Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Occurrence of viruses in sewage sludge: A systematic review

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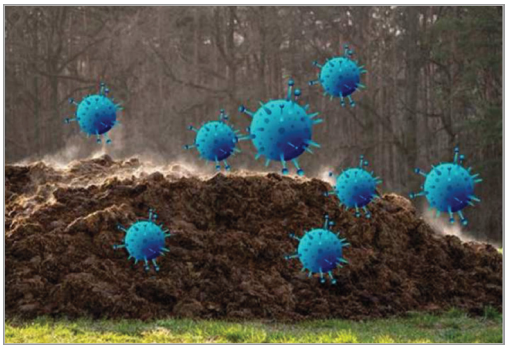
HIGHLIGHTS

- Enteric viruses are highly presented in wastewater and consequently in sludge.
- SARS-CoV-2 removed efficiently by digestion processes from sewage sludge.
- Land application of sewage sludge may pose health risk to exposed individual.

GRAPHICAL ABSTRACT



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ABSTRACT

Enteric viruses are of great importance in wastewater due to their high excretion from infected individuals, low removal in wastewater treatment processes, long-time survival in the environment, and low infectious dose. Among the other viruses, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) surveillance in wastewater systems has received particular attention as a result of the current COVID-19 epidemic. Viruses adhering to solid particles in wastewater treatment processes will end up as sewage sludge, and therefore insufficient sludge treatment may result in viral particles dissemination into the environment. Here, we review data on viruses' presence in sewage sludge, their detection and concentration methods, and information on human health issues associated with sewage sludge land application. We used combinations of the following keywords in the Scopus, Web of Science (WOS), and PubMed databases, which were published between 2010 and January 21th, 2022: sludge (sewage sludge, biosolids, sewage solids, wastewater solids) and virus (enteric virus, viral particles, viral contamination, SARS-CoV-2, coronavirus). The sources were searched twice, once with and then without the common enteric virus names (adenovirus, rotavirus, norovirus, enterovirus, hepatitis A virus). Studies suggest adenovirus and norovirus as the most prevalent enteric viruses in sewage sludge. Indeed, other viruses include rotavirus, hepatitis A virus, and enterovirus were frequently found in sewage sludge samples. Untreated biological sludge and thickened sludge showed more viral contamination level than digested sludge and the lowest prevalence of viruses was reported in lime stabilized sludge. The review reveals that land application of sewage sludge may pose viral infection risks to people due to accidently ingestion of sludge or intake of crops grown in biosolids amended soil. Moreover, contamination of groundwater and/or surface water may occur due to land application of sewage sludge.

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Contents

1. Introduction

People all over the world produce wastewater. In most countries, a high proportion of the wastewater is treated by the activated sludge process (Pepper et al., 2006), resulting in large volumes of sludge or biosolids being produced (millions of tons in each country) (Dubova et al., 2020; Pepper et al., 2006; Wong et al., 2010). Biosolids are defined by the United States Environmental Protection Agency (US EPA) as "the predominantly organic solid product provided by municipal wastewater treatment technologies that can be beneficially recycled" (US EPA, 1995). Sewage sludge is a combination of primary sludge and secondary sludge, produced during the wastewater treatment processes (Deboosere et al., 2012; Pepper et al., 2006). Primary sludge results from the settling of solids as they enter a wastewater treatment plant (Corpuz et al., 2020; Mohapatra et al., 2021; Yin et al., 2018). Secondary sludge results from the conversion of soluble organic matter in the wastewater to bacterial biomass (Bibby and Peccia, 2013a; Pepper et al., 2006; Yin et al., 2018). The two types of sludge are then combined and must either be disposed of or recycled in some manner (Pepper et al., 2006).

In the management of sludge, land application is the most widely used method. Treated sewage sludge, biosolid, is widely used in agricultural and nonagricultural fields as soil amendment since it can enhance the chemical and physical qualities of soil, and provide nutrients that are beneficial for plants growth. Food crops on agricultural lands, such as corn or wheat, and non-food crops like cotton are examples of how land application of biosolids can improve crops growth and yield (Amdiouni et al., 2013; Wong and Xagoraraki, 2012). In addition to farmlands, public parks, golf courses, forests, and cemeteries are examples of non-agricultural land application. It is also possible to use biosolids in order to assist re-vegetation of severely disturbed lands, such as mine tailings or strip mine regions (Pepper et al., 2006). However, biosolids must be treated properly before the land application. Biosolids can be the source of different chemical and biological contaminants such as heavy metals, hydrocarbons, biphenyls, dioxins and pharmaceuticals and pathogenic microorganisms (Assis et al., 2017; Gholipour et al., 2020a; Prado et al., 2014). Biosolids are classified into two categories based on their microbial quality: Class A and Class B (Gerba et al., 2011; Horswell et al., 2010). Class A biosolids result from higher levels of sludge treatment, and no considerable levels of pathogens are found in them. Class B biosolids are the product of a lower level of sludge treatment and are typically contaminated with bacterial, parasite, and viral pathogens (Gerba et al., 2011; Horswell et al., 2010; Pepper et al., 2006; Wong and Xagoraraki, 2012). In developing countries, produced biosolids are mostly placed in the class B (Prado et al., 2014), so the land application of these biosolids may pose health risk to the individuals because of the presence of pathogenic microorganisms. Among the pathogens, enteric viruses are highly presented in wastewater and consequently in sludge. The occurrence of enteric viruses in wastewater is related to the high levels of virus excretion in feces of infected individuals (10^5 to 10^{12} viral particles per gram of feces) (Corpuz et al., 2020). Diseases caused by enteric viruses range from trivial to severe, or even fatal. Several studies have reported the greater health risks of viruses than pathogenic bacteria or protozoa present in wastewater (Farhadkhani et al., 2018; Gonzales-Gustavson et al., 2019; Moazeni et al., 2017). However, health risks associated with viral contamination of land applied biosolids have not been well documented. In addition to enteric viruses, in the recent pandemic, the presence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the feces of infected individuals and consequently in wastewater and sewage sludge has been approved (Bogler et al., 2020; Gholipour et al., 2021a). In contrast to wastewater, there are limited studies about the viruses present in sewage sludge. According to the high accumulation of viruses in biosolids and its wide land application, it seems that a comprehensive review is needed to survey the potential impacts.

In this review, the presence of viruses in sewage sludge/biosolids, methodologies for the extraction and detection of viral particles in sludge samples, and potential viral infection risks associated with the land application of sewage sludge/biosolid are discussed.

2. Materials and methods

2.1. Search strategy

Our literature searches in the Scopus database in the "title, abstract or keywords", in the Web of Science (WOS) in the "all fields" and PubMed published from 2010 to January 21th, 2022, resulted in a total of 1175 articles. According to the search strategy, following keywords were chosen and merged (Boolean operators): sludge, sewage sludge, biosolids, wastewater solids, sewage solids, virus, enteric virus, SARS-CoV-2, coronavirus, viral particle, and viral contamination. Sources search was performed two times, once with common enteric viruses' names (adenovirus, enterovirus, hepatitis A virus, norovirus, and rotavirus) and then without the inclusion of these viruses' names. The Search strategies and inclusion and exclusion criteria are presented in the supplementary material (Appendix A: table S1, S2).

2.2. Screening quality evaluation

The duplicate articles were deleted first, followed by title screening, and then two individuals evaluated the abstracts of all articles retrieved by the keyword search. After full text screening of the article, our study ultimately contained 13 relevant papers. Following that, an initial list of articles was compiled (Fig. 1). A checklist was created to ensure the quality of the included studies and to assess the papers' alignment with the study's

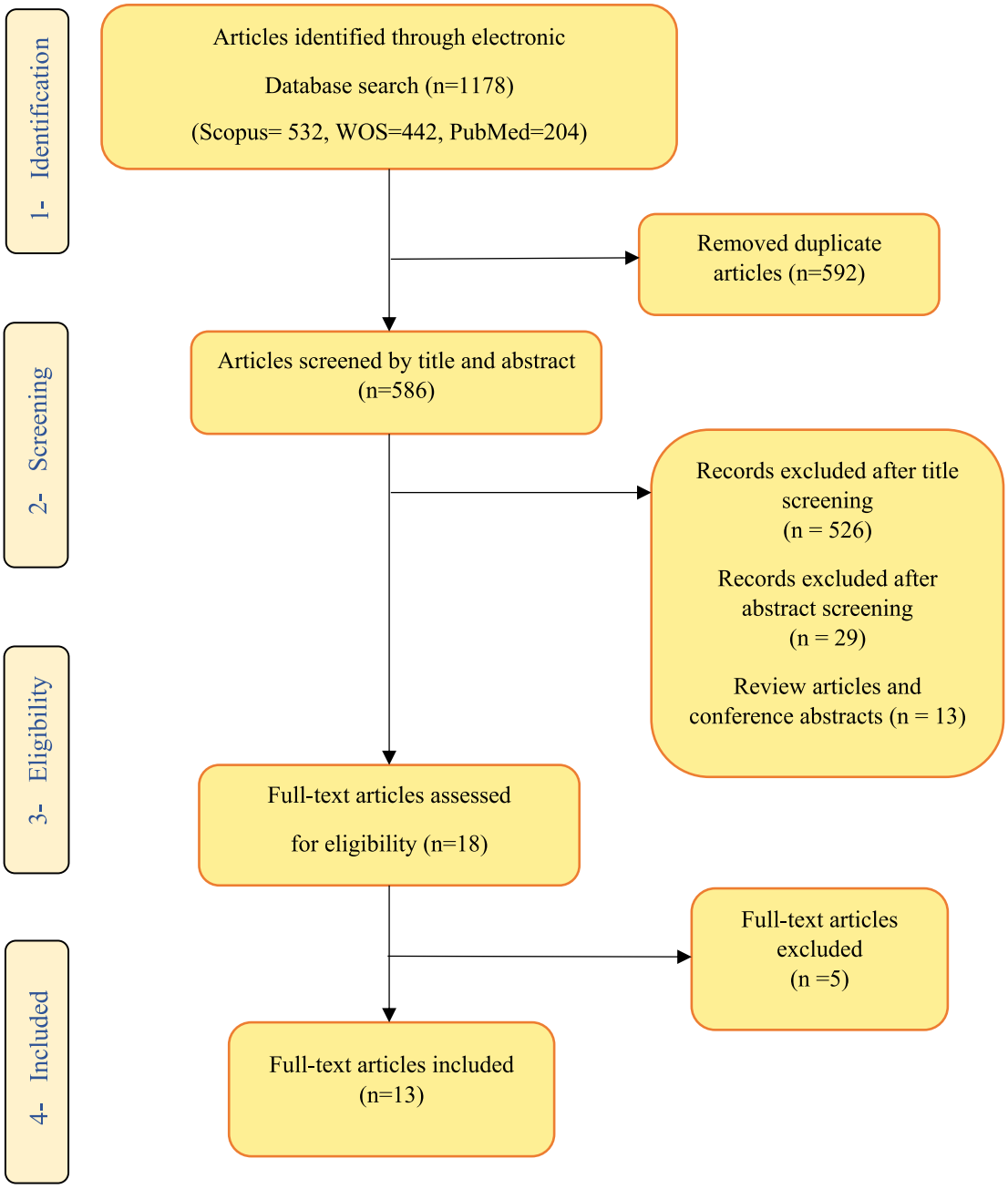

Fig. 1. Flowchart of the database search, selection, and review process of articles.

objectives and questions, as determined by the STROBE checklist (Appendix A: table S3,S4) (Von Elm et al., 2007).

2.3. Data items

The "PICO" strategy for systematic exploratory review was: Population (P) (Virus, Viral particle, Enteric Virus, Adenovirus, Enterovirus, Rotavirus, Norovirus, and Hepatitis A virus), Intervention (I) (Sludge, Sewage sludge, Biosolids, and Digested sludge and Wastewater solids), Comparison (C) (Wastewater Treatment Plant Waste, Activated Sludge), and Outcome (O) (presence of various viral particles in the sludge).

2.4. Data extraction

Two authors (S GH and M R GH) separately extracted data from the included studies and entered them into an MS Excel sheet template. The extracted data was condensed and the following items are presented in this manuscript: The first author's name, the year of the study, country and location, type of sample, number of samples, sampling frequency, detected viruses, virus concentration, frequency of detection and concentration and detection method.

2.5. Data analysis

By following the previous steps, until January 21th, 2022 about 59 published research papers with relevant title, were identified and inspected. Finally, 13 quotable papers were incorporated in the study.

3. Results

As mentioned in the materials and methods section, a total of 585 unique papers were obtained from 3 databases, and after screening based

on the title, abstract, and full text relevance, 13 articles were entered into the study.

Publications in which sewage sludge samples were spiked and artificially contaminated by viral particles include studies investigated the efficiency of virus concentration and extraction methods, the sensitivity and accuracy of detection methods, and virus survival and inactivation rates were excluded from full text articles. Brown et al. (2015) study on viruses' enumeration in sewage sludge samples using the flow cytometry method in which the type of viruses wasn't specified, was also excluded from the remaining articles (Brown et al., 2015).

Extracted data from the literatures is presented in Tables 1 and 2. Articles included in our study were published between 2010 and 2022. In each study, a minimum of two samples have been analyzed from various sludge types. The largest sample sizes (72 samples) related to the Kittigul and

Table 1
Characteristics of included studies.

First author (year)	Country/ location	Type of sample	No. of samples	Investigated viruses (type of virus)	Viral particle extraction method	Detection method	Refrence
Schlindwein et al. (2010)	Brazil/ Florianopolis	Activated sludge	12	Adenovirus, Hepatitis A, Rotavirus, Poliovirus	Elution with beef extract/Adsorption-precipitation $AlCl_3$ & PEG 6000 precipitation	PCR/ICC-PCR/IFA	(Schlindwein et al., 2010)
Wong et al. (2010)	USA/Michigan	Dewatered sludge, mesophilic anaerobically digested	15	Adenovirus, Enterovirus, Norovirus Human polyomavirus, Hepatitis A virus	Elution with beef extract /flocculation	PCR/ICC-PCR	(Wong et al., 2010)
Jebri et al. (2012)	Tunisia/Tunis	Activated sludge	48	Enteroviruses and Hepatitis A virus	Adsorption-precipitation $AlCl_3$ & PEG 6000 precipitation	PCR	(Jebri et al., 2012)
Bibby (2013)	USA	Anaerobically digested sludge	10	Herpesvirus, Papillomavirus, Bocavirus, Parvovirus, Kelassevirus, Coronavirus, Astrovirus, Parechovirus, Saporvirus, Hepatitis C, HIV, Cosavirus, Aichivirus, Rhinovirus, T-lymph virus, Coxackievirus, Rubella virus, Adenovirus, Rotavirus, Enteroviruses and Hepatitis A virus	Elution with glycine/PEG 8000 precipitation	PCR/ Metagenomic	(Bibby and Peccia, 2013a)
Prado et al. (2014)	Brazil/ Rio de Janeiro	Primary sludge	12	Adenovirus, Hepatitis A, Rotavirus, Norovirus	Elution with beef extract/ Ultracentrifugation	PCR	(Prado et al., 2014)
Rhodes et al. (2015)	California, Colorado, Missouri, Texas, Maryland, North Carolina, Ohio, and Utah	Aerobic and anaerobically digested sludge	34	Adenovirus	Flocculating Bacto beef extract	PCR	(Rhodes et al., 2015)
Assis et al. (2017)	Brazil/Rio de Janeiro & Juiz de Fora	Activated sludge	10	Adenovirus	Elution with glycine /Skimmed-milk flocculation	PCR	(Assis et al., 2017)
		Thickened sludge	2				
		Digested Sludge	2				
Kittigul et al. (2019)	Thailand/Bangkok	Lime stabilized sludge	23	Noroviruses	Adsorption-elution with glycine & arginine/ vacuum centrifuge	PCR	(Kittigul et al., 2019)
D'Aoust et al. (2021)	Canada	Post grit sludge	5	SARS-CoV-2	Filtration and PEG concentration	PCR	(D'Aoust et al., 2021)
		Primary clarified sludge	6				
Kittigul (2021)	Thailand/ Bangkok	Lime stabilized sludge	72	Rotavirus	Adsorption-elution with glycine & arginine/ vacuum centrifuge	PCR	(Kittigul and Pombubpa, 2021)
Balboa et al. (2021)	Spain/ Ourense	Primary sludge	5	SARS-CoV-2	Elution with glycine and beef extract/ PEG 8000 precipitation	PCR	(Balboa et al., 2021)
		Biological sludge	10				
		Thickened sludge	10				
		Digested sludge	10				
Serra-Compte et al. (2021)	Spain & France	Non-treated sludge [a]	56	SARS-CoV-2	Elution with glycine and beef extract/ PEG 8000 precipitation	PCR	(Serra-Compte et al., 2021)
		Treated sludge [b]	51				
Pourakbar et al. (2022)	Iran/ East Azerbaijan	Primary sludge	4	SARS-CoV-2	Elution with glycine and beef extract/ PEG 8000 precipitation	PCR	(Pourakbar et al., 2022)
		Activated sludge	4				
		Anaerobically digested sludge	8				

[a] Primary and secondary sludge.
[b] Sludge thickening, anaerobic digestion and anaerobic digestion plus thermal hydrolysis.

Table 2
Detection frequency of viruses in different types of sewage sludge.

First author (year)	Sample type	Frequency (%)							Ref
		CoV[a]	AdV[b]	EnV[c]	NoV[d]	RoV[e]	HeV[f]	PoV[g]	
Schlindwein et al. (2010)[h]	Activated sludge	–	100	–	–	25	16.7	91.7	(Schlindwein et al., 2010)
Wong et al. (2010)[h]	Dewatered sludge	–	100	100	67	–	0	–	(Wong et al., 2010)
	Mesophilic anaerobically digested	–	83	42	50		0	–	
Jebri et al. (2012)	Activated sludge	–	–	7.7	–	–	0	–	(Jebri et al., 2012)
Bibby (2013)[h]	Anaerobically digested sludge	–	100	70	80	83	–	–	(Bibby and Peccia, 2013a)
Prado et al. (2014)	Primary sludge	–	91	–	50	41	0	–	(Prado et al., 2014)
Rhodes et al. (2015)	Aerobic and anaerobically digested sludge	–	100	–	–	–	–	–	(Rhodes et al., 2015)
Assis et al. (2017)	Activated sludge	–	100	–	–	–	–	–	(Assis et al., 2017)
	Thickened sludge	–	100	–	–	–	–	–	
	Digested sludge	–	100	–	–	–	–	–	
Kittigul et al. (2019)	Lime stabilized sludge	–	–	–	73.9	–	–	–	(Kittigul et al., 2019)
D'Aoust et al. (2021)	Post grit sludge	79	–	–	–	–	–	–	(D'Aoust et al., 2021)
	Primary clarified sludge	90	–	–	–	–	–	–	
Kittigul (2021)	Lime stabilized sludge	–	–	–	–	50	–	–	(Kittigul and Pombubpa, 2021)
Balboa et al. (2021)	Primary sludge	80	–	–	–	–	–	–	(Balboa et al., 2021)
	Biological sludge	10	–	–	–	–	–	–	
	Thickened sludge	90	–	–	–	–	–	–	
	Digested sludge	0	–	–	–	–	–	–	
Serra-Compte et al. (2021)	Primary sludge	83.3	–	–	–	–	–	–	(Serra-Compte et al., 2021)
	Secondary sludge	57.1	–	–	–	–	–	–	
	Thickened sludge	69.2	–	–	–	–	–	–	
	Digested sludge	71.4	–	–	–	–	–	–	
	Digested plus thermal hydrolysis sludge	0	–	–	–	–	–	–	
Pourakbar et al. (2022)	Primary sludge	50	–	–	–	–	–	–	(Pourakbar et al., 2022)
	Activated sludge	75	–	–	–	–	–	–	
	Anaerobically digested sludge	0	–	–	–	–	–	–	

[a] SARS-CoV-2.
[b] Adenovirus.
[c] Enterovirus.
[d] Norovirus.
[e] Rotavirus.
[f] Hepatitis A virus.
[g] Poliovirus.
[h] qPCR results are reported.

Pombubpa (2019) study conducted on lime stabilized sludge from a wastewater treatment plant in Bangkok, Thailand (Kittigul and Pombubpa, 2021) and Serra-Compte et al. (2021) study in which 107 sludge samples were taken from 16 wastewater treatment plants in Spain and France (Serra-Compte et al., 2021). Studies have been carried out in different parts of the world on different sewage sludge sample types, including primary sludge, digested and non-digested sludge, dewatered sludge, thickened sludge and lime stabilized sludge. Adenovirus was the most frequently detected enteric virus in sewage sludge samples, followed by rotavirus and norovirus. The presence of SARS-CoV-2, the causative agent of COVID-19 pandemic, was (has been) investigated in four studies (Balboa et al., 2021; D'Aoust et al., 2021; Pourakbar et al., 2022; Serra-Compte et al., 2021). The adsorption-elution method and polyethylene glycol (PEG) precipitation were the most frequently used methods for viral particles extraction in sewage sludge. In 10 of the 13 included studies, polymerase chain reaction (PCR) has been used to identify viruses in sludge samples. However, in two studies, integrated cell culture along with PCR (ICC-PCR) has been used (Schlindwein et al., 2010; Wong et al., 2010), and in the Bibby et al. (2013) study, PCR of selected viruses was used to validate metagenome annotation results.

4. Discussion

This paper highlights the available data on the presence of viruses in sewage sludge. The systematic review process used to find and extract relevant literature benefits from a carefully read selection process that takes into account critical research features such as study relevance and quality (Eftim et al., 2017). Additionally, the review process is quick, reproducible, and adaptable to integrate new data as they become available.

Because sewage sludge contains concentrated human waste from thousands to millions of people, it has a high pathogen diversity potential (Bibby and Peccia, 2013a, 2013b). Raw wastewater and secondary (or biological solids) contain particle-associated viruses, and sewage sludge is the final destination of these viruses (Bibby and Peccia, 2013a).

Land application of sewage sludge/biosolids is becoming more popular around the world since it has the advantage of abating of environmental contamination and providing an extra supply of nutrients to agroecosystems (Sidhu and Toze, 2009). However, there is a growing concern about whether land application of sewage sludge would pose a risk of accidental ingestion by workers, consumption of contaminated agricultural crops, and contamination of groundwater and/or surface water (Horswell et al., 2010).

4.1. Enteric viruses

Because of aggregation and adhesion characteristics of viruses to solid particles, it is believed that the stability and inactivation of enteric viruses in sewage sludge differ from that in wastewater (Bofill-Mas et al., 2006; Sidhu and Toze, 2009). The enteric viruses detected in sewage sludge samples can be categorized into two groups: enteroviruses (poliovirus, coxsackievirus and echovirus) and a heterogeneous group which include rotavirus, human caliciviruses, astroviruses, adenovirus and hepatitis A and E viruses. Human adenoviruses are generally found in all sludge types (Sidhu and Toze, 2009). In all studies conducted on adenoviruses presence in sewage sludge samples, adenovirus was successfully detected in different sludge types with a high prevalence (Assis et al., 2017; Bibby and Peccia, 2013a; Jebri et al., 2012; Prado et al., 2014; Rhodes et al., 2015; Schlindwein et al., 2010; Wong et al., 2010). After a review of existing data on the adenoviruses numbers in sewage sludge, we observed that adenoviruses numbers vary from 2.5×10^3 genomic copy.g^{-1} dry weight in digested sludge (Rhodes et al., 2015) to average around 10^5 genomic copy.ml^{-1} in activated sludge samples (Assis et al., 2017; Schlindwein

et al., 2010). The reported numbers of adenovirus in primary sludge were 10^4 to 10^5 genomic copy.L^{-1} (Prado et al., 2014). In untreated wastewater, human adenoviruses are frequently present in high numbers (10^3 to 10^8 infectious units/L) (Rames et al., 2016). Due to its high resistance and abundance in environmental samples, as well as detection of the infectious virus in chlorinated drinking water and wastewater, adenovirus is considered an emerging pathogen (Hewitt et al., 2013). Adenoviruses because of their non-enveloped structure and double-stranded linear DNA are more resistant to disinfection processes and unfavorable environmental conditions than other viruses, especially RNA viruses (Gholipour et al., 2021b). Adenovirus persistence in environmental samples is associated with a long survival time (132 days in wastewater and > 301 days in viral-contaminated sterilized surface water) (Wong and Xagoraraki, 2012).

Adenovirus has been studied in 6 articles out of 13 included articles. Certain adenoviruses appear to be largely respiratory pathogens, while others appear to be exclusively gastrointestinal pathogens. Adenoviruses, particularly those of type 40/41, are frequent causative agents of diarrhea in infants. Enteric adenoviruses produce devastating infections in immunocompromised patients, with case mortality of up to 50% (Pepper et al., 2006). Identification of adenovirus serotypes would enable more refined evaluation of risks associated with land application of sewage sludge. The adenoviral infection risk associated with the land application of sewage sludge could be discussed from two points of view: gastrointestinal and respiratory infections. Contamination of sewage sludge with a respiratory pathogen, transmitted via inhalation, such as adenovirus 2, may cause respiratory tract infection, while enteric pathogens such as adenoviruses 40/41 may pose gastrointestinal tract risk via ingestion (Rames et al., 2016).

Human noroviruses are the most common cause of acute gastroenteritis, causing significant morbidity and mortality all over the world (Pepper et al., 2006). In one of the studies that was conducted on this topic, norovirus was detected in 74% of lime stabilized sludge samples (Kittigul et al., 2019). In Bibby et al. (2013) study norovirus was detected in anaerobically digested sludge, indicates the low efficiency of thermal treatment on this virus removal from wastewater solids (Bibby and Peccia, 2013a). Norovirus and adenovirus have previously been found in top soil improver samples, including sewage sludge (Tozzoli et al., 2017).

Norovirus, which belongs to the Caliciviridae family, is divided into seven genogroups. Humans generally carry the genogroups GI, GII, and GIV, which can be further classified into more than 40 genotypes (Kittigul et al., 2019). Norovirus genotype GII.4 outbreaks are common in healthcare facilities, whereas water and food-related outbreaks are associated with the GI and non-GII.4 (Kittigul et al., 2019). Norovirus-related waterborne epidemics have been documented and linked to the drinking tap water (Giammanco et al., 2018), contaminated water well (Qin et al., 2016), and wastewater infiltration into the water distribution network (Moreira and Bondelind, 2017).

During rotavirus-associated diarrhea, substantial amounts of viral particles are excreted in the feces. The virus is mainly transmitted by the fecal–oral pathway (Crawford et al., 2017), which in susceptible hosts, only a few virus particles are required to cause disease (Ward et al., 1986). Rotavirus presence in different sludge types has been investigated in 4 studies, with a prevalence of 25% to 83% (Bibby and Peccia, 2013a; Kittigul and Pombubpa, 2021; Prado et al., 2014; Schlindwein et al., 2010). Rotaviruses are widespread, infecting nearly every child in the world by the age of 3–5 years (Gurwith et al., 1981). In 2003, 114 million cases of rotavirus infection in children under the age of five years old were reported worldwide, which 24 and 2.3 million requiring (required) outpatient visits and hospitalization, respectively (Parashar et al., 2003).

Enteroviruses were detected in dewatered sludge (100%) (Wong et al., 2010) activated sludge (7.7%) (Jebri et al., 2012) and digested sludge samples (42% and 70%) (Bibby and Peccia, 2013a; Wong et al., 2010). In Jebri et al. (2012) study, low concentration of enterovirus was associated with low efficiency of its detection method (about 1000 pfu. 100 ml^{-1}).

In study of Schlindwein et al. (2010), hepatitis A virus and poliovirus were detected in 16.7% and 91.7% of activated sludge samples, respectively. They reported that the high prevalence of poliovirus in sewage sludge samples, can be probably originated from vaccine-derived PV strains in Brazil (Schlindwein et al., 2010). All sludge samples in studies of Prado et al. (2014), Jebri et al. (2012) and Wong et al. (2010) were negative for hepatitis A virus (Jebri et al., 2012; Prado et al., 2014; Wong et al., 2010).

Comparison between virus concentrations among different sewage sludge types was not possible because of the difference in reported units. However, a high prevalence of enteric viruses has been reported in, which could be a concern in terms of land application.

4.2. SARS-CoV-2

In our literature review, four studies were found to detect SARS-CoV-2 RNA in sewage sludge samples (Balboa et al., 2021; D'Aoust et al., 2021; Pourakbar et al., 2022; Serra-Compte et al., 2021). D'Aoust et al. (2021) study in Canadian low COVID-19 incidence communities showed that 79% of post grit solids and 90% of primary clarified sludge were positive for the presence of SARS-CoV-2. It indicates primary cleared sludge as a superior solids-rich sample for the detection of SARS-CoV-2 signal during decreasing and low virus load in communities, compared to post grit solids (D'Aoust et al., 2021). Balboa et al. (2021) investigated the presence of SARS-CoV-2 in different sludge types in a wastewater treatment plant located in north-western Spain from 6 to 21-April 2020. Sludge samples were included 5 primary sludge, 10 biological sludge, 10 thickened sludge and 10 anaerobically digested sludge. As is shown in Table 2, SARS-CoV-2 RNA were present in the most of the primary and thickened sludge samples, whereas none of the digested sludge samples were positive. In this wastewater treatment plant, the primary settler and the sludge thickeners act as "concentrators" of SARS-CoV-2 RNA. No viral RNA was found in the digested sludge, which is probably related to the high temperature faced during the anaerobic digestion (Balboa et al., 2021).

Pourakbar et al. (2022) also investigated the fate of SARS-CoV-2 in two wastewater treatment plants in Iran. They observed that the virus has a significant propensity for accumulating in biosolids rather than moving via aqueous phase. However, after studying the fate of virus in sludge, they reported that SARS-CoV-2 is completely destroyed during anaerobic digestion (Pourakbar et al., 2022). This study proves that high retention times and anaerobic processes can effectively break down viral RNA. Despite to the results of Balboa et al. (2021) and Pourakbar et al. (2022), in a study conducted in 16 wastewater treatment plants in Spain and France, SARS-CoV-2 was appeared with high frequency in thickened and anaerobically digested sludge and the only sludge samples without viral RNA were those that had been digested and thermally hydrolyzed (Serra-Compte et al., 2021). However, none of the studies investigated the stability and infectivity of the detected SARS-CoV-2 in sludge samples. Studies have investigated the persistence of enveloped viruses in the environment are rather limited because of enveloped viruses are inactivated faster than non-enveloped viruses in the environment (Gholipour et al., 2020b; Kitajima et al., 2020; Lahrich et al., 2021). However, enveloped viruses can still survive and be infectious for long periods of time depending on the environmental conditions (Lahrich et al., 2021; Rahimi et al., 2020). It is because of surface S-proteins that are deeply anchored and pass through the envelope, so infectivity is maintained even if the envelope is disrupted but the surface S-proteins are intact (Lahrich et al., 2021). Studying the inactivation rate or survival of the SARS-CoV-2 in wastewater treatment plants will assist to improve the sludge treatment requirements and control measures for land application, but to the best of our knowledge, the viability of SARS-CoV-2 in wastewater treatment plants has not been well documented. However, studies have been conducted on other coronaviruses could provide a basic understanding of SARS-CoV-2 persistence. It has been reported that while SARS-CoV-1 persisted for 3 days in water matrices at 20 °C, it was vulnerable to chlorination (Wang et al., 2005). Using a plaque assay or median culture infectious dose (TCID50) technique, Gundy et al. (2009) determined human coronavirus 229E survival in water and wastewater. Thermal condition, biological activity and level of organic matter were effective factors on the coronavirus survival. They

reported that coronavirus was inactivated faster at higher temperatures and lower suspended solids content (Gundy et al., 2009). Therefore, for the estimation of health risks associated with disposal or land application of sludge, further studies are needed to determine the persistence of SARS-CoV-2 in sewage sludge using techniques that determine the infectivity of the virus, such as cell culture assay.

4.3. Concentration and detection methods

Identifying viruses present in sludge is complicated because the land application of sludge and its return to the soil may pose health risks. Some viruses have a low infectious dose. For example, the number of norovirus particles needed to infect a person is estimated at 10–100 (Hamza et al., 2011; Lindesmith et al., 2003). So, even a low number of viruses may pose risks to public health. Developing sensitive methods for monitoring viruses in sewage sludge will help to prevent or reduce potential health risks. Study of viruses in sludge is a complicated procedure that can be divided into two major steps: viral particles extraction from samples and virus detection.

Viral particles should be extracted from environmental samples prior to performing the detection methods. In contrast to virus extraction from aquatic samples, in which viral particles are only concentrated, virus extraction from sludge samples requires an elution step. This is because of the heterogeneity of sludge matrices, the adsorption of viral particles to sludge flocs, and the presence of many different chemical components that are problematic for virus detection methods (Hamza et al., 2011; Yang et al., 2021). A range of various procedures has been used for extraction of viral particles from environmental samples which could be related to the cost of extraction methods, availability of reagents and instruments, and scalability.

The methods used to detect viruses in environmental samples are of two major types: cell culture infectivity and molecular methods (Metcalf et al., 1995; Monpoeho et al., 2001).

Environmental samples, particularly sewage sludge, contain a variety of organic and inorganic compounds (proteins, humic acids, polyphenols, and metal ions) that cause cell cultures to lyse (Monpoeho et al., 2001; Schrader et al., 2012). Additionally, all these compounds are capable of forming complexes with DNA/RNA and inhibiting enzymes responsible for amplification of nucleic acids in molecular methods such as PCR (Monpoeho et al., 2001; Schrader et al., 2012). The accuracy of results of detection methods for viruses therefore depend on the efficiency of inhibitors removal from sludge samples in elution processes and consequently re-concentration of viruses from the eluants in the extraction procedures.

As is shown in Table 1, virus extraction in the included studies was performed by elution of sludge samples with glycine buffer and beef extract buffer followed by different virus concentration processes.

Viral particles was mainly concentrated by PEG (Balboa et al., 2021; Bibby and Peccia, 2013a; D'Aoust et al., 2021; Pourakbar et al., 2022). In two of included studies, samples which precipitated with $AlCl_3$ was mor concentrated by using the PEG (Jebri et al., 2012; Schlindwein et al., 2010). Assis et al. (2017) used skimmed-milk flocculation (Assis et al., 2017) and ultracentrifugation and vacuum centrifugation were also used in some studies (Kittigul et al., 2019; Kittigul and Pombubpa, 2021; Prado et al., 2014). Although, in the included studies the comparison of recovery efficiency of different concentration methods was not possible, there are reports about the high performance of ultracentrifugation for virus concentration (Fumian et al., 2010; Yang et al., 2021). However, ultracentrifugation requires equipment that is not typically available in many laboratories. Some studies reported that PEG precipitation method was more efficient than chemical flocculation methods in viral particles recovery from different environmental samples (Barril et al., 2021; Gholipour et al., 2021a).

As has been mentioned before, cell culture and molecular methods are the main detection methods used in environmental virology. In most of the included studies, viruses have been identified using PCR (Assis et al., 2017; Balboa et al., 2021; Bibby and Peccia, 2013a; D'Aoust et al., 2021; Jebri et al., 2012; Kittigul et al., 2019; Kittigul and Pombubpa, 2021; Pourakbar et al., 2022; Prado et al., 2014; Rhodes et al., 2015), the results of which may be overestimated. In PCR, nucleic acid fragments are targeted by specific primers and extended by the polymerase enzyme. So, PCR is not able to distinguish infectious viruses from non-infectious ones (viable vs non-viable) (He and Jiang, 2005). On the other hand, cell culture, which is known as the gold standard for examining the infectivity of isolated viruses, frequently underestimates the level of viruses. However, viability and infectivity of some viruses in sludge is largely unknown (Sidhu and Toze, 2009). Norovirus can barely be detected by cell culture methods, because the majority of norovirus strains are non-cytopathogenic or develop slowly in cell culture (Ko et al., 2005). Although in none of the studies, direct cell culture was used for detecting viruses in sludge samples, two studies used Integrated Cell Culture followed by PCR (ICC-PCR) to survey the infectivity of isolated viruses. ICC-PCR is a technique that combines cell culture and PCR to detect infectious viruses that grow slowly or do not induce cytopathic effect.

Wong et al. (2010) used quantitative PCR (qPCR) and ICC-PCR to detect adenovirus and enterovirus in sludge samples in which the viruses' detection frequencies were higher using qPCR than with ICC-PCR (A549 cells and BGM cells) (Wong et al., 2010). Schlindwein et al. (2010) quantified the presence of adenoviruses in sludge samples by qPCR and viable ones by ICC-PCR and indirect immunofluorescence assay (IFA). All adenovirus positive samples by PCR were infectious using IFA and ICC-PCR (Schlindwein et al., 2010). In the IFA assay, viral antigen detection is performed by fluorescent staining of viral particles with the virus specific antibodies (Hamza et al., 2011). Techniques like ICC-PCR and IFA shorten the time it takes to detect infectious viruses and overcome to sludge inhibitor components in direct PCR. These approaches, however, may be expensive and do not address the challenge of assessing viral infectivity for viruses such as human noroviruses, that do not infect cell cultures.

5. Conclusions and outlook

The present study is one of the first reviews that reports the global evidence on the occurrence of viruses in sewage sludge. Enteric viruses are more heat resistant than bacteria and their infectious dose is low. So, on-site (workers and children while playing) and off-site (residents and consumers of contaminated crops) exposure to viral particles associated with the land application of sludge/biosolid may pose health risks to individuals. Moreover, ground and surface water contamination related to land application of sewage sludge must be taken into consideration. Viral contamination of sewage sludge emphasizes the importance of application of treatment processes which could effectively reduce viral pathogens in wastewater.

However, limited data on the anthropogenic and environmental factors controlling the occurrence and circulation of viruses in sludge and amended soils are available. Given the significant environmental and economic benefits of land application of sludge/biosolid, further quantitative studies are required to determine the fate and viability of viruses in sewage sludge/biosolid amended soil under a variety of environmental conditions, as well as the associated health risks. It seems, the occurrence of SARS-CoV-2 in treated sludge is low, and may not pose health risks to farmers and consumers, but preventive measures must be taken for wastewater treatment plants workers during treatment processes, to reduce the infection risk.

CRediT authorship contribution statement

Sahar Gholipour: Writing - Original draft preparation, Conceptualization, Writing - Reviewing and Editing.

Mahnaz Nikaeen: Supervision, Writing - Reviewing and Editing.

Mohammad Rezvani Ghalhari & Parichehr Pakzad: Methodology, Investigation, Reviewing and Editing.

Mohammad Bagher Miranzadeh: Writing - Original draft preparation, Supervision.

Davarkhah Rabbani: Writing - Original draft preparation.

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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