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Research Paper

A safe haven of SARS-CoV-2 in the environment: Prevalence and potential transmission risks in the effluent, sludge, and biosolids

Bashir Adelodun [a,b], Pankaj Kumar [c], Golden Odey [a], Fidelis Odedishemi Ajibade [d,e,f], Rahmat Gbemisola Ibrahim [g], Saad A.M. Alamri [h], Sulaiman A. Alrumman [h], Ebrahem M. Eid [h,i], Vinod Kumar [c], Khalid Adeola Adeyemi [a], Ashish Kumar Arya [j], Archana Bachheti [j], Marcos L.S. Oliveira [k], Kyung Sook Choi [a,l,*]

[a] Department of Agricultural Civil Engineering, Kyungpook National University, Daegu 41566, South Korea
[b] Department of Agricultural and Biosystems Engineering, University of Ilorin, PMB 1515, Ilorin 240003, Nigeria
[c] Agro-ecology and Pollution Research Laboratory, Department of Zoology and Environmental Science, Gurukula Kangri (Deemed to be University), Haridwar 249404, Uttarakhand, India
[d] Department of Civil and Environmental Engineering, Federal University of Technology, PMB 704, Akure, Nigeria
[e] Key Laboratory of Environmental Biotechnology, Research Centre for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, PR China
[f] University of Chinese Academy of Sciences, Beijing 100049, PR China
[g] Kwara State Ministry of Health, Ilorin, Kwara State, Nigeria
[h] Biology Department, College of Science, King Khalid University, Abha 61321, Saudi Arabia
[i] Botany Department, Faculty of Science, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt
[j] Department of Environmental Science, Graphic Era (Deemed to be University) Deharadun, 248002 Uttarakhand, India
[k] Department of Civil and Environmental, Universidad De La Costa, Calle 58 #55–66, 080002 Barranquilla, Atlántico, Colombia
[l] Institute of Agricultural Science & Technology, Kyungpook, National University, Daegu 41566, Korea

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ABSTRACT

The novel coronavirus, SARS-CoV-2, which has caused millions of death globally is recognized to be unstable and recalcitrant in the environment, especially in the way it has been evolving to form new and highly transmissible variants. Of particular concerns are human-environment interactions and the handling and reusing the environmental materials, such as effluents, sludge, or biosolids laden with the SARS-CoV-2 without adequate treatments, thereby suggesting potential transmission and health risks. This study assesses the prevalence of SARS-CoV-2 RNA in effluents, sludge, and biosolids. Further, we evaluate the environmental, ecological, and health risks of reusing these environmental materials by wastewater/sludge workers and farmers. A systematic review of literature from the Scopus database resulted in a total of 21 articles (11 for effluents, 8 for sludge, and 2 for biosolids) that met the criteria for meta-analysis, which are then subdivided into 30 meta-analyzed studies. The prevalence of SAR-CoV-2 RNA in effluent and sludge based on random-effect models are 27.51 and $10^{12.25}$, respectively, with a 95% CI between 6.14 and 48.89 for the effluent, and $10^{4.78}$ and $10^{19.71}$ for the sludge. However, the prevalence of SARS-CoV-2 RNA in the biosolids based on the fixed-effect model is 30.59, with a 95% CI between 10.10 and 51.08. The prevalence of SARS-CoV-2 RNA in environmental materials indicates the inefficiency in some of the treatment systems currently deployed to inactivate and remove the novel virus, which could be a potential health risk concern to vulnerable wastewater workers in particular, and the environmental and ecological issues for the population at large. This timely review portends the associated risks in handling and reusing environmental materials without proper and adequate treatments.

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* Corresponding author at: Department of Agricultural Civil Engineering, Kyungpook National University, Daegu 41566, South Korea.
E-mail address: ks.choi@knu.ac.kr (K.S. Choi).

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1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic caused by a novel severe acute respiratory syndrome coronavirus (SARS-CoV-2) has led to a significant paradigm shift on the existing precaution towards handling and exposure to different environmental materials such as water, air, and soil (Cao et al., 2021; Shao et al., 2021; Anand et al., 2022). The prevalence of the SARS-CoV-2 or its genetic material in the environmental materials, especially wastewater samples collected from treatment plants and air samples from the built environment have been well documented in many countries, including the USA (Nemudryi et al., 2020; Peccia et al., 2020; Sherchan et al., 2020; Palmer et al., 2021), Spain (Randazzo et al., 2020; Balboa et al., 2021), Turkey (Kocamemi et al., 2020a, 2020b), Netherlands (Medema et al., 2020), Saudi Arabia (Hong et al., 2021), Australia (Ahmed et al., 2020), Japan (Haramoto et al., 2020; Hata et al., 2021), Iran (Gholipour et al., 2021; Tanhaei et al., 2021), Czech Republic (Mlejnkova et al., 2020), India (Chakraborty et al., 2021; Kumar et al., 2021a), France (Trottier et al., 2020; Wurtzer et al., 2021), United Arab Emirate (Hasan et al., 2021), Germany (Westhaus et al., 2021), Israel (Abu Ali et al., 2021), and Italy (La Rosa et al., 2020a, 2020b; Rimoldi et al., 2020). Similarly, some treated effluents have also been reported to contain the SARS-CoV-2 RNA (Ampuero et al., 2020; Abu Ali et al., 2021; Bhattarai et al., 2021; Carrillo-Reyes et al., 2021; Graham et al., 2021). Although some treatment techniques are highly effective in removing the virus and its genetic material in the wastewater treatment plants (WTTPs) (Balboa et al., 2021; Hasan et al., 2021; Sherchan et al., 2021). However, information on the occurrence of both viable and the fragment material of the SARS-CoV-2 in the receiving environment like soil via effluent and sewage sludge disposal is limited despite the continuous interplay between these media (Adelodun et al., 2021e; Anand et al., 2021b). Sludge or biosolids herein refers to the residuals or solid fraction, depending on the state of stability or digestion, separated during the primary and secondary clarification in the wastewater treatment process or water resource recovery facilities.

Prevalence of SARS-CoV-2 in sludge and biosolids, as environmental materials, have so far received less attention compared to wastewater samples despite being adjudged the potential reservoir of SARS-CoV-2 due to the protection it receives against inactivation from the complex organic matter of the sludge containing 30% to 50% of the pollutants (Yang et al., 2020). Emerging studies have indicated a higher prevalence and longer persistence of SARS-CoV-2 in the sludge and biosolids (Kocamemi et al., 2020b; Balboa et al., 2021; Carrillo-Reyes et al., 2021; D'Aoust et al., 2021), which could aid in filling the knowledge gap on the transmission and public health risks, especially among the workers dealing with such environmental materials, including wastewater workers and farmers (Brisolara et al., 2021). Although there have been continuously raised concerns on the potential transmission risk of the SARS-CoV-2 through different environmental compartments (Adelodun et al., 2021c; Anand et al., 2021a, 2021b; Gwenzi, 2021; Shao et al., 2021), the emergence of the new recalcitrant variants of the virus has heightened this concern due to their high infectious and transmissible nature (CDC, 2020; Abdool Karim and de Oliveira, 2021; Wang et al., 2021). Different variants of SARS-CoV-2 along with the countries of origin were reported in a recent review work by Anand and co-workers (Anand et al., 2021c).

In the rural settings where water and sanitation are currently not appropriately and sufficiently deployed, the reclaimed wastewater effluent is becoming increasingly important to augment the scarcity of freshwater resources for irrigation and other domestic functions. Similarly, sludge and biosolids are being used as a soil amendment to improve soil fertility since agriculture is the mainstay of the rural economy (Eid et al., 2021; Georgin et al., 2021; Kerkhoff et al., 2021; Pereira et al., 2021; Ramos et al., 2021; Sellaoui et al., 2021b; Silva et al., 2021; Vieira et al., 2021). However, the ecological and health risks concern of inappropriate use of reclaimed wastewater and sewage sludge have been recently intensified on their potential escalation of COVID-19 among the wastewater workers and farmers, including in the urban settings (Adelodun et al., 2020b, 2020c, 2021b; Abu Ali et al., 2021; Brisolara et al., 2021; Dada and Gyawali, 2021; Zaneti et al., 2021). It has also been demonstrated that enveloped virus could survive for days in the water environment, depending on the environmental conditions (Bivins et al., 2020; Hokajärvi et al., 2021; Shao et al., 2021; Wurtzer et al., 2021), or treatment processes (Bhattarai et al., 2021; Carrillo-Reyes et al., 2021; Kumar et al., 2021a). Thus, consequently enters the surface and groundwater sources via leaking sewers, runoff, or land application of untreated sewage sludge, thereby leading to transmission by ingestion of such virus-contaminated water resources (Adelodun et al., 2021b). Moreover, previous studies have also demonstrated the risk of viral infections via consumption of vegetables and fruits irrigated with reclaimed virus-contaminated effluent (Maunula et al., 2013; Carducci et al., 2015; Miranda and Schaffner, 2018).

Furthermore, the majority of the wastewater workers do not use adequate protective equipment during different stages of wastewater/sludge treatment and disposal while having direct contact or exposure to raw sludge and biosolids samples, especially in rural settings (Sampson et al., 2017; Gwenzi, 2021). Sludge treatment processes such as oxidation ditch, dewatering, and mechanical agitation have also been reported to generate toxic aerosol containing SARS-CoV-2 in the form of airborne particulate matter (Yang et al., 2020; Brisolara et al., 2021; Dada and Gyawali, 2021; Gholipour et al., 2021), which could remain viable and infectious for several hours in such an environmental medium (Fears et al., 2020; van Doremalen et al., 2020; Kareem et al., 2021). Meanwhile, the guidelines or regulations for the reuse of wastewater and sewage sludge are lacking in many developing and less developed countries, prompting to high vulnerability of viral infections, especially the recalcitrant SARS-CoV-2 variants in these settings (Adelodun et al., 2021b).

The prevalence knowledge of SARS-CoV-2 in environmental materials, most especially in the effluent, sewage sludge, and biosolids, being the final outputs of the wastewater treatment plants that ought to have reached virologically safe status after minimum possible treatments will aid in understanding their fate in the environment and subsequently in assessing the risks of infection and transmission to wastewater/sludge workers, farmers, and other users of these environmental materials. This study, therefore, investigates the prevalence of SARS-CoV-2 in the effluent, sludge, and biosolids. Furthermore, the environmental, ecological, and health risks of the handling and reusing of these environmental materials by wastewater/sludge workers and farmers, extending to the potential transmission of viral infections through the migration of the virus to the soil and uptake by landscape plants, and irrigated crops are analyzed. To the best of our knowledge, this is the first meta-analysis study conducted to investigate the prevalence of SARS-CoV-2 environmental samples or materials, specifically effluent, sludge, and biosolids.

2. Methodology

To address the stated objectives of this study, a meta-analysis was conducted on the studies that quantified the SARS-CoV-2 or its genetic material in the wastewater effluent, sludge, and biosolids to obtain the representative concentration of the virus in the respective environmental media. The systematic review approach of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) for literature search and selecting appropriate and relevant articles, as implemented by (Sano et al., 2016; Adelodun et al., 2021d), was adopted in this study. The Scopus (www.scopus.com), which is the largest abstract and citation database of peer-viewed literature database was searched, with the selection of only applicable articles published in the English Language until September 1, 2021. The relevant keywords, including SARS-CoV-2 and effluent, SARS-CoV-2 and sludge, SARS-CoV-2 and biosolids or digested sludge, COVID-19, wastewater, and health risks, which are in concordance with the topic, scope, and objectives of this study were thoroughly searched with the aid of Boolean search words of 'AND' and 'OR'. However, we excluded articles that only reported the occurrence of SARS-CoV-2 in the effluent, sewage sludge, or biosolids without any quantitative data of the virus or its genetic material. For instance, Nasseri and co-workers in Iran (Nasseri et al., 2021) and Baldovin and co-workers in Italy (Baldovin et al., 2021) only reported the presence of SARS-CoV-2 RNA in treated effluents without providing the quantitative data; hence, these studies were excluded in the meta-analysis. The average value, standard deviation, and sample size were considered for the results only when the reported virus or its genetic material incidence was positive. In some cases where the required data were presented in figures, the WebPlotDigitizer version 4.5 software (Rohatgi, 2021) was used to extract the data to calculate the average value and standard deviation as required.

Furthermore, a meta-analysis was employed to estimate the prevalence of SARS-CoV-2 in the effluent, sludge, and biosolids. The meta-analysis calculation and forest plot construction were performed and constructed using a Microsoft Excel software package developed by Neyeloff et al. (2012) and OriginPro (Origin Corp., USA) software, respectively. Both fixed and random effects models were considered for the three environmental materials. The appropriate model was then determined and selected using the Cochrane Q statistic test with Chi-square (P-value < 0.05) for the existence of statistically significant heterogeneity and the I^2 heterogeneity test for the degree level of heterogeneity, ranging from 0 to 100% (Higgins et al., 2003). The potential risk assessment of using these environmental materials for irrigation, soil amendment, landscaping, and other reuse purposes by wastewater workers, farmers, and other end-users was also discussed.

3. Prevalence of SARS-CoV-2 in the effluent, sludge, and biosolids

The prevalence of SARS-CoV-2 or its genetic material in the effluent, sludge, and biosolids was assessed using meta-analysis based on the available published data. These environmental materials are purposely selected for investigation because of the associated environmental, ecological, and public health risks of their presence in the environment. Moreover, they are expected to be free from the SARS-CoV-2 contamination and other pathogens or at least to a safe limit after being subjected to varying levels of treatments in the wastewater treatment plants. At first, considering the general keywords of SARS-CoV-2 combined with specific keywords such as effluent, sludge, and biosolids using the Scopus database yielded 121 articles. After a thorough screening, however, the articles that met the final criteria with required data were 20 articles. These comprise of 11, 8, and 2 for effluent, sludge (primary, secondary, and activated), and biosolids (digested sludge), respectively, which are sub-divided into 25 studies (14 for effluents, 14 for sludge, and 2 for biosolids) that were meta-analyzed (Fig. 1). The details of the included studies with various treatment processes employed in the wastewater treatment plants are presented in Supplementary Data Table 1.

The meta-analysis using both Q-statistic and I^2 tests indicates high to moderate heterogeneity for the prevalence concentrations of SARS-CoV-2 RNA in effluents and sludge, respectively; thus, random-effects models were selected. For the SARS-CoV-2 RNA prevalence in effluents, the Q-statistic value of 840.946 is greater than the 22.36 Chi-square critical value (13 degrees of freedom at a significance level of 0.05), and a high I^2 value of 98% indicate high heterogeneity. Similarly, the Q-statistic value of 27.703, which is greater than the 22.36 Chi-square critical value (13 degrees of freedom at a significance level of 0.05), and I^2 of 53% also indicate moderate heterogeneity for the sludge (Higgins et al., 2003; Neyeloff et al., 2012). This implies that the prevalence rate of the SARS-CoV-2 in effluent and sludge is affected by different factors, which could include the number of SARS-CoV-2 infected persons and the relative viral shedding within the catchment of the treatment plant, treatment technology adopted in the plant, the virus concentration method, the targeted genes during the sampling, and sampling errors. For instance, it was reported that all the effluents monitored from 11 wastewater treatment plants in the UAE had no SARS-CoV-2 RNA due to the deployed treatment technologies in degrading the viral loads (Kumar et al., 2021a). Zhao and co-workers, however, reported that the three secondary effluent collected from the wastewater treatment plant in China contained positive SARS-CoV-2 RNA (Zhao et al., 2022). The representative prevalence rate of the SARS-CoV-2 RNA in the effluents and sludge based on the random effects models are 27.51 and $10^{12.25}$, respectively, with 95% CI between 6.14 and 48.89 for the effluent and $10^{4.78}$ and $10^{19.71}$ for the sludge (Fig. 2a and b). However, the prevalence of SARS-CoV-2 RNA in the biosolids is found to be homogeneous (less heterogeneity) among the two studies considered as indicated by the Q-statistic value of 0.1735, which is less than the 3.84 Chi-square critical value (1 degree of freedom at a significance level of 0.05) and I^2 of 0%, thus fixed-effect model was used. The smaller number of available studies that reported the prevalence of the SARS-CoV-2 RNA in biosolids, among other factors, could be attributed to the homogeneity indicated by the Q-statistic and I^2 tests. The representative prevalence value of SARS-CoV-2 RNA in the biosolids for the fixed-effect model is 30.59, with 95% CI between 10.10 and 51.08 (Fig. 2c). Although the number of studies that reported the prevalence of SARS-CoV-2 RNA in biosolids is relatively small compared to that of effluent and sludge, the higher prevalence rates of SARS-CoV-2 RNA in biosolids based on the fixed-effect model compared to in the effluent indicates the ability of the viral particles to persist in the solid medium due to the adsorption and protection received from the solid particles despite the further treatment on the digestion or stabilization (Peccia et al., 2020; Balboa et al., 2021).

Several factors contribute to the prevalence of virus concentration in environmental samples, including the number of infected people in the catchment area of the treatment plant, the frequency of viral shedding, RT-qPCR assay, and the concentration method. Hong et al. (2021) opined that consistent detection of SARS-CoV-2 in environmental samples is likely when the number of COVID-19 patients within the catchment of the treatment plant is above 70, 118, and 109 for N1, N2, and N3 genes detection. Similarly, the viral load varies spatially and temporally, while environmental factors could naturally affect the prevalence of the viral loads in the environment (La Rosa et al., 2020a, 2020b). It was reported that the type B ultraviolet (UVB) in sunlight radiation allowed natural inactivation of the SARS-CoV-2 up to T_{90} reduction for less than 10 min considering the latitude, season, and hour (Herman et al., 2021). However, SARS-CoV-2 RNA was detected in abundance in the open-air biological activated sludge tank containing hospital wastewater despite the increased temperature from 41.5 °C to 46.3 °C and solar radiation range of 1184.9 W/m^2 to 1333.0 W/m^2 - (Hong et al., 2021). The recent shreds of evidence of non-detection

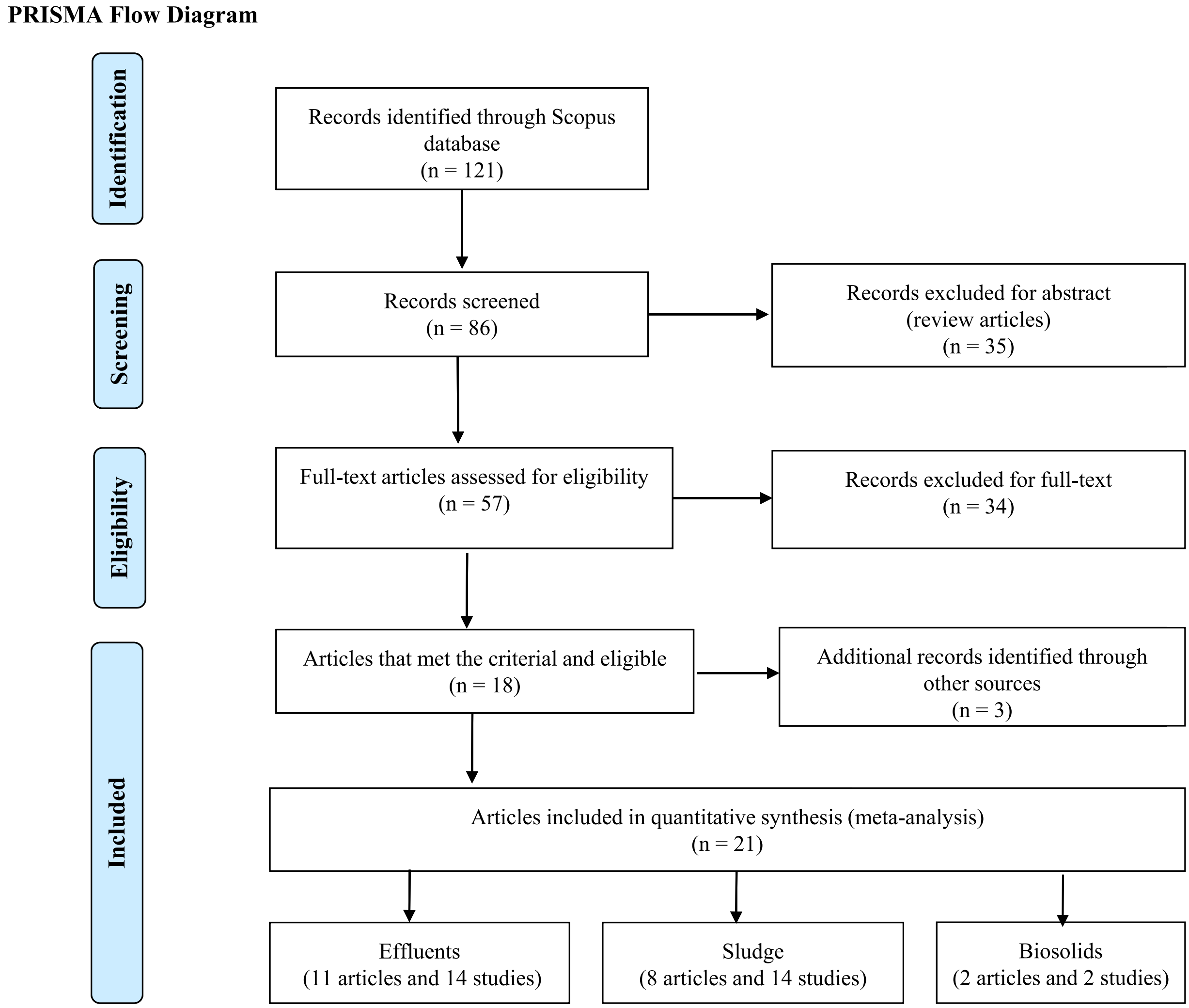

Fig. 1. PRISMA flow diagram of twenty one reviewed papers considered for meta-analysis.

of particular genes of the virus in the environmental materials or the varying efficiencies of the concentration methods pointed that there could be under-reporting of the prevalence of the SARS-CoV-2 or its genetic material. The ORF 1ab and S genes were reported to be more sensitive to treatment than N genes, making the former genes to be significantly reduced than the later gene during the treatment processes (Kumar et al., 2021a). However, among the N genes, Hong et al. (2021) observed that while the N1 gene was more sensitive than N2 and N3 genes, the N3 gene marker as detected by CDC 2019-nCoV-N3 primer-probe pair is not appropriate for environmental surveillance as it may be affected by the nature of the water matrix, temperature, and treatment method employed. Regarding the influence of the concentration method, it was reported that the polyethylene glycol concentration method is superior to the filtration method in terms of virus inhibition performance (Kumar et al., 2021b). Carrillo-Reyes et al. (2021) reported that the adsorption method resulted in a higher value than the ultrafiltration method of concentration for enveloped viruses that are highly associated with solid materials. Haramoto et al. (2020) also attributed the discrepancy of non-detection of SARS-CoV-2 to the volume of environmental water samples used in virus concentration while recommending an appropriate and effective concentration-RNA extraction method for SARS-CoV-2. It was also suggested that the effective prevalence of the SARS-CoV-2 concentration should be based on the presence of multiple genes (Kumar et al., 2021a). Similarly, it was suggested that the digital RT-qPCR (dRT-qPCR) should be deployed for the estimation of SARS-CoV-2 loads in low prevalence areas of COVID-19 (Randazzo et al., 2020), as it has been reported to be more sensitive to inhibitors than the popular RT-qPCR for primary clarified sludge (D'Aoust et al., 2021).

4. Potential of sludge and biosolids to harbor SARS-CoV-2

The enveloped viruses have distinct characteristics different from the non-enveloped viruses due to their genome, structure, replication, pathogenicity, hydrophobicity, persistence, and fate in the environment (Wigginton and Boehm, 2020). The enveloped viruses, with their unique structure, contain a lipid bilayer membrane around the protein capsid, which is responsible for their affinity to adsorb to the solid and colloidal particles to ensure their survival and partitioning behavior in water environment (Gundy et al., 2009; Ye et al., 2016). This has been experimentally demonstrated for two human enveloped virus surrogates, which are murine hepatitis virus and *Pseudomonas* phage ϕ6, in wastewater samples (Ye et al., 2016). The hydrophobic nature of the enveloped viruses allows the viruses to be retained in primary or secondary sludge and biosolids if such materials are not subjected to advanced treatment processes (Wellings et al., 1976; Prado et al., 2014).

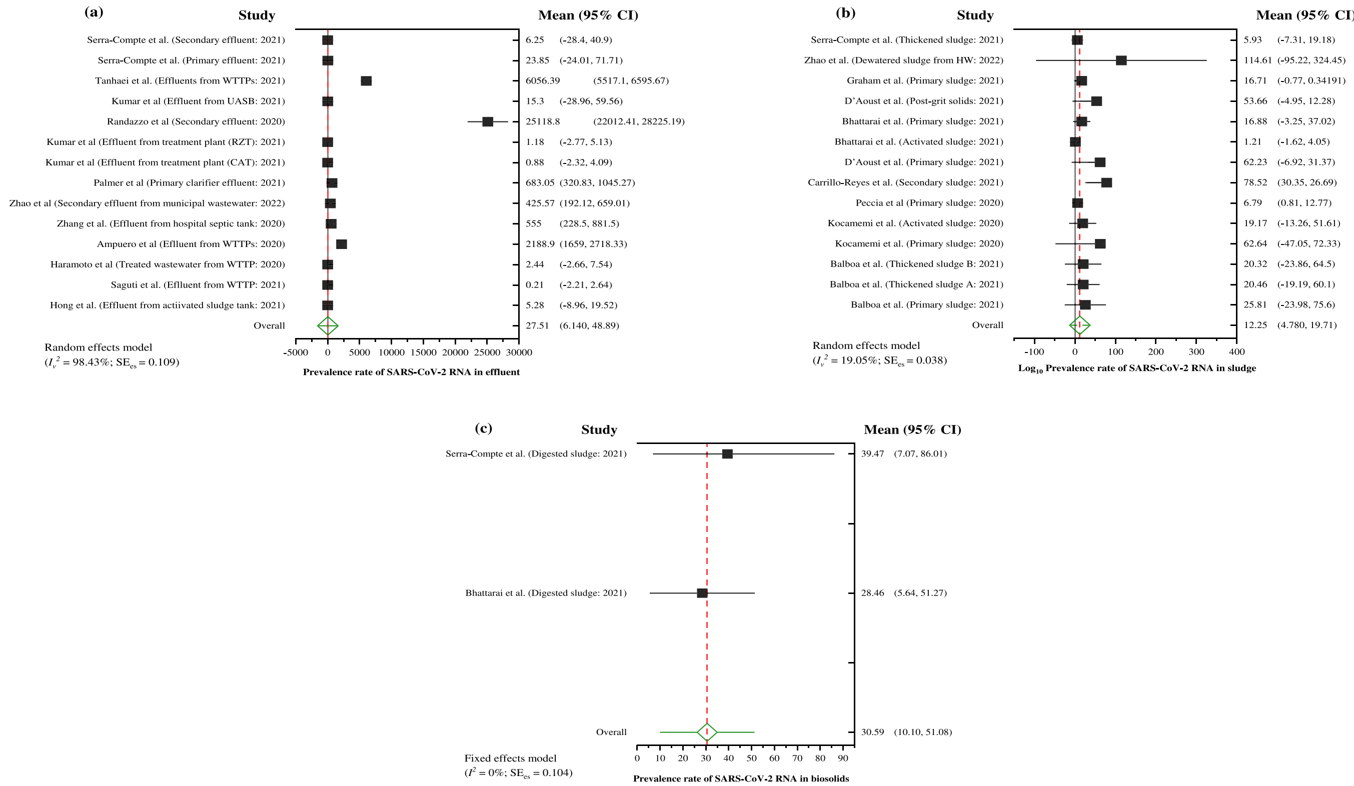

Fig. 2. Forest plot of the prevalence of SARS-CoV-2 in (a) effluents, (b) sludge and (c) biosolids, with corresponding 95% confidence interval (CI).

Based on this available information, it was posited that the SARS-CoV-2 or its genetic material, being an enveloped virus, have a strong affinity towards the sludge/biosolids due to the presence of settled solid materials during the primary and secondary solid separation from the clarified water in the wastewater treatment process, while the virus or its genetic material is also not likely to be detected in the final effluents (Balboa et al., 2021). Moreover, it has been demonstrated that higher solid concentrations and longer retention time enhance the retention of SARS-CoV-2 in the sludge (Balboa et al., 2021). Carrillo-Reyes et al. (2021) reported a higher load of SARS-CoV-2 RNA in secondary sludge compared to in the influent, suggesting the role of solid fraction in adsorbing the viral RNA. The solid concentrations containing the nutrients, organic matters, and ionic strength with appropriate pH level, moisture contents, and temperature can generate hetero-aggregate with the enveloped virus under a sufficient equilibrium time (Katz et al., 2018; Mohan et al., 2021). It has also been reported that as small as 7 mm of suspended solid particles could protect the virus from UV exposure, while 0.3 mm sized solid particles can shield viruses from disinfection, thereby extending their persistence (Templeton et al., 2005; Zhang et al., 2020). The presence of large numbers of viable virions, nonetheless the fragility of the enveloped virus, could pose an infection risk due to its ability to travel more rapidly via porous media under this safe heaven condition of the formed aggregate (Katz et al., 2018). Thus, the capability of a higher concentration of the virus to be retained in the sludge line, even more than in the influent, promotes the use of such an environmental sample for virus surveillance incidence in the population (Peccia et al., 2020).

5. Transmission risks of SARS-CoV-2 through effluents, sludge, and biosolids

The potential transmission pathways of SARS-CoV-2 via effluents, sludge, and biosolids are numerous (Fig. 3), essentially if proper measurements and guidelines are not in place or strictly followed. During the wastewater treatment processes leading to the production of different components, including effluents, sludge, and biosolids, the SARS-CoV-2 have been reportedly evaded the selected treatment stages to reach either effluents or sludge (primary or secondary) (Kocamemi et al., 2020b; Peccia et al., 2020; Balboa et al., 2021; Carrillo-Reyes et al., 2021; D'Aoust et al., 2021; Hong et al., 2021). The long exposure to the raw or partially treated sewage sludge, especially during the extended periods of manual sludge handling such as dewatering indicates a high risk of transmission (Amoah et al., 2020; Dada and Gyawali, 2021).

Similarly, the aerosols containing SARS-CoV-2 and/or other pathogens are generated during the sewage sludge collection (Lu et al., 2020; Madsen et al., 2020), processing and treatment of effluents and sludge (Gholipour et al., 2021; Zaneti et al., 2021), and also during the application or the reuse of effluents and sludge in the field (Rosenberg Goldstein et al., 2014; Sampson et al., 2017). Several other studies have also confirmed the transmission route of SARS-CoV-2 via aerosols (Kim et al., 2020; Shen et al., 2020; van Doremalen et al., 2020), with a relatively high risk of the virus infection by wastewater workers (Gholipour et al., 2021). Furthermore, since the effluents, sludge, and/or biosolids are directly applied to the soil and plant or most times discharge directly into the environment without adequate treatments in some developing and low-income countries, there is a tendency of the virus to desorb and migrate into the subsurface system and groundwater or uptake by the plants (Kumar et al., 2020); thus, leading to possible fecal-oral transmission via either direct contact or ingestion of contaminated crops or water (Adelodun et al., 2020b; Gwenzi, 2021). The inevitable inhalation of the virus-laden aerosols generated during the wastewater and sludge processing activities, especially without adequate protection, could also lead to fecal-oral transmission of the virus.

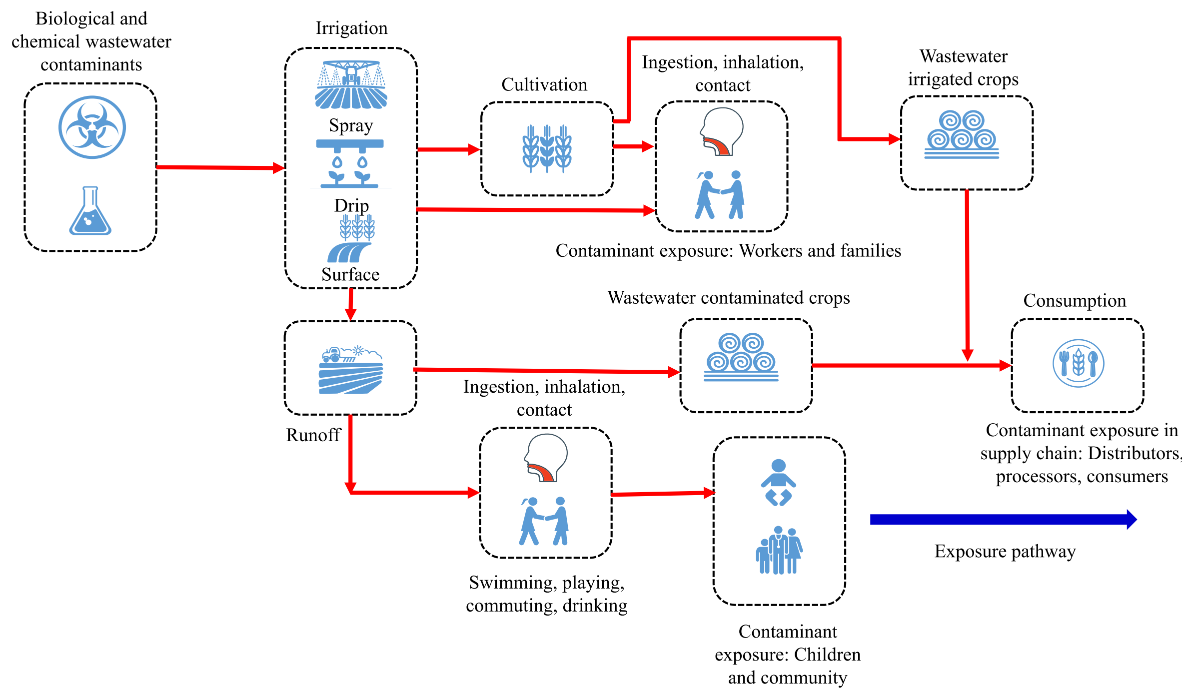

Fig. 3. Exposure pathways associated with the reuse of wastewater.

In fact, new studies have shown that the conventional wastewater treatment is not effective in removing SARS-CoV-2 RNA entirely as the effluents and other products from the treatment plant still contain SARS-CoV-2 genetic material, especially when considering the multiple genes of the virus (Balboa et al., 2021; D'Aoust et al., 2021; Kumar et al., 2021a). Owing to these, the proliferation of the SARS-CoV-2 from influent to effluent and sludge/biosolids could potentially lead to community transmission of the virus via direct contact with the environmental materials. Community transmission of the COVID-19 in the low-income community of Guangzhou in China suggested the plausible sewage transmission route owing to poor sanitation, hygiene conditions, high population density, and inadequate infrastructure (Yuan et al., 2021). Similarly, the possible cluster transmission where eight individuals were infected via bathing in a public bath center at Huai'an in Jiangsu Province, China, was also reported (Luo et al., 2020). Further, a similar coronavirus to SARS-CoV-2, was attributed to a community transmission via toilet systems in Hong Kong during the 2003 SARS epidemic (Yu et al., 2004).

It is worthy to emphasize that the transmission of the SARS-CoV-2 through the reuse of environmental materials, such as effluents and sludge/biosolids is still being debated since the majority of the available studies reported only the genetic material of the virus rather than the infectious and viable virus, which its infectivity and viability have not yet been established. However, the source of concern should also be the health implications of the excessive ingestion of the virus genetic material, as many available studies fail to investigate the infectivity of the SARS-CoV-2 in the environmental samples (Balboa et al., 2021). A recent work by Xu posited the infectivity of the SARS-CoV-2 RNAs and could serve as a putative transmission mechanism (Xu, 2020). Furthermore, the inability to detect the viable SARS-CoV-2 in the environmental samples like effluent and sludge could be attributed to the associated risk of infections and a high level of biosafety requirements to culture and isolate the virus from these samples, leading to the current PCR methods for the detection of only the genetic material of the virus (de Oliveira et al., 2021; Tiamiyu et al., 2021). Since there have been multiple transmission mechanisms leading to the rapid spread of the pandemic, which as of now, could not be linked to a particular route due to the existing knowledge gap on the fate of the SARS-CoV-2 in the environment (Xu, 2020; Gwenzi, 2021). Thus, there is a need to ensure adequate safety and strict compliance with the guidelines regarding the reuse of effluents and sludge/biosolids while new data and knowledge on the transmission mechanism of the SARS-CoV-2 evolve.

6. Global scenario of effluent, sludge, and biosolids generation and management

The global estimate of wastewater generated in 2015 was 359.4 billion m^3/year, out of which 63% were collected and 52% treated (Jones et al., 2021). The use of effluent (treated wastewater) for irrigation is very common in many arid areas like India, Pakistan, China, Australia, Northern Nigeria, and middle eastern countries, including Israel and Saudi Arabia due to the perennial water scarcity in these areas (Jimenez and Asano, 2008; Ajibade et al., 2021a, b; Odey et al., 2021a). In fact, the use of effluent has been substantial in western Europe (16%), and the Middle East and North Africa (15%) out of the total global production (225.6 billion m^3/year), though with low treatment rates before reuse in developing countries (Adelodun et al., 2019, 2020a; Jones et al., 2021). Recently, developed countries like the USA have also adopted wastewater recycling systems and sludge recovery for reuse in agriculture due to the water scarcity induced by climate change (Jimenez and Asano, 2008). Due to the huge amount of sludge production, land application, either directly or after composting, has been the major sludge management around the world, including in different European countries, after incineration (Wei et al., 2020). Although effluent and sludge reuse can address the perennial water scarcity, reduce the water demand, enhance nutrient recycling, improve soil health, mitigate the indiscriminate discharge of pollutants into waterways, and climate change impact; however, these environmental materials need to be carefully managed to prevent the associated environmental, ecological, and public health risks (Hanjra et al., 2012; Elmi et al., 2020; Adelodun et al., 2021d). It is expected that the use of effluent and sludge in agriculture will increase greatly in the future; hence a need for proper guidelines to forestall any associated risks in their usage (Duarte et al., 2019; Ferrari et al., 2019; Akinyemi et al., 2020; Dalmora et al., 2020; Dutta et al., 2020; Oliveira et al., 2020; Sellaoui et al., 2021a).

Management of sewage and sludge generation has been identified as one of the important strategies to prevent and control the transmission of COVID-19 (Yuan et al., 2021). The existing sludge treatment approaches such as thickening, conditioning, dewatering, stabilization, composting, and drying are used to manage the sludge volume, remove the pathogens, recycle the nutrients, and recover the energy, using varying physical, chemical, and biological technologies (Yang et al., 2015). However, these processes are often not carried out when managing the generated sludge in many parts of developing countries. Instead, wastewater treatment is more prioritized, which is, however, less effective in removing emerging pathogens like SARS-CoV-2.

7. Consequences of SARS-CoV-2 contamination in effluents, sludge, and biosolids

7.1. Risk associated with the reuse of virus-contaminated effluent, sludge, and biosolids

The reuse of effluent, sludge, or biosolids has been recognized as an effective strategy to address the scarcity of water shortage through crop irrigation and to supplement the depleted essential nutrients in the soil. However, recent evidence of the prevalence of SARS-CoV-2 RNA on the effluents and sludge samples indicate potential associated environmental, ecological, and health risks on the use of these environmental materials, if they are not properly decontaminated or effectively treated from the novel virus before usage (da Silva et al., 2020; Adelodun et al., 2021a). Indeed, the detection of a high load of SARS-CoV-2 RNA in river water due to direct sewage disposal in low sanitation regions and effluents of wastewater treatment plants is an indication of environmental and public health safety concerns in rural settings (Guerrero-Latorre et al., 2020). Recently, Wiktorczyk-Kapischke and co-workers reported the presence of SARS-CoV-2 RNA with concentrations to range from 205 to 550 copies per gram from the soil samples receiving effluent from the wastewater plant (Wiktorczyk-Kapischke et al., 2021). The wastewater/sewage workers and farmers are the most vulnerable people in this regard due to their direct contact with the environmental materials laden with the virus, and consequently expose them to the COVID-19 (Dada and Gyawali, 2021; Zaneti et al., 2021). The use of certain technology like the sprinkler system for irrigation with wastewater generates considerable aerosols that are dispersed at a large scale, depending on the environmental factors, thereby affecting surrounding communities (Pachepsky et al., 2011). Furrow and flood irrigation systems could also exposure farmers and other farmworkers to contaminated wastewater, thereby increasing risks of infections (Adegoke et al., 2018). The different exposure pathways on the reuse of wastewater, including irrigation, are presented in Fig. 3. The major identified pathways with high risks are the occupational exposure pathway, which is regarded as the main route via direct contact with the environmental materials, and the consumption pathway referred to as an indirect pathway through the ingestion of the contaminated materials, including inhalation and consumption of virus-contaminated produce (Rodríguez-Lázaro et al., 2012; Adegoke et al., 2018). The consequence of occupational exposure to untreated or partially treated wastewater led to viral skin infections among some fish farmers in Hanoi, Vietnam, due to high exposure to the pathogens in wastewaters (Trang et al., 2007).

Further, since foodborne transmission of similar viruses through migration or uptake of the virus from the environmental materials to the irrigated crop have been previously reported (Mullis et al., 2012; Hirneisen and Kniel, 2013; Yépiz-Gómez et al., 2013; Mancuso et al., 2021), the risk of viral infections via the consumption of virus-contaminated farm produce, especially fruits and vegetables can lead to the indirect widespread transmission of the virus. The Bovine coronavirus remained infective on the lettuce leaves for at least 14 days (the entire shelf-life) (Mullis et al., 2012), while human coronavirus 229E was partially inactivated by 0.2 logs after 2 days of storage at 4 °C (Yépiz-Gómez et al., 2013). This scenario of viral infection often occurs where water and sanitation systems are lacking, and the effluents are directly applied for irrigation or sludge, and biosolids are used for soil amendment without proper treatment to meet the regulated guidelines.

To date, no available regulatory limits have been set or defined for occupational exposure to environmental samples laden with SARS-CoV-2. However, considering the environmental and public safety health concerns posed by the use of wastewater, there have been some set guidelines for safe wastewater use in agriculture (World Health Organization (WHO), 2006), which could be adopted for indirect assessment of the exposure level to establish the allowable benchmark value for SARS-CoV-2. Furthermore, to mitigate the exposure and consumption hazard risks of viral infections, it was suggested that renew efforts on micro-irrigation technology where farmers and farm produce would have restricted contact with the wastewater and sewage sludge while safely and efficiently irrigating the soil should be promoted (Oliver et al., 2020).

7.2. Socioeconomic consequences

The rural farmers often result to the use of effluents (partially treated or sometimes untreated) and sludge/biosolids due to the lack of freshwater and the high cost of fertilizer. The lack of water infrastructure to convey freshwater to the farmland, coupled with the high cost of wastewater treatment before reuse have been the limiting factors promoting the use of untreated wastewater, especially for irrigation in rural settings, without considering any form of pathogenic contamination (Hanjra et al., 2012; Ajibade et al., 2021a, b). The reuse of treated wastewater in agriculture reduces freshwater pumping and fertilizer usages; consequently promoting the reduction of the water footprint of food production on the environment while achieving higher yields under reduced cost and carbon footprint (Hanjra et al., 2012; Adelodun and Choi, 2020; Odey et al., 2021b). The reuse of wastewater, rather indiscriminate discharge into the environment contributes immensely to urban food security while improving the urban environment (Goala et al., 2021). Meanwhile, due to the continuous increase in urban migration, peri-urban agriculture has grown tremendously, and the common source of irrigation water is raw wastewater (Ensink et al., 2002), which has been a very common and long-standing practice in developing countries like Indian, Iran, and Pakistan (Thebo et al., 2017).

Unfortunately, the awareness of environmental, ecological, and health risks that are associated with the use of untreated effluents is very low among the farmers, especially when the source and the constituents of the effluents are unknown (Adegoke et al., 2018). These include excess nutrients, pathogen contamination, and heavy metals, among others, which could impact human health, contaminate soil and groundwater resources, and alter the safety of the built and natural environment (Ajibade et al., 2021a,b; Hanjra et al., 2012). A study conducted in Accra, Ghana, indicated an association between the prior knowledge of the irrigation wastewater source and the awareness of health risks, irrespective of age, education, or gender (Antwi-Agyei et al., 2016). The group of people who are vulnerable to exposure with a high risk of viral infections due to wastewater handling and reuse was classified into three. These are farm workers and their family members, crop handlers and other technical staff, consumers of farm produce, and residents in the nearby areas irrigated with wastewater, especially children, aged people, and immunocompromised individuals (Adegoke et al., 2018). Adelodun and co-workers also attributed socioeconomic inequalities, which include poverty, lack of adequate knowledge on associated waterborne viral diseases, and low standard of living, prompting the use of untreated or partially treated wastewater with rural dwellers, women, and children being the vulnerable population group due to their lack of proper sanitation and high level of exposure to contaminated environmental materials (Adelodun et al., 2021b).

8. Challenges associated with virus inactivation and removal in environmental materials future recommendations

The inactivation of pathogens, viruses in particular, in environmental compartments has become a concern of interest due to the hazard risks of COVID-19. Conventional treatment plants, especially in developing and low-income countries, have the primary treatment processes such as screening, trickling filter, coagulation/flocculation, sedimentation, aeration, and disinfection chamber for the sludge and biosolids (Fig. 4). The effluent, primary and secondary sludges produced in this stage are often reused or discharged to the environment without further treatment in the sludge treatment unit. However, as explained in section 4, the enveloped viruses are often retained in primary and secondary sludge due to their hydrophobic nature. The system processes of wastewater and sludge treatment, therefore, require a revisit to cater for the deactivation and removal of pathogens like SARS-CoV-2 and other emerging pollutants (pharmaceuticals and disinfectants) arising from the current and future pandemics. The SARS-CoV-2 virus and other emerging pollutants are of particular concern since some of the treatment plants were not designed to typically monitor or sufficiently remove them during the wastewater treatment processes, thereby posing great risks to human and environmental health. The basic conventional primary treatment processes, including screens, grit chamber, and primary clarifier that are often deployed in rural settings of developing countries have been reported to lack the capacity to remove or inactivate coronaviruses (Balboa et al., 2021; D'Aoust et al., 2021; Palmer et al., 2021).

Similarly, SARS-CoV-2 RNA is recalcitrant during the treatment process due to incorrect doses of disinfectants despite the widely reported capability of disinfectants to successfully inactivate the novel virus (Abu Ali et al., 2021). The authors found that the SARS-CoV-2 RNA was above 100 copies in the secondary effluents after secondary treatment, including chlorination. Similarly, a high concentration of SARS-CoV-2 RNA up to 18.7 copies/mL was present in septic tanks after disinfection with sodium hypochlorite, suggesting a need for a review of disinfection guidelines of $\geq$0.5 mg/L of free chlorine after at least 30 min contact time by WHO (WHO, 2020; Zhang et al., 2020), especially when a higher concentration of solid materials is present. The extended 90% reduction of SARS-CoV-2 infectivity up to 1.5 days in wastewater (Bivins et al., 2020), which is much longer than the typical hydraulic residence time of many urban pipelines and in wastewater treatment plants could pose a challenge to the virus inactivation (Abu Ali et al., 2021). Meanwhile, while a low dose of chlorine or other disinfectants could be effective to inactivate SARS-CoV-2 in the liquid phase of the environmental samples like wastewater, the presence of solid materials in the sludge that shields the SARS-CoV-2 would require a higher quantity or dose of chorine-based disinfectant for effective inactivation (Zhang et al., 2020). Since the recent studies indicate the ineffectiveness of disinfection to inactivate the SARS-CoV-2 when an appropriate dose or quantity is not used, adsorption and coagulation processes were suggested as alternative methods for SARS-CoV-2 removal (Kumar et al., 2021a).

Sludge digestion or stabilization process by combining both thermal hydrolyses with a moderate temperature range of 35–40 °C and long residence time of 10–20 days that are commonly used in large treatment plants has been reported to effectively inactivate the SARS-CoV-2 load during the wastewater treatment process (Balboa et al., 2021). It was also suggested that heating

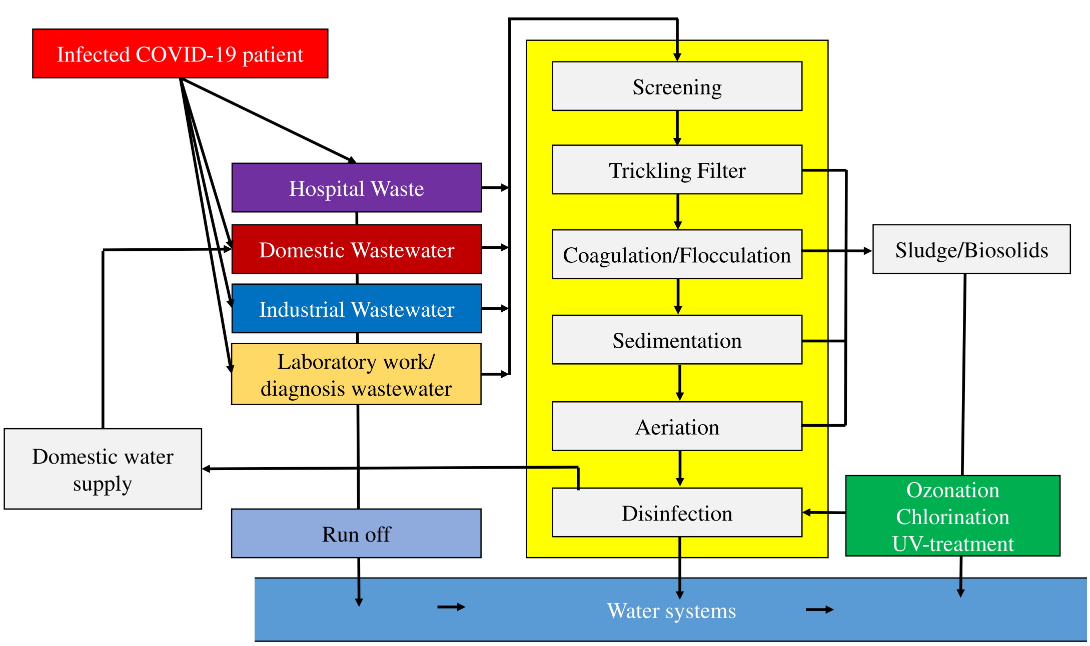

Fig. 4. Schematic of the conventional treatment processes for wastewater and sludge.

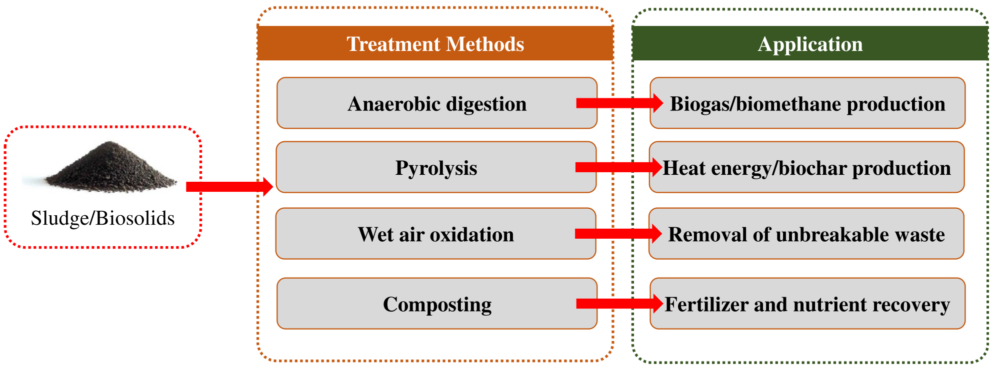

Fig. 5. Treatment methods for sludge and biosolids.

treatments using ultra-temperature aerobic fermentation, thermophilic anaerobic digestion, and sludge incineration could effectively inactivate the SARS-CoV-2 and other forms of coronaviruses (Ji et al., 2020). However, a previous study indicated that mesophilic anaerobic digestion could not effectively inactivate pathogens in the sewage sludge (Viau et al., 2011). Besides, the common treatment plants for sludge only employed heat drying treatment or centrifugation to reduce the water content before disposing of or using for soil amendment (Balboa et al., 2021). Some selected treatment methods for the sludge and biosolids targeting specific applications are, thus, recommended (Fig. 5).

Meanwhile, another issue of concern regarding the SARS-CoV-2 inactivation is the inadequate knowledge on the safe acceptable limit of the virus quantity in the environmental materials. It has been argued that the recommended virus removal of 6 to 7 $\log_{10}$ reduction from wastewater could not be sustained at present due to the evolving knowledge on the viral infections as against the earlier information on the infectivity of the virus population not exceeding 6 $\log_{10}$ per liter in wastewater (Gerba et al., 2017). It is, therefore, necessary to further investigate the safe limit of the virus population in environmental materials by considering the current pandemic. Also, the level at which each treatment process, especially the one that could be easily deployed in the rural settings, could effectively inactivate the virus. The concern on potential ecological risks of an overdose of various disinfectants and other pharmaceuticals that are being used to inactivate and treat the SARS-CoV-2 and consequently mitigate the spread of the COVID-19 has also been raised (Zhang et al., 2020). Some of the disinfection byproducts are known to be highly harmful to the ecosystem, like the algal bloom, and pose serious health challenges such as bladder cancer and miscarriages (Li and Mitch, 2018). It is, therefore, suggested that potential ecological risks should be taken into consideration when developing guidelines to address the challenges of the current and possible future pandemics.

Although some studies reported the absence of SARS-CoV-2 RNA from both wastewater effluent and produced sludge, it is recommended that environmental samples, including wastewater and sludge generated from the hospitals or designated centers for tendering to the COVID-19 patients, should be disposed of following the guidelines of hazardous waste and incineration (Ji et al., 2020; Adelodun et al., 2021a).

9. Conclusion

The studies on the prevalence of SARS-CoV-2 RNA in environmental materials were systematically reviewed, and a meta-analysis was conducted. The prevalence of SARS-CoV-2 RNA in environmental materials followed the order of sludge > biosolids > effluent. In the meta-analysis, only limited available studies on biosolids were investigated based on a fixed-effect model, while the prevalence of the SARS-CoV-2 in effluent and sludge were based on a random-effect model. This is an indication that some treatment plants and treatment processes are lacking in capacity to inactivate and remove the SARS-CoV-2 RNA, while the solid particles in the sludge and biosolids serve as a protecting shield against the degradation of SARS-CoV-2 RNA. The current treatment processes alongside the guidelines on the use of environmental materials like effluent, sludge, and biosolids should be revisited and improved upon to efficiently address the recalcitrant nature of SARS-CoV-2 RNA and other emerging pathogens. Furthermore, adequate protections, including the use of personal protective equipment should be ensured for the wastewater/sludge workers, while the use of reclaimed wastewater and other materials like sludge and biosolids should be done with caution, especially in rural settings of developing and low-income countries where water, sanitation, and hygiene are insufficiently deployed. Similarly, a safe virological standard for the handling and reuse of environmental materials like wastewater for irrigation, and sludge or biosolids for soil amendment with potential SARS-CoV-2 contamination should be established to prevent potential viral outbreaks. Since the genetic material does not imply the infectivity or viability of the virus while health implications of ingesting SARS-CoV-2 RNA are lacking, further studies are highly required to estimate the prevalence of infectious SARS-CoV-2 in environmental materials.

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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Appendix A. Supplementary data

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