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SARS-CoV-2 from faeces to wastewater treatment: What do we know? A review

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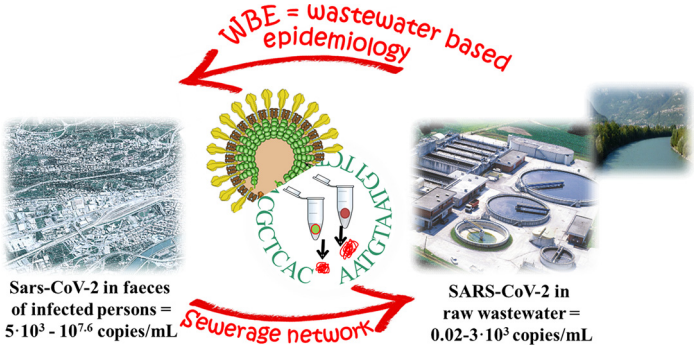
HIGHLIGHTS

- The route of SARS-CoV-2 from faeces to wastewater treatment plants is analysed.
- Viral load in the faeces of positive people for SARS-CoV-2 is $5 \cdot 10^3$–$10^{7.6}$ copies/mL.
- Viral load decreases from 2 copies/100 mL to $3 \cdot 10^3$ copies/mL when entering a WWTP.
- For WBE high uncertainty of viral loads remains, and further research is needed.
- CoVs inactivation in WWTPs is enhanced by tertiary treatments and disinfection.

GRAPHICAL ABSTRACT



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ABSTRACT

SARS-CoV-2, the virus that causes COVID-19, has been found in the faeces of infected patients in numerous studies. Stool may remain positive for SARS-CoV-2, even when the respiratory tract becomes negative, and the interaction with the gastrointestinal tract poses a series of questions about wastewater and its treatments. This review aims to understand the viral load of SARS-CoV-2 in faeces and sewage and its fate in wastewater treatment plants (WWTPs).

The viral load in the faeces of persons testing positive for SARS-CoV-2 was estimated at between $5 \cdot 10^3$ to $10^{7.6}$ copies/mL, depending on the infection course. In the sewerage, faeces undergo dilution and viral load decreases considerably in the wastewater entering a WWTP with a range from 2 copies/100 mL to $3 \cdot 10^3$ copies/mL, depending on the level of the epidemic. Monitoring of SARS-CoV-2 in sewage, although no evidence of COVID-19 transmission has been found via this route, could be advantageously exploited as an early warning of outbreaks. Preliminary studies on WBE seem promising; but high uncertainty of viral loads in wastewater and faeces remains, and further research is needed.

The detection of SARS-CoV-2 in sewage, based on RNA sequences and RT-PCR, requires a shared approach on sample pre-treatment and on-site collection to ensure comparable results. The finding of viral RNA in stools does not imply that the virus is viable and infectious. Viability of CoVs such as SARS-CoV-2 decreases in wastewater - due to temperature, pH, solids, micropollutants - but high inactivation in WWTPs can be obtained only by using disinfection (free chlorine, UVC light). A reduction in the quantity of disinfectants can be obtained by

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implementing Membrane-Bioreactors with ultrafiltration to separate SARS-CoV-2 virions with a size of 60–140 nm. In sludge treatment, thermophilic digestion is effective, based on the general consensus that CoVs are highly sensitive to increased temperatures.

1. Introduction to SARS-CoV-2, the virus responsible for the COVID-19 pandemic outbreak

Until the beginning of this century, coronaviruses (CoVs) were considered minor pathogens for humans. However, CoVs have caused important outbreaks in recent decades: the severe acute respiratory syndrome (SARS) in the years 2002–2003 and the Middle East respiratory syndrome (MERS) in the year 2012 (Yu Chen et al., 2020). At the beginning of the outbreak that occurred in Wuhan at the end of 2019, a novel coronavirus was identified (Lu et al., 2020). It was officially named SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2; formerly called HCoV-19), because the closest relative to the new virus is the SARS-CoV responsible for the SARS outbreak in 2003 (WHO, 2020a; Lu et al., 2020). The genome of the new virus, which has about 80% nucleotide identity with that of human SARS-CoV (Lu et al., 2020; Chan et al., 2020), is more similar to two bat-derived coronavirus strains with 88–89% identity according to Lu et al. (2020) and Chan et al. (2020).

The coronavirus virion is roughly spherical, with a diameter of approximately 60–140 nm and contained within an outer viral envelope covered by projections (9–12 nm) (Zhu et al., 2020) which are arranged in a characteristic external structure similar to a crown (*corona* in Latin) that gives the name to the family. Envelope proteins are involved in several aspects of the virus life cycle, such as assembly, envelope formation, and pathogenesis. Within the envelope there is the helical capsid containing nucleoprotein and the RNA genome. Fig. 1 shows the virion structure of SARS-CoV/SARS-CoV-2.

Phylogenetically, SARS-CoV-2 falls within the subgenus *Sarbecovirus* of the genus *Betacoronavirus*, which is one of the four genera of CoVs belonging to the *Coronavirinae* subfamily. CoVs may infect mammals but also birds or fish, showing diverse tissue tropism. Before 2019, only six CoVs were known to be responsible for infections in humans (Yu Chen et al., 2020; Chan et al., 2020) and as causing respiratory diseases: 229E and NL63 (alpha coronavirus), OC43, HKU1, MERS-CoV, SARS-CoV (beta coronavirus). Owing to the typically high mutation rates of RNA viruses in comparison with both DNA viruses and their hosts, CoVs can quickly increase their virulence and adapt to novel hosts (Duffy, 2018; Elena and Sanjuán, 2005).

The respiratory disease caused by SARS-CoV-2 is called COVID-19 (WHO, 2020a). Patients with COVID-19 typically present fever, cough, rhinorrhea, dyspnea, or severe pneumonia (N. Chen et al. 2020; Yeo et al., 2020; Guan et al., 2020). A large percentage of people may remain asymptomatic even if they have tested positive for SARS-CoV-2 (Lai et al., 2020; P. Yu et al. 2020; Liu et al., 2020; Bai et al., 2020; Rothe et al., 2020; X. Pan et al. 2020). Conventional routes of transmission of SARS-CoV-2 are respiratory droplets and direct contact, or indirect contact with contaminated surfaces (Gu et al., 2020; Yeo et al., 2020), similarly to SARS-CoV and MERS-CoV. Without approved and validated treatment options and without a vaccine, the best practices to limit the disease are protection measures and social distancing.

As of June 16, 2020, about 8 million confirmed cases including >400,000 deaths have been reported worldwide, affecting at least 230 Countries and Territories (https://covid19.who.int/ situation report 147).

The aim of this review is to outline the currently available knowledge about the occurrence of the new coronavirus in wastewater and to highlight the areas where further research is needed to answer the following questions: (i) what are the methods for sampling and identifying SARS-CoV-2 in faeces and wastewater? (ii) how large is the viral load of SARS-CoV-2 in faeces and its capacity of active replication? (iii)

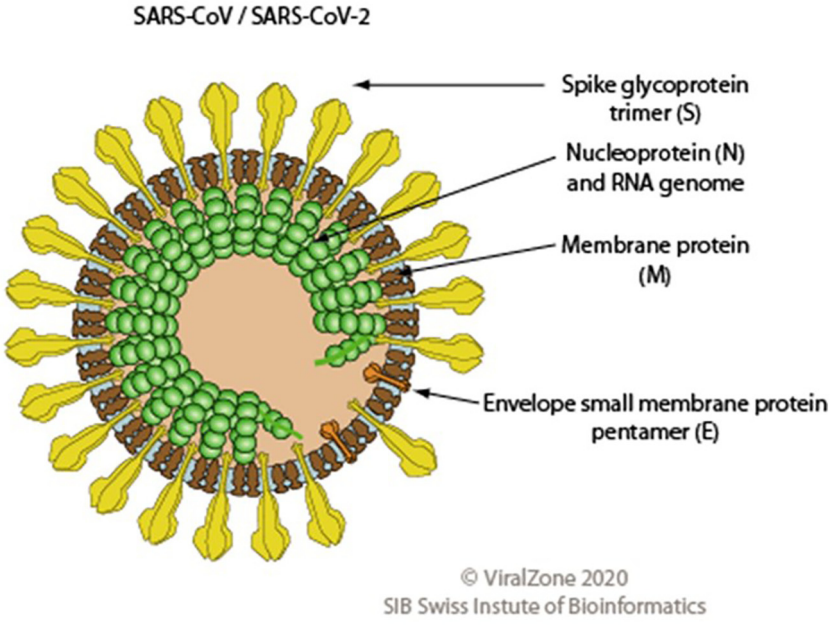

Fig. 1. Virion structure of SARS-CoV/SARS-CoV-2 (permission obtained from Philippe Le Mercier, ViralZone, SIB Swiss Institute of Bioinformatics).

how large is the concentration of SARS-CoV-2 in municipal wastewater and is there evidence of faecal-oral transmission? (iv) is wastewater monitoring useful in the developing field of Wastewater Based Epidemiology (WBE) for early-warning surveillance of the spread of the virus? (v) what is the role of wastewater treatment plants?

Since the recent onset of the COVID-19 pandemic, some of the investigations reviewed here is preliminary and/or ongoing. However, relevant publicly available papers not yet peer-reviewed have been included.

2. Interaction of SARS-CoV-2 with the gastrointestinal tract and its presence in faeces

In general, viruses detected in faeces can derive from: (1) swallowing of respiratory secretions from the upper respiratory tract; in this case the virus can be damaged by the gastric acidity in the stomach, but protection when mixed with food or potential resistance to low pH may allow its passage in the intestine; (2) residues of infected antigen-presenting immune cells; (3) virus replication in intestinal cells (Gu et al., 2020; Xiao et al., 2020), considering that both avian and human influenza viruses are able to replicate in human intestinal epithelial cells (Minodier et al., 2015).

SARS-CoV and MERS-CoV, which caused the previous outbreaks, were associated with gastrointestinal symptoms in a significant proportion of patients (Zhou et al., 2017; Yeo et al., 2020). During the two SARS-CoV epidemic outbreaks, in 2002 and 2003, up to 73% of patients had gastrointestinal symptoms during the development of the disease, and the presence of SARS-CoV RNA was demonstrated in the stool specimens (WHO, 2003; Zhou et al., 2017). For a small percentage of patients, viral RNA was still present after 30 days of illness (K. H. Chan et al., 2004). The ability of active SARS-CoV to replicate was identified in the intestine specimens. It was consequently speculated that the human gastrointestinal tract could be an infection site of SARS-CoV (Ding et al., 2004; Zhou et al., 2017). During the 2012 MERS-CoV outbreak, a quarter of patients reported symptoms, such as diarrhoea or abdominal pain, before severe respiratory symptoms (Assiri et al., 2013; Mackay and Arden, 2015) and MERS-CoV RNA was found in 14.6% of stool samples (Corman et al., 2016). Zhou et al. (2017) demonstrated that intestinal epithelial cells were highly susceptible to MERS-CoV and could support viral replication.

The new SARS-CoV-2 can affect not only the respiratory system with fever, cough, rhinorrhea, dyspnea or severe pneumonia, but may also cause other clinical symptoms like lethargy, muscle ache, headache, neurologic manifestation or gastrointestinal symptoms such as diarrhoea (Guan et al., 2020; L. Pan et al. 2020). Among the first studies from China, Guan et al. (2020) extracted data on 1099 patients with laboratory-confirmed COVID-19 from 552 hospitals in mainland China through January 29, 2020, and among varying degrees of illness, diarrhoea was considered uncommon (3.8% of 1099 patients). Subsequent observations on 204 patients with COVID-19 from January 18th to March 18th, 2020, highlighted that one-third of patients reported diarrhoea (L. Pan et al. 2020), indicating that digestive issues may be a common early symptom of the disease. The differences between the two studies match the findings of Cheung et al. (2020) who observed a significant heterogeneity among the studies and a significant subgroup difference in the data from and outside Hubei Province. In a meta-analysis of 60 studies and 4243 patients, the pooled prevalences of all gastrointestinal symptoms and viral RNA positive stool were 17.6% and 48.1% (Cheung et al., 2020).

Therefore, SARS-CoV-2 positivity can be observed also in faeces of persons in the absence of gastrointestinal symptoms or diarrhoea: in particular, live SARS-CoV-2 was found in the stool of two patients without diarrhoea (W. Wang et al. 2020).

A synthesis of the incidence of viral RNA positivity in the stools of patients infected with SARS-CoV-2 is reported in Table 1. In addition, Table 2 reports the loads of SARS-CoV-2 measured in the faeces detected positive by real-time RT-PCR.

W. Wang et al. (2020) investigated the biodistribution of SARS-CoV-2 among different tissues of patients with confirmed COVID-19 (bronchoalveolar lavage fluid, blood, sputum, faeces, urine, and nasal samples). In faeces, the positive specimens were 44 of 153, corresponding to 29% of the collected specimens. The mean cycle threshold value for stool specimens was 31.4 (st. dev. = 5.1), indicating a viral load $<2.6 \cdot 10^4$ copies/mL, which was significantly lower than the nasal swabs where the copy number was $1.4 \cdot 10^6$ copies/mL (Ct mean 24.3) (W. Wang et al. 2020).

Lin et al. (2020) tested faecal samples of 65 hospitalized patients and approximately one half were positive, including cases with and without gastrointestinal symptoms. The authors concluded that the gastrointestinal tract may be a potential transmission route and target organ of SARS-CoV-2. With regard to this latter observation, SARS-CoV-2 uses the ACE2 protein as a receptor (Wan et al., 2020), which is present not only in the respiratory epithelium, but also in the gastro-enteric mucosa (Hamming et al., 2004).

Xiao et al. (2020) examined the viral RNA in faeces from 73 hospitalized patients with SARS-CoV-2 infection and found that a percentage of 53.4% tested positive for viral RNA in stool. Furthermore, >20% of patients with SARS-CoV-2 continued to remain positive in faeces, even after showing negative results in respiratory samples (Xiao et al., 2020).

Another epidemiological and clinical investigation on ten paediatric SARS-CoV-2 infection cases highlighted that some patients were persistently positive on rectal swabs even after their nasopharyngeal testing had become negative (Xu et al., 2020).

Y. Wu et al. (2020), investigating faecal samples from 74 patients, observed that the faecal samples of over half of patients remained positive for SARS-CoV-2 RNA for a mean of 11.2 days after the respiratory tract samples became negative. In certain cases, this duration in faeces persisted for nearly 5 weeks after the respiratory samples tested negative. Y. Wu et al. (2020) suggest that the virus may be actively replicating in the gastrointestinal tract even after viral clearance in the respiratory tract.

Zhang et al. (2020) investigated the faeces of 23 patients, finding 83% positive, with a median duration of virus shedding of 22.0 days for faeces. During this period, the mean virus titre in faeces was 5623 copies/mL, but the highest titre at the peak reached $10^{5.8}$ copies/mL (Zhang et al., 2020).

As far as we know, only one study - Cheung et al. (2020) - tested viral RNA in stool collected on the day of hospitalization. In this study viral RNA was detected in 15.3% of the patients. In particular, viral RNA was found in 38.5% of patients with diarrhoea and in 8.7% of patients without diarrhoea, confirming again that the presence of SARS-CoV-2 RNA in faeces is not necessarily correlated with diarrhoea. A median viral load of $10^{4.7}$ copies/mL (range $10^{3.4}$–$10^{7.6}$) was found in the stool of 9 positive patients (Cheung et al., 2020). The stool viral load (median values) was $10^{5.1}$ and $10^{3.9}$ copies/mL in the presence or absence of diarrhoea, respectively.

Frequent measurements of viral RNA concentration were performed on the stool of nine hospitalized patients with COVID-2019 during the course of the disease by Wolfel et al. (2020), who found high viral RNA concentrations in initial samples, with a peak during the first week of symptoms. The viral content then declined gradually over time, but stool samples remained RNA-positive for three weeks in six of the nine patients, in spite of full resolution of symptoms (Wolfel et al., 2020). Maximum viral load over 10^7 RNA copies/g faeces was measured in some cases; then a progressive decrease by 2–3 orders of magnitude occurred in the subsequent weeks (Wolfel et al., 2020). These results indicate that the viral load in faeces may be highly variable in the range 10^3–10^7 RNA copies/g faeces, depending on the day of sampling post onset. To change units from #/mL to #/g, the density of wet human faeces, i.e. about 1.06–1.09 g/mL (Penn et al., 2018), is used.

Table 1
Gastrointestinal (GI) symptoms, diarrhoea and viral RNA positivity in stools of patients infected with SARS-CoV-2.

Reference	Patients	GI symptoms		Diarrhoea		Viral RNA posit. in stool		Other findings
	No.	No.	%	No.	%	No.	%	
(W. Wang et al. 2020)	153	–	–	–	–	44/153	29	- live SARS-CoV-2 was observed in the stool sample from 2 patients without diarrhoea - the total number is referred to specimens instead of patients - scarcity of detailed clinical information available
(Lin et al., 2020)	65 95	42/65 58/95	64.6 61		 24.2	31/65	47.7	- 22/42 (52.4%) with positive faeces and GI symptoms - 9/23 (39.1%) with positive faeces but without GI symptoms - GI tract may be a target organ of SARS-CoV-2
(Xiao et al., 2020)	73					39/73	53.4	- duration time of positive stool from 1 to 12 days - 23.3% of patients have positive results in stool after negative results in respiratory samples - ACE2 protein (cell receptor) is abundantly expressed in the glandular cells of gastric, duodenal, and rectal epithelia, supporting the entry of SARS-CoV-2 into the host cells.
(Xu et al., 2020)	10	3/10	30	3/10	30	8/10	80	- ten paediatric cases, only 3 with diarrhoea and no other GI symptoms are indicated - positive cases on rectal swabs even after nasopharyngeal testing proves negative
(Y. Wu et al., 2020)	74	23/74	31			41/74	55	- GI symptoms not associated with faecal sample positivity ($p = 0.45$); - disease severity not associated with duration of faecal sample positivity ($p = 0.60$); - faeces remained positive for a mean of 11.2 days after the respiratory tract samples became negative (up to 5 weeks) - the virus may be actively replicating in the GI tract
(Zhang et al., n. d.)	23					10/12	83.3	- Median duration of virus shedding was 22 days for the faeces - Intestinal samples recommended for diagnosis of COVID-19, especially for monitoring the relapse of discharged patients
(Cheung et al., 2020) - own data	59	15/59	25	13/59	22	9/59	15.3	
(Cheung et al., 2020) - review	4243	–	17.6	–	12.5	–	48.1	- virus RNA found even in stool collected after respiratory samples tested negative - GI symptoms may be under-reported in some studies.
(Wolfel et al., 2020)	9			1/9	11			- profiles of viral RNA during the course of the disease - peak of viral RNA during the first week of symptoms - stool samples remained RNA-positive over 3 weeks without symptoms
(Yu Chen et al., 2020)	42	8	19	7	17	28	67	- patients tested positive in stool: 21% have diarrhoea. - 64.3% of patients tested positive in stool remained positive after the pharyngeal swabs turned negative
(Lo et al., 2020)	10			8	80			- diarrhoea (80%) and nausea (50%) were common symptoms in this cohort of patients - both faecal and respiratory specimens should be tested to aid discharge decision before the role of viral RNA shedding in stool is clarified.
(Guan et al., 2020)	1099	55/1099	5	42/1099	3.8			- data from mainland China through January 29, 2020. - occurrence of diarrhoea is one of the lowest in the literature
(L. Pan et al., 2020)	204	103/204	50.5	35/204	17			- importance of including symptoms like diarrhoea to diagnose COVID-19 early

In a meta-analysis of 60 studies and 4243 patients by Cheung et al. (2020), 70.3% of the stool samples were positive for SARS-CoV-2 even after respiratory specimens tested negative.

Regarding urine, many cases reported negative samples (L. Wang et al. 2020; Yifei Chen et al. 2020; F. Yu et al. 2020; Lescure et al., 2020; Young et al., 2020; Lo et al., 2020; W. Wang et al. 2020). Among the rare positive cases (Xu et al., 2020), viral RNA was found to be still present in urine specimens after throat swabs were negative (Xu et al., 2020).

The presence of SARS-CoV-2 in faeces, based on the detection of viral genetic material, does not necessarily imply viability and infectivity (Y. Wu et al. 2020; W. Wang et al. 2020; Guan et al., 2020; Xiao et al., 2020;

Table 2
SARS-CoV-2 loads and viral RNA Ct values in faecal samples detected positive by real-time RT-PCR.

Reference	No. patients	SARS-CoV-2 load (copies/mL)	Other results
(Wolfel et al., 2020)	9	$>10^7$ (peak) (range 10^3–10^7)	- viral load highly variable, depending on the day of sampling post onset. - peak during the first week from onset
(Zhang et al., n.d.)	23	5623 (mean) $10^{5.8}$ (peak)	- faecal samples detected by rRT-PCR targeting ORF1ab, N and S genes - Ct values of ORF1ab gene from ~25 to 43 - Negative samples for Ct value of 43 (limit of detection) - Ct values of 37.6, 32.64, 29.22, and 25.77 corresponding to 1×10^3, 1×10^4, 1×10^5, and 1×10^6 copies/mL, respectively.
(Cheung et al., 2020)	59	$10^{4.7}$ (median) (range $10^{3.4}$–$10^{7.6}$)	- $10^{5.1}$ copies/mL with diarrhoea - $10^{3.9}$ copies/mL without diarrhoea
(W. Wang et al. 2020)	153	$<2.6 \cdot 10^4$ copies/mL	- mean Ct = 31.4 ± 5.1
(Y. Wu et al., 2020)	41		- faecal viral RNA Ct values in 41 patients with positive faeces - Ct values of the targeted genes RdRp, N, E were 30.33 ± 8.12, 26.85 ± 11.42 and 28.42 ± 6.79, respectively.
(Xu et al., 2020)	10	$2 \cdot 10^3$–$2 \cdot 10^7$ (estimated by us)	- Ct values of Orf1ab and N genes on real-time RT–PCR in rectal swabs from ~23 to 37 - Ct profiles during 1–27 d since admission are available - Ct values of 32.04, 28.81, 25.14 and 21.54 corresponding to 5.27×10^4, 5.27×10^5, 5.27×10^6 and 5.27×10^7 copies/mL

Gu et al., 2020; Yuen et al., 2020; Amirian and Susan Amirian, 2020). At the moment, only few studies have been able to indicate the conditions of viability, infectivity or active replication of SARS-CoV-2 in stool:

- live SARS-CoV-2 was detected in the stool samples of 2 patients who did not have diarrhoea (W. Wang et al. 2020);
- the cultivation of SARS-CoV-2 was observed from a single stool specimen, as reported by China CDC (Chinese Center for Disease Control and Prevention) (Zhang et al., 2020);
- the release of the infectious virions in the gastrointestinal tract and the continuous positive detection of viral RNA in faeces was recently suggested by Xiao et al. (2020) (in unpublished data). This means that, after the entry of the virus, its specific RNA and proteins are synthesized in the cytoplasm of the infected gastrointestinal cells to form new virions, which are then released in the gastrointestinal tract (Xiao et al., 2020);
- the possibility of an active viral replication in the gastrointestinal tract could be supported by the finding that faeces may remain positive for SARS-CoV-2 RNA for weeks even after the respiratory tract has resulted negative (Y. Wu et al. 2020);
- no, or only minimal, active replication in stool was reported by Wolfel et al. (2020) on the basis of observations of viral subgenomic messenger RNAs (sgRNA)-containing cells. In fact, viral sgRNA is an indication of actively-infected cells since it is only transcribed in infected cells and is not packaged in virions. Furthermore, Wolfel et al. (2020) observed that the swallowing of respiratory secretions could not be the only passive origin of the virus in the intestine, because a much higher presence of SARS-CoV-2 RNA was found in stool compared to MERS-CoV during its outbreak. This suggested again a potential active replication in the gastrointestinal tract. However, the authors did not experimentally find a replicating form of the virus in stool samples despite the high levels of viral RNA (Wolfel et al., 2020).

3. SARS-CoV-2 RNA in faeces poses a series of questions about potential faecal-oral transmission

The detection of SARS-CoV-2 in the gastrointestinal tract raises the question of a potential faecal-oral transmission (W. Wang et al. 2020; Guan et al., 2020; Xiao et al., 2020; Gu et al., 2020; Yuen et al., 2020; Amirian and Susan Amirian, 2020). The route begins with the transport of the virus in the sewerage and treatment plants, or in pit toilets used in low-income countries for human excreta disposal, or spread through the practice of "open defecation" which concerns about 900 million people worldwide (WHO, 2017). Inadequate sanitation may be a source of contamination by viruses in soil and groundwater.

In previous outbreaks, the prolonged presence of SARS-CoV and MERS-CoV in the environment suggested that faecal excretion, environmental contamination and fomites might contribute to the viral shedding (Yeo et al., 2020; Zhou et al., 2017; Goh et al., 2013). Hence, also the SARS-CoV-2 could be transmitted via this kind of route, but at present no faecal-oral transmission cases have been documented according to (WHO, 2020b). A framework of possible SARS-CoV-2 faecal-oral transmission routes is described in Heller et al. (2020), who unpack the different pathways that may transmit viruses from faeces to mouth. The critical points of the potential ramifications of the COVID-19 pandemic on waste and wastewater services were highlighted by Nghiem et al. (2020).

In wastewater treatment plants (WWTPs), the current need is to understand the fate of SARS-CoV-2 considering the removal in the treatment stages and the emissions in: (i) effluent wastewater that may become reclaimed water; (ii) excess sludge that may become biosolids; (iii) other by-products; (iv) microbial aerosol originated by forced aeration, mixing, pumping, etc. In these complex systems, a prerequisite for the virus to cause infection is its ability to retain viability. The current knowledge is that CoVs viability decreases in wastewater - due to not optimal temperature, acidic pH, light exposure, high content of particulate solids and pollutants - and this gives confidence that the viral infectivity may be attenuated from faeces to sewage, then to WWTPs and then in the environment (La Rosa et al., 2020a). However, as of June 2020, given the limited information on SARS-CoV-2 in sewage and WWTPs, it would be premature to draw any conclusion.

Other routes, such as solid waste or aerosol from toilets to the sewerage, can be hypothesized to originate faecal–oral transmission of SARS-CoV-2. From the state of the art, it is unlikely that these matrices will become an important transmission pathway for SARS-CoV-2, but they direct attention to the need for much more research in this field.

4. Methods for identification of SARS-CoV-2 in wastewater

The presence of SARS-CoV-2 in human samples is confirmed by the detection of specific viral RNA sequences that are unique to SARS-CoV-2 by qPCR. The viral genes included the nucleocapsid protein gene N, the envelope protein gene E, the spike protein gene S, and the RNA-dependent RNA polymerase gene RdRP (also reported as Orf1ab) (*Laboratory Testing for 2019 Novel Coronavirus (2019-nCoV) in Suspected Human Cases*, n.d.). Internal controls are required to check for biases introduced from the RNA extraction step onward, and they consist of a nontarget RNA sequence, such as a fragment of 412 bases derived from dengue virus type 2 (Medema et al., 2020). Positive controls include, for instance, plasmids containing the complete SARS-CoV-2 nucleocapsid gene (Y. Wu et al. 2020). SARS-CoV-2 RNA copy number, typically expressed as particles/mL or RNA copies/mL, is estimated by cycle threshold values (Ct), which is inversely related to the viral load. These rRT-PCR assays have not only been applied on respiratory tract swabs or samples; they have also been used to detect viral excretion from the gastrointestinal tract (Xu et al., 2020; W. Wang et al. 2020; Y. Wu et al. 2020; Y. Pan et al. 2020; Ma et al., 2020).

Specific pre-treatments steps are normally performed, in particular during wastewater tests, in order to purify and/or concentrate the virus and thus improve the detection efficacy. However, there is still a lack of agreement on a standard protocol. Different viral enrichment approaches used with wastewater samples in recent SARS-Cov-2 investigations and PCR assays used to detect SARS-CoV-2 RNA are included in Table 3.

CDC recommends that virus isolation, inactivation and cultures have a Biosafety Level 3 (BSL-3) laboratory, whereas routine diagnostic testing can be conducted in a BSL-2 lab (CDC, 2020).

An issue that should be carefully considered to ensure a robust and reliable characterization of the viral content in sewage is the sampling procedure. Both flow rates and pollutant concentrations in the influent wastewater are subject to strong fluctuations during the day and thus composite samples collected over time are recommended: generally, a time composite sample is acceptable (Lytle and Sagripanti, 2005). For example, 24-h composite samples are formed by fixed aliquots collected at defined time intervals during the day and represent the average wastewater characteristics during the day. Where possible, depending on the presence of specific flow measurement devices in the WWTP, 24-h flow proportional sampling is preferable (EPA, 2013) because they ensure even more accurate and reliable results. Samples should be in any case maintained at low temperature during the period of collection, because this helps to preserve the viral load and viability (Wang et al., 2005b).

In the first case of detection of SARS-CoV-2 in sewage, Medema et al. (2020) collected 24-h -flow-dependent composite samples stored at 4 °C during sampling. Then samples were transported to the lab in melting ice and RNA was isolated on the day of sampling by using volumes of 40–150 mL (Medema et al., 2020). In the investigation by Ahmed et al. (2020), on two Australian WWTPs and a pumping station, samples were collected using automated samplers or grab sampling technique:

Table 3
Available methods for the concentration of SARS-CoV-2 from wastewater and assays for PCR detection.

Reference	Concentration steps	PCR assays
(F. Wu et al. 2020)	- Filtration through 0.22 μm membrane to remove bacterial cells and debris (initial tests revealed little to no viral RNA on filters) - PEG precipitation, centrifugation at 12,000*g* for 2 h or until a pellet was visible	- US CDC rRT-PCR primer/-probe sets targeting three loci of the nucleocapsid protein gene N (CDC, Centers for Disease Control and Prevention) - Negative controls: samples from the same wastewater treatment facility taken before the first reported COVID-19 case - The rRT-PCR data analysis threshold to call a positive sample included all samples with Ct below 40
(Ahmed et al., 2020)	- Method A: Direct RNA extraction from the electronegative 0.45 μm filter 90 mm diameter (Ahmed et al., 2015) - Method B: Centrifugation at 4750*g* for 30 min. Supernatant centrifuged at 3500*g* for 15 min through a centrifugal filter with a cut-off of 10 kDa	RT-qPCR assays were applied in accordance with the recent literature (Corman et al., 2020; Shirato et al., 2020)
(Medema et al., 2020)	- Centrifugation step at 4654*g* for 30 min to remove large particles (debris, bacteria) - Supernatant filtered with centrifugal ultrafiltration with a cut-off of 10 kDa at 1500 *g* for 15 min	Four primer sets were selected: the N1, N2, N3 sets (US CDC) targetings different regions of the nucleocapsid (N) gene and the set against the envelope protein (E) gene (Corman et al., 2020)
(Wurtzer et al., 2020)	Ultracentrifugation at 200,000*g* for 1 h at 4 °C	- PCR inhibitor removal resins were used. - The RT-qPCR primers were designed within the viral E gene - Positive results were confirmed by amplification of a region from the viral RNA dependent-RNA polymerase (Corman et al., 2020)
(La Rosa et al., 2020b)	Adaptation of the standard WHO protocol (WHO, 2003) for Poliovirus surveillance for enveloped viruses. Briefly: - initial centrifugation of wastewater to pellet the solids - mixing of clarified wastewater with dextran and polyethylene glycol (PEG) and left overnight at 4 °C in a separation funnel - concentrate was added to the pellet Chloroform treatment was omitted to preserve the integrity of the enveloped viruses	RNAs were tested for the presence of SARS-CoV-2 using three different PCR assays: a) a broad range of PCR for Coronavirus detection targeting the ORF1ab (Ar Gouilh et al., 2018) b) a newly designed primer set specific to SARS-CoV-2 c) a published nested RT-PCR for SARS-CoV-2 targeting the spike region (Nao et al., 2020)
Randazzo et al. (2020).	- Concentration method with $Al(OH)_3$ adsorption--precipitation (1 part 0.9 N $AlCl_3$ solution to 100 parts of sample. Incubated for 15 min at room temperature using an orbital shaker) - Centrifugation at 1700 *g* for 20 min - Resuspension of pellet in beef extract and centrifugation at 1900 *g* for 30 min - Pellet resuspension in PBS	RT-qPCR diagnostic panel assays validated by the US Centers for Disease Control and Prevention (CDC, 2020). The first version of the kit with three sets of oligonucleotide primers and probes was used to target three different SARS-CoV-2 regions of the nucleocapsid (N) gene
Authors of this study	PEG precipitation	- Bosphore Novel Coronavirus (2019-nCoV) Detection Kit (Anatolia Geneworks) targeting *orf1ab* and *gene E* regions - positive samples included all samples with Ct below 40, according to (F. Wu et al. 2020)

the samples were transported on ice to the laboratory and stored at 4 °C until further analysis.

5. Concentration of SARS-CoV-2 in wastewater

Faeces reach the sewerage system and undergo a large dilution. Raw wastewater contains organic matter, particulate solids, micropollutants and many pathogens, especially enteric. Viruses contained in faeces may undergo several transformations along the sewer network and possibly a reduction of number and viability, as an effect of solid deposition, decreasing pH, temperature and other factors.

Table 4 summarises the rare studies that have quantified the concentration of SARS-CoV-2 (expressed in copies/mL) in municipal wastewater. F. Wu et al. (2020) reported that the first results in a municipal WWTP in Massachusetts, that could have been affected by a number of factors that are unknown at the moment, were approximately 100 viral particles per mL of sewage, with the lowest observed values of ~10 copies/mL. Wurtzer et al. (2020) measured an increase in the viral load during the exponential growth of the epidemic, with values in the range of $50–3 \cdot 10^3$ copies/mL (calculated by us). Randazzo et al. (2020) estimated an average viral load of $2.5 \cdot 10^2$ copies/mL (recalculated by us) in untreated wastewater. Conversely, the viral loads measured by Ahmed et al. (2020) were 1.9 and 12 copies/100 mL of untreated wastewater, which is significantly lower than the values found by (Y. Wu et al. 2020).

The virus copies in wastewater are largely diluted in comparison to the viral load in the faeces. According to Section 2, viral copies in the faeces of persons testing positive for SARS-CoV-2 varied from $5 \cdot 10^3$ to $10^{7.6}$ copies/mL (Zhang et al., n.d.; Cheung et al., 2020). The dilution of positive faeces in wastewater causes a drop in the concentration by 4–5 orders of magnitude or more. This dilution is due to various factors: the daily flow rate discharged into the sewerage by a person (that is about 80% of the average daily supply of drinking water per capita and makes an approximate 10^3 fold dilution), stormwater or infiltration of parasite inflow in the sewer network. Moreover, not the whole population contributes to the viral load and this depends on the percentage of positive cases among the population served by a WWTP. By way of example, the number of cases in Lombardy, one of the Italian regions most affected to date, was 560 cases in 100,000 people on the 10th of April 2020; this produced a further dilution of $5.6 \cdot 10^3$ fold.

6. Can the SARS-CoV-2 abundance in wastewater be used for COVID-19 surveillance?

When capillary and frequent individual testing in a population is not possible, aggregate information about the level of the outbreak could be useful for monitoring its evolution and the effectiveness of containment measures. Together with other relevant approaches, additional information could be extrapolated from the viral load in municipal wastewater. This is the focus of the developing field of WBE, which in broad terms means “the application and development of using the quantitative measurement of human biomarkers in sewage to evaluate lifestyle, health and exposure at the community level” (https://score-cost.eu/). It has been so far quite extensively used in studies related to drugs or

Table 4
Type of raw wastewater collected in different studies and concentrations of SARS-CoV-2 in raw wastewater (n.a. = not available).

Reference	Geographical area	Points of raw wastewater sampling	No. of samples	SARS-CoV-2 load
F. Wu et al. (2020)	Massachusetts (USA)	A major WWTP (split into 2 catchment areas: Southern and Northern). Samples were collected in 7 dates for each catchment.	14 (4 samples before the first known US SARS-CoV-2 case + 5 samples in March 2020)	- ~100 copies/mL - lowest values of ~10 copies/mL.
Ahmed et al. (2020)	South-East Queensland (Australia)	- 1 suburban pumping station - 2 WWTPs representing urban catchments	2	1.9 and 12 copies/100 mL of untreated wastewater
(Medema et al., 2020)	Netherlands	- sewage of 6 cities - Amsterdam Airport Schiphol	24	Detected, load n.a.
(Wurtzer et al., 2020)	Paris (France)	- 3 WWTPs of the Parisian area	23	Range 50–$3 \cdot 10^3$ eq/mL (calculated by us; eq/mL = equivalent viral genomes per mL)
(La Rosa et al., 2020a)	Milan and Rome (Italy).	- 2 WWTPs in Milan - 1 WWTP in Rome that received pipelines from two different districts of the city	12	Detected, load n.a.
Randazzo et al. (2020).	Region of Murcia (Spain)	- 6 WWTPs serving the major municipalities	42	5.4 ± 0.2 $\log_{10}$ copies/L on average ($2.5 \cdot 10^2$ copies/mL, recalculated)

pharma consumption, but also for poliovirus (Nakamura et al., 2015) and infectious disease surveillance and early warning (Sims and Kasprzyk-Hordern, 2020).

Daughton (2020) suggested that WBE could help in quickly determining an increasing or decreasing trend of SARS-CoV-2 spread. Several research groups are directing the effort in monitoring wastewater for SARS-CoV-2 RNA specifically for this purpose. A WBE tool could be helpful also in regards to the second wave of virus infection or in the case of other future viral epidemics. The level of infection and the temporal trends could be determined by comparing (or associating) the viral load in wastewater with the served population (Daughton, 2020).

However, to elucidate if WBE can be successfully applied, coordination of methodologies and data sharing among different scientists are imperative. Standardization of sampling methods (see Section 4) and sharing analytical methods and collected information would provide a solid basis for estimating the consistency of the population served. In addition to flow and conventional macropollutant loads (COD, TSS, TKN, etc.) largely used to calculate the served population, the inclusion of tracers strictly connected to human discharges could improve the estimation accuracy.

The study performed in Massachusetts in March (F. Wu et al. 2020) on the stool of confirmed COVID-19 patients and on the wastewater produced by the municipality, strongly showed a titre of SARS-CoV-2 in wastewater higher than in clinically confirmed cases. Explaining such discrepancy is not easy, since many factors may be involved such as the underestimation of total positives (asymptomatic) and the population excretion rate.

(Lodder and de Roda Husman, 2020) suggested that wastewater could be a sensitive surveillance system and may act as an early warning tool. Indeed, wastewater samples collected in the Netherlands were found positive within a few days after the first cases of COVID-19 (Lodder and de Roda Husman, 2020).

KWR Netherlands researchers have recently investigated the presence of SARS-CoV-2 in wastewater entering some WWTPs that served 2 large and 3 medium sized cities and the main airport (Medema et al., 2020). They obtained positive signals for the selected primers when the observed COVID-19 prevalence was in the order of 1.0 to 3.5 cases among 100,000 people or more, although not always consistently. On this basis they suggested that, even in cases where the COVID-19 prevalence is low, the monitoring of sewage could be sensitive to predict the circulation of the virus in a community (Medema et al., 2020). Sewage surveillance could complement the current clinical surveillance, which is mostly concentrated on patients with COVID-19 symptoms, while infected but asymptomatic individuals are excluded.

A detailed proof-of-concept study of the WBE approach has been described by Ahmed et al. (2020), who tested 9 wastewater samples, collected from two WWTPs and a pumping station for a period of six days. This study quantified the SARS-CoV-2 RNA copies in raw wastewater, with the aim of estimating, via Monte Carlo simulation, the number of infected individuals in the catchment area. The work by Ahmed et al. (2020) draws attention to the uncertainty of some input parameters. In fact, the viral load in the stool of infected persons is not constant as described in Section 2, and it appears to be a critical parameter. The model of Ahmed et al. (2020) estimated a median range of 171–1090 infected persons in the catchment basin of 600,000 inhabitants. Despite this variation in the estimate, the authors said that this result was in reasonable agreement with the clinical observations that reported the median of 450 cases and a 95% upper confidence bound of 764 cases (Ahmed et al., 2020).

Wurtzer et al. (2020) demonstrated that the increase of the average viral load of SARS-CoV-2 in wastewater samples over time accurately followed the increase in the number of fatal cases of COVID-1. Moreover, the authors indicated that the presence of SARS-CoV-2 was detected before the beginning of the exponential growth of the epidemic (Wurtzer et al., 2020). This aspect was also confirmed by Randazzo et al. (2020) who observed experimentally the circulation of SARS-CoV-2 in wastewater even before the first cases were reported by authorities.

Briefly, a simplified theoretical approach of WBE methodology starts from the concentration of SARS-CoV-2 (converted into copies/m^3) measured in municipal wastewater taken along the network or at the inlet of a WWTP, but at a point that represents a known urbanized area drained by the sewer system. The daily viral load in wastewater (expressed in copies/d) is then calculated by multiplying the concentration by the daily flow rate of wastewater (expressed in m^3/d). This load is then compared with the viral copies in the faeces of persons testing positive for SARS-CoV-2, to obtain an estimation of the number of positive cases in the urbanized area. Unfortunately, analytical data have demonstrated that the viral load in faeces is highly variable. It is so for up to 4 orders of magnitude, from $5 \cdot 10^3$ to $10^{7.6}$ copies/mL (see Section 2), and further research is needed to propose reasonable values that can be used as a reference.

Despite these difficulties, wastewater monitoring could be proposed also as a semi-quantitative detection system or at worst for detecting presence/absence in the early surveillance of COVID-19 diffusion (Nghiem et al., 2020).

In synthesis, WBE could be a promising tool for COVID-19 surveillance, but extensive and highly coordinated studies are required, including the quantification of individual virus load in stool and during the

disease, because this information is very uncertain at the moment but is fundamental for obtaining accurate estimations.

7. Fate of SARS-CoV-2 in wastewater treatment plants

Wastewater originating from domestic, commercial and industrial sites flows along the sewerage network with a hydraulic retention time of a few hours and reaches the WWTP. When viruses have survivability (T_{90}, i.e. the time required for the virus titre to decrease by 90%) of hours or days - with T_{90} that becomes longer at low temperatures - the still infective viruses excreted in faeces can reach the WWTP (Ye et al., 2016).

At present, no comprehensive studies on the fate of SARS-CoV-2 along the entire chain of a WWTP including digested sludge or biosolids are available. Two recent studies reported the investigation of SARS-CoV-2 in treated wastewater (Wurtzer et al., 2020; Randazzo et al., 2020); main findings are summarised in Table 5. In particular, in the study of (Wurtzer et al., 2020), 6 out of 8 samples of treated wastewater were positive for SARS-CoV-2; the viral load was 1–10^2 copies/mL and was reduced by 100 times compared to the raw wastewater entering the plant (Wurtzer et al., 2020). In this study, some results were near the quantification limit set at 10^3 equivalent viral genomes per litre. Randazzo et al. (2020) found positivity only in secondary treated wastewater, with a viral load of 5.40 $\log_{10}$ copies/L, while tertiary treated effluents were all negative. The tertiary treatment in the WWTPs investigated by Randazzo et al. (2020) was based on coagulation/flocculation and/or sand filtration, while all plants implemented disinfection with UV or NaClO. The tertiary treated effluent was directed to reuse for public domain or irrigation.

These findings indicate that secondary wastewater treatment may contribute to reduce the virus concentration in wastewater, thanks to the adverse environmental conditions that the virus encounters, but removal is largely variable and thus, to enhance the level of virus inactivation in WWTPs, for example for reuse, disinfection has an important role.

Current knowledge about SARS-CoV-2 in WWTPs is largely based on what is known from the similar CoVs (Nghiem et al., 2020), which are severely affected by several environmental factors (i.e. temperature, solids, pH) or disinfectants. There is evidence that CoVs are less stable in the environment than enteric viruses - such as adenoviruses, norovirus, rotavirus and hepatitis A - for which a wide literature exists in WWTPs (Simmons and Xagoraraki, 2011; Ye et al., 2016; Gundy et al., 2009). In wastewater, T_{90} ranges from days to months for nonenveloped viruses, whereas it is several hours or days for enveloped viruses (Ye et al., 2018). Viruses of avian influenza, SARS, MERS, Ebola and SARS-CoV-2 are enveloped (Bibby et al., 2017). At the moment, the mechanisms explaining the higher susceptibility of enveloped viruses to be inactivated in aqueous environments are mostly unknown in the literature (Ye et al., 2018). Some environmental factors that may affect the stability of SARS-CoV-2 in water are summarised in Table 6.

Disinfection treatments implemented in WWTPs are based on hypochlorous acid (free chlorine), chlorine dioxide, ozone and peracetic acid or UV radiation (Naddeo and Liu, 2020). Due to the phylogenetic similarities between SARS-CoV-1 and SARS-CoV-2, disinfection technologies adopted during the SARS epidemic can be implemented also for the inactivation of SARS-CoV-2 in wastewater (J. Wang et al. 2020). The enveloped viruses, having a fragile outer membrane, are more susceptible to oxidant disinfectants, such as chlorine, than nonenveloped human enteric viruses (WHO, 2020c). Among chemical disinfectants, free chlorine proves more effective in inactivating SARS CoV than chlorine dioxide (Wang et al., 2005b), but a continuous determination of the residual chlorine content should be implemented on the effluent, to adjust the disinfectant dosage accordingly. In fact, a free chlorine residual is important to ensure the removal of the virus, but excessive disinfectant applications may cause potential adverse environmental effects, for example on ecosystems or in agriculture (Bruins and Dyer, 1995).

A reduction in the quantity of disinfectants and by-products can be obtained implementing Membrane-Bioreactors equipped with ultrafiltration (UF). The absolute pore size (defined according to Simmons and Xagoraraki, 2011) of UF is from 0.01 μm, permitting to separate SARS-CoV-2 virions with size of 60–140 nm (Table 6).

Water contaminated with sewage discharged from combined sewer overflows (CSO) during events of heavy rainfall, is another issue that poses potential risks, including the definition of specific exposure scenarios (Bibby et al., 2017). During CSO, the flow rate that is above a threshold is discharged directly into the receiving water bodies in order to reduce the impact on public health, since the mix of sewage and rainfall may contain pathogenic microorganisms and other pollutants.

8. Conclusions and research needs

The recent global outbreak of SARS-CoV-2 has highlighted scant knowledge about CoVs in the field of sewage and WWTPs. This review has collected the scientific literature currently available on the route of SARS-CoV-2 from faeces to WWTPs, although the research available in this field is very limited, fragmented or still in the early stage of development.

A percentage from 15 to 83% of patients infected with SARS-CoV-2 have detectable viral RNA in faeces, even in the absence of gastrointestinal symptoms or diarrhoea. Patients may continue to remain positive in the stool, even when respiratory tract samples become negative. Conversely, urine is often negative. The load of SARS-CoV-2 in faeces is highly variable, in the range $5 \cdot 10^3$–$10^{7.6}$ RNA copies/mL, depending on various factors (i.e. time from onset). This viral load decreases remarkably when faeces are diluted in municipal wastewater, where the

Table 5
Studies that report quantification of SARS-CoV-2 in treated wastewater (n.a. = not available).

Reference	Geographical area of WWTPs	Configuration of WWTPs	Type and No. of samples	SARS-CoV-2 load
(Wurtzer et al., 2020)	3 WWTPs of the Parisian area (France)	n.a.	8 samples of treated wastewater	From the limit of detection (1 eq/mL) to the maximum value of 10^2 eq/mL (eq/mL = equivalent viral genomes per mL)
Randazzo et al. (2020).	6 WWTPs serving the major municipalities in the region of Murcia (Spain)	Secondary treatment based on activated sludge	18 samples of secondary effluents	2 out of 18 samples were positive (1 with 5.40 $\log_{10}$ copies/L and 1 below quantification limit).
		Tertiary treatment based on: - coagulation, flocculation in 3 plants - sand filtration in 3 plants - disinfection in all plants (UV or NaClO)	12 samples of tertiary effluents	All negative

Table 6
Factors of influence and treatments that contribute to a significant reduction of SARS-CoV-2 or CoVs in WWTPs.

	Factor of influence	Experimental observations about SARS-CoV-2 or derived from other CoVs	Principles, mechanisms and laws
Environmental factors affecting stability	Stability in water is affected by temperature	• at 4 °C SARS-CoV-2 is highly stable, with an around 0.7 log-unit reduction of infectious titre after 14 d (Chin et al., 2020) • at 20 °C CoVs remain infectious for 2 d, when seeded in sewage (Wang et al., 2005a) • at 70 °C for 5 min, SARS--CoV-2 is inactivated (Chin et al., 2020)	• The decline in infectivity of CoVs (surrogates for SARS-CoV) followed a typical first-order kinetic at rate of 1.5–2.0 log per week at 25 °C, while at 4 °C the rate was slower and approximately 0.2–0.3 log per week (Casanova et al., 2009).
	Stability in water is affected by contaminants and solids	• the time required for CoVs (surrogates for SARS-CoV) infectivity reduction in sewage was approximately half that in reagent-grade water (Casanova et al., 2009) • a ~ 2 log reduction of CoV was obtained after 2 d in primary effluent in comparison to 7–8 d in tap water (Gundy et al., 2008) • CoV declined rapidly in wastewater, with a reduction of 99.9 in 2–4 d (Gundy et al., 2008)	• physicochemical constituents in sewage may accelerate the inactivation processes • T_{90} of enveloped viruses is reduced in highly contaminated matrix (demonstrated by models, Brainard et al., 2017)
	Stability in aerosol	• the half-life of SARS-CoV-2 in aerosol was approximately 1.1 h, very similar to SARS-CoV-1 (van Doremalen et al., 2020) • during the outbreak of SARS in Hong Kong the SARS viral aerosols in the building plumbing were drawn into a large apartment complex (Amoy Gardens) primarily through the air (McKinney et al., 2006) • To date there is no evidence that the COVID-19 virus has been transmitted via sewerage systems and wastewater treatments, but for a precautionary principle a certain caution should be taken	• WHO (WHO, 2020c) developed specific guidance for workers • workers wear PPE that protect from the exposure to pathogens including SARS-CoV-2 (Patel, Jernigan and 2019-nCoV CDC Response Team, 2020); US EPA: https://www.epa.gov/coronavirus/should-wastewater-workers-take-extra-precautions-protect-themselves-covid-19-virus
Stages of treatment in WWTPS	Treatment by membrane biological reactors	• 5.5, 5.1 and 3.9 log reduction of human adenovirus, human enterovirus and norovirus, respectively was obtained in MBRs with absolute pore size of 0.1 μm (Simmons et al., 2011)	• ultrafiltration (>0.01 μm), is advised in virus separation • microfiltration (>0.1 μm) is partially efficient and may require a further step of disinfection
	Treatment by disinfection with free chlorine or chlorine dioxide	• SARS-CoV in wastewater is more susceptible to sodium hypochlorite and chlorine dioxide than *Escherichia coli* (Wang et al., 2005b) • to control the virus, the dosage of hypochlorous acid (free chlorine) should ensure a free residue chlorine over 0.5 ppm, to confirm that chlorine has not been completely depleted and ensure complete inactivation of SARS-CoV (Wang et al., 2005b) • Free residue chlorine over 2.2 mg/L is needed for chlorine dioxide for complete inactivation of SARS-CoV	• Inactivation is due to the reaction with proteins in the nucleocapsid instead of genome or membrane lipids (Ye et al., 2018)

(continued on next page)

Table 6 (*continued*)

Factor of influence	Experimental observations about SARS-CoV-2 or derived from other CoVs	Principles, mechanisms and laws
	(while *E. coli* is not completely inactivated)	
Treatment by UV disinfection (UVC light)	• Enveloped viruses do not seem to have a higher susceptibility to UVC light than non-enveloped viruses (Wigginton and Boehm, 2020)	• Inactivation primarily targets the genome, while lipid membrane do not protect viruses from the radiation (Wigginton and Boehm, 2020)
Treatment by primary and secondary settling	• 26% of enveloped viruses adsorbed to solids, compared to 6% of nonenveloped (Ye et al., 2016) • a significant part of CoVs, is likely to be present in the primary or secondary sludge • inactivation in sludge is similarly as in wastewater (3 log in 2–4 d) (Gundy et al., 2008)	• The hydrophobicity of the viral envelope makes enveloped viruses less soluble in water and they tend to adhere to solids and to concentrate in sludge (Gundy et al., 2008)
Treatment by mesophilic and thermophilic anaerobic digestion	• Human CoV were detected in sludges pre and post anaerobic digestion (Bibby et al., 2011; Bibby and Peccia, 2013) • SARS-CoVs infectivity is lost at 56 °C for 90 min (temperature of thermophilic anaerobic digestion) (Duan et al., 2003) • in anaerobic digestion at 28 °C, poliovirus lose infectivity and ammonia may act as a virucidal agent (Ward and Ashley, 1979)	• higher inactivation of CoVs is expected in anaerobic digestion because CoVs are much more sensitive to warm temperature than poliovirus • thermophilic aerobic digestion is much more effective against nonenveloped viruses than mesophilic digestion (Wigginton et al., 2015).

concentration of SARS-CoV-2 drops to a range from 2 copies/100 mL to $3 \cdot 10^3$ copies/mL, depending on the level of the epidemic. Quantitative data on viral load in faeces and wastewater and their relationship, currently uncertain, are fundamental for WBE applications to be used for the early warning of outbreaks. In particular, the large uncertainty in the viral load in faeces makes it difficult to determine a typical value that could be extremely useful in WBE.

The fate of SARS-CoV-2 in WWTPs, because of the actual scarcity of analytical data, is predicted on the basis of similar CoVs that are severely affected by environmental factors (e.g. temperature, solids, pollutants, pH). However, current findings indicate that faecal-oral transmission is not proven. By analogy with the previous studies on SARS and MERS outbreaks, there are reasons to conclude that the viral content may be controlled in WWTPs.

CRediT authorship contribution statement

Paola Foladori: Conceptualization, Methodology, Writing. **Francesca Cutrupi:** Methodology, Writing. **Nicola Segata:** Conceptualization, Methodology, Writing. **Serena Manara:** Methodology, Writing. **Federica Pinto:** Methodology, Writing. **Francesca Malpei:** Conceptualization, Methodology, Writing. **Laura Bruni:** Methodology, Writing. **Giuseppina La Rosa:** Conceptualization, Methodology, Writing.

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.

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