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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 ($R_0$SARS-CoV-2 $> R_0$SARS-CoV $> R_0$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
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 virus 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. G.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 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 T90 (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 T90 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
coronavirus T<sub>99.9</sub> (time for 99.9% of virus titer decrease) growing from temperature (23 °C), the same authors observed a higher T<sub>99.9</sub> for the effluent from wastewater treatment plant at the same environmental temperature. Moreover, comparing filtered and unfiltered primary 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 (4log<sub>10</sub> 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 (2log<sub>10</sub> 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<sub>50</sub>). 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...
| Experimental Conditions | Investigated Viruses | Concentration/Detection Methods | Main Results on Virus Persistence in Wastewaters | 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 (25,000-50,000 ml) | SARS-CoV | Positively charged filter media particles | RT-PCR assay | No presence of infectious SARS-CoV | Wang et al. (2005a) |
| Sewage samples collected before disinfection (2500 ml) and after disinfection (25,000-50,000 ml) from Xiao Tang Shan Hospital, 309th Hospital and housing estate | Bacteriophage f2 (as coronavirus model) and SARS-CoV | Positively charged filter media particles | RT-PCR assay | No presence of infectious SARS-CoV | 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 f2 (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 | 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 of coronaviruses) | – | – | 99% decrease in infectious titer equal to 22 d for TGEV and 17 d for MHV at 25 °C | 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 TCID50 | 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 | Gandy 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 7log10 reduction after day 3 and detection limit at day 4 | Casanova and Weaver (2015) |
### Table 1 (continued)

| Virus persistence main results | Detection method | Concentration method | Experimental |
|-------------------------------|------------------|---------------------|--------------|
| Plaque assay                  | Ye et al. (2016)  | MHV and Pseudomonas \(\phi_6\) | Virus suspended and pretreated with artificial and natural quenchers from wastewater to confirm the integrity of viral particles | 5 |
| - Slower decrease in purified wastewater compared to unpurified one | - Pseudomonas \(\phi_6\) at 23 °C | - Pseudomonas \(\phi_6\) at 23 °C | - Fast deactivation in wastewater with removed solids for MHV (53%) and Pseudomonas \(\phi_6\) in the untreated wastewater | 5 |
| - Faster decay observed for MHV and Pseudomonas \(\phi_6\) in the untreated wastewater and no significant differences observed in wastewater with removed solids | - T\(_{25}\) survival at 10 °C | - T\(_{25}\) equal to 36 h for MHV and 28 h for Pseudomonas \(\phi_6\) at 25 °C | - 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 | 5 |
| - T\(_{20}\) survival within 1 h | - MS2 significant concentration decrease in wastewater with removed solids | - No significant T\(_{3}\) decrease in both untreated wastewater and wastewater with removed solids | - No significant T\(_{3}\) decrease in both untreated wastewater and wastewater with removed solids | 5 |
| - Faster decay observed for MHV and Pseudomonas \(\phi_6\) in the untreated wastewater and no significant differences observed in wastewater with removed solids | - MS2 significant concentration decrease in wastewater with removed solids | - Faster decrease in viral particle level followed by a slower decay of viral concentration | - Faster decrease in viral particle level followed by a slower decay of viral concentration | 5 |

### 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. HCQ and CQ belong to the quinolone family and exert their action by blocking toll-like receptors and statistically proper number of virus particles on a layer of immobilized cells (Cooper, 1962). The TCID\(_{50}\) inturns, 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 transcribe 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.
present a concerning environmental issue. CQ and HCQ are highly persistent in surface water through wastewater treatment plant effluents, representing a cumulative at high release extent in the environment potentially resulting in aquatic media. An additional environmental element to be considered is the potential environmental harm posed by the environmentally persistent and/or bioaccumulative nature of these contaminants. Therefore, future researches are necessary to assess the magnitude of the environmental risk posed by CQ and HCQ in wastewater treatment plants. Moreover, 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 and HCQ in wastewater effluents in Nigeria. 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.

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, 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. 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. 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. 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

M. Race: Conceptualization, Supervision, Writing - original draft. A. Ferrararo: Conceptualization, Writing - original draft, Writing- Reviewing and Editing. E. Galdiero: Supervision, Writing - original draft. M. Guida: Supervision. A. Núñez-Delgado: Writing- Reviewing and Editing. F. Pirozzi: Funding acquisition, Supervision. A. Siciliano: Writing - original draft. M. Fabbricino: Project administration, Writing- Reviewing and Editing.

### Table 2

| Compound              | Organism     | Species                        | Endpoint (exposure time)   | EC50 (ppm) | Reference                      |
|-----------------------|--------------|--------------------------------|-----------------------------|------------|--------------------------------|
| Tocilizumab           | Desmodesmus subspicatus | Growth rate inhibition (72 h)    | > 100                       |            | Roche safety data sheet (2018) |
| Chloroquine           | Alivibrio fischeri | Biomimicinence Inhibition (24 h) | 132.1                       |            | Zurita et al. (2005)           |
| Hydroxychloroquine    | Raphidcocel subcapitata | Growth rate (72 h)              | 3.1                         |            | FASS safety data sheet (2019)  |

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

Acree, W.E., Grubbs, L.M., 2012. Prediction of toxicity, sensory responses and biological responses with the Abraham model. Toxic. Drug Test. 261–296.
Ashour, H.M., Elkhath, W.F., Rahman, M., Elshabrawy, H.A., 2020. Insights into the recent 2019 novel Coronavirus (SARS-CoV-2) in light of past human coronavirus outbreaks. Pathogens 9, 186.
Babić, S., Dabić, D., Ćurković, L., 2017. Fate of Hydroxychloroquine in the Aquatic Environment.
Barcelo, D., 2020. An environmental and health perspective for COVID-19 outbreaks: meteorology and air quality influence, sewage epidemiology indicator, hospitals disinfection, drug therapies and recommendations. J. Environ. Chem. Eng. 10, 40406.
Bärneca, M., Oostergetel, G.T., Baranellic, W., Faus, F.G.A., Verkleij, A., Rottier, P.J.M., Kotsia, A.J., Bosch, R.J., 2009. Cryo-electron tomography of mouse hepatitis virus: insights into the structure of the coronavirus. Proc. Natl. Acad. Sci. Unit. States Am. 106, 582–587.
Bosch, A., Pintó, R.M., Abad, F.X., 2006. Survival and transport of enteric viruses in the environment. In: Goyal, S.M. (Ed.), Viruses in Foods. Food Microbiology and Food Safety. Springer, Boston, MA, pp. 151–187. https://doi.org/10.1007/0-387-29251-9_6.
Bosch, B.J., van der Zee, P., de Haan, C.A.M., Rottier, P.J.M., 2003. The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. J. Virol. 77, 8801–8811.
Cantalojas, P.G., Calgua, B., Zhao, G., Hendrix, A., Wier, A.D., Katz, J.P., Grabe, M., 2011. Raw sewage harbors diverse viral populations. mBio https://doi.org/10.1128/mBio.00180-11.
Cao, Z., Zhang, Q., Lu, X., Pfeiffer, D., Jia, Z., Song, H., Zeng, D.D., 2020. Estimating the Effective Reproduction Number of the 2019-nCoV in China. medRxiv.
Carducci, A., Federigi, I., Liu, D., Thompson, J.R., Verani, M., 2020. Making Waves: Coronavirus detection, presence and persistence in the water environment: state of the art and knowledge needs for public health. Water Res. 179, 115907. https://doi.org/10.1016/j.watres.2020.115907.
Carter, M., Shieh, J., 2015. Molecular cloning and recombinant DNA technology. In: Guide to Research Techniques in Neuroscience. Academic Press. pp. 219–237. https://doi.org/10.1016/S0097-8987(12)80051-1 0-00101-1.
Casanova, L., Rutala, W.A., Weber, D.J., Sobsey, M.D., 2009. Survival of surrogate coronaviruses in water. Water Res. 43, 1895–1898. https://doi.org/10.1016/j.watres.2009.02.002.
Casanova, L.M., Leom, S., Rutala, W.A., Weber, D.J., Sobsey, M.D., 2010. Effects of air temperature and relative humidity on coronavirus survival on surfaces. Appl. Environ. Microbiol. 76, 2712–2717. https://doi.org/10.1128/AEM.02941-09.
Casanova, L.M., Weaver, S.R., 2015. Inactivation of an envelaged coronavirus in human sewage. Environ. Sci. Technol. Lett. 2, 76–78. https://doi.org/10.1021/acs.estlett.5b00029.
Casella, M., Rajind, M., Cuomo, A., Dableeboon, S.C., Di Napoli, R., 2020. Features, evaluation and treatment coronavirus (COVID-19). In: StatPearls [Internet]. StatPearls Publishing.
Chen, V., 2013. Diagnosis disparities in testing for hydroychloroquine (HQC)-related ocular toxicity. Invest. Ophthalmol. Vis. Sci. 54, 1564.
Chen, Y., Liu, Q., Guo, D., 2020. Emerging coronaviruses: genome structure, replication, and future. Biocatal. Agric. Biotechnol. 20, 418–423.
Cooper, P.D., 1962. The plaque assay of animal viruses. In: Advances in Virus Research. Academic Press, pp. 319–378. https://doi.org/10.1016/S0065-3527(08)60689-2.
Corman, V.M., Landt, O., Kaiser, M., Molenkamp, R., Chu, D.K.W., Bleicker, T., Brünink, S., Schneider, J., Schmidt, M.L., Mulders, D.G.J.C., Haagmans, B.L., van der Velden, B., van den Brink, S., Wijman, L., Goderski, G., Romette, J.-L., Ellis, J., Zambon, M., Peiris, M., Gossens, H., Reusken, C., Koopmans, M.P.G., Drosten, C., 2020. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 25, 2000045. https://doi.org/10.2807/1369-5986.CD.2020.25.3.2000045.
Cucinotta, D., Vanelli, M., 2020. WHO declares COVID-19 a pandemic. Acta Bio-medica – CUP E65D18000820006.
Daughton, G., Bonadonna, L., Lucinetti, L., Kennese, S., Surrndell, F., 2020. Coronavirus in water environments: occurrence, persistence and concentration methods - a scoping review. Water Res. 179, 115899. https://doi.org/10.1016/j.watres.2020.115899.
Daughton, G., Fratini, M., della Libera, S., Iaconelli, M., Muscillo, M., 2012. Emerging and potentially emerging viruses in water environments. Ann. Ist. Super Sanita 48, 315–329. https://doi.org/10.4103/0393-4664.105924.
Liu, C., Shih, T-P., Bo, C.-C., Chang, H.-J., Hsieh, P.-R., 2020. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and corona virus disease-2019 (COVID-19): the epidemic and the challenges. Int. J. Antimicrob. Agents 105924.
Lee, P.-L., Hsieh, P.-R., 2020. Emerging threats from zoonotic coronaviruses from SARS and MERS to 2019-nCoV. Biochem. Biophys. Res. Commun. 530, 365–367. https://doi.org/10.1016/j.bbrc.2020.02.001.
Liu, J., Cao, R., Xu, M., Wang, X., Zhang, H., Hu, M., Li, Y., Hu, Z., Zhong, W., Wang, M., 2020. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell Discov 6, 1–4.
Liu, Y., Gayle, A.A., Wilder-Smith, A., Rocklöv, J., 2020. The reproductive number of 2019-nCoV is higher compared to SARS coronavirus. J. Trav. Med. 27. https://doi.org/10.1177/1354800020918753.
Lodder, W., de Roda Husman, A.M., 2020. SARS-CoV-2 in wastewater: potential health risk, but also data source. Lancet Gastroenterol. Hepatol. 5, 533–534. https://doi.org/10.1016/S2468-1253(20)30087-X.
Liu, G., Wang, Q., Gao, G.F., 2015. Bat-to-human: spike features determining ‘host jump’ of coronaviruses SARS-CoV, MERS-CoV, and beyond. Trends Microbiol. 23, 468–478.
Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., Wang, S., Song, H., Huang, B., Zhu, N., 2020. Characterization and epidemiology of 2019-novel coronavirus: implications for virus origins and receptor binding. Lancet 395, 565–574.
Núñez-Delgado, A., 2020. What do we know about the SARS-CoV-2 coronavirus in the environment? Sci. Total Environ. 138647. https://doi.org/10.1016/j.scitotenv.2020.138647.

Makino, T., 2020. Novel wastewater surveillance strategy for early detection of COVID-19 hotspots. Curr. Opin. Environ. Sci. Heal. 17, 8–13. https://doi.org/10.1016/j.coesh.2020.05.003.

Wang, X.-W., Li, J.-S., Guo, T.-K., Zhen, B., Kong, Q.-X., Yi, B., Li, Z., Song, N., Jin, M., Wu, X.-M., Xiao, W.-J., Zuo, X.-M., Gu, C.-Q., Yin, J., Wei, W., Yao, W., Liu, C., Li, J.-F., Ou, G.-R., Wang, M.-N., Fang, T.-Y., Wang, G.-J., Qiu, Y.-H., Wu, H.-H., Chao, F.-H., Li, J.-W., 2020a. Excretion and detection of SARS coronavirus and its nucleic acid from digestive system. World J. Gastroenterol. 11, 4390–4395. https://doi.org/10.3748/wjg.v11.i37.4390.

Wang, X.-W., Li, J.-S., Guo, T.-K., Zhen, B., Kong, Q.-X., Yi, B., Li, Z., Song, N., Jin, M., Xiao, W.-J., Zuo, X.-M., Gu, C.-Q., Yin, J., Wei, W., Yao, W., Liu, C., Li, J.-F., Ou, G.-R., Wang, M.-N., Fang, T.-Y., Wang, G.-J., Qiu, Y.-H., Wu, H.-H., Chao, F.-H., Li, J.-W., 2020b. Excretion and detection of SARS coronavirus and its nucleic acid from digestive system. World J. Gastroenterol. 11, 4390–4395. https://doi.org/10.3748/wjg.v11.i37.4390.

Wang, X.-W., Li, J.-S., Jin, M., Zhen, B., Kong, Q.-X., Song, N., Xiao, W.-J., Yin, J., Wei, W., Wang, G.-J., Si, B., Gao, B.-Z., Liu, C., Ou, G.-R., Wang, M.-N., Fang, T.-Y., Chao, F.-H., Li, J.-W., 2020c. Study on the resistance of severe acute respiratory syndrome-associated coronavirus. J. Virol. Methods 126, 171–177. https://doi.org/10.1016/j.jviromet.2020.05.005.

Wigginton, K.R., Boehm, A.B., 2020. Environmental engineers and scientists have important roles to play in stemming outbreaks and pandemics caused by enveloped viruses. Environ. Sci. Technol. 54, 3736–3739. https://doi.org/10.1021/acs.est.0c01174.

Wigginton, K.R., Ye, Y., Ellenberg, R.M., 2015. Emerging investigators series: the source and fate of pandemic viruses in the water cycle. Environ. Water Technol. 1, 735–746. https://doi.org/10.1201/b18665/70863.

Yeo, C., Kaushal, S., Yeo, D., 2020. Enteric involvement of coronaviruses: is faecal-oral transmission of SARS-CoV-2 possible? Lancet Gastroenterol. Hepatol. 5, 335–337. https://doi.org/10.1016/s2468-1253(20)30048-0.

Zurita, J.L., Moulin, L., 2020. Evaluation of Lockdown Impact on SARS-CoV-2 Dynamics Through Viral Genome Quantification in Paris Wastewaters. medRxiv.

2020. Effective treatment of severe COVID-19 patients with tocilizumab. China J. Infect. Dis. 20, 400–402. https://doi.org/10.1073/pnas.2005615117.

2020. Novel wastewater surveillance strategy for early detection of COVID-19 hotspots. Curr. Opin. Environ. Sci. Heal. 17, 8–13. https://doi.org/10.1016/j.coesh.2020.05.003.

2020. Excretion and detection of SARS coronavirus and its nucleic acid from digestive system. World J. Gastroenterol. 11, 4390–4395. https://doi.org/10.3748/wjg.v11.i37.4390.

2020a. Excretion and detection of SARS coronavirus and its nucleic acid from digestive system. World J. Gastroenterol. 11, 4390–4395. https://doi.org/10.3748/wjg.v11.i37.4390.

2020b. Excretion and detection of SARS coronavirus and its nucleic acid from digestive system. World J. Gastroenterol. 11, 4390–4395. https://doi.org/10.3748/wjg.v11.i37.4390.

2020c. Study on the resistance of severe acute respiratory syndrome-associated coronavirus. J. Virol. Methods 126, 171–177. https://doi.org/10.1016/j.jviromet.2020.05.005.

2015. Emerging investigators series: the source and fate of pandemic viruses in the water cycle. Environ. Water Technol. 1, 735–746. https://doi.org/10.1201/b18665/70863.

2020. Environmental engineers and scientists have important roles to play in stemming outbreaks and pandemics caused by enveloped viruses. Environ. Sci. Technol. 54, 3736–3739. https://doi.org/10.1021/acs.est.0c01174.

2015. Emerging investigators series: the source and fate of pandemic viruses in the water cycle. Environ. Water Technol. 1, 735–746. https://doi.org/10.1201/b18665/70863.