



## Challenges to detect SARS-CoV-2 on environmental media, the need and strategies to implement the detection methodologies in wastewaters

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### ARTICLE INFO

Editor: Dr. Z. Wen

#### Keywords:

SARS-CoV-2

Sewage line sampling

Environmental media

COVID-19

Cost-effective outbreak monitoring

Post-pandemic decision making tool

### ABSTRACT

Understanding risks, putting in place preventative methods to seamlessly continue daily activities are essential tools to fight a pandemic. All social, commercial and leisure activities have an impact on the environmental media. Therefore, to accurately predict the fate and behavior of viruses in the environment, it is necessary to understand and analyze available detection methods, possible transmission pathways and preventative techniques. The aim of this review is to critically analyze and summarize the research done regarding SARS-CoV-2 virus detection, focusing on sampling and laboratory detection methods in environmental media. Special attention will be given to wastewater and sewage sludge. This review has summarized the survival of the virus on surfaces to estimate the risk carried by different environmental media (water, wastewater, air and soil) in order to explain which communities are under higher risk. The critical analysis concludes that the detection of SARS-CoV-2 with current technologies and sampling strategies would reveal the presence of the virus. This information could be used to design systematic sampling points throughout the sewage systems when available, taking into account peak flows and more importantly economic factors on when to sample. Such approaches will provide clues for potential future viral outbreak, saving financial resources by reducing testing necessities for viral detection, hence contributing for more appropriate confinement policies by governments and could be further used to define more precisely post-pandemic or additional waves measures if/ when needed.

### 1. Introduction

According to the latest World Health Organization Situation Report the novel coronavirus disease 2019 (COVID-19) pandemic has severely affected the globe with more than 50 Million cases and up to 2.8 million deaths, as of April 1st 2021. This virus belongs to the family of RNA based coronaviruses (CoV) [119]. It contains membrane, spike (S), nucleocapsid (N) and envelope (E) proteins similar to other coronaviruses. It uses the host receptors to bind their spike proteins and ultimately infect the host [14,69]. Besides the fact that the virus infects their hosts with the same mechanism as other coronaviruses, it is also

genetically related to the coronavirus responsible for the severe acute respiratory syndrome (SARS) outbreak of 2003 and therefore can be recognized as (SARS-CoV-2) [85].

The intermediate source of origin and transfer of SARS-CoV-2 is unclear, but [8], provides two possible scenarios. Natural selection in an animal host before zoonotic transfer and selection in humans following zoonotic transfer. Furthermore, it is known to be passed through human interaction rapidly [96]. Also, several studies suggested the possibility of transmission via environmental media [7,12,55]. Official publications on this subject have been made by many international organisms including the World Health Organization (WHO) in July 2020 [116].

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<https://doi.org/10.1016/j.jece.2021.105881>

Received 10 November 2020; Received in revised form 15 April 2021; Accepted 13 June 2021

Available online 29 June 2021

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SARS-CoV-2 was recognized to be airborne by the WHO, strongly providing evidence of environmental factors that may/will affect its transmission [115]. Environmental factors are of special importance since they can extend the COVID-19 transmission period that is currently known to begin even when no symptoms are showing [98].

This review analyzes and summarizes the research related to SARS-CoV-2 and related viruses, aiming to explain how the SARS-CoV-2 is tracked, transmitted and inactivated in water/wastewater, surfaces and other environmental media. In order to determine the risk of the transmission from environmental media (water, air and soil/sludge) technologies for treatment were discussed. Similarly, how the environmental media conditions affect people in vulnerable social and economic conditions were also addressed. The review addresses the limitations of the detection methods and provides novel suggestions on how to use the viral detection methodology in wastewater to create a low-cost system able to track the viral presence in communities. Those types of systems could be used to oversee viral outbreaks, track the viral presence during pandemics and to make pandemic and post-pandemic decisions that could also affect the confinement strategies.

Relevant publications on the field found in Google Scholar, Scopus and Web of Science were employed. While choosing the work to be cited peer-reviewed papers were preferred. Previous literature on SARS was included as well as the current findings related to pandemics/epidemics.

## 2. Challenges in the detection of SARS-COV-2

### 2.1. Challenges of sampling and testing of SARS-COV-2 presence in water/wastewater

Sampling and testing for virus detection in water/ wastewater has been suggested and implemented in urban areas for routine surveillance purposes before the pandemic [37]. These ideas were further extended to be used for SARS-CoV-2, to monitor current epidemics and prognosis of possible outbreaks [110]. The global pandemic's unforeseen situation has created a worldwide urgency for the development of tests on a scale unseen before [97]. The development of standardized viral detection methods is challenging for natural bodies of waters or wastewaters due to problems such as the high variability of viral presence in a water/wastewater sample, limitations of adapting methods previously developed for clinical purposes [6,59], and intrinsic viral biology as its high mutation rates [58].

The most common water-borne viral pathogens are non-enveloped viruses (i.e., adenovirus, hepatitis B), hence the majority of tests for recovery of viruses on waters are designed for them. These viruses differ biochemically and structurally from enveloped viruses such as the SARS-CoV-2 [9]. Viral concentration method and test assay methods were the main methods used to detect SARS-CoV-2 on waters [59] that were initially used for non-enveloped viruses. These two methods are well known to be plagued with uncertainties, including sampling size, DNA/RNA stability in the sample, and primer accuracy. Additionally, the use of traditional non-enveloped viral methods on SARS-CoV-2 would have unexpected results like low recovery rates [9,118].

Concentration of the viral counts after sampling is the other challenge that viral detection in waters has [6]. An analysis of several studies on the detection of SARS-CoV-2 on wastewater shows that the concentration methods used to trap the virus are not yet standardized, since there is no systematic comparison among the various studies performed [3,5,74,117] and that the concentration method significantly varies between enveloped and non-enveloped viruses [75]. Surrogate viruses are used to study SARS-CoV-2 as an indicator of the recovery; however, their recovery values in relation to the surrogates vary too much and differ not only between wastewater samples but also between liquid and solid phase of the wastewater hence a standardized method has not been developed yet [75]. Similarly, the RNA extraction method is an important parameter to determine the RNA extraction efficiency, which will also affect the concentration and detection of the SARS-CoV-2 in

wastewater.

Studies report different quantitative values or even show discrepancies in determining the presence of SARS-CoV-2 on water/ wastewater. These discrepancies could be attributed to the volume of the sample or the COVID-19 cases in the region that relates to the viral presence in the sample [75]. Studies with untreated wastewater and tap water found differences between using direct RNA extraction via electronegative membranes and ultrafiltration [3–5,53]. Positive samples were obtained by the electronegative membrane method on SARS-CoV-2 and negative on the ultrafiltration, followed by second samples showing the inverse pattern. Additionally, electronegative membrane-vortex (EMV) methods and adsorption-direct RNA extraction methods exhibited differences in detection levels with better performance for the EMV method [50].

Despite the differences in detection methods of SARS-CoV-2, other methods employed successful detection in waters. For concentration, the electronegative membrane centrifugal filter method (the Centricon® Plus-70) and the polyethylene glycol (PEG) method were successfully used [74,117]. A study comparing the efficiency of concentration methods using a murine hepatitis virus (MHV), an RNA Betacoronavirus as control, also reported adsorption-extraction methods as optimal for enveloped viruses [3,5]. This study was the first to evaluate coronaviruses' different concentration methods, an essential step for decision-makers when implementing wastewater-based epidemiology monitoring systems. Finally, SARS-CoV-2 was detected using a skimmed milk organic flocculation method in Ecuador's rivers during the June 2020 outbreak [37]. In general, developing a methodology for estimating viral presence in rivers where dilution is very high is essential for environmental asset management and future impacts of SARS-COV-2 [47]. Up to date several papers investigating the concentration of SARS-CoV-2 from wastewater (Table 2) have been published. A summary of previous findings shows that many methods available to measure SARS-CoV-2 recovery have been tested with wastewaters from different global wastewaters. The results indicated that similar to surrogate studies, there is not a standard protocol to concentrate SARS-CoV-2 for complete or even high recovery.

Another key aspect of detecting SARS-CoV-2 on water is the selection of the assay and the criteria for a positive result. The real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) test is a governing technology employed in environmental sampling. Similarly, it is one of the most widespread methods for detecting the virus from nasopharyngeal swabs, throat swabs, or even saliva [95]. The detection assays consist of targeting the genes for the envelope (E), nucleocapsid (N), spike (S), RNA-dependent RNA polymerase (RdRp), and ORF1 [95]. In an assay developed by the United States Centers for Disease Control and Prevention (CDC) the N region is targeted at three different regions called N1, N2, and N3. The N1 and N2 are specific to detect the SARS-CoV-2, and the N3 is for the general Sarbecovirus, including the SARS-CoV-2 [67]. The Japanese National Institute of Infectious Disease (NIID) test also targets different regions in the N gene region. A comparison study of the main kits and test assays [70] found that the N2 assays of the US CDC and the NIID are the most sensitive. They also found the CDC N1 assay kit and the envelope protein E assay [30] to perform well. The combined literature suggests that primers targeting the N region of SARS-CoV-2 are going to be the most accurate ones for testing the viral presence in complex environmental media [30,70]. Studies performed up to date showed that successful RNA extraction could be done with commercial kits and N1 and N2 genes could successfully be used for the viral detection [10]. However, N\_Sarbeco assay reported positive results for SARS-CoV-2 did not provide the same results when performed with NIID\_2019-nCoV\_N assay suggesting that N region targeted assays also needs to be optimized or different regions in the SARS-CoV-2 genome needed to be used for detection [3,5,30,99].

The assays focusing on different genomic materials of SARS-CoV-2 were compared with results revealing inconsistency in terms of viral count. Differences among the N1 and N2 CDC assays and the E\_Sarbeco

**Table 1**Persistence of coronaviruses on different surfaces under different temperatures (inoculum of all the surfaces is either equal to or above  $10^3$  viral titer).

Virus	Surface/Media	Persistence	Temperature (°C)	Relative humidity (%)	Reference	
MERS-CoV	Steel	48 h	20		[108]	
		8–24 h	30		[108]	
	Plastic	48 h	20		[108]	
		8–24 h	30		[108]	
	Copper	>28 days	4		[108]	
		48 h	20		[108]	
SARS-CoV	Metal	2–24 h	30		[108]	
		5 days	20–22		[34]	
	Steel	50 h	21–23	40	[107]	
	Copper	20 h	21–23	40	[107]	
	Wood	4 days	20–22		[34]	
	Paper	<5 min up to 5 days	20–22		[34]	
	Cardboard	8 h	21–23	40	[107]	
	Disposable gown	1 h- 2 days	20–22		[64]	
	Plastic	4–9 days	22–25		[23,34,91]	
	75 h	21–23	40		[107]	
HCoV	Steel	5 days	21		[113]	
	Copper	<5 min	21		[113]	
	Aluminum	2–8 h	21		[100]	
	Glass	5 days	21		[113]	
	Plastic	2–6 days	20–22		[91]	
	PVC	5 days	21		[113]	
	Silicon rubber	5 days	21		[113]	
	Latex surgical glove	<8 h	21		[100]	
	Ceramic	5 days	21		[113]	
	Teflon	5 days	21		[113]	
	SARS-CoV-2	Paper	3 h	22	65	[25]
		Tissue paper	3 h	22	65	[25]
Wood		2 days	22	65	[25]	
Cloth		2 days	22	65	[25]	
Glass		4 days	22	65	[25]	
Banknote		4 days	22	65	[25]	
Copper		10 h	21–23	40	[107]	
Cardboard		35 h	21–23	40	[107]	
Stainless steel		7 days	22	65	[25]	
		60 h	21–23	40	[107]	
		7 days	22	65	[25]	
Plastic		80 h	21–23	40	[107]	
		7 days	22	65	[25]	
		7 days	22	65	[25]	
Mask inner layer		>7 days	22	65	[25]	
Mask outer layer	>7 days	22	65	[25]		
Hand soap (2%)	15 min	22	65	[25]		

**Table 2**

Recovery of SARS-CoV-2 from wastewater using concentration methods targeting N1 and N2 region via rt-PCR.

Methods for concentration	Volume of the sample used (mL)	SARS-CoV-2 seeded (GC/mL)	Sampling region	Mean amplification cycles with rt-PCR for minimum detection	Recovery after concentration (%)	Fold change after concentration	Reference
Ultrafiltration	8	none	Italy	36.38		3.9	[41]
	250	none	Spain		8–23		[87]
Double ultrafiltration	8	none	Sweden	36.64–38.15		1.1–6.2	[41]
Polyethylene glycol (PEG) precipitation	200	$4.3 \times 10^3$	Argentina		2.7–16.7		[13]
	200	$4.3 \times 10^2$	Argentina		0–26.4		[13]
Aluminum polychloride (PAC) flocculation	200	none	Argentina	Not detected (<45)			[13]
	200	none	Spain		38–52.8		[84]
	200	$4.3 \times 10^3$	Argentina		0.7–21.0		[13]
	200	$4.3 \times 10^2$	Argentina		7.8–93.6		[13]
	200	none	Argentina	Not detected (<45)			[13]
Skimmed milk flocculation	200	none	Spain		30.2–42		[84]
	250	none	Spain		23–37		[87]
Electropositive filtration	100	none	China		0		[29]
	100	$1.0 \times 10^2$	China		1		[29]
	100	$1.0 \times 10^3$	China		21.4		[29]

assay in different samples were found in the Netherlands [74]. La Rosa et al. [63] found that the use of open reading frames (ORF) targets differ from the use of spike protein for SARS-CoV-2 detection on wastewater samples taken from an Italian site. Randazzo et al. [93] also observed a differential pattern on the use of the N1, N2, and N3 assays of the CDC. They found positive but different results among the three N1, N2, and

N3 RT-qPCR assays in water treatment plant on different regions of Spain. The study also found two positive cases over 18 samples of secondary treated waters using the CDC assays, one positive for the three N1, N2, and N3 CDC assays, and the other was only positive for the N2 assay. Those results clearly state that the environmental assays' methods drive the positive results of the viral presence.

The size and viral content in a sample also makes the minimal load of detection (LOD) one of the most important criteria when sampling from the environment. In the specific case of a study made in Australia [3,5] differences between positive results were observed. The possible cause of these differential results between assays could be explained by presence of minimum LOD value with N\_Sarbeco assay and lack of it with the NIID\_2019-nCoV\_N assay. Further, the automated diagnostic equipment currently used for COVID19 testing, commonly works with high LOD compared to the actual viral load at water bodies. Considering this low viral load levels in water is essential to define if this parameter would likely be used as an alert system for future outbreaks or even for detecting cases in a community. For such measures to be valid, a standardized quality controlled viral measure needs to be developed.

The challenges for detection of SARS-CoV-2 in environmental samples could include the rate of mutations that causes mismatches [58,83] or the false-negative rate (FNR) changes within time [61]. The FNR of the tests for detecting SARS-CoV-2 is around 30% [61,119]. One of the reasons for this FNR percentage is that the N gene presents multiple mutation hot-spot loci [119]. The existence of mutation hot spots supports the stress on targeting different viral genome regions of the different detection assays to mitigate the loss of sensitivity of our testing [83]. These challenges are considered intrinsic to SARS-CoV-2 biology for testing purposes and are extensively covered in clinical literature. However, they are not considered in the environmental detection studies. This multitargeting genome practice will be critical in water, where it is notably observed that results will vary with the type of viral concentration technique, and the detection assay elected for the analysis.

## 2.2. Detecting SARS-COV-2 on surfaces and environmental media

One of the main concerns that COVID-19 brought to society was the possibility of spreading not only from symptomatic or non-symptomatic patients but also from surfaces (like packaging materials, fruits and vegetable skins, etc.) and media like air and water. To understand how viral survival will depend on environmental media and the effect if the type of the media has any effect on its survival, analysis of the viral survival needs to be made on different types of media. Previous studies focusing on the survival rates and persistence of different types of coronaviruses on diverse surfaces concluded that the amount of virus present on a surface affected their persistence, being higher viral titer related to longer virus persistence [64]. Similarly, strain type within the same type of coronaviruses (SARS-CoV) showed differences in persistence [57] even if the same amount of viral titer had been applied to the same surface [23,34,64,91]. These studies suggest that persistence changes are based on virus strain. Even though the persistence times varies for each type of coronaviruses in different materials, these differences for most materials were not drastically different. Hence, it can be concluded that coronaviruses (including COVID-19 data) show more or less similar characteristics of survival on solid surfaces. Temperature seemed to be the driving effect on the survival rates on surfaces. Also, increasing the temperature from 4 °C to 30 °C lowered persistence (Table 1). Now, focusing on analyses done on SARS-CoV-2 survival on surfaces, it is concluded that less smoother the surface was, the longer the virus survived [25]. This finding was consistent with the viral survival and could be explained by exposability of the virus to the environment. In other words, the virus is less protected in smoother surfaces, and therefore more exposed to potentially toxic compounds that could inactivate it [109]. The conclusion raises a concern that SARS-CoV-2 will be viable to remain longer on uneven surfaces such as: porous vegetables and fruits and also suggests that SARS-CoV-2 would survive a lot longer in environmental samples like soil and sludge that have very irregular surfaces. The results were similar to a test conducted with bovine coronaviruses using adsorbent environmental materials like clay, charcoal, kaolinite and others [27]. This study showed that coronaviruses will be adsorbed to adsorptive materials (above 95% absorption)

and will not be adsorbed by the non adsorptive materials like sand [27]. Survival of the virus in the environment also presents an obstacle with detection of SARS-CoV-2 in wastewater. Because the double life of the virus at ambient conditions changes from 4 to 8 h [6] composite sampling is a necessity for detection. Similarly the temperature of storing the composite creates variations in the detection of the virus and shorter storing times decreases the detection limits suggesting that the sample should not be concentrated immediately [6].

Wastewater is a residue that plays an important role in society, because the exposure to wastewater and its byproducts is inevitable. Up to this date, wastewater in pandemics has been considered to be used as a tool [66,74,92] for early detection; however it also carries a danger for transmitting the virus [46,121]. Hence, understanding the persistence of the coronavirus is extremely important in environmental media like water, wastewater, wastewater related sludge and aerosols. Available data reveals that coronavirus persistence varies in different environmental media (Table 3). Similar to solid surfaces, temperatures being below 30 °C increased the persistence of coronaviruses. Further studies with SARS-CoV-2 revealed that the virus was very stable (more than 7 days) on temperatures below 37 °C and the stability of the virus decreased as temperature increased [25]. The effect of pH was assessed on SARS-CoV-2 survival and it was concluded that pH did not have an effect on the virus stability [25]. This finding was consistent with previous coronavirus studies reporting high variability of pH dependence on viral fusion by variation of three amino acids in the viral glycoprotein [42]. Overall comparison of coronaviruses persistence showed that viruses could survive a lot longer on water, wastewater and sludge than any surface. This information revealed that the risk of virus transmission is a lot higher from wastewater contaminated areas than from contaminated surfaces.

Since wastewater creates aerosols, it is essential to know what the survival rates of SARS-CoV-2 are in wastewater created aerosols, both to evaluate the risk of the employees of waste management facilities and wastewater treatment plants and also to anybody that might be exposed to or near any wastewater related sewage [28,80]. Transmission being reported to be mainly via air [112] the survival of SARS-CoV-2 and some coronaviruses was tested on air via aerosols (Table 3). The analyses concluded that the virus survival rate in aerosols is relatively long and it can persist for more than 3 h. However, no correlation was found with relative humidity. Future studies are needed to understand what affects the survival rates of the virus in the environment and especially on aerosol particles if the main transmission mechanism is via air.

Wastewater treatment systems produce sludge, which is normally treated and applied as soil aggregate in agricultural sites. Therefore, it is important to evaluate the virus transmission risk within the sludge [16, 81,93]. At this point, no conclusive correlation has been made between the coronaviruses survival rates in sludge versus water (Table 3). The studies up to this point revealed that the presence of disinfectants drive the survival rates of coronaviruses in water and sludge [62]. Hence, the risk in the environmental media is still present especially because water and sludge applied or discharged to the environment cannot contain high levels of disinfectants. The practices of using untreated municipal sludge or raw wastewater is a common practice in urban and peri-urban areas of the developing world [68]. Therefore, the COVID-19 transmission risk is quite high in the areas where untreated sludge from wastewater treatment plants or raw wastewater is disposed or reused.

While estimating the risk of the SARS-CoV-2 presence in the environmental media, the quantity of the viral presence should be taken into consideration [54]. Based on the studies anywhere from 1 to 10 infectious viral particles per litre of water (or gram of soil) can become a health risk, since lower numbers of viral count would not carry a high risk in environmental media [54]. Recent studies on primary sludge instead of wastewater showed that SARS-CoV-2 virus could be tracked successfully in a community via qRT-PCR using the same N1 and N2 primer sets employed in COVID-19 individual testing [90]. Testing sludge in a vulnerable area had viral copies ranging from 1.7 Å~103



**Table 3**

Persistence of coronaviruses on different media related to wastewater under different temperatures (inoculum of all the surfaces is either equal to or above  $10^6$  viral titer).

Virus	Media	Persistence	Temperature (°C)	Relative humidity (%)	Reference
SARS-CoV	Sterilized water	3–4 days	21–23		[34]
	Dechlorinated tap water	>14 days	4		[112]
	Dechlorinated tap water	2 days	20		[112]
	Wastewater	2 days	20		[112]
	Domestic sewage	>14 days	4		[112]
	Domestic sewage	2 days	20		[112]
	Aerosol	>3 h	21–23	65	[107]
HCoV	Sludge	96 h	22		[20]
	Tap water	>390 days	4		[48]
	Tap water	10–13 days	23		[48]
TGEV	Wastewater	2–4 days	23		[48]
	Reagent grade water	>49 days	4		[21]
	Reagent grade water	>15 days	25		[21]
MHV	Lake water	>14 days	4		[21]
	Wastewater	>9 days	23–25		[21]
	Pasteurized sludge	>35 days	4		[21]
	Reagent grade water	>15 days	4		[21]
	Reagent grade water	>49 days	25		[21]
SARS-CoV-2	Lake water	>49 days	4		[21]
	Wastewater	>7 days	23–25		[21]
	Pasteurized sludge	>35 days	4		[21]
	Aerosol	>3 h	21–23	65	[107]
	Aerosol –tissue culture media	180 min	19–22	68–88	[101]
SARS-CoV-2	Aerosol- tissue culture media	360 <sup>a</sup> min	19–22	40–60	[101]
	Aerosol- artificial saliva	600 <sup>a</sup> min	19–22	68–88	[101]
	Aerosol- artificial saliva	240 min	19–22	40–60	[101]

<sup>a</sup> Extrapolated based on the data plots

$\text{mL}^{-1}$ – $4.6 \times 10^5$ – $10^5 \text{ mL}^{-1}$  of primary sludge [90] which is also a health threat. Therefore, while monitoring water/ wastewater/ sludge the viral quantity affecting human health should be considered, specially when monitoring waterborne disease outbreaks [44].

### 3. Managing SARS-CoV-2 in wastewater and sewage systems

Measurements of SARS-CoV-2 survival reveal the virus is inactivated in less than 5 min when disinfectants like conventional bleach (1%, 2%), ethanol (70%), povidone iodine (7.5%), chloroxylenol (0.05%), chlorhexidine (0.05%) and benzalkonium chloride (0.1%) are applied and less than 15 min when hand soap (2%) is used [25]. However, the application of these high concentrations to wastewater is difficult due to the high quantities required.

Due to the widespread pandemics, the transmission risk is not only confined to hospital waste systems, but to the sewage system and wastewater plants. This situation makes areas exposed to wastewater and its residues vulnerable to transmission also.

#### 3.1. Treating the virus: Coronavirus inactivation in wastewater and wastewater residues

Even though virus removal is not a design parameter for domestic wastewater treatment plants due to the lack of viral regulations, it has been reported viral removal in wastewater treatment plants in primary and secondary treatment, as well as with membrane bioreactors (MBR). [120]. Viruses are inactivated by disinfection at the tertiary treatment prior to discharge or reuse of the effluent water [22].

Most of the viral presence in wastewater is expected to be in the sludge [52], with reported viral count in activated sludge to be ranging from 0.7 to 2.9 Log [52]. Therefore, the virus removal process has been explained by flock formation in the sludge, followed by settling. For the case of MBRs, the settling process is replaced by flock removal by the membrane filters. Therefore, the removal of viruses will not happen in sewage systems, sludge lines or in small treatment facilities like septic tanks. This situation makes specially vulnerable small communities or rural areas.

The disinfection for virus removal in a conventional wastewater treatment plant is achieved either by chlorination, ozonation or UV application. One study conducted in China revealed that concentrations of 6700 mg/L of sodium hypochlorite (equivalent to 350 mg/L of chlorine dose) are needed to inactivate SARS-CoV-2 and viral RNA, for them to not be observed in the hospital wastewater effluent. This high dosage also brings issues of high trichloromethane, tribromomethane, bromodichloromethane and dibromochloromethane concentrations in the effluent [121]. Studies focusing on removal of coronaviruses from municipal wastewater were previously conducted with SARS-CoV with results demonstrating that coronavirus survival is dependent not only on the dose applied but also on the time of exposure (Table 4). Even though increasing the concentration dose decreases the contact time needed for full inactivation, it also increases the residual chlorine concentration [112]. It was also shown that the inactivation of SARS-CoV could be achieved at 20 °C and retention times of 30 min with residual chlorine or chlorine dioxide at concentrations above 0.5 mg/L and 2.19 mg/L respectively [24].

One of the most promising technologies for disinfection and inactivation 'mkm of viruses' is UV. Virus reduction can be achieved in environmental media using a UV dose between 1 and 200 J/m<sup>2</sup> at 254 nm based on the type of virus that needs to be inactivated as long as transmittance of water and the exposure of outer layers of porous bio-solids are considered. The measured inactivation dose for coronaviruses was estimated to be achieved with 40 J/m<sup>2</sup> in air samples [31]. However, tests conducted with SARS-CoV-2 revealed contradictory information, showing that the virus is viable even after being exposed to UV for more than 60 min [31]. Nevertheless, this study [31] was not conducted under a UV generator for sterilization but was just placed in a UV laminar hood in a laboratory. Aerosol containing HCoV under different

**Table 4**  
Doses and time needed for total inactivation of SARS-CoV [112].

SARS-CoV	Chlorine		Chlorine dioxide	
	Dose (mg/L)	Time (min)	Dose (mg/L)	Time (min)
	10	10	10	> 30
	20	<1	20	5

UV wavelengths showed that doses required to inactivate the virus ranged between 1.2 and 1.7 J/m<sup>2</sup> [18]. Even though particular studies have not been done directly with wastewater, known technologies can generate dosages between 1 and 200 J/m<sup>2</sup> and could be easily modified to dose sufficient UV especially at 254 nm as long as the transmission of UV to the wastewater considered the turbidity and UV transmittance for successful virus elimination [45]. The advantage of employing UV for wastewater treatment for SARS-CoV-2 is the absence of residues resulting in toxic byproducts.

### 3.2. Designing sewage systems that ease the SARS-Cov-2 / COVID-19 detection

Presence of active SARS-CoV-2 has not been observed at the effluent of wastewater treatment plants [38]. Contrarily, viral presence in the untreated wastewater has been observed in many places globally [28]. The viral presence does not always mean infection risk in the wastewaters, it has been reported that SARS-CoV-2 RNA could be present in wastewater samples while the virus is inactivated [3,5]. However, the analysis of viral presence in wastewater could provide a tool to determine the physical areas where transmission or the number of infected people is high [38]. Currently, sampling sewage entering wastewater treatment plants has been either planned for large urban areas, or is currently being applied to have an idea of viral presence and/or monitor possible waves of viral transmission [1,26,75,76].

Unless designed for a very small community, sampling from a wastewater treatment plant influent, would not narrow down a specific area to take any preventative actions. Hence, recent discussions focused on sewer line detection strategies put forward the idea of monitoring infection status in urban areas by wastewater based epidemiology [28, 33]. Even some candidate biomarkers and metagenomic analysis specifically for SARS-CoV-2 have been published lately [79].

The solutions found in literature point to combining principles of wastewater epidemiology with those of environmental engineering by defining sewer sampling points for estimating viral counts. The main challenge is how to determine the minimum household number that SARS-CoV-2 can be detected in the wastewater at the sampling points. Economic, demographic and public health aspects of the region need to be considered while establishing the minimum household requirement. Existing sewer system characteristics need to be known not only to determine the possible flow and sampling availability, but also to consider possible dilution of biological material with rainwater for the case of combined sewage systems. The available points in the sewage system for sample analysis are usually open channels, pumping stations and inspection chambers. Sample points location will depend on the geography, sampling availability and the number of people covered. Risk assessment needs to be done in terms of how many households and occupants will be benefited by having such a sampling and assessment system.

The proposed solution is to develop an optimal model with continuous monitoring of every sewage line is compared with the number of people that needs to be tested including economical factors and time based strategies. Such a model could help to make pandemic and post-pandemic decisions [105]. Testing populations through wastewater production would be especially useful in countries with limited resources, providing a global approach to keep wastewater testing and to inform confinement strategies [11]. An example of the proposed system is provided on Fig. 1 with a hypothetical sewage line. Based on this model, a sampling analysis frequency of two times a day on peak flows is proposed. Representative available sewage line sampling points were selected based on 1) location (distance of each other and distance of the community), 2) joint points to determine differences in different areas and 3) sampling size representing the maximum number of houses that could provide meaningful representation. On this figure every sampling point is assumed to contain no more than 100 houses contributing to the sewage stream line for minimal detection, the sampling number was

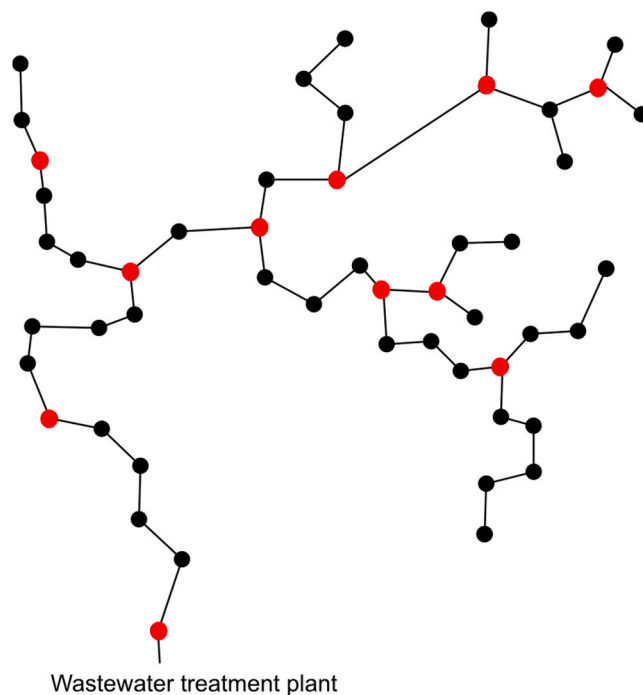


Fig. 1. Proposed sampling points (red) of a sewage line on a hypothetical sewer map. Every dot represents a sewage line entrance of a community with minimum 10 houses where length of the lines are representing the distances of each sewage line from each other. Sampling was proposed to be twice a day (morning and evening) on the peak flow. Every dot represents an available sampling line.

chosen considering 10,000 viral counts per sick person and assuming average of 4 people living in every household to get sufficient RNA for PCR [12]. This figure is provided to clarify and explain the idea mentioned and is hypothetical. Similar maps should be obtained with optimization models for every location separately for further applications.

### 4. Societal issues and risks of COVID-19 on rural / impoverished populations

Poverty and inequality go hand in hand [19]. Dwellers in non-consolidated informal settlements do not count on having a community treatment plant. Usually, the provision of such service does not begin until many years later along their settlement, once the process of the consolidation of formality begins. The only option is to let wastewater run through their premises, affecting their and neighbors' health conditions, especially those of children [82]. Under such circumstance common waterborne diseases and even SARS-CoV-2 aerosols might impact the population [2,88].

More interestingly, not having direct clean access to water, can also be the source for water-borne diseases, some of which may have a detrimental effect on health for life or lead to death. A serious issue with not having in-house water sources, is that accessing water through other means may increase the exposure to disease risk [77,104]. When the water does not reach a household directly, going out and getting it will expose the individuals to sudden site overcrowding, which may become a hazardous scenario for any infectious diseases and specially for COVID-19 [102].

Usually, viruses and water are seen related during water-borne virus outbreaks. For example in the US between 1971 and 2006, 64 outbreaks were reported and 136 in Europe from 2000 to 2007 [39]. However, even though COVID-19 might not be considered a water-borne virus, water is at the center of prevention. Having access to clean water is

critically important in order to comply with WHO [116] recommendations of keeping hands washed thoroughly and constantly, as well as for washing food and keeping spaces and objects clean. For all of those chores and other aspects of family sanitation, not only quality but quantity of clean water matters. Therefore, the COVID-19 pandemic should bring a fresh reminder of the importance of prioritizing investments in water infrastructure, water treatment, food contamination testing and proper hygiene campaigns [49].

It is a known fact that there is a strong positive correlation between testing and the number of reported COVID-19 cases [94]. However, for developing countries where genomic testing facilities will be hard to establish or equip, it will be critical to develop water-based epidemiology which can in turn be a key factor to determine the health of a community [32]. This becomes even more critical in countries where up to 60% of the wastewater end up in the urban and rural water system used as drinking water sources due its infiltration [16]. An example of this situation is brought forward in [103], where the case of Sub-Saharan African case is presented. The authors pose the question on how to make SARS-CoV-2 water-based surveillance systems on places that do not have wastewater treatment plants, and some practical solutions are given, some of them are paper based wastewater tests and the use of portable devices for testing [72]. Several different technologies have also been developed for SARS-CoV-2 being a center of interest. Sensing technologies based on surface visualization/characterization tools such as AFM, EM, XRD were used to detect SARS-CoV-2 based on the viral shape [17,73]. Point of care detection of Sars-Cov-2 methodologies were used with blood or urine to detect the virus via cost effective lateral flow assays. Similar methods were used for biosensors tests and nanotechnology-based approaches using nanoparticles with DNA and protein based technologies with upper respiratory specimens, blood or urine to detect Sars-Cov-2 [56]. Biosensor tests include chip-based, paper-based, film-based, thread-based, graphene-based and black phosphorous based biosensors which are focusing on rapid and affordable detection that could be further coupled with smartphone analyses [35]. Further work has been done to improve the PCR analyses via rapid kit testing or use aerosol samples to further detect SARS-CoV-2 presence to prevent direct contact and have analyses for low viral presence [65]. The most recent efforts were done by using extensive databases to predict COVID-19 cases [60] and use machine learning tool such as Logistic Regression, Support Vector Machine, Gradient Boosted Decision Tree, and Neural Network methods to model the COVID-19 outbreak [43].

It is under this scope that considerations on how to improve access to clean water can follow the policies introduced by the Sustainable Development Goals [114]. Which also include actions geared to reduce economic and social inequality. In this regard, policy makers must address three issues necessary for water provision and to be able to reduce COVID-19 and other risk problems: 1) development, adaptation and/or improvement of city-wide, country-wide if possible, potable water distribution networks; 2) ensuring the quality, quantity, water pressure, strengthening of flow, of the water service; and 3) governance of the water resources.

The issue with water and wastewaters also become one societal issue in nature, with current methods providing a measure of the presence of the virus, however not answering if this discovery should be applied to nearby sources, or to the whole population of a city, regardless of closeness to hospitals, or the access to sewage systems. Moreover, it needs to be said that the risk assessment has been chiefly performed from a biological and epidemiological perspective. However, more research is needed to understand the interface between environmental conditions and issues that affect people in highly vulnerable social and economic conditions. Water and wastewater management needs further consideration in the strategies in place to gain control over the COVID19 pandemic. Not only because potable water is a vital resource for a healthy life and its role in the household's sanitation routines, but also especially because low-income communities have serious issues with

wastewater treatment. This is a problem that also encompasses the access to clean and reliable water sources [16,102].

## 5. Concluding remarks

COVID-19 has become a staple of society for good and for bad, changing the way we interact, travel and carry about our normal lives. There are several factors fueling this pandemic, among them, the ease of spreading from human to human and a great deal of environmental factors that need to be unmasked [89]. This article evaluates the research related to SARS-CoV-2 and the possibility of detection in the environment and in sewage lines.

The severe effects of COVID-19 in humans may have prevented more thorough environmental studies from being carried out. Most of the studies describing the issue come from the medical field analyzing the outcomes of patients and their comorbidities, and are usually performed in the clinical settings in hospitals and health facilities. Therefore, understanding viruses closely related to SARS-CoV-2 could help researchers to devise novel methods to limit its transmission via environmental media. More importantly relevant studies can help manage risks of the current pandemic.

Understanding environmental factors, applying the existing technologies and predicting risks due to environmental contact is essential to overcome the pandemic effects. It also can help society prepare for post pandemic issues. For instance, many political, sociological and psychological problems that became evident after the SARS epidemic [40, 51,71] have now been reported now for COVID-19 pandemic [86,111].

In terms of SARS-CoV-2 detection in samples of either human or environmental media, it seems that some technologies are better suited than others, with no standardized method. Various detection methods can be reduced to just answering the question of presence. However, the issue of quantification becomes one of where to put the threshold of determination. For sludges this issue boils down to how to extract the material to actually measure viral presence in such porous surfaces. RNA based detection techniques in solids or solid containing mixtures not only face problems with rapid RNA degradation in the samples but also difficulties in RNA extraction from samples containing high humic acid as well as low efficiency of RNA to cDNA conversion. Therefore, methodologies of rapid RNA extraction with humic acid cleaning steps may provide data for the virus but that data will not exactly be quantitative. Moreover, the detection methodologies up to date are good to determine the presence of SARS-CoV-2 RNA in wastewater or in sludge, however this is not necessarily indicative of the virulence of the virus itself, because the SARS-CoV-2 RNA could be present, but the virus can be inactivated.

Beside looking at SARS-CoV-2 RNA fragments, recently researchers began to look at structural proteins, N (nucleocapsid), M (membrane), S (spike), and E (envelope), shedding that can be found in higher copy numbers in wastewaters and sludges are in general are more stable than viral RNAs [78]. Using proteins as a markers based on antigens and could reduce the risk of false positives obtained with DNA techniques like PCR because they cannot be directly amplified. Direct detection of the SARS-CoV-2 can be done by enzyme-linked immunosorbent assay (ELISA), Chemiluminescence immunoassay (CLIA) and COVID antigen assay that is based on antigens with its specific antibodies for COVID-19 based on ELISA or CLIA. This assay could fail when antibodies against the proteins are not available or alternative affinity ligands are not present [35,36]. Therefore, this western blot-based methodology still has its ways to go, to become a Mass Spectrometry based solution or to be comparable to PCR methods and to newer CRISPR, Digital PCR and Lab-on-chip technologies [12].

Further advanced molecular genetic tools that were developed for gene editing were also used for SARS-CoV-2 detection. Those techniques are based on RNA and can be summarized as CRISPR-Cas12/13-based SHERLOCK, DETECTR, CARVER and PAC-MAN, antisense oligonucleotides, antisense peptide nucleic acids, ribozymes, aptamers, and RNAi

silencing. Among those methods ASO or peptide nucleic acid are considered to be the most applicable ones because of the low cost [15, 35]. Other RNA based techniques for SARS-CoV-2 detections are Nucleic Acid Hybridization Using Microarray and Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP). This technique is based on cDNA amplified from RNA and Amplicon-Based Metagenomic Sequencing using different sequencing methods such as MinION or Illumina [35].

In conclusion, SARS-Cov-2 detection capability could be used to predict pandemic cases, this could be especially helpful to monitor what are otherwise asymptomatic carriers. Moreover, the wastewater sampling points should be engineered focusing on pipe joints of water arriving from smaller communities, this will enhance the in-site data collection. This information could be used to design systematic sampling points throughout the sewage systems when available, taking into account peak flows caused by rain flow or other common events. Such an approach will be able to provide an insight of the viral outbreak [106]. More importantly will save financial resources that otherwise will be spent in testing every person in the community. Hence it can contribute with the definition of confinement policies, and governments can have the systems fixed in place to be used as post-pandemic measures if/when needed. The only caveat is that sewage systems need to be present for this to happen. Hence, vulnerability of the wastewater carrying the virus should be highly considered in low-income areas and counties where sewage cannot be handled.

With the mutation of the SARS-CoV-2 more strains with different viral counts per sick individual have been observed. Difficulty of providing and implementing vaccines globally indicates that viral presence in waste and wastewaters will be a concern. Further research is needed to define what the minimum number of detected genes means for the transmission, to standardize testing methodologies in waste and waste waters. Fulfilling the needs could be used to define proper legislations to implement treatment and handling of waste/wastewater that will prevent further viral contingency.

#### CRedit authorship contribution statement

**Javier E. Sanchez-Galan**: Writing - original draft, Writing - reviewing & editing, **Grimaldo Ureña**: Writing - original draft, Critically analyzing detection methodologies **Luis F. Escovar**: Reviewing and editing the wastewater treatment technologies, **Jose R. Fabrega**: Editing and reviewing, **Alexander Coles**: Drafting the socioeconomic relationships, Review & editing. **Zohre Kurt**: Writing - original draft, Critically analyzing the literature for persistence, Detection and treatment technologies, Writing - reviewing & editing, Providing article idea, initiating the work, gathering the author team and assessing the gaps in the literature.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgments

The authors thank the Sistema Nacional de Investigación (SNI) of National Secretariat for Science, Technology and Innovation, Government of Panama which supports research activities by Z.K., J.R.F-D and J.E.S.-G. Part of this project was financially supported by the Secretaría Nacional de Ciencia, Tecnología e Innovación (SENACYT; IOMA17-010).

#### Conflicts of Interest

The authors declare no conflict of interest.

#### References

- [1] Abu-Ali, H., Yaniv, K., Bar-Zeev, E., Chaudhury, S., Shaga, M., Lakkakula, S., Ronen Z., Kushmaro A., & Nir, O. (2020). Tracking SARS-CoV-2 RNA through the wastewater treatment process. medRxiv.
- [2] B. Adelodun, F.O. Ajibade, R.G. Ibrahim, H.O. Bakare, K.-S. Choi, Snowballing transmission of COVID-19 (SARS-CoV-2) through wastewater: any sustainable preventive measures to curtail the scourge in low-income countries? *Sci. Total Environ.* 742 (2020), 140680.
- [3] W. Ahmed, N. Angel, J. Edson, K. Bibby, A. Bivins, J.W. O'Brien, P.M. Choi, M. Kitajima, S.L. Simpson, J. Li, B. Tscharke, R. Verhagen, W.J.M. Smith, J. Zaugg, L. Dierens, P. Hugenholz, K.V. Thomas, J.F. Mueller, First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community, *Sci. Total Environ.* 728 (2020), 138764.
- [4] W. Ahmed, V. Harwood, P. Gyawali, J. Sidhu, S. Toze, Comparison of concentration methods for quantitative detection of sewage-associated viral markers in environmental waters, *Appl. Environ. Microbiol.* 81 (2015) 2042–2049.
- [5] W. Ahmed, P. Bertsch, A. Bivins, K. Bibby, K. Farkas, A. Gathercole, J. Mueller, Comparison of virus concentration methods for the RT-qPCR-based recovery of murine hepatitis virus, a surrogate for SARS-CoV-2 from untreated wastewater, *Sci. Total Environ.* 739 (2020), 139960.
- [6] N. Alygizakis, A.N. Markou, N.I. Rousis, A. Galani, M. Avgeris, P. G. Adamopoulos, N.S. Thomaidis, Analytical methodologies for the detection of SARS-CoV-2 in wastewater: protocols and future perspectives, *Trends Anal. Chem.*: TRAC 134 (2021), 116125.
- [7] I.D. Amoah, S. Kumari, F. Bux, Coronaviruses in wastewater processes: source, fate and potential risks, *Environ. Int.* 143 (2020), 105962.
- [8] K.G. Andersen, A. Rambaut, W.I. Lipkin, E.C. Holmes, R.F. Garry, The proximal origin of SARS-CoV-2, *Nat. Med.* 26 (4) (2020) 450–452.
- [9] C. Annalaura, F. Ileana, L. Dasheng, V. Marco, Making waves: coronavirus detection, presence and persistence in the water environment: state of the art and knowledge needs for public health, *Water Res.* (2020), 115907.
- [10] C. Ambrosi, C. Prezioso, P. Checconi, D. Scribano, M. Sarshar, M. Capannari, D. Limongi, SARS-CoV-2: comparative analysis of different RNA extraction methods, *J. Virol. Methods* 287 (2021), 114008.
- [11] A. Atalan, Is the lockdown important to prevent the COVID-19 pandemic? Effects on psychology, environment and economy-perspective, *Ann. Med. Surg.* 56 (2020) 38–42.
- [12] D. Barceló, Wastewater-based epidemiology to monitor COVID-19 outbreak: present and future diagnostic methods to be in your radar, *Case Stud. Chem. Environ. Eng.* 2 (2020), 100042.
- [13] P.A. Barril, L.A. Pianciola, M. Mazzeo, M.J. Ousset, M.V. Jaureguiberry, M. Alessandrello, J.M. Oteiza, Evaluation of viral concentration methods for SARS-CoV-2 recovery from wastewaters, *Sci. Total Environ.* 756 (2021), 144105.
- [14] S. Belouzard, J.K. Millet, B.N. Licitra, G.R. Whittaker, Mechanisms of coronavirus cell entry mediated by the viral spike protein, *Viruses* 4 (2012) 1011–1033.
- [15] B. Berber, C. Aydin, F. Kocabas, G. Guney-Esken, K. Yilancioglu, M. Karadag-Alpaslan, C. Tastan, Gene editing and RNAi approaches for COVID-19 diagnostics and therapeutics, *Gene Ther.* 28 (2021) 290–305.
- [16] S.K. Fanourakis, J. Peña-Bahamonde, P.C. Bandara, D.F. Rodrigues, Coronavirus disease 2019 (COVID-19) outbreak: some serious consequences with urban and rural water cycle, *Clean. Water* 3 (2020) 1–8.
- [17] N. Bhalla, Y. Pan, Z. Yang, A.F. Payam, Opportunities and challenges for biosensors and nanoscale analytical tools for pandemics: COVID-19, *ACS nano* 14 (7) (2020) 7783–7807.
- [18] M. Buonanno, D. Welch, I. Shuryak, D.J. Brenner, Far-UVC light (222 nm) efficiently and safely inactivates airborne human coronaviruses, *Sci. Rep.* 10 (2020) 1–5.
- [19] F. Bourguignon, The poverty-growth-inequality triangle, *Indian Counc. Res. Int. Econ. Relat.* (2004).
- [20] F. Carraturo, C. Del Giudice, M. Morelli, V. Cerullo, G. Libralato, E. Galdiero, M. Guida, Persistence of SARS-CoV-2 in the environment and COVID-19 transmission risk from environmental matrices and surfaces, *Environ. Pollut. (Barking, Essex: 1987)* 265 (2020), 115010.
- [21] L. Casanova, W.A. Rutala, D.J. Weber, M.D. Sobsey, Survival of surrogate coronaviruses in water, *Water Res.* 43 (2009) 1893–1898.
- [22] C. Chahal, B. Van Den Akker, F. Young, C. Franco, J. Blackbeard, P. Monis, Pathogen and particle associations in wastewater: significance and implications for treatment and disinfection processes, in: *Advances in applied microbiology*, 97, Elsevier, 2016, pp. 63–119.
- [23] K.-H. Chan, J.M. Peiris, S. Lam, L. Poon, K. Yuen, W.H. Seto, The effects of temperature and relative humidity on the viability of the SARS coronavirus, *Adv. Virol.* 2011 (2011), 734690, 2011.
- [24] C. Chen, X.-J. Zhang, Y. Wang, L.-X. Zhu, J. Liu, Wastewater disinfection during SARS epidemic for microbiological and toxicological control, *Biomed. Environ. Sci.*: BES 19 (2006) 173–178.
- [25] A. Chin, J. Chu, M. Perera, K. Hui, H.-L. Yen, M. Chan, M. Peiris, L. Poon, Stability of SARS-CoV-2 in different environmental conditions, *Lancet Microbe* 1 (2020) 10.
- [26] Centers for Disease Control and Prevention (CDC). (2020, Oct). National Wastewater Surveillance System (NWSS). National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases. (<https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance.html>).



- [27] K. Clark, A. Sarr, P. Grant, T. Phillips, G. Woode, In vitro studies on the use of clay, clay minerals and charcoal to adsorb bovine rotavirus and bovine coronavirus, *Vet. Microbiol.* 63 (1998) 137–146.
- [28] M.C. Collivignarelli, C. Collivignarelli, M.C. Miino, A. Abbà, R. Pedrazzani, G. Bertanza, SARS-CoV-2 in sewer systems and connected facilities, *Process Saf. Environ. Prot.* 143 (2020) 196–203.
- [29] Colosi, L.M., Barry, K.E., Kotay, S.M., Porter, M.D., Poulter, M.D., Ratliff, C., & Mathers, J. (2020). Development of wastewater pooled surveillance of SARS-CoV-2 from congregate living settings. medRxiv.
- [30] V.M. Corman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D.K. Chu, T. Bleicker, S. Brünink, J. Schneider, M.L. Schmidt, D.G. Mulders, B.L. Haagmans, B. van der Veer, S. van den Brink, L. Wijsman, G. Goderski, J.L. Romette, J. Ellis, M. Zambon, M. Peiris, H. Goossens, C. Reusken, M.P. Koopmans, C. Drosten, Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, *Eur. Surveill.: Bull. Eur. Sur Les. Mal. Transm. = Eur. Commun. Dis. Bull.* 25 (2020), 2000045.
- [31] M.E. Darnell, K. Subbarao, S.M. Feinstone, D.R. Taylor, Inactivation of the coronavirus that induces severe acute respiratory syndrome, SARS-CoV, *J. Virol. Methods* 121 (2004) 85–91.
- [32] C.G. Daughton, Wastewater surveillance for population-wide Covid-19: the present and future, *Sci. Total Environ.* 736 (2020), 139631.
- [33] C.G. Daughton, Monitoring wastewater for assessing community health: Sewage Chemical-Information Mining (SCIM), *Sci. Total Environ.* 619 (2018) 748–764.
- [34] J.H.X.-P. Dong, Stability of SARS coronavirus in human specimens and environment and its sensitivity to heating and UV irradiation, *Biomed. Environ. Sci.* 16 (2003) 246–255.
- [35] A. Eftekhari, M. Alipour, L. Chodari, S. Maleki Dizaj, M.R. Ardalan, M. Samiei, M. Cucchiari, A comprehensive review of detection methods for SARS-CoV-2, *Microorganisms* 9 (2) (2021) 232.
- [36] W. Feng, A.M. Newbelling, C. Le, B. Pang, H. Peng, Y. Cao, X.C. Le, Molecular diagnosis of COVID-19: challenges and research needs, *Anal. Chem.* 92 (15) (2020) 10196–10209.
- [37] X. Fernandez-Cassi, N. Timoneda, S. Martínez-Puchol, M. Rusiñol, J. Rodríguez-Manzano, N. Figuerola, S. Bofill-Mas, J.F. Abril, R. Girones, Metagenomics for the study of viruses in urban sewage as a tool for public health surveillance, *Sci. Total Environ.* 618 (2018) 870–880.
- [38] P. Foadori, F. Cutrupi, N. Segata, S. Manara, F. Pinto, F. Malpei, L. Bruni, G. La Rosa, SARS-CoV-2 from faeces to wastewater treatment: What do we know? A review, *Sci. Total Environ.* 743 (2020), 140444.
- [39] M. Forde, R. Izurieta, B. Ormezi, M. Arellano, K. Mitchell, *Agua y salud, Calid. Del. Agua En. las Américas* (2019) 29.
- [40] H. Füller, Pandemic cities: biopolitical effects of changing infection control in post-SARS Hong Kong, *Geogr. J.* 182 (2016) 342–352.
- [41] M.H. Jafferli, K. Khatami, M. Atasoy, M. Birgersson, C. Williams, Z. Cetecioglu, Benchmarking virus concentration methods for quantification of SARS-CoV-2 in raw wastewater, *Sci. Total Environ.* 755 (2021), 142939.
- [42] T. Gallagher, C. Escarmis, M. Buchmeier, Alteration of the pH dependence of coronavirus-induced cell fusion: effect of mutations in the spike glycoprotein, *J. Virol.* 65 (1991) 1916–1928.
- [43] Y. Gao, G.Y. Cai, W. Fang, H.Y. Li, S.Y. Wang, L. Chen, Q.L. Gao, Machine learning based early warning system enables accurate mortality risk prediction for COVID-19, *Nat. Commun.* 11 (1) (2020) 1–10.
- [44] K.E. Gibson, Viral pathogens in water: occurrence, public health impact, and available control strategies, *Curr. Opin. Virol.* 4 (2014) 50–57.
- [45] J. Gibson, J. Drake, B.U.V. Karney, Disinfection of Wastewater and Combined Sewer Overflows. *Ultraviolet Light in Human Health, Diseases and Environment*, Springer, 2017, pp. 267–275.
- [46] M. Gormley, T.J. Aspray, D.A. Kelly, COVID-19: mitigating transmission via wastewater plumbing systems, *Lancet Glob. Health* 8 (2020), 643.
- [47] L. Guerrero-Latorre, I. Ballesteros, I. Villacres, M.G. Granda-Albuja, B. Freire, B. Rios-Touma, First SARS-CoV-2 detection in river water: implications in low sanitation countries, *Sci. Total Environ.* 743 (2020) 1–5.
- [48] P.M. Gundy, C.P. Gerba, L.L. Pepper, Survival of coronaviruses in water and wastewater, *Food Environ. Virol.* 1 (2009) 10.
- [49] W. Gwenzi, Leaving no stone unturned in light of the COVID-19 faecal-oral hypothesis? A water, sanitation and hygiene (WASH) perspective targeting low-income countries, *Sci. Total Environ.* 753 (2021), 141751.
- [50] E. Haramoto, B. Malla, O. Thakali, M. Kitajima, First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan, *Sci. Total Environ.* 737 (2020), 140405.
- [51] L. Hawryluck, W.L. Gold, S. Robinson, S. Pogorski, S. Galea, R. Styra, SARS control and psychological effects of quarantine, Toronto, Canada, *Emerg. Infect. Dis.* 10 (2004) 1206–1212.
- [52] C.J. Hurst, C.P. Gerba, Fate of viruses during wastewater sludge treatment processes, *Crit. Rev. Environ. Sci. Technol.* 18 (1989) 317–343.
- [53] L.A. Ikner, M. Soto-Beltran, K.R. Bright, New method using a positively charged microporous filter and ultrafiltration for concentration of viruses from tap water, *Appl. Environ. Microbiol.* 77 (2011) 3500–3506.
- [54] T.R. Julian, K.J. Schwab, Challenges in environmental detection of human viral pathogens, *Curr. Opin. Virol.* 2 (2012) 78–83.
- [55] I. Ihsanullah, M. Bilal, M. Naushad, Coronavirus 2 (SARS-CoV-2) in water environments: current status, challenges and research opportunities, *J. Water Process Eng.* 39 (2021), 101735.
- [56] S.K. Kailasa, V.N. Mehta, J.R. Koduru, H. Basu, R.K. Singhal, Z.V. P. Murthy, T. J. Park, An overview of molecular biology and nanotechnology based analytical methods for the detection of SARS-CoV-2: promising biotools for the rapid diagnosis of COVID-19, *Analyst* (2021).
- [57] G. Kampf, D. Todt, S. Pfander, E. Steinmann, Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents, *J. Hosp. Infect.* 104 (2020) 246–251.
- [58] K.A. Khan, P. Cheung, Presence of mismatches between diagnostic PCR assays and coronavirus SARS-CoV-2 genome, *R. Soc. Open Sci.* 7 (2020), 200636.
- [59] M. Kitajima, W. Ahmed, K. Bibby, A. Carducci, C.P. Gerba, K.A. Hamilton, E. Haramoto, J.B. Rose, SARS-CoV-2 in wastewater: State of the knowledge and research needs, *Sci. Total Environ.* 739 (2020), 139076.
- [60] N.E. Kogan, L. Clemente, P. Liautaud, J. Kaashoek, N.B. Link, A.T. Nguyen, M. Santillana, An early warning approach to monitor COVID-19 activity with multiple digital traces in near real time, *Sci. Adv.* 7 (10) (2021) eabd6989.
- [61] L.M. Kucirka, S.A. Lauer, O. Laeyendecker, D. Boon, J. Lessler, Variation in false-negative rate of reverse transcriptase polymerase chain reaction–based SARS-CoV-2 tests by time since exposure, *Ann. Intern. Med.* 20 (2020) 1–7.
- [62] G. La Rosa, L. Bonadonna, L. Lucentini, S. Kenmoe, E. Suffredini, Coronavirus in water environments: occurrence, persistence and concentration methods—a scoping review, *Water Res.* 179 (2020), 115899.
- [63] G. La Rosa, M. Iaconelli, P. Mancini, G. Bonanno Ferraro, C. Veneri, L. Bonadonna, L. Lucentini, E. Suffredini, First detection of SARS-CoV-2 in untreated wastewaters in Italy, *Sci. Total Environ.* 736 (2020), 139652.
- [64] M.Y. Lai, P.K. Cheng, W.W. Lim, Survival of severe acute respiratory syndrome coronavirus, *Clin. Infect. Dis.* 41 (2005) e67–e71.
- [65] J.A. Lednický, S.N. Shankar, M.A. Elbadry, J.C. Gibson, M.M. Alam, C. J. Stephenson, C.Y. Wu, Collection of SARS-CoV-2 virus from the air of a clinic within a university student health care center and analyses of the viral genomic sequence, *Aerosol air Qual. Res.* 20 (6) (2020) 1167–1171.
- [66] W. Lodder, A.M. de Roda Husman, SARS-CoV-2 in wastewater: potential health risk, but also data source, *Lancet Gastroenterol. Hepatol.* 5 (2020) 533–534.
- [67] X. Lu, L. Wang, S.K. Sakhthivel, B. Whitaker, J. Murray, S. Kamili, B. Lynch, L. Malapati, S.A. Burke, J. Harcourt, A. Tamin, N.J. Thornburg, J.M. Villanueva, S. Lindstrom, US CDC real-time reverse transcription PCR panel for detection of severe acute respiratory syndrome coronavirus 2, *Emerg. Infect. Dis.* 26 (2020) 1654.
- [68] J. Mateo-Sagata, L. Raschid-Sally, A. Thebo, Global wastewater and sludge production, treatment and use. *Wastewater*, Springer, 2015, pp. 15–38.
- [69] N.J. Matheson, P.J. Lehner, How does SARS-CoV-2 cause COVID-19? *Science* 369 (2020) 510–511.
- [70] Y. Matsumura, T. Shimizu, T. Noguchi, S. Nakano, M. Yamamoto, M. Nagao, Comparison of 12 molecular detection assays for SARS-CoV-2, *bioRxiv* (2020) 1–21.
- [71] R.G. Maunder, W.J. Lancee, K.E. Balderson, J.P. Bennett, B. Borgundvaag, S. Evans, C.M. Fernandes, D.S. Goldbloom, M. Gupta, J.J. Hunter, L. McGillis Hall, L.M. Nagle, C. Pain, S.S. Peczenik, G. Raymond, N. Read, S.B. Rourke, R. J. Steinberg, T.E. Stewart, S. VanDeVelde-Coke, G.G. Veldhorst, D.A. Wasylenko, Long-term psychological and occupational effects of providing hospital healthcare during SARS outbreak, *Emerg. Infect. Dis.* 12 (2006) 1924–1932.
- [72] S. Mavrikou, G. Moschopoulou, V. Tsekouras, S. Kintzios, Development of a portable, ultra-rapid and ultra-sensitive cell-based biosensor for the direct detection of the SARS-CoV-2 S1 spike protein antigen, *Sensors (Basel)* 20 (2020) 3121.
- [73] Mao, K., Zhang, H., & Yang, Z. (2020). Can a Paper-Based Device Trace COVID-19 Sources With Wastewater-Based Epidemiology?
- [74] G. Medema, L. Heijnen, G. Elsinga, R. Italiaander, A. Brouwer, Presence of SARS-coronavirus-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in the Netherlands, *Environ. Sci. Technol. Lett.* 7 (2020) 511–516.
- [75] I. Michael-Kordatou, P. Karaolia, D. Fatta-Kassinos, Sewage analysis as a tool for the COVID-19 pandemic response and management: the urgent need for optimised protocols for SARS-CoV-2 detection and quantification, *J. Environ. Chem. Eng.* 8 (5) (2020), 104306.
- [76] I. Michael-Kordatou, P. Karaolia, D. Fatta-Kassinos, Sewage analysis as a tool for the COVID-19 pandemic response and management: the urgent need for optimised protocols for SARS-CoV-2 detection and quantification, *J. Environ. Chem. Eng.* 8 (5) (2020), 104306.
- [77] M. Motoshita, N. Itsubo, A. Inaba, Development of impact factors on damage to health by infectious diseases caused by domestic water scarcity, *Int. J. Life Cycle Assess.* 16 (2011) 65–73.
- [78] N. Neault, A.T. Baig, T.E. Graber, P.M. D’Aoust, E. Mercier, I. Alexandrov, D. Crosby, S. Baird, J. Mayne, T. Pounds, M. MacKenzie, D. Figeys, A. MacKenzie, R. Delatolla, SARS-CoV-2 protein in wastewater mirrors COVID-19 prevalence, *medRxiv* (2020).
- [79] A. Nemudryi, A. Nemudraia, T. Wiegand, K. Surya, M. Buyukyoruk, C. Cicha, K. K. Vanderwood, R. Wilkinson, B. Wiedenheft, Temporal detection and phylogenetic assessment of SARS-CoV-2 in municipal wastewater, *Cell Rep. Med.* 1 (2020), 100098.
- [80] L.D. Nghiem, B. Morgan, E. Donner, M.D. Short, The COVID-19 pandemic: considerations for the waste and wastewater services sector, *Case Stud. Chem. Environ. Eng.* 1 (2020), 100006.
- [81] A. Núñez-Delgado, What do we know about the SARS-CoV-2 coronavirus in the environment? *Sci. Total Environ.* 727 (2020), 138647.
- [82] J. Parkinson, K. Tayler, Decentralized wastewater management in peri-urban areas in low-income countries, *Environ. Urban.* 15 (2003) 75–90.
- [83] L. Peñarrubia, M. Ruiz, R. Porco, S.N. Rao, M. Juanola-Falgarona, D. Manissero, M. López-Fontanals, J. Pareja, Multiple assays in a real-time RT-PCR SARS-CoV-2

- panel can mitigate the risk of loss of sensitivity by new genomic variants during the COVID-19 outbreak, *Int. J. Infect. Dis.: IJID: Off. Publ. Int. Soc. Infect. Dis.* 97 (2020) 225–229.
- [84] A. Pérez-Cataluña, E. Cuevas-Ferrando, W. Randazzo, I. Falcó, A. Allende, G. Sánchez, Comparing analytical methods to detect SARS-CoV-2 in wastewater, *Sci. Total Environ.* 758 (2021), 143870.
- [85] N. Petrosillo, G. Viceconte, O. Ergonul, G. Ippolito, Petersen E. COVID-19, SARS and MERS: are they closely related? *Clin. Microbiol. Infect.* 26 (2020) 729–734.
- [86] B. Pfefferbaum, C.S. North, Mental health and the Covid-19 pandemic, *N. Engl. J. Med.* 383 (2020) 510–512.
- [87] M. Rusiñol, S. Martínez-Puchol, E. Forés, M. Itarte, R. Girones, S. Bofill-Mas, Concentration methods for the quantification of coronavirus and other potentially pandemic enveloped virus from wastewater, *Curr. Opin. Environ. Sci. Health* 17 (2020) 21–28.
- [88] E.E. Odih, A.O. Afolayan, I. Akintayo, I.N. Okeke, Could water and sanitation shortfalls exacerbate SARS-CoV-2 transmission risks? *Am. J. Trop. Med. Hyg.* 103 (2020) 554–557, [tpmd200462](https://doi.org/10.1186/s12875-020-0462-2).
- [89] G. Qu, X. Li, L. Hu, G. Jiang, An imperative need for research on the role of environmental factors in transmission of novel coronavirus (COVID-19), *Environ. Sci. Technol.* 54 (2020) 3730–3732.
- [90] J. Peccia, A. Zulli, D.E. Brackney, N.D. Grubaugh, E.H. Kaplan, A. Casanovas-Massana, S.B. Omer, Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics, *Nat. Biotechnol.* 38 (10) (2020) 1164–1167.
- [91] H. Rabenau, J. Cinatl, B. Morgenstern, G. Bauer, W. Preiser, H. Doerr, Stability and inactivation of SARS coronavirus, *Med. Microbiol. Immunol.* 194 (2005) 1–6.
- [92] Randazzo W., Cuevas-Ferrando E., Sanjuan R., Domingo-Calap P., Sanchez G. Metropolitan Wastewater Analysis for COVID-19 Epidemiological Surveillance. Available at SSRN 3586696. 2020.
- [93] W. Randazzo, P. Truchado, E. Cuevas-Ferrando, P. Simón, A. Allende, G. Sánchez, SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area, *Water Res.* 181 (2020), 115942.
- [94] M. Sambridge, A. Jackson, National COVID numbers-Benford's law looks for errors, *Nature* 581 (2020) 384, 384-384.
- [95] N. Sethuraman, S.S. Jeremiah, A. Ryo, Interpreting diagnostic tests for SARS-CoV-2, *Jama* 323 (2020) 2249–2251.
- [96] M.A. Shereen, S. Khan, A. Kazmi, N. Bashir, R. Siddique, COVID-19 infection: origin, transmission, and characteristics of human coronaviruses, *J. Adv. Res.* 24 (2020) 91–98.
- [97] C. Sheridan, Coronavirus and the race to distribute reliable diagnostics, *Nat. Biotechnol.* 38 (2020) 382–384.
- [98] Y. Shi, Y. Wang, C. Shao, J. Huang, J. Gan, X. Huang, E. Bucci, M. Piacentini, G. Ippolito, G. Melino, COVID-19 infection: the perspectives on immune responses, *Cell Death Differ.* 27 (2020) 1451–1454.
- [99] K. Shirato, N. Nao, H. Katano, I. Takayama, S. Saito, F. Kato, et al., Development of genetic diagnostic methods for novel coronavirus 2019 (nCoV-2019) in Japan, *Jpn. J. Infect. Dis.* (2020). [JJID. 2020.061](https://doi.org/10.1186/1475-2875-2020-061).
- [100] J. Sizun, M. Yu, P. Talbot, Survival of human coronaviruses 229E and OC43 in suspension and after drying on surfaces: a possible source of hospital-acquired infections, *J. Hosp. Infect.* 46 (2000) 55–60.
- [101] S.J. Smither, L.S. Eastaugh, J.S. Findlay, M.S. Lever, Experimental aerosol survival of SARS-CoV-2 in artificial saliva and tissue culture media at medium and high humidity, *Emerg. Microbes Infect.* (2020) 1–9.
- [102] C. Staddon, M. Everard, J. Mytton, T. Octavianti, W. Powell, N. Quinn, J. Geere, Water insecurity compounds the global coronavirus crisis, *Water Int.* 45 (5) (2020) 416–422.
- [103] R. Street, S. Malema, N. Mahlangeni, A. Mathee, Wastewater surveillance for Covid-19: an African perspective, *Sci. Total Environ.* 743 (2020), 140719.
- [104] A.F. Trevett, R.C. Carter, S.F. Tyrrel, The importance of domestic water quality management in the context of faecal-oral disease transmission, *J. Water Health* 3 (2005) 259–270.
- [105] J. Trottier, R. Darques, N. Ait Mouheb, E. Partiot, W. Bakhache, M.S. Deffieu, R. Gaudin, Post-lockdown detection of SARS-CoV-2 RNA in the wastewater of Montpellier, France, *One Health* 10 (2020), 100157.
- [106] J.G. Tundisi, J.A. Garcia, T. Matsumura-Tundisi, J.E.M. Tundisi, L.J. Patrizzi, F. Ghiglieno, Monitoring wastewater for assessing community health in cities: perspectives and a proposal, *Braz. J. Biol.* 80 (2020) 946–947.
- [107] N. Van Doremalen, T. Bushmaker, D.H. Morris, M.G. Holbrook, A. Gamble, B. N. Williamson, A. Tamin, J.L. Harcourt, N.J. Thornburg, S.I. Gerber, J.O. Lloyd-Smith, E. de Wit, V.J. Munster, Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1, *N. Engl. J. Med.* 382 (2020) 1564–1567.
- [108] N. Van Doremalen, T. Bushmaker, V. Munster, Stability of middle east respiratory syndrome coronavirus (MERS-CoV) under different environmental conditions, *Eur. Surveill.: Bull. Eur. Sur Les. Mal. Transm. = Eur. Commun. Dis. Bull.* 18 (2013) 20590.
- [109] P. Vasicokova, I. Pavlik, M. Verani, A. Carducci, Issues concerning survival of viruses on surfaces, *Food and Environ. Virol.* 2 (2010) 24–34.
- [110] A. Venugopal, H. Ganesan, S.S. Sudalaimuthu Raja, V. Govindasamy, M. Arunachalam, A. Narayanasamy, P. Sivaprakash, P. Rahman, A. V. Gopalakrishnan, Z. Siama, B. Vellingiri, Novel wastewater surveillance strategy for early detection of coronavirus disease 2019 hotspots, *Curr. Opin. Environ. Sci. Health* 17 (2020) 8–13.
- [111] C. Wang, R. Pan, X. Wan, Y. Tan, L. Xu, C.S. Ho, R.C. Ho, Immediate psychological responses and associated factors during the initial stage of the 2019 coronavirus disease (COVID-19) epidemic among the general population in China, *Int. J. Environ. Res. Public Health* 17 (2020) 1729.
- [112] X.-W. Wang, J.-S. Li, M. Jin, B. Zhen, Q.-X. Kong, N. Song, W.-J. Xiao, J. Yin, W. Wei, G.-J. Wang, B. Si, B.-Z. Guo, C. Liu, G.-R. Ou, M.-N. Wang, T.-Y. Fang, F. H. Chao, J.-W. Li, Study on the resistance of severe acute respiratory syndrome-associated coronavirus, *J. Virol. Methods* 126 (2005) 171–177.
- [113] S.L. Warnes, Z.R. Little, C.W. Keevil, Human coronavirus 229E remains infectious on common touch surface materials, *MBio* 6 (2015), 01697-15.
- [114] J. Weststrate, G. Dijkstra, J. Eshuis, A. Gianoli, M. Rusca, The sustainable development goal on water and sanitation: learning from the millennium development goals, *Soc. Indic. Res.* 143 (2019) 795–810.
- [115] World Health Organization, Modes of Transmission of Virus Causing COVID-19: Implications for IPC Precaution Recommendations: Scientific Brief, 27 March 2020, World Health Organization,, 2020.
- [116] World Health Organization, Water, Sanitation, Hygiene, and Waste Management for SARS-CoV-2, the Virus that Causes COVID-19: Interim Guidance, 29 July 2020, World Health Organization,, 2020.
- [117] F. Wu, A. Xiao, J. Zhang, X. Gu, W.-L. Lee, K. Kauffman, et al., SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases, [medRxiv \(2020\)](https://doi.org/10.1101/2020.07.20.20156840), 2020.
- [118] Y. Ye, R.M. Ellenberg, K.E. Graham, K.R. Wigginton, Survivability, partitioning, and recovery of enveloped viruses in untreated municipal wastewater, *Environ. Sci. Technol.* 50 (2016) 5077–5085.
- [119] X. Yuan, C. Yang, Q. He, J. Chen, D. Yu, J. Li, S. Zhai, Z. Qin, K. Du, Z. Chu, P. Qin, Current and perspective diagnostic techniques for COVID-19, *ACS Infect. Dis.* 6 (2020) 1998–2016.
- [120] C.-M. Zhang, L.-M. Xu, P.-C. Xu, X.C. Wang, Elimination of viruses from domestic wastewater: requirements and technologies, *World J. Microbiol. Biotechnol.* 32 (2016) 69.
- [121] D. Zhang, H. Ling, X. Huang, J. Li, W. Li, C. Yi, T. Zhang, Y. Jiang, Y. He, S. Deng, X. Zhang, X. Wang, Y. Liu, G. Li, J. Qu, Potential spreading risks and disinfection challenges of medical wastewater by the presence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) viral RNA in septic tanks of fangcang hospital, *Sci. Total Environ.* 741 (2020), 140445.