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A review of the presence of SARS-CoV-2 RNA in wastewater and airborne particulates and its use for virus spreading surveillance

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ABSTRACT

According to the WHO, on October 16, 2020, the spreading of the SARS-CoV-2, responsible for the COVID-19 pandemic, reached 235 countries and territories, and resulting in more than 39 million confirmed cases and 1.09 million deaths globally. Monitoring of the virus outbreak is one of the main activities pursued to limiting the number of infected people and decreasing the number of deaths that have caused high pressure on the health care, social, and economic systems of different countries. Wastewater based epidemiology (WBE), already adopted for the surveillance of life style and health conditions of communities, shows interesting features for the monitoring of the COVID-19 diffusion. Together with wastewater, the analysis of airborne particles has been recently suggested as another useful tool for detecting the presence of SARS-CoV-2 in given areas. The present review reports the status of research currently performed concerning the monitoring of SARS-CoV-2 spreading by WBE and airborne particles. The former have been more investigated, whereas the latter is still at a very early stage, with a limited number of very recent studies. Nevertheless, the main results highlights in both cases necessitate more research activity for better understating and defining the biomarkers and the related sampling and analysis procedures to be used for this important aim.

1. Introduction

The first detection of severe acute respiratory syndrome (SARS) disease caused by SARS-CoV-2 (officially designated by the Coronavirus Study Group of the International Committee on Taxonomy of Viruses) occurred in the city of Wuhan (China) in late December 2019 (CDC, 2020). Since that date, the Coronavirus Disease 2019 (COVID-19) has grown rapidly, and exists in 235 countries and territories and have resulted in more than 39 million confirmed cases and 1.09 million deaths globally as of October 16 (WHO., 2020a). Currently, the most credited even if not fully demonstrated transmission pathway of SARS-CoV-2 virus is the one-based on respiratory droplets, with an average diameter ≥5 μm, that could be generated by a sneeze, cough, breath or during normal speaking of infected subjects (Lewis, 2020; National Research Council, 2020; Yu et al., 2018; WHO, 2020b). However, the presence of the virus within airborne droplet nuclei with a diameter ≤5 μm and hence transmissible to distances > 1m is still a matter of debate (Anderson et al., 2020; Lewis, 2020; Morawska and Cao 2020; National Research Council, 2020; Yu et al., 2018; WHO, 2020b). During the pandemic peaks, the presence of SARS-CoV-2 was detected in

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the aerosol samples collected in the internal air of two hospitals of Wuhan (Liu et al., 2020). The presence of the virus was also reported by Santarpia et al. (2020) in analysing air samples of 13 isolation rooms for patients infected by SARS-CoV-2 at Nebraska University Hospital. Both authors agreed on staying in open spaces and effective room ventilation as two effective methods for decreasing the spreading of the virus. Additionally, a high number of recent studies supported the aerosols and droplets diffusion/transmission route capacity of SARS-CoV-2 (Becchetti et al., 2020; Buonanno et al., 2020; Mizukoshi et al., 2020; Moreno et al., 2020; Noorimotlagh et al., 2020; Stadnytskyi et al., 2020; Tang et al., 2020). Differently, no presence of SARS-CoV-2 was detected by Faridi et al. (2020) in air samples collected from 2 m to 5 m of distance from the beds of patients affected by COVID-19. Despite all air samples were negative, the authors suggested *in vivo* experiments to be conducted using actual patient cough, sneeze, and breath aerosols for obtaining airborne size carrier aerosols. In some recent research, the idea that outdoor particulate matter (PM) could be another carrier for the spreading of the COVID-19 pandemic was proposed (Coccia 2020; Bontempi 2020a; Setti et al., 2020a). This was based on the evidence of the correlation between the number of cases and the PM concentration in the air in the Italian regions where the larger number of cases were registered during the first pandemic peak of March 2020. In this context, PMs may act as physical carriers of the virus or as a possible infection-boosting factor (Comunian et al., 2020). However, some scientific debate is still going on since the absence of a demonstrated causal nexus (Altman and Krzywinski, 2015).

In analysing the Italian context, Bontempi (2020a,b) detected a significant correlation between commercial exchanges, that are strictly connected also with social interactions, and COVID-19 diffusion/-transmission. This correlation resulted also higher than the one detected for the PM.

Similarly, SARS-CoV-2 was also detected in wastewater and sewage (Medema et al., 2020; Núñez-Delgado, 2020; Ahmed et al., 2020a), representing another concern for the spreading of the virus in particular by aerosols generated in wastewater treatment plants (WWTPs). In any case, also for this last aspect, there is a lack of definitive scientific evidence.

Wastewaters generated by hospitals, but in particular, those generated by domestic users, have been largely investigated in the last months (Núñez-Delgado, 2020; Medema et al., 2020; Randazzo et al., 2020; Ahmed et al., 2020a). The persistence of SARS-CoV-1 in hospital and domestic wastewater up to 2 days at 20 °C and >14 days at 4 °C was already detected by Wang et al. (2005) when no chlorine was added. More recently, Randazzo et al. (2020) found SARS-CoV-2 RNA in six Spanish wastewater treatment plants. Other possible routes for the diffusion of the virus were also considered by Nghiem et al. (2020) and Di Maria et al. (2020) concerning the municipal solid waste streams. All this suggested wastewater-based epidemiology (WBE) (Barcelo, 2020a) for the early detection of virus presence and for the surveillance and monitoring of COVID-19 (Farkas et al., 2020a).

WBE was already successfully adopted for the surveillance of life style and health conditions of communities concerning the use and/or abuse of both licit and illicit drug (Choi et al., 2018; Daughton, 2001). A similar role in COVID-19 detection and management seems to be played also by the monitoring of the airborne particles generated due to both symptomatic and asymptomatic individuals (Mizumoto et al., 2020; Oran and Topol, 2020; Wang et al., 2020a,b,c,d). Furthermore, the noninvasive nature of both WBE and airborne particle sampling can also help to prevent any ethical issue of confidentiality and potential stigmatization of infected patients (Choi et al., 2018; Bagcchi, 2020).

However, the efficient use of this apporach for SARS-CoV-2 monitoring in both water environment and airborne particles requires specific standardization of viral sampling procedures and methodologies along with the appropriate identification of specific biomarkers. The biomarkers are pivotal to providing the required epidemiological information for the early detection of the virus in the environmental compartments of water and air media to forestall further transmission. The present review aims to report about the scientific evidence of SARS-CoV-2 spreading via aerosols and wastewater carriers and on the criticisms concerning the WBE application for an effective monitoring and prevention of the virus outbreak. Particular attention was also focused on the virus transmission and interaction with the host along with the indication of future research needs.

2. Coronaviruses description

Coronaviruses (CoVs) belong to the subfamily Coronavirinae, subdivided into four genera Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus based on their phylogenetic properties (Cui et al., 2019). The members of Alphacoronavirus and Betacoronavirus possess the ability to infect mammals. CoVs possess spike-like projections on its surface like a crown, thereby the name. Attributes of the viruses belonging to the family Coronaviridae include the presence of an envelope with a positive-sense single-stranded RNA genome of 26–32kilo bases (Fig. 1) (Weiss, 2005). Electron microscopic analysis demonstrated the spherical shape of the virus with an estimated diameter of 60–140 nm. In addition, the open reading frame (ORF)1a/b and the remaining ORFs of all coronaviruses encode for 16 non-structural proteins (NSPs) and envelope (E), membrane (M), nucleocapsid (N) and spike (S) proteins which constitute the structural proteins respectively (Fig. 1) (Su et al., 2016). The structural proteins form an integral part of the virus membrane and aid in functions like fusion with the host cell, assembly, and release of virions; moreover, the NSPs function in virus replication and transcription. The first 21st century outbreak associated with CoVs was caused by SARS-CoV in 2002–2003 followed by a second pandemic caused by MERS-CoV in 2012 (Jakhmola et al., 2020) https://doi.org/10.1016/j.heliyon.2020.e05706. The SARS-CoV genetically resembled the bat-CoV and was predicted to be transmitted to humans through intermediate hosts like palm civets, raccoon dogs, and Chinese ferret (Weiss, 2005). Moreover, the MERS-CoV transmitted to humans viadromedary camels, primary reservoirs of MERS-CoV (Conzade et al., 2018) https://doi.org/10.3390/v10080425. Investigations have revealed that SARS-CoV-2 is genetically closer to SARS-CoV. Moreover, a phylogenetic study sorted the various genomes of SARS-CoV-2 into three lineages (*i.e.,* A, B, and C). America and Europe predominantly consisted of the A and C types, respectively (Forster et al., 2020). The B type was mostly confined to East Asia (Forster et al., 2020). The complete 29,903-bp–long RNA genome sequence of the SARS-CoV-2 (GenBank: MN908947.3) contained 5′-capped and 3′-polyadenylated ends and many ORFs (Wang et al., 2020a,b,c,d). Notably, the Hemagglutinin-esterase gene, prevalent in lineage A Betacoronaviruses was absent in SARS-CoV-2 (Chan

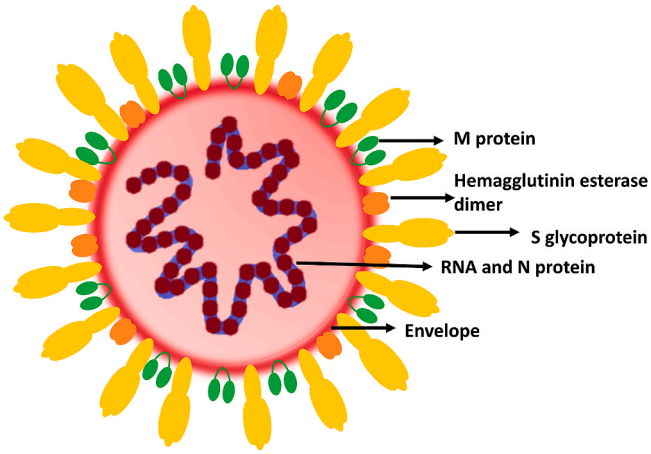

Fig. 1. Coronaviruses schematic.

et al., 2020). Like its distinct relative SARS-CoV, the SARS-CoV-2 also uses S protein to interact with the host ACE-2 receptor for cellular entry. However, another cell surface protein Neuropilin-1 has also been shown to act as entry factor for SARS-CoV-2. A study based on cryoelectron microscopy elaborated that the SARS-CoV-2 S protein has 20-fold higher affinity towards ACE-2 than SARS-CoV and this is due to 30% difference in the S protein of these two viruses. Unlike SARS-CoV or SARS-CoV-2, the MERS-CoV S protein engages with host dipeptidyl peptidase 4 (DPP4, also known as CD26) for the purpose of entry. The viral protein and host receptor interaction induces conformational changes in the S protein and facilitates entry. After interaction with the host receptor the virus is either endocytosed inside the cell or releases the genome in cytosol through membrane fusion. Once in the cytoplasm, RNA is translated into a polypeptide which is further cleaved by the proteases. Briefly, two viral encoded cysteine proteases; papain- and 3C-like proteases, cleave the polypeptides encoded by ORF1a/b into 15 NSPs (Chan et al., 2020). The virus encoded RNA dependent RNA polymerase (RdRp) performs discontinuous transcription and synthesizes sub genomic RNAs. These RNAs further get translated into various viral proteins like S, M, E, N etc. Among these the SARS-CoV-2 E protein, the smallest among all structural proteins, is localized to the endoplasmic reticulum and Golgi complex. The M protein is the most abundant transmembrane glycoprotein (Nieto-Torres et al., 2011; Siu et al., 2008) and prominently functions in the viral assembly with E and N proteins (Siu et al., 2008). Taking advantage of the host cytoplasmic membrane system, the viral RNA and proteins are assembled into virions. Subsequently, these virions are exocytosed through vesicles infecting the nearby cells. These different steps of viral life cycles can be used as drug targets for the treating the infection (Indari et al., 2021). However, adding up to the difficulties of therapeutic interventions are the newly developed alterations in the SARS-CoV-2 genome.

3. Airborne and wastewater carriers: focus on the virome

3.1. Coronaviruses in wastewater: Sources and occurrence and potential risk of infection

The role that water can play as a vehicle for pathogen spread as a consequence of faecal contamination was historically recognized for several microorganisms and diseases. Among these, the most important viruses such as *Norovirus*, *Enterovirus*, *Hepatitis* A *virus*, and *Adenovirus* belong to the Calicivirade, Picornaviridae, and Adenoviridae families (WHO, 2017). These viruses were detected in several types of water (wastewater, seawater, freshwater, and drink water) and hence associated with disease outbreaks (Bonadonna and La Rosa, 2019; Gall et al., 2015; La Rosa and Fratini, 2012; Moreira and Bondelind, 2017; Girones, 2017). SARS-CoV-2 is an enveloped virus belonging to the *Betacoronavirus* genus in the *Coronaviride* family characterized by positive-sense and single-strained RNA. A significant portion of a patient infected by SARS-CoV-2 showed gastrointestinal symptoms with consequent virus shedding into faeces above those from the respiratory tract (Xiao et al., 2020a; Wu et al., 2020). In addition, Xiao et al. (2020a) reported that the virus can infect glandular epithelial cells, and the virions can be secreted from intestinal cells albeit there is limited evidence of the potential infectivity of the virus detected in faecal samples (Xiao et al., 2020b). Faeces was charged to be the main carrier of the virus in wastewater and hence a further potential route of COVID-19 pandemic spreading. As reported in Table 1 the percentage of patients affected by SARS-CoV-2 representing gastrointestinal symptoms resulted quite high, in general, $> 10\%$ up to 62.5%, as the number of patients presenting SARS-CoV-2 RNA fragments in the stools. Furthermore, for those cases investigated, the SARS-CoV-2 load in the samples of stools analyzed resulted in general $> 2\ E^3$ (copies/ml).

Differently from other microorganisms (*e.g.,* bacteria), viruses showed a very limited survival period without hosting cellules. Furthermore, due to the damage susceptible by the labile lipid envelope,

Table 1
Patients monitored, number of gastrointestinal symptoms, SARS-CoV-2 RNA fragments detected in stools, and related SARS-CoV-2 loads.

Reference	Number of patients	Gastrointestinal symptoms	Stools with RNA	SARS-CoV-2 load (copie/ml)
Wang et al., (2020a,b,c,d)	153	–	44	<2.6E4
Lin et al. (2020)	160	100	31	–
Xiao et al. (2020a)	73	–	39	–
Xu et al. (2020b)	10	3	8	2E3-2E7 (range)
Wu et al. (2020)	74	23	41	–
Zhang et al. (2020)	23	–	10	5.6E3 (mean)
Cheung et al. (2020)	4243	747	530	10E3.8-10E7.6 (range)
Wolfel et al. (2020)	9	1	–	10E3-10E75 (range)
Chen et al. (2020)	42	8	28	–
Lo et al. (2020)	10	–	–	–
Gaun et al. (2020)	1099	55	–	–
Pan et al. (2020)	204	103	–	–

SARS-COV-2 resulted inactivated in the aqueous environment even if this process could result not so rapid. The occurrence of SAR-CoV-2 in municipal wastewater was investigated in the Netherlands by Medema et al. (2020). Presence of RNA fragments was detected in 6 out of 8 wastewater treatment plants monitored. Similar results were also reported by Wu et al. (2020b) in monitoring one of the major wastewater treatment plants in Massachusetts. Ahmed et al. (2020a) monitored one pumping station and two wastewater treatment plants in Southeast Queensland (Australia), reporting 2 out of 9 samples with the presence of fragments of SARS-CoV-2 RNA. Similar studies were also performed by Wurtzer et al. (2020) and La Rosa et al. (2020) for France and Italy, respectively. All these findings suggested that wastewater could be used for COVID-19 surveillance, but, in any case, no evidence of SARS-CoV-2 infection spreading via water and wastewater was currently detected. Wang et al. (2005) reported a persistence of SARS-CoV in hospital wastewater, domestic sewage, and tap water for 2 days at 20 °C and 14 days at 4 °C. The same authors also reported that at 20 °C, the persistence of the virus in faeces and urine was of 3 days and 17 days, respectively, whereas a concentration of 10 mg/L of chlorine with a contact time ≥10 min resulted able to completely inactivate SARS-CoV. For a chlorine concentration of 20 mg/L the minimum contact time decreased to 1 min. Concerning the survival of coronavirus in tap and wastewater, Gundy et al. (2019) compared HCoV-229E and animal FIPV coronavirus with Poliovirus-1 (PV-1). In wastewater at 23 °C, HCoV resulted inactivated in less than 4 days, whereas PV-1 lasted for about 11 days in primary wastewater and about 6 days in secondary effluents. In tap water, both HCoV and FIPV lasted for about 12 days, whereas at 4 °C the persistence was estimated to achieve up to 100 days.

The specific RNA sequences for SARS-CoV-2 reported in the study were detected by qPCR (Foladori et al., 2020). For improving the efficiency and effectiveness of RNA detection, liquid samples were generally pre-treated for both increasing its purity degree and/or for increasing the virus concentration. By the way, there is a lack of consensus on the protocols and standard procedures to be adopted for these analyses. Among the available methods for the SARS-CoV-2 concentration, Wu et al. (2020) proposed a filtration using 0.22 μm

membranes followed by PEG precipitation, centrifugation at 12,000 rpm for 2h or until the pellet was visible. Ahmed et al. (2020a) proposed two methods: a first method based on direct RNA extraction from the electronegative 0.45 -μm filter 90 mm diameter; a second method based on centrifugation at 4750 rpm for 30 min followed by centrifugation of the supernatant at 3500 rpm for 15 min through a centrifugal filter with a cut of 10 kDa. Wurtzer et al. (2020) proposed ultracentrifugation at 200, 000 rpm for 1h at 4 °C. For RNA detection, La Rosa et al. (2020) used three different PRC assays: a broad range of PCR for Coronavirus detection targeting ORF1ab; a nested RT-PCR targeting the spike regions for SARS-CoV-2 proposed by Nao et al. (2020). For the same purpose, Randazzo et al. (2020) adopted an RT-qPCR diagnostic panel assay proposed by CDC (2020). All these differences in the procedures adopted leaded to some difficulties in performing direct comparison between the results reported in the studies as the consequent correct and univoque interpretation of the data.

3.2. SARS-CoV-2 in airborne particulate matter

Recently, 239 scientists from 32 countries have written an open letter to the World Health Organization (WHO) arguing the importance of airborne as a transmission route for COVID-19 (Morawska and Milton, 2020). However, at the moment the airborne mechanisms of transport of the virus, including the role of particulate matter, are not clear. In this context, the number of studies concerning the role of $PM_{10}/PM_{2.5}$ as carriers of virus spreading is rapidly growing. Airborne particles containing the virus can be emitted by infected individuals during sneeze, cough, shouting, speaking and normally breathing as already reported for other respiratory viruses belonging or not to the Coronavirinae subfamily (Milton et al., 2013; Yan et al., 2018; Leung et al., 2020). Settling rapidity of larger size respiratory droplets (*i.e.* > 5 μm) resulted higher than their evaporation rapidity. Oppositely, the evaporation rapidity of lower size respiratory droplets resulted higher than the settling one leaving residuals called droplet nuclei. These latter may be rich of viruses aggregate and other compounds as mineral salts and proteins (Asadi et al., 2020; Borouiba, 2020). Due to their dimensions, droplet nuclei can float in the air for long periods potentially contributing to the airborne transmission mechanism (Allen and Marr, 2020; Morawska and Cao, 2020; Martano, 2020). Air quality also in terms of humidity and temperature was already indicated as another relevant aspect in the COVID-19 spreading (Barcelo, 2020b). Ma et al. (2020) reported during January and February 2020 a decrease of deaths due to SARS-CoV-2 in the city of Wuhan as both air temperature and humidity increased. Opposite findings were reported by Zhou and Xie (2020) for 122 Chinese cities during the same period indicating the absence of evidence between increase in ambient temperature and decrease of infection spreading. By the way, is worthy to be noted that smaller size droplet nuclei can remain suspended in the outdoor air for several days (Brito et al., 2018) before being eventually inhaled by other individuals. This facilitates its dilution below the infective inhalation dose (about 10^5-10^6) but also the inactivation and/or complete degradation of the SARS-CoV-2 and higher temperature can facilitate this process. In any case, low air quality can affect the respiratory apparatus of exposed individuals making them more vulnerable to the virial aggression (Barceló, 2020b). With specific reference to the role of $PM_{10}/PM_{2.5}$ as potentially carriers of infection spreading, the methodological approach adopted in such studies can be classified into two main categories: (a) statistical inference between the concentration of PM and the number of infections and (b) lab analysis of the presence of RNA in the particulate matter. Bibliography research in Scopus database was performed using the keywords as "COVID-19" or "SARS-COV" or "Coronavirus" and "aerosols" or "droplets" or "PM" or "air pollution" or "air transmission" or "airborne. The research was limited to articles with open access published in 2020 and the English language. Among more than 700 papers, only 6 can be referred to the role of PM as a carrier of COVID-19 and all of them can be referred to the first approach category. Besides, the investigation of the references listed in the most relevant papers led to detect further 3 papers based on the statistical approach and 1 based on lab analysis.

Concerning the "statistical" approach, several studies (Bontempi, 2020; Borro et al., 2020; Chennakesavulu and Reddy, 2020; Coccia, 2020; Delnevo et al., 2020; Fronza et al., 2020; Li et al., 2020; Setti et al., 2020a) found a correlation/association between the PM concentrations (in some cases in terms of number of exceeding of regulated concentration of air quality standards) in several cities in Italy and China. Spearman's correlation coefficient was the most used tool implemented for statistical analysis. However, it remains controversial the cause-effect relationship between infection and PM concentration since as Altman and Krzywinski (2015) explained correctly in their work "Correlation is not causation". Besides, confounding variables are not sufficiently investigated. In the specific case where a mere association or correlation between particulate concentration (variable - X) and the number of infected people (variable - Y) occured, the relationship cannot be interpreted a priori as a cause-effect relationship (X cause Y), because they would overlook other phenomena. For example, the number of infected people could be caused by a third factor (variable - Z) and air pollution would play a role as not the cause but as an amplifier to ("boost") of the virus effects on the respiratory system as some studies have identified (Bontempi et al., 2020; Comunian et al., 2020; Setti et al., 2020a). Cities with ≤100 and > 100 days with the concentration of PM10 higher than the limits indicated by EU air quality standards, showed an increase in the number of infections of about 0.25% and 85% respectively (Coccia, 2020). Additionally, Chennakesavulu and Reddy (2020) highlighted how the concentration of PM2.5 can affect the distance in which the virus can be transmitted because SARS-CoV-2 droplets absorb PM2.5 and form a solid/liquid (PM2.5/SARS-CoV-2) interface. Zhao et al. (2019) found a similar result through a modelling analysis applied to the virus of Pathogenic Avian Influenza (HPAI). They stated that PM2.5 might transport the virus for longer distances than PM10, due to the lower deposition velocity. However, higher distances contribute to a lower probability of infection because of the decrease of the concentration of virus/particles. Concerning the RNA analysis approach, only the study of Setti et al. (2020b) was identified. Here, 34 PM_{10} samples were collected from an industrial site in the province of Bergamo in Northern Italy, over three weeks (from February 21st to March 13th, 2020) and in 7 samples have identified the presence of SARS-COV-2 RNA. About this approach, some general considerations should be reported:

- The extraction method has not yet been well standardized;
- The number of samples is statistically low to deduct general considerations and further studies are necessary;
- The evidence of viral RNA is not indicative of the presence of active viruses that could be effectively transmitted.

In conclusion, based on what emerged from the analysis of the scientific literature, there is no scientific evidence of a possible spread of COVID-19 infection through atmospheric particulate matter. Furthermore, these findings resulted characterized by a high level of uncertainty preventing any definitive scientifically valid conclusions.

4. Wastewater-based epidemiology (WBE): SARS-CoV-2 monitoring and its detection techniques

Water-borne diseases such as cholera, diarrhoea, dysentery, typhoid, and polio resulting death rate increase day-by-day globally. It is due to more than 2 billion people using drinking water sources contaminated with faeces in the worldwide (WHO, 2017). Most of these infections are from the water environment through the improper treatment of viruses in the sewage and wastewaters. In the last two decades, SARS-CoV-2 is the third coronavirus to spread worldwide (Guarner, 2020). As already reported in Table 1, several studies detected the presence of SARS-CoV-2

in stools of infected patients using as conformity test the rectal swab method (Lamers et al., 2020; Xu et al., 2020b; Zang et al., 2020). Moreover, a duration and survival of this virus in such media from 15 day up to 42 days was also reported (Jiang et al., 2020; He et al., 2020; Wölfel et al., 2020). These evidences suggested the use of wastewater monitoring for the surveillance of the presence of the virus and of the health conditions of given communities adopting the WBE approach (Medema et al., 2020; Randazzo et al., 2020) (Fig. 2). Several studies reported about the detection of SARS-CoV-2 RNA in wastewater samples in Nethelands (Medema et al., 2020), in Spain (Randazzo et al., 2020), in USA (Sherchan et al., 2020; Wu et al., 2020), in Australia (Ahmed et al., 2020a), in India (Kumar et al., 2020) and in Germany (Wasthaus et al., 2020). Of particular interest was the study or Ahmed et al. (2020b) reporting the use of WBE for SARS-CoV-2 in wastewater in commercial passenger aircraft and cruise ships. One of the clinical suggests WBE as a good tool for the detection of variations in viral strains through phylogenetic analysis recognising virus trees over time and among various regions. Only a few studies are available for SARS-CoV-2 through phylogenetic analysis of wastewater samples. Nemudryi et al. (2020), found strains of SARS-CoV-2 in the wastewater of Bozeman, Montana. Rimoldi et al. (2020) revealed similarities among the isolated viral strains with those found in Europe and the Lombardy region in northern Italy through genome sequencing and phylogenetic analysis. WBE was already successfully used to monitor both life style and health of communities *e.g.* by the detection of the use and/or abuse of both licit and illicit drugs (Choi et al., 2018; Daughton, 2001). Some contrasting results were reported about the detection of SARS-CoV-2 in treated wastewater and surfaces water. If from one hand Naddeo and Liu (2020) observed SARS-CoV-2 survival and diffusion in both wastewater and received surface waters, from the other hand no presence in treated wastewater was reported by other authors (Singer and Wray, 2020; Wang et al., (2020a,b,c,d; Wurtzer et al., 2020). However, these results can be influenced by some factors as low concentration levels and RNA extraction spread rates requiring more research efforts for the definition of large scale WBE protocols (Hart and Halden, 2020). O'Brien and Xagoraraki (2019, 2020) model-based spatial and temporal evolution of viral diseases through wastewater data collection. Mao et al. (2020) proposed a small, portable paper-based device for the detection of SARS-CoV-2. In Italian based sewage wastewater and river samples, Rimoldi et al. (2020) demonstrated SARS-CoV-2 RNA detection through real-time RT-PCR.

The continuously human excreta received from the different sewer systems offer near-real-time outbreak data and these data were containing viruses from infected humans. This can be also related to their symptomatology status like no symptoms, paucisymptomatic or subclinical, and presymptomatic. By the way, it is worthy to mention that Chen et al. (2020) reported about patients with positive faecal specimen but negative pharyngeal and sputum test.

WBE identified viral concentrations in wastewater during different seasonal fluctuations. However, these concentrations were analyzed in comparison with other virus protocols. For example, the highest concentration of noroviruses was found during the winter and summer seasons (Nordgren et al., 2009). Similarly, Li et al. (2011) and Katayama et al. (2008), reported the concentration levels of rotavirus and norovirus were higher from November to April. This variation in COVID-19 spread also occurs in sewage at locations falling inside of the climatologically favoured zones (4–12 °C) (Poole, 2020). Due to SARS-CoV-2 is a potentially lethal virus to humans, biosafety guidelines and procedures for concentrating such virus through specific filtration are given by the Center for Disease Control and Prevention (CDC, 2020). Several virus surrogates are used which depends on the experiment type, murine hepatitis virus is used as a SARS-CoV-2 surrogate (Ahmed et al., 2020a) in wastewater samples. Transmissible gastroenteritis (TMV) virus, enveloped bacteriophage, non-enveloped *Escherichia*, and feline infectious peritonitis viruses were used to test the persistence and disinfection of human coronaviruses in water and wastewater (Boehm et al., 2019; Silverman and Boehm, 2020; De Carvalho et al., 2017; Zielińska and Galik, 2017). Shirasaki et al. (2017) concluded that plant virus (Pepper mild mottle virus) is a suitable surrogate for the human virus. Lesimple et al. (2020) reviewed recent techniques for virus removal from wastewater like algal ponds, nanomaterials, membranes, and other treatment techniques. They have reviewed these techniques to develop WBE for both sampled (localised infection clusters) and quantified.

4.1. Protocols for WBE

The practical exploitation of WBE requires the definition of clear and agreed methodologies including all different phases from wastewater sampling to the final correlation of biomarker concentration and health status. Based on the current aim of this analysis (*i.e.,* lifestyle or disease surveillance) and on the biomarkers used for this goal, the methodology proposed in the literature showed some differences. In any case, a common preliminary step of all the methodologies consists in the study of the area and of the population served by the sewage system for the exact identification of the size of the population interested and the presence of other wastewaters different from the urban ones entering in

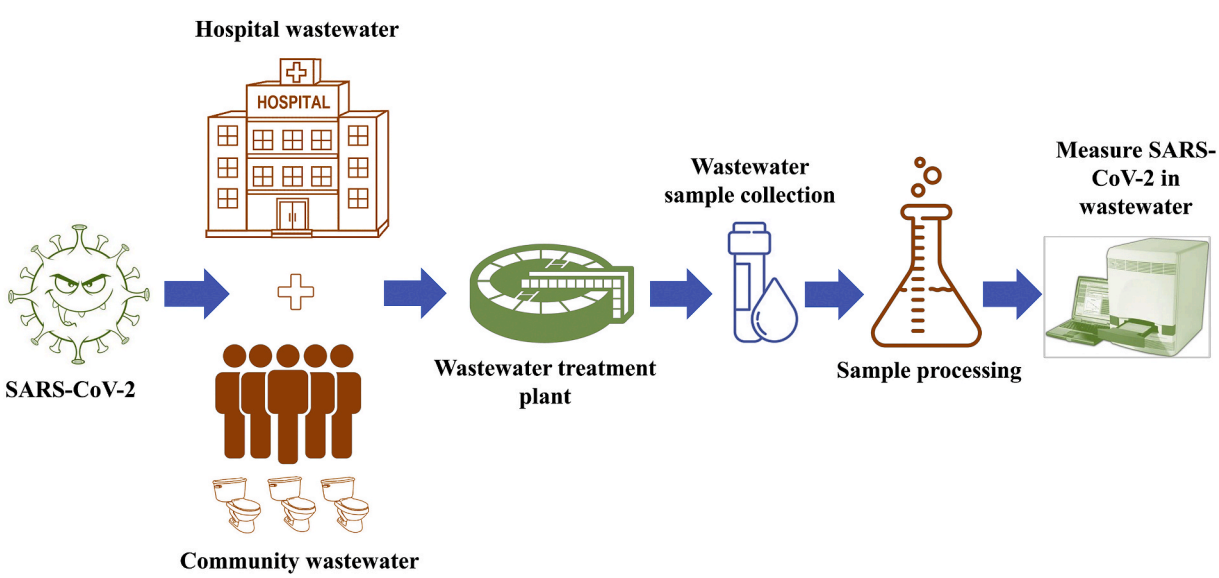

Fig. 2. Wastewater based epidemiology (WBE) concept.

the sewage system (McCall et al., 2020).

4.1.1. Lifestyle surveillance

A relevant aspect concerns the selected biomarkers. These have to be a major and exclusive excretion metabolite, chemical or biological product of the specific disease under investigation, characterized by adequate stability in wastewater and the successive sampling and storage process (Sims and Kaspryzk-Hordern, 2020; Zuccato et al., 2008). In investigating community drug abuse, Zuccato et al. (2008) excluded from the target biomarkers adopted in their study the most abundant excretion products (*i.e.,* Glucuronic acid-conjugated metabolites) since their high instability in wastewater. Once these two main aspects have been defined, these authors suggested that the wastewater specimen has to be realized by the collection of wastewater samples at the wastewater plant inlet every 20 min for 24 h. This composite sample has to be pooled and performed by an automatic computer-controlling device, in a time-proportional mode for considering the rate fluctuations during different hours of the day. It is also recommended to withdraw more samples on consecutive days for ensuring the correct reproducibility of the tests. Before being analyzed, the samples have to be properly stored for avoiding the degradation of the biomarkers. Maida et al. (2017) suggested filtration the same day of collection and storage at 4 °C for a

Table 2
Biomarkers exploitable for the monitoring of life style and health at the community level through wastewater.

Detection goal	Biomarker	Disease	Amount/ Concentration detected	Reference
Illicit drugs				
Cocaine	BE Cocaine	–	267-390 (mg/day/ 1000 people) 109-157 (mg/day/ 1000 people)	Zuccato et al. (2008)
Heroin	Morhpine 6-Acetylmorphine	–	32-173 (mg/day/ 1000 people)	Zuccato et al. (2008)
Amphetamine	Amphetamine	–	2.5–24 (mg/day/1000 people)	Zuccato et al. (2008)
Methamphetamine	Methamphetamine	–	2.4–4.5 (mg/day/ 1000 people)	Zuccato et al. (2008)
Ecstasy	Ecstasy	–	3.4–7.3 (mg/day/ 1000 people)	Zuccato et al. (2008)
Cannabis	THC-COOH	–	20-50 (mg/day/1000 people)	Zuccato et al. (2008)
Antibiotics				
	Azithromycin *n-Demethyl azithronicin*	Pneumonia, middle ear infection, strep throat, intestinal infection	269-22,730 mg/L 30–74 mg/L	Senta et al. (2019)
	Clarithromycin *n-Demethyil clarithromycin*	Pneumonia, skin infection, H, Pylori infection, Lyme disease	111-10,491 mg/L 13-1559 mg/L	Senta et al. (2019)
	Ciprofloxacin	Respiratory tract infection, skin infection, gastroenteritis	17-2500 mg/l	Guerra et al. (2014)
	Trimethoprim	Urinary tract infection	464-6796 mg/L	(Kasprzyk-Hordern et al., 2009; Roberts and Thomas, 2006)
Antivirals				
	Oseltamivir phosphate *Oseltamivir carboxylate*	Flu virus (influenza)	5–529 ng/L 28–1213 ng/L	(Leknes et al., 2012; Takanami et al., 2012)
	Acyclovir *Carboxy-acyclovir*	Herpes simplex virus infections, chicken pox, shingles	1780 ng/L 490–3420 ng/L	(Funke et al., 2016; Prasse et al., 2010)
	Lamivudine, *Carboxy lamivudine*	HIV/AIDs, hepatitis B	52–720 ng/L 25–84 ng/L	(Funke et al., 2016; Prasse et al., 2010)
	Zidovudine	HIV/AIDs	310–380 ng/L	Prasse et al. (2010)
Painkillers				
	Acetaminophen	Painkiller	5529–500,000 ng/L	(Guerra et al., 2014; Roberts and Thomas, 2006)
	Ibuprofen	Painkiller	968–45,000 ng/L	(Guerra et al., 2014; Kasprzyk-Hordern et al., 2009; Roberts and Thomas, 2006)
Pathogenic organisms				
	Bacterial DNA *Klebsiella pneumoniae*	Pneumonia, UTI, bacteremia and endophthalmitis	6.31–6.56 log gene copies/100 mL	Shannon et al. (2007)
	Bacterial DNA *Pseudomonas aeruginosa*	Pneumonia, UTI, gastrointestinal infections	4.31–4.38 log gene copies/100 mL	Shannon et al. (2007)
	Bacterial DNA *Enterococcus faecalis*	UTIs, bacteremia, septicemia	4.66–4.85 log gene copies/100 mL	Shannon et al. (2007)
	Viral DNA/RNA Norovirus (GI)	Gastroenteritis	<10–3500 viral genomes/L	Hellmer et al. (2014)
	Viral DNA/RNA Influenza A	Respiratory infection	2.6 × 105 genome copies/L	Heijnen and Medema (2011)
	Viral DNA/RNA Hepatitis A	Liver infection	<10–1500 viral genomes/L	Hellmer et al. (2014)
	Viral DNA/RNA Severe acute respiratory syndrome (SARS CoV)	Respiratory infection	–	Poon et al. (2004)
	Fungal DNA Candida species	Candidiasis	–	Assress et al. (2019)
	Fungal DNA Aspergillus (Aspergillus fumigatus, Aspergillus niger and Aspergillus flavus)	Chronic pulmonary aspergillosis, pulmonary and nasal allergies, asthma, pneumonitis	–	Assress et al. (2019)

maximum of 2 days. The analytical procedure differs based on the origin of the biomarkers. In the case of drugs, the targeted biomarkers are urine metabolites and/or the unchanged parents of specific drugs (Table 2). These can be measured by a fully validated procedure (Castiglioni et al., 2006; Zuccato et al., 2008; Maida et al., 2017) consisting in the following main steps: spiking of the water samples with internal standards, acidification and extraction of the solid phase using reverse-phase/cation-exchange cartridges. Cartridges were preventively conditioned with methanol, water, and 0.01 N HCl solution. Successively, the liquid eluted obtained by methanol and 2% ammonia in methanol was analyzed by liquid chromatography-tandem mass spectrometry. Quantitative analyses were performed in the selected reaction-monitoring mode by measuring the fragmentation products of the protonated or deprotonated pseudo-molecular ions of each compound and deuterated analog. Instrumental limits, repeatability, recoveries, and limits of detection have to be also calculated (Castiglioni et al., 2006). Another validated procedure, with some minor differences to the one proposed by Castiglioni et al. (2006) was proposed by Lai et al. (2011; 2015). Once the concentration of the targeted biomarkers was determined, the whole amounts of biomarkers excreted by the population served by the wastewater treatment plant can be calculated by simply multiplying the concentration by the whole amount of wastewater collected from the plant. Since the population served by the plant was preventively investigated, it is possible to assess the amounts of biomarkers excreted per inhabitant per day (mg/inhabitant/day). The evaluation of the daily consumption of drugs per inhabitant is hence possible by multiplying the amount of mg/inhabitant/day by a corrective factor that represents the percentage of drug dose excreted multiplied by the molar mass ratio of the parent drug/targeted biomarker.

4.1.2. Disease surveillance

As already discussed in the previous section 4.1.1, also in this case the stability of the biomarkers and the adequate concentrations are important issues able to affect the results of the whole analysis. If the targeted biomarkers are represented by viral DNA/RNA, the water samples were filtered by nano-ceramic filters eluted for 2 min with 1 L 1.5% w/w beef extract as indicated in EPA's protocol (U.S. EPA, 2001) within 24 h. The pH of the obtained solution was adjusted to 3.5 ± 0.1. After being flocculated for 30min. and centrifuged at 2500g for 15 min at 4 °C, the solid phase was resuspended by 30 mL of 0.15M sodium phosphate, pH 9.0–9.5, and centrifugated at 7000g for 10 min at 4 °C. After centrifugation, the supernatant was firstly neutralized to a pH of about 7.25 and the filtrated using 0.45 -μm and 0.22 -μm syringe filters. Extraction of nucleic acid was performed with 140 mL of purified virus concentrate using a commercial kit. The nucleic acid was stored at −80 °C until being further processed. The quantification of the targeted viral DNA/RNA is usually performed by quantitative PCR (qPCR) or reverse transcription (RT) qPCR (McCalla et al., 2020).

4.2. SARS-CoV-2 detection

In general, as reported by several authors (Ahmed et al., 2020a; Fongaro et al., 2020; Rimoldi et al., 2020; Medema et al., 2020; La Rosa et al., 2020a, b; Trottier et al., 2020; Bar-Or et al., 2020; Randazzo et al., 2020; Kocamemi et al., 2020; Green et al., 2020; Sherchan et al., 2020; Wu et al., 2020; Kumar et al., 2020; Haramoto et al., 2020), more information resulted necessary concerning the survival and persistence of the SARS-CoV-2 in water and wastewater. In any case, its detection and quantification in this media was performed by RT-qPCR method, which is based on the presence of TaqMan detection probe assay are reported by different authors (Wurtzer et al., 2020; Fongaro et al., 2019; Nemudryi et al., 2020; Medema et al., 2020; Bar-Or et al., 2020; Randazzo et al., 2020; Kocamemi et al., 2020; Green et al., 2020; Wu et al., 2020; Haramoto et al., 2020). The choice of this method is based on its reliability due to its specificity and sensitivity properties (Bustin and Nolan, 2020). Michael-Kordatou et al. (2020) reviewed different methodology protocols for the SARS-CoV-2 quantitative analysis in wastewater. Stachler and Bibby (2014) reported that several viruses were abundantly available, such as pepper mild mottle virus (PMMoV), adenovirus, human polyomavirus, torque teno virus, and norovirus, CrAssphage in the sewage of the United States and European Union countries. Many of the researchers were found CrAssphage appears to be human-associated; however, it has been detected in a limited number of seagulls, dog, duck, pig, and chicken faecal samples (Malla et al., 2019; Stachler et al., 2017). Countries like United States, United Kingdom, Australia, Japan, Thailand, and Nepal were successfully deployed CrAssphage to detect faecal pollution in environmental waters (Stachler et al., 2019; Farkas et al., 2020; Kongprajug et al., 2019; Bivins et al., 2020).

A recent study by Haramoto et al. (2020) found detection of SARS-CoV-2 RNA in wastewater through qPCR technique and none of the samples of river water was found positive for SARS-CoV-2 RNA. They found virus detection in the secondary treated wastewater was 1.4×10^2–2.5×10^3 copies/L. The survival and stability of viruses in wastewater depend on physicochemical conditions such as temperature, pH, solids, and the presence of micropollutants (Fongaro et al., 2019). Auffret et al. (2019) showed that the detrimental effect on virus persistence with high alkalinity levels of wastewater. Bivins et al. (2020) reported a 90% reduction of viable SARS-CoV-2 in wastewater and tap water through RT-qPCR detection technique at room temperature. Other researchers have reported persistence of viruses at different temperatures. For example Casanova et al. (2009) found a 99% reduction of TGEV and MHV at the temperature of 25 °C in the sewage. Ye et al. (2016) reported a 90% reduction of MHV in unpasteurized wastewater in 13 h at 25 °C. Gundy et al. (2009) found to be 99.9% reduction of feline infectious peritonitis virus and human coronavirus 229E in the primary effluent at 23 °C in 2 and 4 days, respectively. In the case of wastewater from biological treatment processes, the survival and persistence of SARS-CoV-2 RNA are unclear due to insufficient experimental data (Oliver et al., 2020). Guerrero-Latorre et al. (2020) found SARS-CoV-2 RNA in the river samples at concentrations of 2.07×10^5 to 3.19×10^6 gene copies/L. Ahmed et al. (2020) reported that among five different RT-qPCR assays (CDCN1, CDC N2, N_Sarbeco, NIID_2019-nCoV_N, and E_Sarbeco), the CDC N1 assay is the most sensitive one while the N_Sarbeco showed the least sensitivity, while positive samples among wastewater samples of a cruise ship and three aircraft were near the assay LOD (37–40 cycles), providing inconsistent results among the tested assays.

Wastewater and aerosols are ideal surveillance materials for assessing qualitative and quantitative molecular epidemiological information on population exposure to SARS-CoV-2, including biological and chemical markers associated with human activities within a community or a catchment (Carducci et al., 2020; Polo et al., 2020). Chirizzi et al. (2021) measured the concentrations of SARS-CoV-2 distribution in outdoor air samples from North and South of Italy. The authors found that the less than 0.8 copies for each size range, indicating less possible transmission through this medium, especially in a less crowded environment. Fig. 3 depict the bioaerosols sampling and WBE surveillance concept for viral particles in outdoor particulate matter, indoor aerosol, and wastewater treatment plants. One of the challenges of virus identification, unlike other microorganisms, is the inability to mutate outside the living host cells (Polo et al., 2020). The detection of human viruses in the environment can only be facilitated when excreted through faeces and urine or passed out through breath and cough or sneeze with significant concentrations and a half-life long enough to identify and characterize them.

Some studies have also demonstrated the presence of SARS-CoV-2 RNA in aerosol samples. Chia et al. (2020) identified positive SARS-CoV-2 through the environmental sampling of hospital rooms. Santarpia et al. (2020) confirmed the presence of SARS-CoV-2 in the air samples from the hospital and isolation rooms. Liu et al. (2020) detected higher concentrations of SARS-CoV-2 RNA in the aerosol samples from the toilets used by the patients compared to the isolation room. Ong

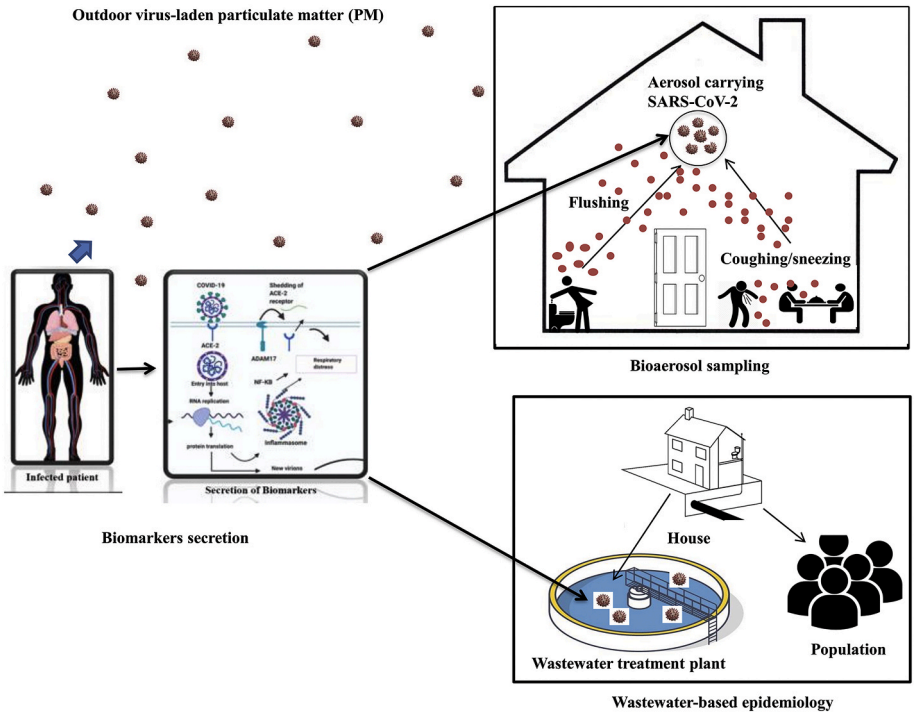

Fig. 3. Possible virus transmission schemes from infected to not infected individuals by outdoor and indoor aerosols and wastewater.

et al. (2020) detected small virus-laden droplets at the air exhaust outlet from the swab samples. Srivastava et al. (2021) also reported the prospect of rapid detection of SARS-CoV-2 in aerosol using nanomaterials enabled biosensing technique. The detection of the genetic material of SARS-CoV-2 is enabled by patient-generated airborne biomarkers of pathogens found in aerosols and other related volatile compounds, which can be exploited for making point-of-care devices (Bhalla et al., 2020; Gould et al., 2020). However, highly advanced technology is required to detect the chemical markers from environmental samples such as odor, breath, and aerosolized pathogens like SARS-CoV-2 generated by the infected patient (Fung and Mykhaylova, 2014; Bhalla et al., 2020). While the majority of the studies that were conducted to detect SARS-CoV-2 RNA in either wastewater or aerosol are based on reverse transcription-polymerase chain reaction (RT-PCR) and reverse transcription real-time polymerase chain reaction (RT-qPCR) techniques for qualitative and quantitative information, respectively, and digital PCR (Amoah et al., 2020; Polo et al., 2020; Santarpia et al., 2020; Liu et al., 2020), there have been instances where inconsistent results were obtained (Ahmed et al., 2020a; Rimoldi et al., 2020). This was due to the diverse contents of the PCR inhibitors and different concentration protocols being adapted to detect the existing molecular markers (Gibson et al., 2012; Hart and Halden, 2020). On this note, there is a need to identify appropriate biomarkers specific for SARS-CoV-2 detection in the environment, which have been scantly researched.

4.2.1. *Biomarkers exploitable for WBE*

WBE aims to assess the population's health by the detection and quantification of endogenous and exogenous urinary human biomarkers found in wastewater (*i.e.* wastewater-based epidemiology) (Table 2). Exogenous biomarkers have been assessed as population markers, whereas endogenous chemicals excreted by human metabolism characterized by low variance have been indicated as those suitable for estimating the population size (Choi et al., 2018). The use of WBE as a non-intrusive and non-incriminating method for obtaining real-time data truly reflecting the usage of drugs by wide communities was suggested for the first time by Daughton (2001). In this case, the authors proposed the investigation of domestic sewage for identifying the presence of illicit drugs and synthesis by-products excreted in human's faeces and urine and consequent evaluation of the amount of drug used and of the number of users.

One of the first practical applications of this approach was reported by Zuccato et al. (2005) for estimating the use of cocaine in a broad area of Northern Italy based on wastewater analysis. Based on the detection by mass spectrometry of cocaine and its main urinary metabolite (benzoylecgonine), the authors assessed an average daily use of cocaine of about 27 doses every 1000 inhabitants. Since that date, the use of this approach for the non-intrusive and wide investigation on the use of both licit and illicit drugs, pesticides, pharmaceutical and personal care products (PPCPs), alcohol consumption, and general health by oxidative stress markers, was largely exploited (Been et al., 2014; Choi et al., 2018; Kankaanpää et al., 2016; Kim et al., 2015; Lai et al., 2016; Reid et al., 2011; Rousis et al., 2017; Ryu et al., 2015). Since the success has already demonstrated in monitoring the above-described aspects, there is a large potential of using WBE for monitoring also other biomarkers related to infectious diseases (Sims and Kasprzyk-Hordern, 2020). King et al. (2009) reported the presence in the aquatic environment of Mastadenovirus A-F, responsible for gastroenteritis, ear infection, respiratory illness, and conjunctivitis, and Torque Teno Virus, responsible for hepatitis. The presence of Hepatitis A virus, Bk, MC and JC polyomavirus, responsible for gastroenteritis, hepatitis, cancer, and infection of respiratory, urinary tract and skin was reported by Moens et al. (2017). Similarly, the presence of in wastewater of Rotavirus A and Betacoronavirus (a novel coronavirus) was reported by Cook et al. (2004) and Chan et al. (2015), respectively.

4.2.2. *Characteristics of biomarkers for SARS-CoV-2 surveillance in wastewater*

Biomarkers are measurable and reproducible biological and chemical indicators containing pathogenic deoxyribonucleic acid (DNA) and/or ribonucleic acid (RNA), proteins, inflammation resistance genes, and metabolites emanating from any biological system for assessing the potential environmental risk through its early detection (Sims and Kasprzyk-Hordern, 2020). Daughton (2012) highlighted examples of biomarker indicators to include human excretion products such as

blood, faeces, urine, and other clinical samples. Since SAR-CoV-2 has shown to have similar clinical and genomic characteristics of protein sequence to SAR-CoV (Xu et al., 2020a), prompting to possible similar biomarkers in the environment (Michael-Kordatou et al., 2020; van Doremalen et al., 2020). However, recent studies have shown that, unlike the original SARS-CoV, SARS-CoV-2 has multifaceted infections, including respiratory, gastrointestinal tract, nervous system, cardiovascular, and other body organ-related infections (Zhang and Guo, 2020). This implies the production of more viral particles from infected parts, subsequently disseminating into the environment, including wastewater and air through urine, faeces, breath, and possibly odor (Gould et al., 2020; Adelodun et al., 2020a). Although more studies are still required to conclude on the frequency of SARS-CoV-2 shedding through faeces and urine and other metabolic activities in infected individuals, some studies confirmed the shedding of viral genetic particles in faeces before the onset and after the disappearance of COVID-19 symptoms (Wang et al., 2020a,b,c,d; Gupta et al., 2020; La Rosa et al., 2019), leading to the detection of SARS-CoV-2 RNA in wastewater before the actual reported COVID-19 case (Medema et al., 2020). Reid et al. (2014) highlighted the essential characteristics of compound suitability as biomarkers to include stability within the wastewater system, sufficiently present in high concentrations, sufficiently excreted in a high level of observability and must be excreted in urine without much solid partitioning. Similarly, Daughton (2012) identified some essential attributes of biomarkers to include a high level of persistence in sewage, sensitive to biological changes and or health status of the infected individual. Among the main features identified, the most relevant were represented by: the minimal variance in daily excretion among the intra-individual; minimal interference of exogenous factors; high level of detectability, and ability to identify the biological pathway or physiological system of origination. While the majority of these characteristics were used to describe the drug biomarker, the same can be attributed to SARS-CoV-2 since they are both found to exhibit through metabolism and excretion, considering the currently limited data on the pathogenesis of SARS-CoV-2. Sims and Kasprzyk-Hordern (2020) further noted some desirable characteristics of biomarkers that can be fitted by WBE techniques. The excretion of the biomarker must be through urine with concentration measured in ng/L and high enough to be detected at the downstream of the wastewater. As already mentioned before, the stability of the biomarker in the wastewater during the process of sampling and characterization is essential. Furthermore, the biomarker should be unique to the human metabolism of interest to distinguish it from exogenous biomarkers originating from other sources such as microbes or animal contamination in wastewater (Barceló, 2020a). However, it was argued that the genetic materials of SARS-CoV-2 decays naturally below the limits of detection in the environment, including wastewater and aerosol (Hart and Halden, 2020b), which could affect the quantity and quality of biomarker characteristics in these media.

4.2.3. Potential biomarkers for SARS-CoV-2 in wastewater

The selection of appropriate biomarkers can be challenging due to the complexity of the water and wastewater matrix, which requires extensive preparation of samples and pre-concentration of target analytes (Sims and Kasprzyk-Hordern, 2020; Pandopulos et al., 2020a,b). Wastewater contains complex microbial communities with diverse information on biological and chemical compounds, which are difficult to extract and characterize (Adelodun et al., 2020b). Similarly, the use of volatile compounds for the biosampling of airborne viruses has so far received less attention due to the challenges in identifying virus-specific volatile compounds (Gould et al., 2020). Nevertheless, significant progress has been recorded on biomarkers for drug/pharmaceutical monitoring (Daughton, 2020; Sims and Kasprzyk-Hordern, 2020). SARS-CoV-2 infections are found to cause serious alterations in the respiratory, pulmonary, and gastrointestinal microbiomes (Villapol, 2020; Lamote et al., 2020), thereby causing changes of metabolites which can be detected in the faeces or breath (Gould et al., 2020). The viruses contain proteins, RNA, and lipids, which are excreted or expelled via metabolic activity, making them potential marker candidates for viral infection (Gould et al., 2020). Few studies have also been conducted on the biomarkers for SARS-CoV-2. Zhang and Guo (2020) highlighted three specific biomarker targets for SARS-CoV-2 detection, and these include the viral RNA genome, spike protein, and glycans. Conticini et al. (2020) suggested chemokines and inflammatory cytokines as potential biomarkers for acute respiratory distress syndrome, an elevated condition in COVID-19 resulting from airborne particulates of SARS-CoV-2. Table 3 presents other potential biomarkers proposed in the literature that could be adopted for monitoring SARS-CoV-2 in wastewater and airborne particles using previously identified biomarkers with similar indicators to COVID-19.

5. Conclusions and future perspectives

The detection of specific pollutants in wastewater related to human lifestyle and health issues is an effective non-intrusive, and non-incriminating system for monitoring and returning relevant information for preserving human health and legacy for communities. This can also be an effective way of helping in the early warning of outbreaks as those caused by viral infections. Although, wastewater based epidemiology has been successfully exploited for investigating the use and abuse of both licit and illicit drugs related to lifestyle and health of communities, the criticisms remain in its use for detecting viral infections and disease. Adequate concentration and stability of the specific biomarkers in both wastewater and during the sampling and storing procedure resulted in the first critical aspects that are able to affect the liability of the successive measurements. Another criticism was observed in the absence of standardized and largely agreed methodologies and protocols, in particular for SARS-CoV-2, which which could lead to uncomparable results. Traces of SARS-CoV-2 RNA in the airborne particulate matter were also detected by some studies in the literature, suggesting this medium as another possible way for the virus spreading. However, the limited number of studies, the absence of adequate knowledge, and several criticisms concerning the analytical procedures and methodologies, indicates that much more research activity necessitate the need for better understanding of this phenomenon. Better identification of biomarkers with suitable features for viral diseases detection and definition of standardized and adequate procedures for sampling, storage, and analysis of both wastewater and airborne samples are the main aspect requiring further research activity. In view of the above, the interdisciplinary approach, and multi-dimensional research activities capable of examining the initial diffusion of the virus as well as its outbreak are strongly recommended. This requires a multiple perspectives approach, including, among others, biomedical, epidemiological and environmental expertise together with engineering, economic, political, social and demographic ones.

Credit author statement

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Table 3
Potential biomarkers for monitoring SARS-CoV-2 in wastewater.

Biomarker	Type	Metabolism description	Indicator	Concentration	References
Cotinine	Exogenous	Nicotine	Urine	5.0 ng/L–3.0 μg/L	Chen et al. (2014)
5-hydxyindoleacetic acid (5-HIAA)	Endogenous	Serotonin	Urine	30 ng/L - 10 μg/L	Chen et al. (2014)
Homovanillic acid (HVA)	Endogenous	Catecholamine	Wastewater	1.70 ± 0.88 mg/day	Pandopulos et al. (2020a)
Vanillylmandelic acid (VMA)	Endogenous	Catecholamine	Urine	1.94 ± 1.03 mg/day	Pandopulos et al. (2020a)
C-reactive protein	Endogenous	Matrix metalloproteinase	Urine	0.54–2.76 μg/mL	Stuveling et al. (2003)
Coprostanol	Endogenous	Cholesterol	Faeces	30 μg/L - 10 mg/L	Chen et al. (2014)
Cortisol	Endogenous	Steroid hormone	Faeces	30 ng/L - 10 μg/L	Chen et al. (2014)
Cholesterol	Endogenous	Lipid molecule, and components of cell membranes	Faeces	30 μg/L - 10 mg/L	Chen et al. (2014)
Androstenedione	Endogenous	Sex hormone precursor	Urine	30 ng/L - 10 μg/L	Chen et al. (2014)
Acesulfame	Exogenous	Artificial sweetener	Urine	9.59–30.13 μg/L	Rico et al. (2017)
Lamivudine	Endogenous	Trans-sulfoxide	Urine	2.2–579.1 ng/L	Hou et al. (2020)
Atenolol	Exogenous	Hypertension (drug) better blocker	Urine	15.21–90.11 μg/L	Rico et al. (2017)
Isoprostanes	Endogenous	15-F_{2t}-isoP	Urine	57–390 ng/g	Janicka et al. (2010)
8-isoprostanes	Endogenous	15-F_{2t}-isoP	Exhale breath condensate	16–110 pg m/L	(Syslová et al., 2008; Psathakis et al., 2006)

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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