. 2020). SARS-CoV-2, an enveloped virus, does not survive easily in water and is able to eliminate and inactivate itself efficiently. International and local regulations have launched treatment requirements so that waterborne pathogens, such as viruses, do not attain drinking water systems. The survival of SARS-CoV-2 in wastewater treatment and drinking water supplies is a global concern (García-Ávila et al. 2020).

The World Health Organization (WHO) noted that the accessibility of drinking water is a fundamental condition for the safety of people. It is widely documented how unsafe water has been suspected to be the cause of epidemics since ancient times(Balacco et al. 2020). The absence of evidence on the survival of COVID-19 in drinking water is valuable because it could improve the hygienic conditions of people and prevent the spread of the virus (Balacco et al. 2020; WHO 2021).

Further investigation is urgently required to determine the persistence and infectivity of SARS-CoV-2 in polluted water and sewage as well as the potential of disease transmission via exposure to contaminated water matrices. This might be of critical importance in controlling COVID-19 in vulnerable communities and crisis zones with poor access to water, sanitation, and hygiene (Carraturo et al. 2020; Kassem and Jaafar 2020).

## The urban and rural water cycle

Waterborne pathogens, which are divided into three main categories, i.e., viruses, bacteria, and parasites (Bridle 2014), can be released into the urban water cycle through domestic sewage, urban runoff, agricultural runoff, and wastewater discharges (Bar-Or et al. 2020).

Appropriate management of the urban and rural water cycle is essential to contain the spread of SARS-CoV-2 since the disease it causes can spread through fecal-oral routes. Correct disinfection of drinking water and wastewater treatment plants and measures such as prevention of sewage leakage into freshwater resources are essential to reduce human exposure to the virus (Naddeo and Liu 2020; Sharifi and Khavarian-Garmsir 2020).

SARS-CoV-2 can maintain its viability in sewage and the urban-rural water cycle, originating from the fecal discharge of infected patients and moving to different bodies of water through the pathways shown in Fig. 3 (Bhowmick et al. 2020).

Several studies have reported the presence of SARS-CoV-2 in urban and rural sewerage systems. This sewage has the possibility to contaminate freshwater; it can cross untreated effluent discharges to surface water or leaks, as well as affect the supply of traditionally treated graywater, and thus these recycled urban waters also represent possible transmission methods (Mukherjee et al. 2020). Table 3 shows these studies in urban and rural sewage.

According to the table above, multiple authors have detected SARS-CoV-2 in the sewage from urban and rural areas, which confirms its mobility and presence in the water cycle, represented in Fig. 3.

The quantification method used in all studies was real-time polymerase chain reaction (RT-PCR), and virus concentrations were reported as cycle thresholds (Ct) and copy numbers per liter. Ct < 29 values corresponded to raw sewage in India, while countries such as Israel, the USA, and Spain presented Ct > 30 but below cycle 40. It is important to note that a sample is considered positive when the cycle threshold (Ct) took place below cycle 40. In the Northeastern U.S. metropolitan area, a primary sewage sludge exhibited the highest virus RNA copies per liter, and the sewage in Quito contained the lowest copies per liter.

The detected concentrations of SARS-CoV-2 in different countries indicate its high persistence in sewage, and thus the monitoring of the virus in these effluents is extremely important because it can provide information on the prevalence,

**Table 2** Different viruses are found in drinking water samples and treatments to reduce these pathogens

| Sample | Virus | Treatment | Findings | Ref |
|---|---|---|---|---|
| Drinking water | MS2 virus | Pre-coagulation and filtration through ultrafiltration (UF) and microfiltration (MF) membranes.<br>Two commercial aluminum-based coagulants (ALG and PAX) were used. | - ≤ 1–30 pfu/mL.<br>- (> 7.4 logs) by UF and MF membrane filtration.<br>- 5 mg Al/L dose<br>- The virus removal was obtained with PAX of 3 mg Al/L.<br>- MF membrane filtration was slightly less (6.7 logs). | (Fiksdal and Leiknes 2006) |
| Surface water and drinking water in Portugal | Hepatitis E virus (HEV) | Not reported | - Samples positive for HEV RNA (77.8% in surface water and 66.7% in drinking water) or infectious HEV (23.0% in surface water and 27.7% in drinking water).<br>- Effective virological control for human consumption and activities. | (Salvador et al. 2020) |
| Municipal subsurface drinking water supplies | Enteric viruses<br>Norovirus GI qPCR | Not reported | - Norovirus was not detected in over 90% of samples, which emphasizes the need to consider the spatially and/or temporally intermittent patterns of enteric pathogen contamination in subsurface water supplies. | (Emelko et al. 2019) |
| Drinking-water supply of primary schools of Sindh, Pakistan | Hepatitis A virus | Not reported | - The highest daily risk of HAV infection for schoolchildren was calculated as 35.0 per 10,000 schoolchildren in the Karachi district with an annual risk of 66%, whereas the lowest risk was 3.0 per 10,000 schoolchildren in Larkana with 7.8% of the annual risk. | (Ahmed et al. 2020b) |
| Drinking water | Bacteriophages, MS2, and ΦX174 | Secuential electrocoagulation-electrooxidation.<br>200-mL batch reactors.<br>Four 1020 steel electrodes for EC.<br>EO reactors BDD/Si anode.<br>EC electrodes were polarized at 100 mA in 3 mM sodium bicarbonate solution for 10 min.<br>The BDD anode and titanium cathode were polarized at 100 mA for 10 min in 0.1 M $H_2SO_4$. | - Both surface water (Lake Michigan and Mississippi River) tended to favor the dual process of EC-EO, with optimal charge allocated to EC of 47% (both MS2 and Φ favor the dual process of EC- ΦX174 | (Heffron et al. 2019) |
| Drinking water | MS2 bacteriophage | Microfiltration membranes modified with a cationic polymer | - The membrane modification resulted in~ 22% loss of the membrane permeability while an increase of ≥ 3 $\log_{10}$-units (≥ 99.9%) in MS2 reduction was observed.<br>- MF membrane led to substantial viral reductions with a significant flux of 5000 L/m2 in approximately 2.5 h. | (Sinclair et al. 2018) |
| Drinking water | Hepatitis E virus | UV disinfection and flocculation-chlorination | - The HEV-p6-Kernow strain was replicated in the HepG2/C3A cell line and quantified genome number using qRT-PCR and infectivity using an immunofluorescence assay (IFA).<br>- UV irradiation tests using low-pressure radiation showed inactivation kinetics for HEV of 99.99% with a UV fluence of 232 J/m$^2$. | (Guerrero-Latorre et al. 2016) |
| Drinking water | H3N8 influenza virus | Not reported | - The finding of a novel genotype of the H3N8 virus in drinking water highlighted the importance of strengthening the surveillance of avian influenza. | (Dong et al. 2013) |
| Drinking water | Enteric virus | Tank with a capacity of 300 L and a 36-W UV lamp coupled, with controlled temperature. | - Recombinant human adenovirus (AdHu5-GFP) and murine norovirus (MNV-1) infectivity were assessed after 0, 3, 6, and 12 h of water recirculation. 99.99% inactivation was reached after 12 h for AdHu5-GFP and before 6 h for MNV-1. Chlorine concentration had a decay of 0.77 mg/L after 12 h. | (Garcia and Barardi 2019) |
| Drinking water (Japan) | Pepper mild mottle virus (PMMoV) as a process indicator | Coagulation-sedimentation (CS), rapid sand filtration (RSF), ozonation, and biological activated carbon treatments. | - PMMoV, the most abundant virus in raw water, was also determined during the CS, RSF and advanced treatment processes in two full-scale drinking water treatment plants.<br>- The $\log_{10}$ reduction of PMMoV in CS and ozonation contributed to the global $\log_{10}$ reduction. | (Kato et al. 2018) |
| Drinking water | Hepatitis A virus (HAV) | This method includes a two-step procedure: concentrating the virus using a microporous electropositive filter (47-mm diameter, 0.45-µm pore size). And the Zetaporfilter, which had charged membrane with a pore size of 0.45 µm. | - The recovery rate of HAV and norovirus ranged between 3.47% and 62.41% with the 0.45-µm electropositive filter. | (Hennechart-Collette et al. 2020) |

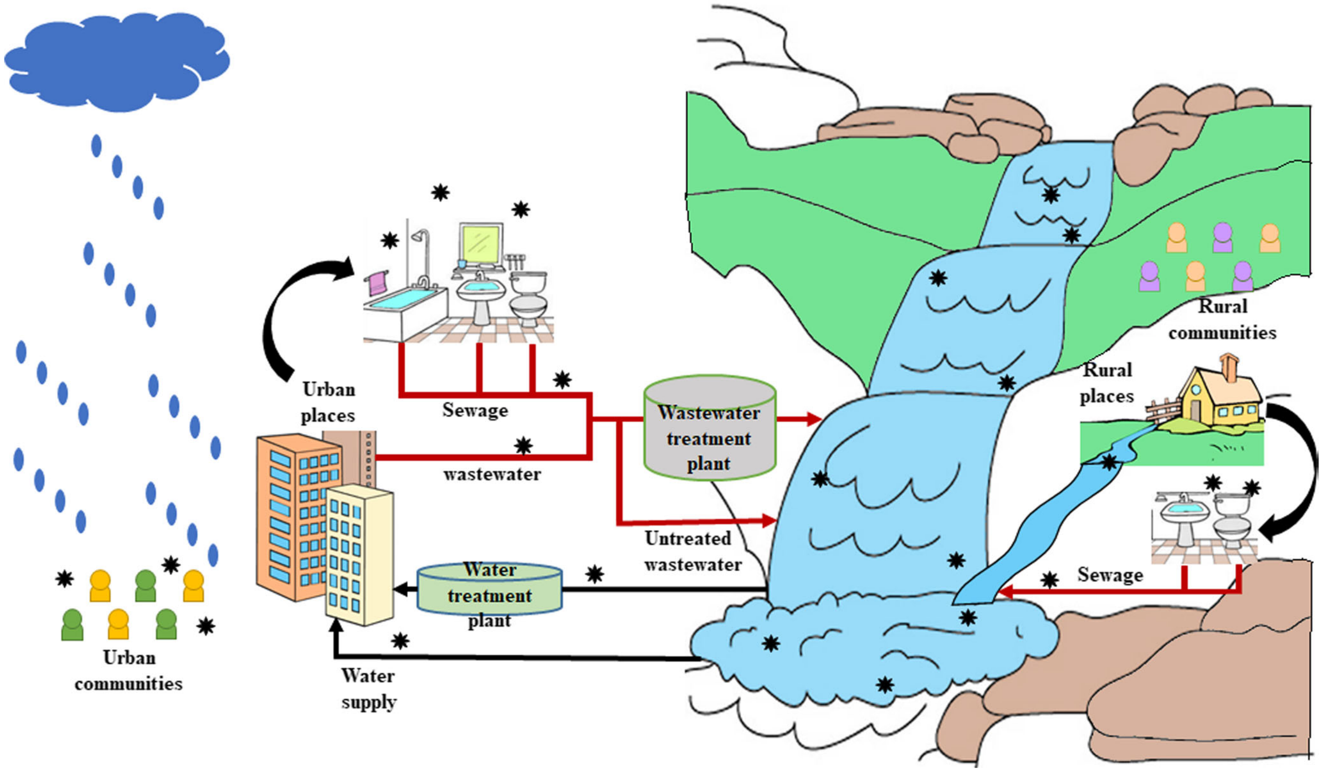


**Fig. 3** Contamination system of SARS-CoV-2 in the rural and urban water cycle with human exposure

distribution in communities, and possible infection dynamics to prevent future outbreaks and evaluate virus elimination.

Urban sewer systems are usually more representative samples of communities than wastewater because wastewater is collected from the population through interceptors that are used to divide the study people. If higher viral loads are observed in one interceptor than in the rest, the corresponding service area will be of greater concern for a possible viral outbreak (O'Brien and Xagoraraki 2019). Taking samples in rural areas is more complex than in urban areas due to the nonexistence of wastewater collection systems and the proximity of discharges to surface waters (Polo et al. 2020).

The COVID-19 pandemic influences multiple aspects of urban and rural water areas, such as engineering, sanitary, economic, and social aspects, which will have important effects in the future (Poch et al. 2020). Table 4 summarizes the publications that report the impacts of COVID-19 on the management of the water cycle in urban and rural areas.

As seen in Table 4, there are several impacts of the COVID-19 pandemic on the management of the urban and rural water cycles. In rural communities, impacts such as water supply programs, water scarcity and quality, disruption of activities in agriculture and supply chains, and ensuring water resources to basic needs are mentioned.

On the other hand, urban areas have shown these impacts: the COVID-19 pandemic affects economic-social water aspects, water policies must be improved for equitable distribution and sewage is a critical tool for monitoring the COVID-19 pandemic because SARS-CoV-2 can maintain its viability in sewage. However, a positive effect that some studies report is that the COVID-19 lockdown has reduced water contamination in multiple urban cities.

## Surface water

SARS-CoV-2 presence in surface water, including both saltwater and freshwater, was confirmed in some regions with a high prevalence of COVID-19 disease. Moreover, the presence of coronaviruses from anthropogenic activities was confirmed in different water bodies (La Rosa et al. 2020a; Sivakumar 2020).

It is important to understand the mobility of SARS-CoV-2 in the water environment to ensure that public health protection measures are properly established (Naddeo and Liu 2020). However, there are limited data on the presence of viral loads in water bodies due to sewage discharged (Cahill and Morris 2020). It is important to conduct studies that address these issues to develop solutions that help developing countries with poor water and sewage infrastructure (Al Huraimel et al. 2020).

SARS-CoV-2 can be spread to water ecosystems due to leaking sewers or deficient removal after sewage treatment (Wurtzer et al. 2020a). Moreover, rainfall events can increase virus concentrations in natural water systems through

**Table 3** Detection of SARS-CoV-2 in urban-rural sewage by several authors

| Sample type/country | Quantification method | Concentration methods | Virus concentration | Key findings | Reference |
|---|---|---|---|---|---|
| Raw sewage/Israel | PCR system | Polyethylene glycol (PEG) or alum precipitation | Tel Aviv: qPCR Ct of 33 and 33.6 Bnei Brak city: qPCR Ct of 33-37. Beer Sheva and Haifa: negative (Ct > 40). | - This study shows a proof-of-concept for the detection of SARS-CoV-2 RNA in sewage. - Results will enable early identification and spatial-based monitoring of future outbreaks and be used to confirm virus elimination. | (Bar-Or et al. 2020) |
| Primary sewage sludge/Northeastern U.S. metropolitan area | Quantitative reverse transcriptase--polymerase chain reaction (qRT-PCR) | Not reported | Samples ranged from $1.7 \times 10^3$ to $4.6 \times 10^5$ virus RNA copies $mL^{-1}$. The lower concentration in this range corresponds to a qRT-PCR cycle threshold (Ct) value of 38.75. 96.5% of all CT values were less than 38. | - SARS-CoV-2 RNA is present in the stool of COVID-19 patients and thus in raw sewage. - Monitoring it in a community's collection system can provide information on the prevalence and dynamics of infection for the population. | (Peccia et al. 2020) |
| Raw sewage/Turkey | Quantitative reverse transcription PCR (RT-qPCR) | Ultracentrifugation, polyethylene glycol adsorption, electronegative membrane, and ultrafiltration methods | Ambarli, Pasakoy, Kadikoy, Terkos, Buyukcekmece, Baltalimani and Tuzla points: $8.26 \times 10^3$, $1.80 \times 10^4$, ND, ND, $3.73 \times 10^3$, $4.95 \times 10^3$, $2.89 \times 10^3$ SARS-CoV-2 copy numbers per liter, respectively. | - SARS-CoV-2 in raw sewage can be used as a tool in wastewater-based epidemiology and it can provide information about SARS-CoV-2 distribution in the wastewater of various districts of Istanbul. | (Kocamemi et al. 2020a) |
| Raw sewage/India | RT-PCR analysis | Filtration and PEG/NaCl adsorption method | Four samples with Ct of 25.5, 34.1, 23.7, and 25.9 | - SARS-CoV-2 RNA is present in hospital sewage samples of India. - These findings demonstrate the applicability of WBE or sewage surveillance as an early indicator of the persistence of the virus in the community and the risk associated with wastewater handling. | (Arora et al. 2020) |
| Primary sewage sludge/Spain | RT-PCR detection | Ultrafiltration and PEG/NaCl precipitation | April 7: Ct of 34.3, 36.0 and 39.8 April 16: Ct of 33.4, 35.9, and 36.6 | - Based on the destination of the viral particles, ideal places must be identified to detect COVID-19 incidence and monitor its evolution. - The primary and thickened sludge showed higher concentrations, suggesting that COVID-19 incidence could be monitored in the sludge line. | (Balboa et al. 2021) |
| Municipal sewage/The San Francisco Bay Area, USA | RT-qPCR assay | Ultrafiltration | Cq values ranged from 29.5 to 36.2 (~ 2 to ~ 553 genome copies/µL of RNA). | - This study sequenced RNA directly from sewage collected by municipal utility districts to generate complete SARS-CoV-2 genomes. - Genomic sequencing can be used to profile the viral genetic diversity across infected communities. | (Crits-Christoph et al. 2021) |
| Sewage pools/China | Quantitative real-time reverse transcription PCR (qRT-PCR) method | Not reported | Cycle threshold (Ct) values of 29.3, 30.5, 32.4 (inlets of pre-processing disinfection pool), and 33.5 (outlet of | - SARS-Cov-2 is present in the sewage from pre-processing disinfection pool of Chinese hospitals. | (Wang et al. 2020a) |

**Table 3** (continued)

| Sample type/country | Quantification method | Concentration methods | Virus concentration | Key findings | Reference |
|---|---|---|---|---|---|
| | | | pre-processing disinfection pool).<br>Not detected (final outlet of sewage disinfection pool). | - The SARS-CoV-2 RNA results demonstrated that routine disinfection measures of sewage in the hospital were sufficient and the hand hygiene of staff was effective. | |
| Urban rivers of Quito impacted by the discharge of sewage/Ecuador | qRT-PCR analysis | The skimmed milk flocculation method | SARS-CoV-2 N1 region: 3.19 × $10^6$, 2.84 × $10^5$, and 2.91 × $10^6$ GC/L.<br>SARS-CoV-2 N2 region: 2.23 × $10^6$, 2.07 × $10^5$, and 8.55 × $10^5$ GC/L. | - Viral loads of SARS-CoV-2 were detected from rivers in urban streams of Quito.<br>- -The presence of the virus can be used as a surveillance tool for an early warning.<br>- -A system using main sewage discharges along the city helping to control the pandemic.<br>- -The method implemented can be used in other cities where sewage is not possible to sample and wastewaters are discharged to streams or rivers. | (Guerrero-Latorre et al. 2020) |

*Considering a positive sample when the cycle threshold (Ct) took place below cycle 40

combined sewer overflows or failures in wastewater infrastructure (Bogler et al. 2020).

Previous studies reported that viral loads of SARS-CoV-2 are present in the water environment. However, some authors did not detect viral RNA, and others provided a method for the rapid assessment of the SARS-CoV-2 transmission risk, as shown in Table 5.

As seen in Table 5, SARS-CoV-2 was detected in surface water. Rimoldi et al. (2020) reported positive results in three rivers in Milan, Italy; however, the concentration of the virus was not measured.

Guerrero-Latorre et al. (2020) found viral loads in the discharge of raw sewage into urban rivers of Quito, Ecuador, and Weidhaas et al. (2021) detected SARS-CoV-2 RNA in small facilities areas, such as Price River WID.

On the other hand, some authors have studied the presence of SARS-CoV-2 in natural water but have not reported the presence of viral concentrations. Haramoto et al. (2020) reported negative results in samples of river water in Japan, and in the results of Desdouits et al. (2021), none of the seawater samples tested positive for SARS-CoV-2 RNA in different areas of France. Samples tested negative can be affected by dilutions made by an incorrect concentration method, or SARS-CoV-2 did not reach the water environment during sampling at significant levels.

Kumar et al. (2021b) and Shutler et al. (2021) reported viral concentrations in natural water bodies through an assessment of the SARS-CoV-2 transmission risk by modifying pollution analysis methods. Viral load values were very different, < 100 copies/L for small proportions and > 4000 copies/L for large natural water systems, probably due to the large influence of volume.

The detection of SARS-CoV-2 in surface water, such as saltwater and freshwater in rivers, streams, or lakes, has not been sufficiently studied, and the information available is limited, as shown in the table. Moreover, several countries treat their wastewater, so viral loads are considerably lower, although there are also places where wastewater can be discharged into rivers, producing negative impacts due to the presence of human viral pathogens.

The presence and mobility of SARS-CoV-2 in water must be considered because viral RNA copies that are discharged into freshwater and saltwater are a risk of infection for the population in contact with these water bodies (Mahlknecht et al. 2021; Mordecai and Hewson 2020). In addition, studies of water systems could be used to assess the risk of transmission to aquatic and human life and identify countries that are exposed to a high risk of transmission. Additionally, data sources can help to develop viral detection methods to reduce impacts on the environment.

## Wastewater

SARS-CoV-2 has been quantified in wastewater through genomic copying (GC), and the air at wastewater treatment plants (WWTPs) has been monitored to determine its presence

**Table 4** Impacts of COVID-19 in urban-rural areas related to water cycle management

| Country/place | Key findings | Reference |
|---|---|---|
| Rural areas and developing countries | - Impact of COVID-19 on the SDG number 6: clean water and sanitation due to supply disruptions and inadequate access.<br>- Rural water supply programs that provide communities with deep boreholes and public hand pumps, sanitation campaigns, and biosand filters for household water treatment. | (Barbier and Burgess 2020) |
| Urban-rural areas | - During COVID-19 lockdown (i)Venice waters cleared due to fewer boat and tourist activities, and (ii) water utilities from Germany and Austria report that the daily peak of water consumption in the morning is 1.5 to 2 h.<br>- Cities with high tourist activity will exhibit an important reduction in water consumption.<br>- Industrial water consumption has decreased, and the agricultural sector has high water demand. | (Cheval et al. 2020) |
| Countries in the Global South (scarcity). Saudi Arabia, Jordan, Egypt, or Lebanon (quality). | - The water scarcity and quality are aggravated by the impacts of COVID-19.<br>- Competition for water by the different consuming sectors is also happening between the rural and urban areas, mainly in water-scarce economies.<br>- The COVID-19 pandemic is an accelerator of the existing water crisis. | (Keulertz et al. 2020) |
| Urban communities | - COVID-19 exist and can maintain their viability in sewage and the urban water cycle, originating from the fecal discharge of infected patients. Therefore, water contaminated by coronaviruses is a potential vehicle for human exposure.<br>- The use of chlorine still represents the best economic solution for disinfectant and inactivation in the water of coronavirus. | (Naddeo and Liu 2020) |
| Pakistan (urban area) | - The impact of COVID-19 on the informal urban population is a threat to human lives and the health sector, which faces an increasing number of serious cases.<br>- 34 million people live in urban informal settlements, where water is scarce for basic needs, so government policies have to integrate urban design and water-smart cities for them.<br>- Covid-19 has impacted SDG 11: make cities and human settlements inclusive, safe, resilient, and sustainable. Water has a direct impact on the resilience and habitability of cities. | (Neal 2020) |
| India (rural area) | - The COVID-19 pandemic has interrupted activities in agriculture and supply chains.<br>- Water management should focus on the need to guarantee the availability and access to water for subsistence needs and domestic food production. | (Neal 2020) |
| Urban areas | - The COVID-19 pandemic affects urban water aspects such as engineering processes, sanitary, economic, and social aspects.<br>- Monitoring of SARS-CoV-2 in human sewage is used to map its spread and scale community outbreaks.<br>- Sewage tracking from the hospital and pooled human samples indicate the epidemic severity.<br>- Sewage is a critical tool for human health monitoring due to the COVID-19 pandemic. | (Poch et al. 2020) |
| Bangladesh and many parts of Africa (rural and peri-urban areas) | - International institutions have to ensure resources were deployed to meet the basic needs of rural communities, such as water to enable hand washing.<br>- The covid-19 outbreak could still be tackled in rural areas. | (Ranscombe 2020) |
| Urban and rural areas | - Water systems have perceived positive impacts because of the reduction of pollutant loading from input of vehicle emission, and industries. Also, a reduction in the demand for coliform and biochemical oxygen in rivers and lakes.<br>- Sewer systems and freshwater sources in hospitals or public places may be contaminated with COVID-19. | (Rashed et al. 2020) |
| France, Italy, Los Angeles, Spain, and Wuhan city of China | - The COVID-19 lockdown has reduced water pollution in many urban cities.<br>- Due to the clear water and the free movement of wild animals, humans began to feel the recovery of the environment. | (Rupani et al. 2020) |
| Urban cities | - The main impacts of the pandemic for the management of the urban water cycle are (i) the quality of water resources has improved, (ii) water contamination increased due to drugs for COVID-19 treatment, and (iii) water treatment to reduce the transmission of COVID-19 through fecal matter is necessary.<br>- The presence of COVID-19 in the sewage system provides information about the infection hotspots and efficacy of control and spread patterns through regular testing. | (Sharifi and Khavarian-Garmsir 2020) |
| Countries, such as China, India, and the USA (urban-rural areas) | - Controlling the spread of COVID-19 will increase water demand and worsen water quality. | (Sivakumar 2020) |

Table 4 (continued)

| Country/place | Key findings | Reference |
|---|---|---|
| | - The water demands will increase in the domestic, industrial, and recreational sectors.<br>- COVID-19 will have serious impacts on water quantity and water quality.<br>- This pandemic will bring challenges related to water sources, infrastructure for distribution, sewer/drainage structures, wastewater treatment, and disposal. | |

(Medema et al. 2020). Some stages of wastewater treatment processes can generate aerosols, derived from the collision between water sheets in a mixing, aeration, or grease separation stage. Transmission by exposure to wastewater aerosols is possible in wastewater treatment plants (Cruz-Cruz et al. 2020; Gholipour et al. 2021) (Fig 1).

The presence of viral RNA in wastewater has been determined, even when the prevalence of COVID-19 is low, establishing a correlation between the concentration in wastewater and the reported prevalence of COVID-19 (Medema et al. 2020). Monitoring of SARS-CoV-2 in wastewater represents pandemic status, a wastewater-based epidemiological approach (WBE) (W. Ahmed et al. 2021), and has been developed worldwide as an environmental surveillance approach to inform health authority decision-making (Table 6). This epidemiological basis could help to identify specific areas of increased epidemiological activity; however, its use would be limited to cities with a wastewater collection or sewerage system, without clandestine discharges or open drains.

Although the virus has been determined in wastewater and its concentration is correlated with pandemic behavior, recent studies suggest that the risk of accidental occupational exposure to SARS-CoV-2 in raw sewage, through inhalation in a treatment plant environment, is negligible, with less than 0.3% of the population served by the plant actively infected (Dada and Gyawali 2021). However, other studies indicate that the risk increases as a function of the fraction of the population with an active infection and warn that greater exposure to aerosols may occur if the sewage and wastewater collection system is inadequate. This is a common situation in underdeveloped countries, which may be subject to routes of exposure to the virus by direct ingestion and inhalation of bioaerosol (Zaneti et al. 2021). It is recommended that workers take biosafety measures to reduce risks, such as disinfection of work surfaces with 0.1% hypochlorite, ventilated work areas, keeping areas free of clutter and personal belongings, and use of long-sleeved gowns, biosafety goggles, and disposable gloves (WHO 2020).

The methodology for SARS-CoV-2 RNA quantification may have some limitations; large differences in catchment size, pipe networks, wastewater characteristics, and subsequently hydraulic retention times can modify the viral concentration (W. Ahmed et al. 2021). Therefore, it is important to use the right concentration method. Viral concentration has been observed to improve with precipitation, filtration, ultrafiltration, and ultracentrifugation (Table 6) (Amoah et al. 2020). The detection of viruses in wastewater and drinking water requires methods that are sensitive, resistant to false-positive results, and must be fast and inexpensive (Lahrich et al. 2021). Viral concentration methods are an essential step to accurately detect SARS-CoV-2 RNA in wastewater (Gonçalves et al. 2021).

The quantification of the virus may vary according to the physicochemical characteristics of the wastewater (Table 6). The concentration of SARS-CoV 2 in the wastewater in GC per liter can be variable between 500 GC/L and 2200 GC/mL (Medema et al. 2020; Zhang et al. 2020). According to Medema et al. (2020), this may be related to pandemic status during the analysis. Large particles can protect and contain the virus inside (Wang et al. 2020a), causing unreliable quantification. The sampling method is critical, and 24 h composite samples may be more reliable in showing the daily average (Sherchan et al. 2020). Sampling protocols should consider the inactivation time of the coronavirus and the effect of storage temperature (Cervantes-Avilés et al. 2021).

Due to the risk of hospital wastewater, treatment with sodium hypochlorite has been proposed (Table 6) at concentrations of 800 $g/m^3$; under this condition, the virus concentration was 500 GC/L. To achieve maximum removal, a concentration of 6700 $g/m^3$ was used. Due to the organic matter load of the wastewater matrix and the high concentration of hypochlorite used, the formation of trichloromethane, tribromomethane, bromodichloromethane, and dibromochloromethane compounds was observed, which represents an environmental risk (Wang et al. 2020b).

## Wastewater treatment polluted with SARS-CoV-2

Removal of the SARS-CoV-2 virus through chlorine disinfection treatment has been evaluated, and the ecological risks of disinfection byproducts need to be carefully considered. Trichloromethane, tribromomethane, bromodichloromethane, and dibromochloromethane concentrations were 332, 1.9, 5.1, and 0.6 μg/L in the effluents, respectively (Table 7). They show high ecological risks and challenge the surrounding environment receiving disinfected medical wastewater,

**Table 5** Detection of SARS-CoV-2 in surface water by several authors

| Sample type/country | Quantification method | Concentration method | Virus concentration | Key findings | Reference |
|---|---|---|---|---|---|
| River water/Yamanashi, Japan | PCR assays | Electronegative membrane-vortex (EMV) and adsorption direct RNA extraction | Not detected | - None of the river water samples tested positive for SARS-CoV-2 RNA. | (Haramoto et al. 2020) |
| Natural water bodies | Quantitative microbial risk assessment (QMRA) | Not applied | SARS-CoV-2 RNA in receiving water bodies does not probably exceed < 100 copies/L.<br>SARS-CoV-2 RNA in receiving water bodies exceeds > 100 copies/L in an urban river that consists of a large proportion of treated wastewater from infected regions. | - The use of QMRA could be useful to manage the potential risk of SARS-CoV-2 in water bodies.<br>- Discharge of untreated wastewater from combined sewer overflows (CSOs) is very common in Central Europe (around 70% of systems are combined sewer systems) and in the United States. | (Kumar et al. 2021a) |
| Three river samples/Milan, Italy | RT-PCR analysis | Not reported | 14/04/2020:<br>Vettabbia river (+)<br>Lambro Meridionale river (+)<br>Lambro river (+)<br>22/04/2020:<br>Vettabbia river (−)<br>Lambro Meridionale river (−)<br>Lambro river (+) | - Positive detection of SARS-CoV-2 RNA in the receiving rivers in the Milano Metropolitan Area.<br>- The presence of the SARS-CoV-2 genome in rivers indicated the inefficiency of the sewerage system of the Milano Metropolitan Area. | (Rimoldi et al. 2020) |
| Spain, UK, and Morocco | Relative risk of transmission | Not applied | Spain:<br>633 copies per 100 mL<br>UK:<br>468 copies per 100 mL<br>Morocco:<br>459 copies per 100 mL | - Obtaining a method that quickly assesses the risk of SARS-CoV-2 transmission in water systems contaminated with feces.<br>- Interactions between river water and wastewater spills should be minimized to reduce the risk of infection. | (Shutler et al. 2021) |
| Urban rivers of Quito impacted by the discharge of sewage/ Ecuador | qRT-PCR analysis | The skimmed milk flocculation method | N1 region:<br>$3.19 \times 10^6$, $2.84 \times 10^5$, and $2.91 \times 10^6$ GC/L<br>N2 region:<br>$2.23 \times 10^6$, $2.07 \times 10^5$, and $8.55 \times 10^5$ GC/L | - Viral loads of SARS-CoV-2 were detected from rivers in urban streams of Quito.<br>- The presence of the virus can be used as a surveillance tool for an early warning.<br>- A system using main sewage discharges along the city helping to control the pandemic.<br>- The method can be used in other cities where sewage is not possible to sample and wastewaters are discharged to streams or rivers. | (Guerrero-Latorre et al. 2020) |
| Price river water improvement district/Utah | Reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) | Membrane filtration | No. of samples/% positive:<br>11/27<br>AVG of SARS-CoV-2:<br>86 GC/L | - SARS-CoV-2 RNA was detectable in different influents during nine weeks.<br>- SARS-CoV-2 RNA was detectable in small areas (< 100 K people) such as Price River WID.<br>- Facilities in areas that serve more than 100,000 people had higher detection frequencies as compared to facilities serving smaller communities. | (Weidhaas et al. 2021) |
| Seawater/France | RT-PCR kit | Negative-charged membrane filtration (MF) and $FeCl_3$ flocculation (FF) | SARS-CoV-2 positive samples:<br>0/9 (Normandy area)<br>0/22 (Brittany area)<br>0/16 (Atlantic area)<br>0/9 (Mediterranea area) | - None of the water samples were found contaminated by SARS-CoV-2.<br>- SARS-CoV-2 did not reach the French coastal environment during summer 2020 at significant levels.<br>- The detection of SARS-CoV-2 in the coastal environment, using shellfish may help to monitor the viral diffusion in seaside communities. | (Desdouits et al. 2021) |

possessing threats to the ecological system and human health (Wang et al. 2020a).

Conventional wastewater treatment plants (WWTPs) have shown effectiveness in the removal of SARS-CoV-2 (Table 2), which may be related to the removal of solids. These solids can protect the virus, and the treatment must contribute to the removal of solids. Carrillo-Reyes et al. (2021) indicated that the virus may have an affinity for solids. SARS-CoV-2 concentrations are higher in both primary and waste-activated sludge (Kocamemi et al. 2020a). Virus removal in an aseptic tank was low due to the high concentration of solids; this process does not separate and reduces the solid content (Wang et al. 2020a).

The hydrophobic envelope of the virus could explain the affinity of the virus for the solid matrix of the wastewater (Ahmed et al. 2020c), which is explained by the fact that

**Table 6** Reports of SARS-CoV-2 in wastewater: key findings

| Country | Sample type | Method of quantification | Method of concentration | Concentration | Key findings | Ref |
|---|---|---|---|---|---|---|
| Australia | Wastewater | RT-qPCR | Adsorption-extraction with electronegative membrane | 135–11,992 GC/100 ml | - Wastewater-based Epidemiology (WBE) approaches the potential of WBE as an early warning system.<br>- The observed prevalence of SARS-CoV-2 RNA in WWTP.<br>- The viral load of the influent varied among the wastewater basins, which could be due to differences in basin size, pipe networks, and hydraulic retention times. | (W. Ahmed et al. 2021) |
| Iran | Wastewater and air | RT-qPCR | Concentrated by aluminum hydroxide and polyethylene glycol | $10^4$ GC/L | - Detection of SARS-CoV-2 in air samples of WWTP demonstrated that wastewater aerosols may contribute to the transmission. | (Gholipour et al. 2021) |
| Slovenia | Hospital wastewater | RT-qPCR | Ultra-centrifugal (10kDA) | No available quantitative*. | - WBE is important in cases where the ability to perform clinical tests is limited because the concentration of the virus in a large population corresponds to its presence in municipal wastewater. | (Gonçalves et al. 2021) |
| China | Hospital wastewater | RT-qPCR | Centrifuged at 10,000g for 30 min | 0.5–18.7 × $10^3$ copies/L | - The unexpected presence of SARS-CoV-2 viral RNA in septic tanks after disinfection with 800 g/m$^3$ of sodium hypochlorite.<br>- The effluents showed negative results for SARS-CoV-2 viral RNA when overdosed with sodium hypochlorite (6700 g/m$^3$) but had high a level of disinfection by-product residuals, possessing significant ecological risks. | (Zhang et al. 2020) |
| Netherlands | Wastewater | qRT-PCR | Ultrafiltration | 2.6–30 GC/mL<br>790–2200 GC/mL | - The increase in GC correlated with the increase in COVID-19 prevalence reported by health authorities.<br>- The detection of virus RNA in sewage was low when the prevalence of COVID-19 in the population was low.<br>- A correlation was established between the concentration of virus in wastewater and the reported prevalence of COVID-19. | (Medema et al. 2020) |
| USA | Wastewater | RT-qPCR | Precipitated with polyethylene glycol 8000 | 100 viral particles/mL | - Where in-person testing may be unavailable or limited, a cross-sectional analysis of wastewater can provide population-level estimates of the presence of the SARS-CoV-2 virus. | (Wu et al. 2020) |
| USA | Municipal wastewater | RT-qPCR | Centrifugal filtration | 34–528 copies/L | - SARS-CoV-2 has been detected in feces, which indicates that wastewater may be used to monitor viral prevalence in the community. | (Nemudryi et al. 2020). |
| France | Wastewater | RT-qPCR | Centrifugated at 200 000 × g for 1 h at + 4 °C | 50 × $10^4$–3.1 × $10^6$ genome units/L$^-$ | - Wastewater's survey may provide an alternative and possibly early tool to detect pathogens in populations when investigations in humans are difficult to conduct for logistic, ethical, or economic reasons, notably in poor countries that are strongly exposed to the COVID-19 epidemic. | (Wurtzer et al. 2020b) |
| Spain | Wastewater | RT-qPCR | Aluminum hydroxide adsorption-precipitation | 5.15, 5.53, and 5.49 logs<br>198 GC/L | - The detection of SARS-CoV-2 in wastewater in the early stages of the spread of COVID-19 highlights the relevance of this strategy as an early indicator of the infection within a specific population. | (Randazzo et al. 2020) |
| Italy | Municipal wastewater | RT-PCR | Extran and polyethylene glycol | No available quantitative*. | - In this study, thermal treatment of samples (30 min at 56°C) was included before concentration to increase the safety of the laboratory personnel during sample manipulation. | (La Rosa et al., 2020a) |
| France | Municipal wastewater | RT-PCR | Ultrafiltration (1500 × g for 15 min) and on PEG 6000 precipitation. Stored at − 20 °C. | ~ $10^4$ GC/L to ~ $10^2$ GC/L | - A concentration method combining ultrafiltration, phenol-chloroform- isoamyl alcohol purification.<br>- Data are provided to validate a link between a COVID-19 outbreak in a population and fecally excreted virus concentrations in wastewater. | (Bertrand et al. 2021) |
| India | Wastewater | RT-PCR | - PEG 9000 (80 g/L).<br>- Incubated at 17 °C, 100 rpm overnight.<br>- Centrifuged at 14000×g 90 min. | ~ 924.5 copies/L<br>~ 897.5 copies/L | - WBE surveillance to predict the fluctuation of COVID-19 cases.<br>- This study suggests that wastewater surveillance needs to be included as an integral part of COVID-19 pandemic monitoring, which can help water authorities identify hotspots within a | (Kumar et al. 2021c). |

**Table 6** (continued)

| Country | Sample type | Method of quantification | Method of concentration | Concentration | Key findings | Ref |
|---|---|---|---|---|---|---|
| | | | | | population and can provide up to a 2-week head start in generating interventions. | |
| Turkey | Wastewater | RT-PCR | Polyethylene glycol 8000 (PEG 8000) adsorption electronegative membrane electronegative membrane | $8.26 \times 10^3$, $1.80 \times 10^4$, $3.73 \times 10^3$, $4.95 \times 10^3$, $2.89 \times 10^3$ virus titer/L | - Continuous monitoring of wastewater for SARS-Cov-2 may provide early warning signs before an epidemic starts in case of infection resurge. | (Kocamemi et al. 2020b) |

*Present but non-quantifiable in the last sample

RNA is easier to quantify in the sludge without employing a concentration method, and sludge monitoring has been suggested to serve as an indicator for SARS-CoV-2 surveillance (Carrillo-Reyes et al. 2021). The more efficient the treatment may be in removing solids, the more efficient it will be in removing the virus. The most commonly used methods to improve quantification of the virus in wastewater employ polyethylene glycol to concentrate the viral load (La Rosa, Bonadonna, et al. 2020; Peccia et al. 2020; Zhang et al. 2020).

Virus envelope proteins play a very important role in the process of infection and spread, they can contribute to the strong or weak adhesion of SARS-CoV-2 to its host cell surface, which mainly involves hydrophobic interactions(Jakhmola et al. 2021). These proteins are mostly of a quaternary structure (Duart et al. 2021) and are characterized by hydrophobic interactions (Bhagavan 2002).

The virus concentration can be equal in raw water and primary sludge, and after biological digestion of the sludge, the virus has not been detected (Balboa et al. 2021). Despite the effectiveness of virus removal in raw water and virus denaturation in sludge, chlorination of treated water has been recommended as a final treatment step (Carrillo-Reyes et al. 2021). The combined treatment of thermal hydrolysis and anaerobic digestion prevented the detection of SARS-CoV-2 in sludge effluent of the plant (Balboa et al. 2021).

Although more studies are needed to demonstrate the transmission of the virus via untreated wastewater, the presence of the virus has been demonstrated and poses a risk. Therefore, WWTPs play an important role in controlling the pandemic, as do sewage systems that allow for maximum wastewater collection.

## Future trends

The primary mechanism of virus transmission is person-to-person contact (Rothan and Byrareddy 2020); however, we have discussed other possible routes that represent a risk condition. In the future, it will be important to demonstrate the viral load necessary to generate COVID-19. These secondary mechanisms (Fig. 1) represent a risk, but the particular viral load condition for developing the disease is not clear.

Different efforts to control the spread of COVID-19 will increase the water demand and worsen the water quality, leading to additional challenges in water planning and management (Sivakumar 2020). Further studies are needed to determine the survival of SARS-CoV-2 in the environment, clearer mechanisms of transmissibility through sewage, and the potential to infect humans via the fecal-oral route. (Dhama et al. 2021).

The detection and quantification of the virus have confronted difficulties due to the complex composition of the wastewater. The concentration method is an important factor in determining the virus (La Rosa, Iaconelli, et al.

**Table 7** SARS-CoV-2 removal in wastewater treatment systems

| Country | Treatment | Condition treatment | Removal concentration | Key findings | Ref |
|---|---|---|---|---|---|
| India | Upflow anaerobic sludge blanket (UASB) | Primary treatment: Clarifier HRT of 2.5 h<br>Secondary treatment: UASB 6 aeration tanks<br>HRT: 5 h<br>pH: ~ 7 to 8.5.<br>Sludge thickening unit: retention time 20 days<br>Secondary clarifier: HRT 2.5 h | Raw wastewater: $1.8 \times 10^3$ copies/L and $3.5 \times 10^3$ copies/L<br>Final effluent: RNA was not detected at all in the final effluent | - PEG (polyethylene glycol) method performed better in removing materials inhibiting RT-qPCR.<br>- A conventional treatment system seems to be effective in reducing the SARS-CoV-2 genes. | (Kumar et al. 2020) |
| China | Aseptic tank chlorination | Sodium hypochlorite was not regularly added to the final concentration of 800 g/$m^3$ and it increased to 6700 g/$m^3$.<br>1.5-h contact and the mixing | Raw wastewater: $7.5 \times 10^3$–$14.7 \times 10^3$copies/L<br>Final effluent: RNA was not detected to 6700 g/$m^3$ of sodium hypochlorite | - Trichloromethane, tribromomethane, bromodichloromethane, and dibromochloromethane were 332, 1.9, 5.1, and 0.6 μg/L in the effluents, respectively. | (Zhang et al. 2020) |
| United Arab Emirates (UAE) | Wastewater treatment plants conventional | Preliminary, primary, secondary (ASP/clarification), and tertiary (and filtration, disinfection, chlorination) | Raw wastewater: $7.5\times10^2$–$3.4\times10^4$ gene copies/L<br>Final effluent: not detected | - Wastewater treatment technologies implemented in the UAE are efficient in the removal of SARS-CoV-2 and confirm the safety of the treated re-used water across the country. | (Hasan et al. 2021) |
| México | Dual (biofilter/-activated sludge). | Coarse and fine screening, biological treatment and disinfection process (chlorinated) | Raw wastewater: $1.6 \times 10^4$–$5.2 \times 10^4$ gene copies/L<br>Final effluent: not detected | - Secondary sludge from the WWTP showed virus RNA levels eight orders of magnitude higher than in the influent, suggesting a migration of genetic material from the liquid to a solid matrix in the wastewater treatment process. | (Carrillo-Reyes et al. 2021) |
| México | Activated sludge | Coarse and fine screening and biological treatment | Raw wastewater: $1.8 \times 10^4$–$3.8 \times 10^4$ gene copies/L<br>Final effluent: not detected | - The detection of virus RNA in the sludge was determined to be due to migration of the genetic material, an affinity of the virus for solids was observed. | (Carrillo-Reyes et al. 2021) |
| Spain | WWTP conventional | Primary sludge<br>Biologic sludge<br>Thickened sludge<br>Digested sludge | In waterline:<br>Raw wastewater: < $7.5 \times 10^3$ copies/L<br>Outflow primary: < $7.5 \times 10^3$ copies/L<br>Treated effluent: negative<br>In sludge line:<br>Primary sludge: < $7.5 \times 10^3$ copies/L<br>Biologic sludge: negative<br>Thickened sludge: $15\times10^3$–$20 \times 10^3$ copies/L<br>Digested sludge: negative<br>Final effluent: not detected | - The affinity of the SARS-CoV-2 virus for biosolids was observed to be associated with sludge currents in WWTPs.<br>- The combined treatment of thermal hydrolysis and anaerobic digestion prevented the detection of SARS-CoV-2 in sludge leaving the plant. | (Balboa et al. 2021) |
| Turkey | Activated sludge | Primary sedimentation tanks.<br>Waste activated sludge (WAS) | $1.17 \times 10^4$ to $4.02 \times 10^4$ viruses per liter.<br>Final effluent: not determined | - In this study, it was observed that SARS-CoV-2 virus concentrations were higher in both primary and activated sludge. | (Kocamemi et al., 2020a) |

2020a; Carrillo-Reyes et al. 2021; Dhama et al. 2021). Therefore, more sensitive, easy to perform, accessible, and economical techniques are needed to further monitor the pandemic and allow sanitary control to ensure public health. Devices have been suggested for trace detection of SARS-CoV-2 in water. One of the main challenges in the detection/quantification of SARS-CoV-2 in wastewater samples is to generate optimized and standardized protocols (Kitajima et al. 2020), where the risk of transmission is high, as shown in Fig. 4.

WWTPs have shown effectiveness in virus removal (Arora et al. 2020; Jiao Wang et al. 2020a); however, more knowledge is needed about virus sharing through the different stages of the treatments to more clearly determine the most effective removal or denaturation method against the virus.

Disinfection methods used in the drinking water treatment process highly inactivate and efficiently destroy SARS-CoV-2 in water (Tran et al. 2021). However, there is a need to investigate and improve the performance of disinfection technologies to be adopted for the inactivation of SARS-CoV-2 in municipal and hospital wastewater to reduce the related risk. (Mandal et al. 2020; Tran et al. 2021).

The studies compiled in this review are solid antecedents that demonstrate the presence of the SARS-CoV-2 in natural

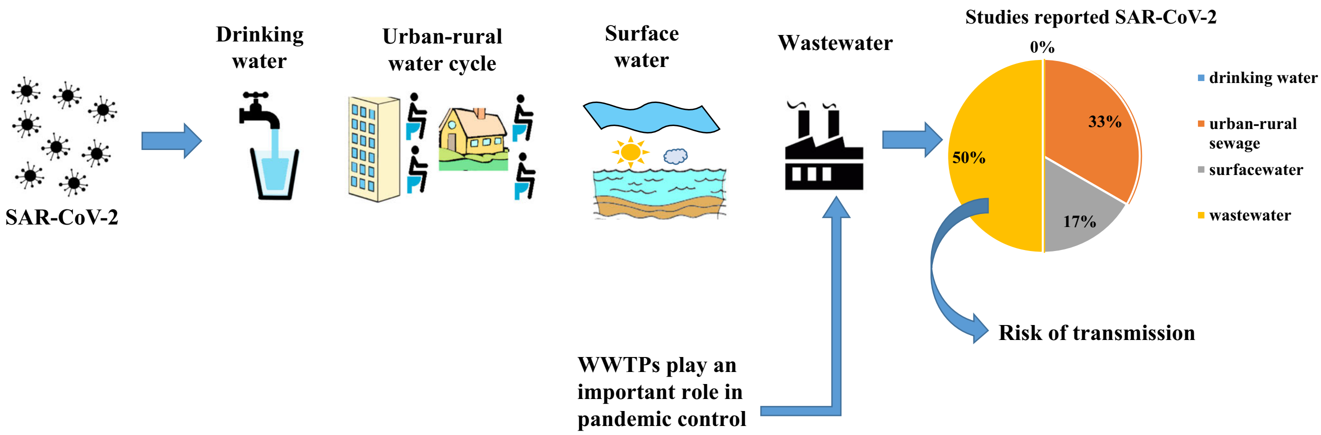


**Fig. 4** Potential risk of transmission of SARS-CoV-2 in water

water and wastewater. Some data found, such as the study areas and concentrations of viral loads, would help to quickly relate the presence of the virus in water with the epidemiological and sanitary situation in the future, which could determine the economic and social factors that they intervene in the duration of the disease in different rural or urban communities

Identifying those geographic areas that report the presence of SARS-CoV-2 in water could support future research to predict or control re-outbreaks in surrounding areas. In Italy, viral loads were detected in contiguous rivers and traveled through densely populated areas near the Milan metropolitan area last year and then a re-outbreak occurred in the neighboring city (Lazio).

The need for further research to establish the behavior of the SARS-CoV-2 virus in aquatic systems is a priority to establish efficient methods to concentrate and detect enveloped viruses (and coronavirus in particular) from water matrices and the estimation of the survival of these viruses in natural conditions, at different temperatures and in different types of water. Additionally, the information collected on SARS-CoV-2 in this review aims to facilitate ideas that improve the monitoring of COVID-19 in natural and wastewater in future studies, which would allow investigating the development of the disease in the population. This information would help to determine the stability of the virus in water bodies and to know its possible dynamics in space and time.

The findings discussed in this review would generate options that mitigate the presence of the virus and with it its transmission in drinking water, natural water, and wastewater. Finally, these findings could be used to develop new preventive measures, in addition to healthy distance, temperature measurement, and the use of antibacterial gel, related to the pathways of spread of SARS-CoV-2 in water.

## Conclusions

After reviewing 169 references, it was possible to analyze the characteristics of the coronavirus, its transfer routes, detection methods, and its impact on drinking water, surface water, wastewater, and systems that are effective for wastewater treatment.

The presence of SARS-CoV-2 has been demonstrated in the water cycle in both urban and rural areas. One of the routes of transmission of the virus is through the sewage system of domestic origin, i.e., gray water, sewage, soaps and detergents, and toilet paper.

The monitoring of SARS-CoV-2 in wastewater can be a tool that can monitor the behavior of the pandemic. Conventional treatment systems are effective in removing the virus, and a relationship between solid removal and viral load has been observed. WWTPs play an important role in pandemic control.

The transmission of SARS-CoV-2 may be greater in rural areas. Public policies surrounding the urban-rural water cycle have been exposed as deficient and bad in several countries, mainly third world countries.

Some studies have reported the presence of water pathogens including viruses in drinking water. However, SARS-CoV-2 has not yet been found in any drinking water facilities, and it is safe to comment that the risk to drinking water supplies is low.

There is still little information available regarding the existence of SARS-CoV-2 in surface waters; however, more study is needed as its detection in different bodies of water, which are usually in direct contact, is important. These studies should include different natural ecosystems, both terrestrial and aquatic.

The presence of SARS-CoV-2 in surface water should be further studied to determine whether its transmission to different living beings, including humans, is possible and whether it is important to reduce or eradicate possible loads of viruses found in these waters.

Finally, future trends focus on the scope of the present information collected by several authors about the impact of coronavirus SARS-CoV-2 in water to answer the question of how this review could guide future research.

**Abbreviations** *ASP,* Activated sludge process; *ARDS,* Acute respiratory distress syndrome; *ALG,* Alum coagulant; *ACE2,* Angiotensin-converting enzyme 2; *BDD,* Boron-doped diamond; *BBD/Si,* Boron-doped diamond/silicon; *CS,* Coagulation-sedimentation; *CSOs,* Combined sewer overflows; *cDNA,* Complementary DNA; *COVID-19,* Coronavirus disease 2019; *Ct,* Cycle threshold; *DIC,* Disseminated intravascular coagulation; *EC-EO,* Electrocoagulation-electro-oxidation; *EMV,* Electronegative membrane-vortex; *MS2 virus, Escherichia* virus MS2; *FF,* $FeCl_3$ flocculation; *GI qPCR,* Gastrointestinal qPCR; *GC/L,* Genomic copies per liter; *HAV,* Hepatitis A virus; *HEV,* Hepatitis E virus; *HEV-p6,* Hepatitis E virus strain Kernow (clone P6); *AdHu5-GFP,* Human adenovirus type 5-green fluorescent protein; *HCOVs,* Human coronaviruses; *HepG2/C3A* , Human hepatocellular carcinoma cells; *HRT,* Hydraulic retention time; *IFA,* Immunofluorescence assay; *MF,* Microfiltration; *MERS-CoV,* Middle East respiratory syndrome coronavirus; *MNV-1,* Murine norovirus 1; *PMMoV,* Pepper mild mottle virus; *PEG,* Polyethylene glycol; *PAX,* Prepolymerized aluminum coagulant; *QMRA,* Quantitative microbial risk assessment; *qPCR,* Quantitative polymerase chain reaction; *RSF,* Rapid sand filtration; *RT-PCR,* Reverse transcription-polymerase chain reaction; *RNA,* Ribonucleic acid; *SARS-COV-2,* Severe acute respiratory syndrome coronavirus 2; *SDG,* Sustainable development goals; *TMPRSS2,* Transmembrane serine protease 2; *UF,* Ultrafiltration; *UAE,* United Arab Emirates; *UASB,* Upflow anaerobic sludge blanket; *ϕX174 virus,* Virus that infects the bacterium *E. coli*; *WAS,* Waste-activated sludge; *WWTP,* Wastewater treatment plants; *WBE,* Wastewater-based epidemiology; *WID,* Water improvement district; *WHO,* World Health Organization

**Authors' contributions** RGN: devised the project, the main conceptual ideas, conducting a research and investigation process. ILH: devised the project, the main conceptual ideas, acquisition of the financial support for the project leading to this publication. LACS: supervised the project, revised it critically for important intellectual content. All authors provided critical feedback and helped shape the research, analysis, and manuscript. All authors read and approved the final manuscript.

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## Declarations

**Competing interests** The authors declare no competing interests.

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