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Current emerging SARS-CoV-2 pandemic: Potential direct/indirect negative impacts of virus persistence and related therapeutic drugs on the aquatic compartments

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ABSTRACT

The purpose of the present work is to provide a complete overview of possible direct/indirect implications on the quality of aquatic compartments due to the recent SARS-CoV-2 outbreak. With this aim, the environmental impacts are mainly related to i) the virus persistence in sewage and wastewaters, and ii) possible fate in aquatic compartments of drugs tested and administered to SARS-CoV-2 infected patients. Because SARS-CoV-2 spread is very recent, and there is a lack of specific studies on this strain, the virus persistence in wastewaters, the parameters influencing the persistence, as well as the detection methodologies are referenced to the general coronaviruses group. However, the present detailed report of up-to-date knowledge on this topic can provide a useful source for further studies focusing on more deepened investigations of SARS-CoV-2 behaviour in the environment. Such a perspective is significant not only for the control of virus diffusion but also represents a crucial point for the identification of produced alteration to the environmental quality.

1. Introduction

At the end of December 2019, the first detection of SARS-CoV-2 virus occurred in Wuhan (Hubei Province, China) creating a big concern related to a possible outbreak (Zhu et al., 2020). The SARS-CoV-2 is a viral strain from a wide viruses group identified as coronaviruses in the sixties (Woo et al., 2010). The group includes strains such as MERS-CoV and SARS-CoV, which have been responsible for a widespread diffusion in the last 20 years (Lee and Hsueh, 2020; Lu et al., 2015). However, the current SARS-CoV-2 showed a wider and faster diffusion than the previous coronaviruses. In fact, its basic reproduction number (R_0) is almost one order of magnitude higher compared to the others of the same group ($R_{0\text{-SARS-CoV-2}} > R_{0\text{-SARS-CoV}} > R_{0\text{-MERS-CoV}}$) (Cao et al., 2020; Liu et al., 2020; WHO, 2003, 2019). Because of the SARS-CoV-2 worldwide diffusion, the World Health Organization (WHO) declared the state of pandemic on the last March 11, and each country all over the world adopted specific containment measures on the population and economic activities (Cucinotta and Vanelli, 2020; WHO, 2020a). It resulted in a global social restrictive action with no precedents. To date, the presence of the virus is confirmed in more than 200 countries with a number of confirmed infected individuals not far from **7**.0 millions (WHO, 2020b). However, these numbers are likely to be much higher, as they do not include positive asymptomatic patients (Pan et al., 2020).

Because of their nature of respiratory pathogens, over the years the main concern about coronavirus strains transmission has been focused on aerosols and droplets, coming from infected individuals (Chen et al., 2020). Indeed aerosols and droplets control are primary means for the coronavirus spreading and play a key role for the prevention and containment of the contagion extension (Lai et al., 2020; Motta Zanin et al., 2020). Nonetheless, to fully control the outbreak, many researchers moved their attention on the potential correlation between the coronavirus spread and its survival in the environment outside the human host (Núñez-Delgado, 2020). In particular, different studies focused on the presence endurance of the virus in sewage and wastewaters identifying this environmental compartment as a potential mean of exposure to the contagion (Daughton, 2020; Reusken et al., 2020; Wigginton and Boehm, 2020). This concern is mainly due to the

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coronaviruses structure which is common to other viruses (such as H1N1 "Spanish flu", avian influenza H5N1 and H7N9, SARS-CoV, MERS-CoV) detected in the stools and urine of infected individuals (Wigginton et al., 2015). For instance, Wang et al. (2005c) observed that SARS-CoV persistence in body fluids (stools and serum) was up to 96 h. Similarly some studies recently reported traces of the SARS-CoV-2 RNA in the wastewater of several European urban centres, during the current pandemic (Reusken et al., 2020; Wurtzer et al., 2020).

This is certainly the main aspect of the direct relationship existing between the current SARS-CoV-2 pandemic and the aquatic compartments (Venugopal et al., 2020; Zambrano-Monserrate et al., 2020). Nonetheless, there is an indirect relationship, which is worth highlighting too, related to the use of drugs administered to infectious individuals. In particular, several scientific studies are currently focusing on specific drugs already used for other medical diseases therapy in order to identify effective treatments, which could mitigate the dangerous effects of coronavirus infection (Guo et al., 2020). All these drugs are released, in massive amount, into the wastewater through the body fluids of the infected patients, potentially causing a dramatic alteration of the final aquatic receptor compartments and exposed biota (Richardson, 2012).

Therefore, the aim of the present manuscript is to provide a wide and detailed report about all the potential consequences of the current pandemic, which can be directly or indirectly exerted on the aquatic compartments. Accordingly, the manuscript focuses on three main sections analysing i) the correlations existing between the SARS-CoV-2 virus and the previously identified strains of the same coronavirus group; ii) the persistence characteristics of coronaviruses in wastewater, and the related methodologies for its detection; iii) the negative impact on water bodies, related to the release of therapeutic drugs for coronavirus infection treatments.

2. Genetics and virology

Coronaviruses are positive single stranded RNA viruses, having a diameter of about 60–140 nm (Bárcena et al., 2009; Cascella et al., 2020). They are named after their shape, observed at the electron microscope, which reminds a crown (in latin *corona*) because of the presence of spike proteins on the surface. Coronaviruses are common in many animal species and, in some cases, can be transmitted to humans, leading to zoonotic diseases (Su et al., 2016). Sequencing and phylogenetic analyses have shown that the novel SARS-CoV-2 is closely related (similarity rate of 88%) to a group of human SARS-like coronaviruses and bat SARS-related coronaviruses (bat-SL-CoVZC45, bat-SL-CoVZXC21).

The viral composition of coronaviruses includes pericapsid envelope, which consists of a double layer of phospholipids and glycoproteins (Ashour et al., 2020). The several structural proteins (Fig. 1) are represented by the membrane (M), the envelope (E), the spike (S), and the nucleocapsid (N) one (Fehr and Perlman, 2015). The N protein is sited into the virus particle core and interacts with the virus RNA. The stabilization of this latter is due to the M protein, which is bound to the N protein. The structural M protein is the most abundant one and confers the shape to the virus (Nal et al., 2005). It forms the virion internal core, and together with the E protein, the mature viral envelopes. The amount of the E protein in the envelope is not high but, during the replication cycle, its presence inside the infected cells is reported to be remarkable. Moreover the E protein plays a fundamental role in the virus production (Siu et al., 2008). The S protein is responsible for the formation of homotrimeric spikes on the viral particle surface. It is a highly glycosylated protein, which mediates viral entry into the host cells. In certain coronaviruses, each S protein monomer can be present on viral particle as two subunits (i.e. S1 and S2). This is caused by the S protein separation occurring in the virus replication, due to the host furin-like proteases (Bosch et al., 2003; Izaguirre, 2019). In other strains, such as SARS-CoV, the S protein does not separate and forms the S1 and the S2 domains (Ashour et al., 2020; Xiao et al., 2003). In S1, the receptor-binding domain (RBD) is responsible for the mediation with the related host cell receptor while the S2 domain allows the fusion between the two cell membranes (host and viral) which is necessary for the entry of coronaviruses into the host cells (He et al., 2006).

It has been reported that some cellular receptors can be identified as coronaviruses receptor such as the case of angiotensin-converting enzyme 2 (ACE2) for the SARS-CoV (Wrapp et al., 2020). Moreover, Lu et al. (2020) observed similarities in the RBD between SARS-CoV and SARS-CoV-2. According to this, ACE2 could be a cellular receptor also for the new SARS-CoV-2 (Wrapp et al., 2020). ACE2 in humans is well expressed in the respiratory tract, so when the virus enters it is able to quickly replicate in the target cells, causing strong respiratory infections (Jia et al., 2006).

Also relevant, taking into account that cell entry of coronaviruses depends on binding of the viral S proteins to cellular receptors and on S protein priming by host cell proteases, Hoffmann et al. (2020) found that SARS-CoV-2 uses the SARS-CoV receptor ACE2 for entry and the serine protease TMPRSS2 for S protein priming.

3. Direct environmental impact on wastewater

3.1. Persistence of coronavirus in wastewaters

Because of the limited time span occurred from the beginning of the COVID-19 pandemic, very scarce research studies dealing with the presence of the SARS-CoV-2 in wastewaters are yet available (Lodder and de Roda Husman, 2020; Medema et al., 2020). However, the methodologies applied in the past for the detection of other strains of the same coronavirus group, such as virus RNA detection, Most Probable Number method, etc. can be considered applicable also for the present situation, and might turn out useful for monitoring the presence of the SARS-CoV-2 in wastewaters (Barcelo, 2020; C. C.G. Daughton, 2020; La Rosa et al., 2020; Orive et al., 2020).

Generally speaking, research studies concerning the fate of viruses in aquatic compartment, have been mainly focused on nonenveloped enteric viruses, as these viruses are characterized by higher resistance in various environmental conditions (Carducci et al., 2020). The number of studies concerning the fate of enveloped viruses in aquatic compartments, instead, is quite limited, as enveloped viruses are predisposed to deactivate in waters (Wigginton et al., 2015). Nonetheless, in the last 10–15 years, some authors have reported, the possible presence of enveloped viruses, such as coronaviruses in sewage systems (Cantalupo et al., 2011; Ikehata et al., 2009). Indeed, despite the faster inactivation rate of enveloped viruses compared to the nonenveloped ones, the survival time of enveloped viruses can be not negligible, according to the specific environmental conditions (Table 1). Main parameters affecting the potential survival of coronaviruses in wastewater are represented by temperature, relative humidity (Casanova et al., 2010; La Rosa et al., 2012) as well as suspended solids content and disinfection products in waters. For instance, Ye et al. (2016) observed a T_{90} (time for 90% virus titer decrease) equal to 7 h for enveloped *Pseudomonas* phage ϕ6 and equal to 13 h for murine hepatitis virus (MHV) in unpasteurized water at 25 °C. The value of T_{90} increased to 28 h for ϕ6 and to 36 h for MHV at 10 °C. On the contrary, Casanova and Weaver (2015) reported a faster inactivation of bacteriophage ϕ6 in sewage, increasing the temperature. In more details, the limit of virus detection occurred after 4 observation days at 30 °C, and after 10 observation days at 22 °C. In general, low temperatures and humidity can be favourable conditions for a prolonged persistence and more concerning viability of coronaviruses (Yeo et al., 2020). Comparing different waters (tap water filtered and unfiltered) or wastewaters (primary effluent filtered and unfiltered and secondary effluent), experimental results always indicated a decrease of coronavirus survival with increasing temperatures (Gundy et al., 2009) reporting a

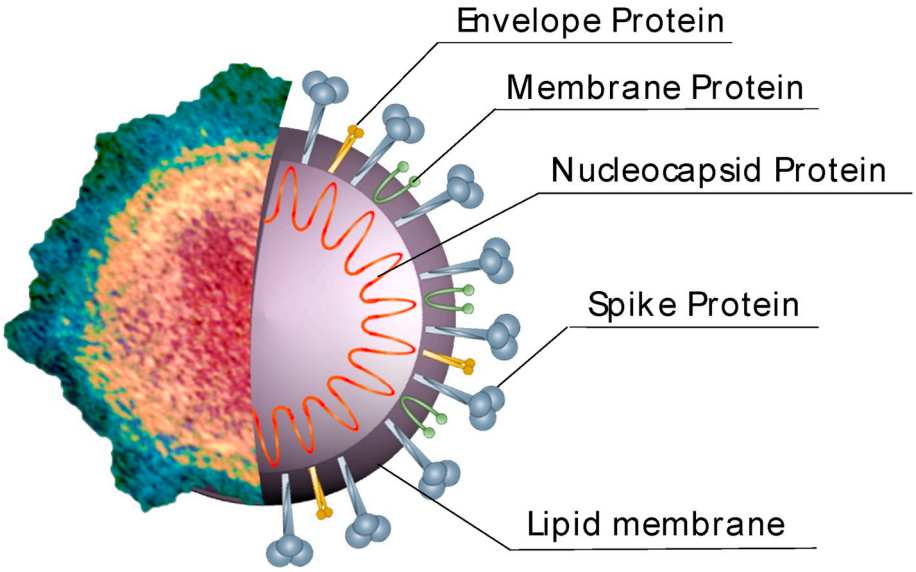

Fig. 1. Transmission electron microscope image (left side) and structural schematic representation (right side) of SARS-CoV-2.

coronavirus $T_{99.9}$ (time for 99.9% of virus titer decrease) growing from 10 to above 100 d with temperature decreasing from 23 to 4 °C in filtered tap water. Moreover, comparing filtered and unfiltered primary effluent from wastewater treatment plant at the same environmental temperature (23 °C), the same authors observed a higher $T_{99.9}$ for the unfiltered wastewater than for the filtered one (Gundy et al., 2009). Such a result suggested that a higher solid content in water/wastewater samples can provide a higher protection to the virus, for its longer survival in the environment. This is expected considering the hydrophobicity of the coronavirus envelop, which leads to a lower virus solubility and to a potentially higher rate of virus adhesion onto solid particles (Gundy et al., 2009). Similar inactivation trend were observed for MHV and transmissible gastroenteritis virus (TGEV) in pasteurized settled sewage at two different temperatures (25 and 4 °C) (Casanova et al., 2009). A faster inactivation was observed for both viruses at 25 °C with a 99.99% of virus titer decrease ($4\log_{10}$ viral reduction kinetic) of 19 d for TGEV and 14 d for MHV. On the contrary, half of the time was required in order to achieve a 99% of virus titer decrease ($2\log_{10}$ viral reduction kinetic) for both viruses.

Further information concerning the survival of coronaviruses in aquatic environment can be found in the report of the World Health Organization (WHO, 2003), dealing with a significant SARS spreading occurred in a housing block of Hong Kong. According to a preliminarily investigation, in fact, the contagion was attributed to the contaminated air, flowing inside the apartments through the ventilation system located in the building bathrooms, having a flawed plumbing system. This hypothesis led to a pilot-scale investigation conducted with a model organism, *Pseudomonas putida,* aimed at assessing the potential virus transmission via building plumbing systems (Gormley et al., 2017). The experimental observations confirmed that the organisms spreading could have been favoured by the transportation through the ventilation system. Such an event allowed identifying the interconnectedness of a plumbing system and its conditions, as two fundamental factors to be monitored in order to prevent infection diffusion, especially in high risk location, such as hospitals (Gormley et al., 2020).

Strongly related to the high potential of sanitary structures to contribute to viruses spreading, are the two research studies conducted by Wang et al. (Wang et al., 2005a, 2005b). In these studies the RNA of SARS-CoV was isolated from patients' stools, no RNA was found in their urine. Moreover, no live viruses were isolated from stools samples, suggesting the possibility of no infectious SARS-CoV excretion from digestive system of infected patients (Wang et al., 2005a). Also, the virus nucleic acid was mainly found in sewage samples collected before the disinfection treatment while less RNA occurrence was detected in disinfected sewage samples (Wang et al., 2005b). However, despite the RNA of SARS-CoV was detectable in sewage for 8 d, no active virus could be found (Wang et al., 2005b). Indeed, SARS-CoV virus showed a marked sensibility to inactivation due to disinfection products in wastewater. Experimental tests proved that both sodium hypochlorite and chlorine dioxide may exert an inactivation effect on the virus (Wang et al., 2005c). However, free chlorine from sodium hypochlorite dissolution resulted more efficient as inactivating agent, recommending its use as preferred disinfecting agent for hospital wastewater (Tsai and Lin, 1999; Wang et al., 2005c). It has to be highlighted that, besides chlorinated compounds, several other organic compounds, including alcohols (ethanol, and isopropanol), aldehydes (formaldehyde), and phenolic compounds (creosol soap) were found to be very efficient for some coronaviruses inactivation, such as MHV and canine coronavirus (Wolff et al., 2005). This generally highlights that an important approach to limit possible exposure to the virus infection could be represented by new disinfection technologies and upgrading of wastewater treatment plants for the remediation of wastewaters deriving from specific buildings (such as hospitals) (Naddeo and Liu, 2020).

3.2. Methodologies for detection of coronaviruses persistence in wastewater

Detection methods for viruses in the environment must be preceded by a suitable concentration technique especially in case of very low virus levels. Various concentration methods have been tested, each presenting some advantages and some disadvantages. Among others can be cited the methods based on adsorption-elution (negatively/positively charged filters, glass powder or fiber), precipitation (organic flocculation, ammonium sulphate precipitation), ultracentrifugation, ultrafiltration, and lyophilisation (Bosch et al., 2006).

Detection methods are often tested on artificially contaminated samples. A well-assessed practice includes the use of surrogate viruses. This practice is useful to overcome problem related to viruses which are not easily cultivable (Bosch et al., 2006). For instance, MHV and TGEV have been successfully used as surrogate viruses to identify the coronaviruses persistence at various ambient temperatures and in different water environments (Casanova et al., 2009).

Common traditional methods for virus determination are represented by plaque assay or, for viruses not forming plaques, by the 50% tissue culture infective dose ($TCID_{50}$). In the plaque assay, it is possible to determine the viral titer in terms of plaque forming units (pfu). The assay is conducted using petri dishes, inoculating countable

Table 1
Information related to experimental conditions, investigated viruses, concentration/detection methods, and main results on virus persistence in wastewaters reported in literature studies.

Experimental	Virus	Concentration method	Detection method	Virus persistence main results	Reference
21 stool and urine samples collected from Xiao Tang Shan Hospital and 309th Hospital; sewage samples collected for 7 d before disinfection (2500 ml) and after disinfection (25,000-50000 ml)	SARS-CoV	Positively charged filter media particles	RT-PCR assay	• No presence of infectious SARS-CoV • SARS-CoV RNA detection in stool samples (7 on 11) of symptomatic patients • No RNA detection in urine samples and in stool of recovered patients • RNA detection in sewage samples before disinfection and RNA detection in sewage after disinfection only in 3 d	Wang et al. (2005a)
Sewage samples collected before disinfection (2500 ml) and after disinfection (25,000-50000 ml) from Xiao Tang Shan Hospital, 309th Hospital and housing estate	Bacteriophage f_2 (as coronavirus model) and SARS-CoV	Positively charged filter media particles	RT-PCR assay	• No presence of infectious SARS-CoV • SARS-CoV RNA detection in sewage samples before the disinfection • RNA detection in sewage after disinfection only in 3 d • Average f_2 recovery from hospitals samples of 79.2 and 85.8% before disinfection and 61.2 and 85.5% after disinfection	Wang et al. (2005b)
Samples of stool (3) and urine (2) from Xiao Tang Shan Hospital; wastewater samples from 309th Hospital; sewage samples from housing estate; disinfection tests on wastewater with different chlorine (by dissolution of sodium hypochlorite) or chlorine dioxide concentration and disinfection time	Bacteriophage f_2 (as coronavirus model) and SARS-CoV	–	RT-PCR assay	• Persistence of SARS-CoV in wastewater and sewage samples for 2 d at 20 °C and 14 d at 4 °C • Persistence of 3 d in stool and 17 d in urine at 20 °C and persistence > 17 d at 4 °C • Complete SARS-CoV inactivation with 10 ppm of chlorine after 10 min • Reduced effectiveness in presence of chlorine dioxide • Total inactivation with 20 ppm of chlorine in 1 min and with 40 ppm of chlorine dioxide in 5 min	Wang et al. (2005c)
Wastewater samples collected from wastewater treatment plant and pasteurized; comparison with reagent-grade and lake water; tests on temperature effect carried out at 23–25 °C and 4 °C	TGEV and MHV (as surrogates coronaviruses)	–	–	• 99% decrease in infectious titer equal to 22 d for TGEV and 17 d for MHV at 25 °C • No significant decrease over 49 d at 4 °C in reagent-grade water and 99% decrease in infectious titer equal to 13 d for TGEV and 10 d for MHV at 25 °C • $1\log_{10}$ decline for TGEV and no decline for MHV after 14 d at 4 °C in lake water • 99% decrease in infectious titer equal to 9 d for TGEV and 7 d for MHV at 25 °C in pasteurized settled water • 99% decrease in infectious titer equal to 49 for TGEV and 70 d for MHV at 4 °C in pasteurized settled water	Casanova et al. (2009)
Samples of unfiltered tap water tested at 23 °C and filtered tap water tested at 23 and 4 °C; samples of filtered and unfiltered primary effluent tested at 23 °C; samples of unfiltered secondary (activated sludge) effluent tested at 23 °C	Feline infectious peritonitis virus (FIPV), Human coronavirus 229 E (HCoV) and Poliovirus 1 LSc-2ab (PV-1)	–	Plaque assay or $TCID_{50}$	• 99% virus titer decrease of 6.76 d (for HCoV and FIPV) and 43.3 d (for PV-1) in filtered tap water at 23 °C • Higher persistence in unfiltered tap water at 23 °C equal to 8.09 d for HCoV, 8.32 d for FIPV, and 47.5 d for PV-1 • Persistence in filtered tap water at 4 °C equal to 392 d for HCoV, 87 d for FIPV, and 135 d for PV-1 • 99% virus titer decrease of 1.57 d for HCoV, 1.60 d for FIPV, and 23.6 d for PV-1 in filtered primary effluent at 23 °C • Higher persistence in unfiltered primary effluent at 23 °C equal to 2.36 d for HCoV, 1.71 d for FIPV, and 7.27 d for PV-1 • Persistence in secondary effluent at 23 °C equal to 1.85 d for HCoV, 1.62 d for FIPV • Lower 99% virus titer decrease required for PV-1 in secondary effluent (3.83 d) • Similar trends for 99.9% virus titer decrease	Gundy et al. (2009)
Samples of wastewater collected and pasteurized at 70 °C for 3 h artificially spiked with enveloped virus surrogate; virus titer decrease tested at 22 and 30 °C	Bacteriophage ϕ6 (as surrogate of enveloped human viruses)	–	–	• Predominant linear virus inactivation observed at 30 °C with $7\log_{10}$ reduction after day 3 and detection limit at day 4 • Slower and nonlinear inactivation from day 0–3 • Accelerated inactivation occurred from day 4–6 followed by slower average inactivation from day 7–9 at 22 °C (detection limit achieved at day 10)	Casanova and Weaver (2015)

(*continued on next page*)

Table 1 (*continued*)

Experimental	Virus	Concentration method	Detection method	Virus persistence main results	Reference
Unpasteurized and pasteurized samples of wastewater from wastewater treatment plant artificially spiked and incubated at 10 and 25 °C for viruses survival tests; artificial spiking of untreated wastewater and centrifuged wastewater for solids removal incubated at 4 °C for viruses partitioning tests	MHV and *Pseudomonas* ϕ6 (as surrogates of enveloped human viruses); MS2 and T3 (as nonenveloped bacteriophages)	Polyethylene Glycol (PEG) precipitation method, ultracentrifugation method and ultrafiltration method	Plaque assay	• Slower viruses infectivity decrease in pasteurized wastewater compared the unpasteurized one • T_{90} in unpasteurized wastewater equal to 13 h for MHV and 7 h for *Pseudomonas* ϕ6 at 25 °C • T_{90} equal to 36 h for MHV and 28 h for *Pseudomonas* ϕ6 at 10 °C • T_{90} equal to 121 h for MS2 at 25 °C in unpasteurized wastewater • Longer T3 survival at 25 °C in unpasteurized wastewater and no significant differences observed at 10 °C • Fast deactivation in wastewater with removed solids for MHV (53%) and *Pseudomonas* ϕ6 (23%) • Faster decay observed for MHV and *Pseudomonas* ϕ6 in the untreated wastewater within 1 h • MS2 significant concentration decrease in wastewater with removed solids over 3 d with faster decrease in liquid phase followed by a slower decay kinetic • No significant T3 decrease in both untreated wastewater and wastewater with solids removed • Best recovery of enveloped viruses from wastewater with optimized ultrafiltration method and worse recovery of enveloped viruses from wastewater with PEG precipitation and ultracentrifugation methods	Ye et al. (2016)

and statistically proper number of virus particles on a layer of immobilized cells (Cooper, 1962). The $TCID_{50}$, in turns, allows to determine the sample dilution value corresponding to the occurrence of 50% cytopathic effect (CPE) (Gundy et al., 2009). Together with these traditional methods, nowadays more modern molecular techniques are frequently adopted. The most used ones are the polymerase chain reaction (PCR) and the reverse transcription polymerase chain reaction (RT-PCR). In particular, the RT-PCR is a technique used to transcript the RNA in a DNA chain through the reverse transcriptase enzyme and successively amplify the DNA fragment through the PCR technique in order to indirectly determine RNA species (Carter and Shieh, 2015). Real-time RT-PCR has been recently proposed as reliable technology to institute new diagnostic tests in the current pandemic emergency related to the SARS-CoV-2 (Corman et al., 2020). In this work, a validated diagnostic workflow has been suggested to detect the current coronavirus and methodology design/validation was allowed due to the genetic connection between the SARS-CoV-2 and the previous SARS-CoV.

Further methodology suggested for rapid and economic pathogens diagnosis is represented by paper-based devices (Magro et al., 2017). These devices are small and easily transportable analytical tools which can integrate various processes useful for tests on nucleic acid (from the extraction to the amplification and visual detection) (Mao et al., 2020). Paper-based devices could therefore potentially represent an useful tool for virus fast detection in wastewaters and fundamental monitoring system useable in emergency circumstances such as the current SARS-CoV-2 infection spreading.

4. Potential environmental impact of administered drugs

During an outbreak, the lack of information on effective antiviral drugs (AVs) or vaccines leads to nonspecific therapy for the minimization of the mortality rate. In the current pandemic, the existing drugs are being administered in much larger amount so representing an important threat to the quality of the receiving water bodies. Besides the treatment of disease due to coronaviruses, drugs are administered against a broad spectrum of viral infections such as HIV, herpes, hepatitis, Ebola and Malaria as well as autoimmune diseases such as lupus and rheumatoid arthritis (Babıć et al., 2017; Stebbing et al., 2020).

As part of clinical trials on drugs for the treatment of COVID-19 disease, the use of humanized monoclonal antibody like tocilizumab (TCZ) has been approved by FDA (Food and Drug Administration) for various therapies including those related to rheumatologic disease and lymphoproliferative disorder (Xu et al., 2020). TCZ is a recombinant monoclonal antibody against the interleukin-6 receptor (IL-6R) produced by recombinant DNA technology. IL-6R is a cytokine adopted in the development of immunological and inflammatory reactions. TCZ recognizes the IL-6 binding site on the cell membrane inhibiting the IL-6 transduction signalling (Kallen, 2002; Venkiteshwaran, 2009).

TCZ is considered a Protein and Peptide Therapeuticals (PPTs) not associated to environmental concern by the European Medicines Evaluation Agency (EMEA) guideline on environmental risk assessment (ERA) for human pharmaceuticals (EMEA, 2006). Although ecotoxicological data are not available, the half maximal effective concentration (EC50), as reported by the safety data sheet from the supplier, showed no TCZ adverse effects for concentrations higher than 100 ppm (Table 2). Biodegradability and acute ecotoxicity studies on TCZ report rapid biodegradability in sewage and surface waters as well as low ecotoxic characteristics (RCC Ltd, 2006a, 2006b; 2006c, 2006d; Straub, 2010).

The hydroxychloroquine (HCQ) and the chloroquine (CQ) represent another class of disease-modifying anti-rheumatic drug (DMARD) also added to the list of trial drugs in the guidelines for the diagnosis and treatment of COVID-19 (J. Liu et al., 2020). These drugs have also been used for years in antimalarial prevention. HCQ and CQ belong to the quinolone family and exert their action by blocking toll-like receptors

Table 2
Toxic effects of drugs used for the COVID-19 disease treatment on selected models and biomarkers.

Compound	Organism	Species	Endpoint (exposure time)	EC50 (ppm)	Reference
Tocilizumab	Alga	*Desmodesmus subspicatus*	Growth rate inhibition (72 h)	> 100	Roche safety data sheet (2018)
	Alga	*Desmodesmus subspicatus*	Biomass inhibition (72 h)	> 100	
	Crustacean	*Daphnia magna*	Immobility (48 h)	> 100	
	Fish	*Danio rerio*	Embryotoxicity (96 h)	> 100	
Chloroquine	Bacteria	*Aliivibrio fischeri*	Bioluminescence Inhibition (24 h)	132.1	Zurita et al. (2005)
	Alga	*Chlorella vulgaris*	Growth Inhibition (24 h)	133.3	
	Crustacean	*Daphnia magna*	Immobility (24 h)	21.5	
	Topminnow	PLHC-1 cell line	Protein content (24 h)	158.3	
	Basket willow	Salix viminalis	Relative transpiration (NRT) (117 h) (pH from 6 to 8)	7–28	Rendal et al. (2011)
	Crustacean	Daphnia magna	Immobility (48 h) (pH from 7 to 9)	4–30	
Hydroxychloroquine	Alga	Raphidocelis subcapitata	Growth rate (72 h)	3.1	FASS safety data sheet (2019)
	Crustacean	Daphnia magna	Immobility (48 h)	14	

(TLR) and reducing the activation of dendritic cells, with consequent mitigation of the inflammatory process.

The possible increasing release due to human excreta of HCQ and CQ in surface water through wastewater treatment plant effluents represents a concerning environmental issue. CQ and HCQ are highly soluble in water with partition coefficient octanol/water (log K_{ow}) equal to 4.67 for HCQ and 3.03 for CQ. Moreover, available literature studies indicate that these drugs are only partially transformed inside the human body and can be almost completely excreted through urine and stools. It is reported that CQ is excreted unaltered at a percentage variable from 40 to 70% through kidney, and at a percentage variable from 5 to 10% through urine (Haładyj et al., 2018). Similarly, percentages of unaltered excreted HCQ range from 40 to 60% through kidney and from 8 to 25% through stools (Babić et al., 2017).

CQ and HCQ can be also considered as persistent and/or bioaccumulative at high release extent in the environment potentially representing new emerging contaminants (Daughton, 2014; Howard and Muir, 2011; Zurita et al., 2005). Nevertheless, data on CQ and HCQ concentrations in the environment are very scarce. Chen et al. (2013) reported the CQ and HCQ detection in surface sediments near three rivers in southeast China. Similarly, Olaitan et al. (2017) found CQ in wastewater effluents in Nigeria (Olaitan et al., 2017). The general findings (Table 2) confirmed that CQ and HCQ compounds could be risky for the environment and should be classified as harmful to aquatic organisms (Ramesh et al., 2018; Zurita et al., 2005).

From an environmental perspective, different considerations should be made for the AVs used in COVID-19 disease therapies. The real concern related to AVs use and their environmental persistence, in analogy to the development of antibiotic resistance, is the potential formation of resistant strains through chronic exposure. This could consequently entail more adverse effects to human health than other classes of drugs (Jain et al., 2013). Moreover, further drawbacks are represented by AVs low biodegradability and their increasing use during pandemic outbreaks (Funke et al., 2016; Hill et al., 2014; Russo et al., 2017).

Most of the AVs are excreted as unchanged parent compounds with highly bioactive characteristics which are resistant to conventional treatments in wastewater treatment plants. Moreover, they can react with organic and inorganic constituents during wastewater treatment and can be transformed in additional molecules characterized by higher persistence (Funke et al., 2016; Jain et al., 2013). Despite no effective AV has been specifically approved for the treatment of COVID-19 disease, recent studies are focusing on the possible use of Lopinavir and Remdesivir (Grein et al., 2020). Regarding the Lopinavir, the environmental risk assessment in hospital effluents has been evaluated through the determination of the Predicted Environmental Concentration (PEC) and the Predicted No-Effect Concentration (PNEC) (Acree and Grubbs, 2012). The result from the risk assessment showed that PEC value of Lopinavir was higher than its PNEC value (0.26 and 0.05 ppb, respectively) therefore indicating a potential environmental harm. Moreover, the Lopinavir was listed among the top ten ranked active pharmaceutical ingredients (API), mainly due to its high bioaccumulation potential (log K_{ow} > 3.9) (Daouk et al., 2015).

The Remdesivir is a nucleoside analog, which incorporates into nascent viral RNA chains and inhibits viral RNA polymerases. This AV has broad-spectrum activity against members of the filoviruses, coronaviruses, and paramyxoviruses. The EMEA has recommended for compassionate use of the Remdesivir although information on the related environmental risk (ecotoxicity and degradability in the environment) are lacking. According to this, further researches are necessary to assess the magnitude of the environmental risk posed by the Remdesivir.

5. Conclusions

Since the beginning of globalization era, COVID-19 disease has been the first pandemic characterized by such a wide and significantly fast spread. This global emergency took all the world countries unawares. The occurrence of the infection spread also in poorly industrialized areas with limited resources for epidemic containment and healthcare systems represents an even more concerning issue.

In this context, the scientific community should not only pay close attention to the health aspect but also inevitably consider the environmental one. In fact, fundamental importance should be given to further deepened studies aimed at identifying accurate monitoring and analysis systems for prompt detection of potential viruses diffusion through aquatic media. An additional environmental element to be taken into account is related to the consumption of drugs for the coronavirus related disease therapy, which could lead to risky release of toxic substances in the receiving water bodies. To date, the removal efficiencies of these new contaminants from wastewaters through feasible treatments have been poorly investigated. Therefore, future researches should focus on the environmental fate of these contaminants, and should evaluate the effectiveness of tertiary treatments (such as advanced oxidation processes) on their removal.

Credit author statement

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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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