

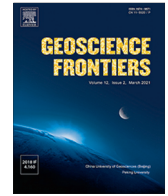
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Research Paper

Physiological characteristics, geochemical properties and hydrological variables influencing pathogen migration in subsurface system: What we know or not?

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ABSTRACT

The global outbreak of coronavirus infectious disease-2019 (COVID-19) draws attentions in the transport and spread of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in aerosols, wastewater, surface water and solid wastes. As pathogens eventually enter the subsurface system, e.g., soils in the vadose zone and groundwater in the aquifers, they might survive for a prolonged period of time owing to the uniqueness of subsurface environment. In addition, pathogens can transport in groundwater and contaminate surrounding drinking water sources, possessing long-term and concealed risks to human society. This work critically reviews the influential factors of pathogen migration, unravelling the impacts of pathogenic characteristics, vadose zone physiochemical properties and hydrological variables on the migration of typical pathogens in subsurface system. An assessment algorithm and two rating/weighting schemes are proposed to evaluate the migration abilities and risks of pathogens in subsurface environment. As there is still no evidence about the presence and distribution of SARS-CoV-2 in the vadose zones and aquifers, this study also discusses the migration potential and behavior of SARS-CoV-2 viruses in subsurface environment, offering prospective clues and suggestions for its potential risks in drinking water and effective prevention and control from hydrogeological points of view.

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1. Introduction

Since the beginning of the 21st century, there are increasing emerging diseases caused by various pathogens, including swine-origin pandemic 2009 A influenza (H1N1), Ebola and Nipah (Murphy, 1998; Morse et al., 2012). The recent outbreak of coronavirus infectious disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and becomes a public health emergency of international concerns causing over 240 million cases and nearly 5 million deaths by 18th October 2021 in 195 countries. As most pathogens can be released by patients into the surrounding environment and survive for a prolonged period of time, increasing concerns have been raised

for their potential transport and spread via environmental media. For instance, noroviruses causing acute gastroenteritis mainly infect humans by contaminated foods (Cheng et al., 2005; Widdowson et al., 2005) and are frequently detected in water environment after an outbreak (Haramoto et al., 2005; Rutjes et al., 2005). SARS-CoV-2 transmits mainly through aerosols, fomites (Sosnowski, 2021) and respiratory droplets (Kampf et al., 2020). Additionally, there is increasing evidence showing the potential spillover of pathogens from natural hosts in their wildlife habitats to human societies across the ecological barriers (Zhang et al., 2021a), or from dedicated hospitals to surrounding public communities via aerosols (Zhang et al., 2021b) or wastewater (Randazzo et al., 2020; Zhang et al., 2020). Thus, it is of urgency to study the migration of pathogens, especially those causing severe pandemics like Nipah and SARS-CoV-2, in natural environment for effective prevention and control of emerging infectious diseases.

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Groundwater is widely used for drinking water supply around the world because of freshwater scarcity (Murphy et al., 2017; Panda et al., 2020). Approximately 2.2 billion people globally rely on groundwater for daily use (Murphy et al., 2017), and over 70% of drinking water supplies in California's San Joaquin Valley are groundwater (Dieter and Maupin, 2017). Due to the intensive irrigation and improper sewage treatment, pathogens can migrate into subsurface environment and are frequently detected in aquifers, causing serious pollution and imposing risks for drinking water safety (Flury and Aramrak, 2017; Haveman, 2002; Makowska et al., 2021). Contaminated groundwater will be ingested as drinking water, during recreation or via irrigated crops (Oliveira et al., 2016). Pathogens in groundwater are estimated to cause 35.2–59.4 million cases of acute gastrointestinal illness and over 2 million deaths worldwide annually (Pandey et al., 2014). Waterborne enteric pathogens are responsible for diarrhea which is the second leading diseases causing infant deaths worldwide (Wardlaw et al., 2010). Over 21 thousand cases of water-borne diseases occurred in USA during 1999–2019, including vector-borne diseases and water-borne toxins (Rhoden et al., 2021), and contaminated groundwater accounts for about 64% of the drinking water outbreaks between 1989 and 2002 (Fong et al., 2007). The norovirus outbreak in Wisconsin in June 2007 is caused by fecal pathogens in contaminated aquifers (Borchardt et al., 2011). In Delhi, about 28% of the household were affected by water-borne diseases (Bidhuri et al., 2018). In Norway, the proportion of water-borne outbreaks related to groundwater reflects the proportion of groundwater works from 1984 to 2007, and the proportion of disease cases caused by groundwater corresponds to the proportion of persons supplied by groundwater (Kvitsand and Fiksdal, 2010). Therefore, pathogens in groundwater are key sources of infectious diseases and threaten the public health, and it is necessary to study the existence and migration of pathogens in subsurface environment.

Extensive studies have reported the occurrence of pathogens in groundwater, including bacteria and viruses (Hynds et al., 2014). In Jiangnan Plain of China, 193 potentially pathogenic bacterial species are identified in groundwater, and opportunistic pathogenic *Acinetobacter baumannii*-like and enteric pathogenic *Vibrio cholerae*-like bacteria are present in 99% and 32% of groundwater samples, respectively (Wu et al., 2019). Frequently detected pathogenic viruses in groundwater include enterovirus, hepatitis virus, norovirus, adenovirus and rotavirus, and they are identified in 20%–30% of 550 wells in the United States and urban aquifers in the United Kingdom (Foster and Chilton, 2004). From 1992 to 2013, one or more viruses are detected in 15% of 2273 groundwater samples from 746 public drinking water systems in Canada (Fout et al., 2017), and human enterovirus (polyomavirus, adenovirus A, and GI norovirus) are detected in 10 of 22 wells (Allen et al., 2017). In Korea, 35.3%–48.3% of 71 groundwater sites are positive for waterborne norovirus (Lee et al., 2011). Particularly, the living pathogenic viruses are even detected in aquifers up to 40 m at depth in the UK (Cronin et al., 2003).

Besides the distribution and composition of pathogens in groundwater, many studies also focus on their migration and transport in subsurface environment. Pathogen migration from above-ground is believed as the main source of groundwater pathogenic contamination (Alegbeleye and Sant'Ana, 2020), and the vertical transport is mainly accompanied with the rainfall and wastewater infiltration by agricultural irrigation and improper sewage discharge (Bellou et al., 2015; Syngouna et al., 2017; Alegbeleye and Sant'Ana, 2020). The abundance of *Escherichia coli* in groundwater increases by one to three orders of magnitude after rainfall in karst terrain (Buckerfield et al., 2019, 2020). The agricultural irrigation and pit latrine are the main sources of underground pathogens in developing countries (Liu et al., 2021a), and norovirus

is detected in 1% (7 of 773) of irrigation waters in South Korea (Shin et al., 2019). In addition, pathogens are abundant in feces or urine of humans or animals (Hellmér et al., 2014; Gerba et al., 2017; Ong et al., 2020; Sun et al., 2020), and the urban sewage or animal wastes are the main routes of pathogenic transmission into the environment (Fumian et al., 2010). Globally, about 100 billion tons of animal wastes are produced annually as soil conditioners or crop fertilizers (Pennington, 2010), and many pathogens might be released into agricultural fields. During the COVID-19, studies have confirmed the presence of SARS-CoV-2 in untreated wastewater (Ahmed et al., 2020; La Rosa et al., 2020; Randazzo et al., 2020; Sherchan et al., 2020; Zhang et al., 2021c). Considering the potential transmission from the urban sewage to humans and animals via natural environment (Rizzo et al., 2013; Fouz et al., 2020) and leakage into the underground environment directly in rural area (Rashid and Pandit, 2017; Weststrate et al., 2019; Buckerfield et al., 2020; Li et al., 2021), it is important to study the mechanisms of pathogen migration and transport in the vadose zone.

Pathogenic transport in subsurface environment depends on many geochemical variables (e.g., grain size, porosity, heterogeneity, moisture content, pH, ionic strength, temperature) and hydrodynamics (e.g., flow rate and direction) (Jin and Flury, 2002; Bradford et al., 2013; Qin et al., 2020; Zhang et al., 2021e). The activities of some pathogens decreases with temperature (Balboa et al., 2021; Gundy et al., 2009; Pinon and Vialette, 2018), and pathogen migration is benefited by a higher pH value (Torkzaban et al., 2006). In the vadose zone, the migration of pathogens is closely related to the grain size and moisture content (Anders and Chrysikopoulos, 2006; Bai et al., 2016), whereas in heterogeneous aquifer media, it depends mainly on the flow rate and flow direction (Luo et al., 2020). In the presence of preferential migration pathway in vadose zone or aquifer, pathogens can even migrate faster (Corapcioglu et al., 2006). In addition, pathogen migration is also strongly associated with pathogenic physiological characteristics like size, morphology, and hydrophobicity (Ghanem et al., 2016; Pang et al., 2021). Pathogens with smaller size, higher hydrophilic and spherical shapes are more favored for migration (Weiss et al., 1995; Schinner et al., 2010; Whitehead and McCue, 2010; Farkas et al., 2015; Schijven and Hassanizadeh, 2000; Pang et al., 2021). Nevertheless, most studies only focus on and compare limited pathogenic species or influential factors, and there is still lack of a comprehensive and complete understanding on the influential factors and risks of pathogen migration in subsurface environment.

This work critically reviews the influential factors of pathogen migration in subsurface environment, attempting to discuss the impacts of pathogenic physiological characteristics and geochemical/hydrological variables on the transport and spread of typical pathogens in the vadose and saturated zones. More importantly, we propose a database of pathogenic physiological characteristics and an assessment algorithm to evaluate the migration ability and risks of pathogens in subsurface environment. Our findings can predict the potential behavior of SARS-CoV-2 in subsurface environment, provide prospective clues for its potential risks in groundwater and drinking water, and offer suggestions for effective prevention and control of emerging infectious diseases from hydrogeological points of view.

2. Pathogen physiological characteristics

With the recent development of virology and genetics, the unique morphological characteristics and genetic diversity of many pathogens have been widely uncovered and linked to their behavior in environment (Dion et al., 2020; Zimmerman et al.,

2020). Generally, pathogen size, morphological features, surface charge, surface hydrophobicity and membrane structure are the most investigated and accepted physiological characteristics affecting pathogen migration in subsurface environment.

2.1. Pathogen size

Pathogen size is a critical factor for their transport in porous media. As particles with smaller size pass filters such as soils and sediments more easily, pathogenic biocolloids with larger size migrate slower by colliding with and adhering to porous medium (Schijven and Hassanizadeh, 2000; Pelley and Tufenkji, 2008; Aronino et al., 2009; Schinner et al., 2010). For bacteria which in general have larger sizes (0.5–3 μm) than viruses (20–90 nm) (Walshe et al., 2010), they are more readily to be retained in porous media by straining. Viruses therefore normally migrate faster in porous media, generally two to three times than pathogenic bacteria (Robertson and Edberg, 1997). Sinton et al. found a significantly different transport and attenuation behavior between bacterial *Escherichia coli* (1.0–1.5 μm in diameter and 3.0–5.0 μm in length) and viral F-RNA bacteriophage (26 nm in diameter, the same as follows) (Sinton et al., 2010). The transport of viruses MS-2 and PRD-1 in porous media had a faster peak emergence than bacteria *Salmonella* and *Klebsiella*, although *Salmonella* and *Klebsiella* survive for a longer time (dropped by 1–6 log units in 32 d, whereas viruses below the detection limit within 10 d) (Dowd and Pillai, 1997). For bacterial pathogens of similar size like *Escherichia coli* (1.11 μm), *Klebsiella* sp. (1.56 μm) and *Rhodococcus rhodochrous* (2.31 μm), their transport is more dependent on grain size (Bai et al., 2016). Although smaller colloids with size <1 μm may be easier to deposit on medium surfaces for their greater Brownian diffusion, most studies document that viruses with smaller size migrate faster. Particularly, 60 nm is reported as the critical diameter of viral transportation, below which viral migration is in a considerably longer distance (Cao et al., 2010; Chrysikopoulos et al., 2010; Walshe et al., 2010) and predominantly affected by viral size rather than other factors (Dowd et al., 1998). For instance, coliphage MS2 (27–29 nm) are preferably to migrate (55%–79% of infiltration rate) than human adenoviruses (70–90 nm, 1%–31% of infiltration rate) (Wong et al., 2014; Kokkinos et al., 2015).

2.2. Morphological features

The morphological features of pathogens are another key influential factor on their migration (Ghanem et al., 2016). Bacterial morphological shapes include filamentous, helical, rod-shaped, ellipsoid and ovoid (Balkwill and Ghiorse, 1985). As rod-shaped colloids (width-length ratio = 1:6) are reported to have a slower migration rate than spherical ones (Ma et al., 2020), the elongated cells are more likely to adhere to particles than spherical ones (Feldner et al., 1983; Van Loosdrecht et al., 1987) and the length-width ratio is inversely proportional to bacterial migration rate (Salerno et al., 2006; Jiang and Bai, 2018). For instance, round bacterial cells have stronger migration ability comparing to elongated ones and >90% of the infiltrated bacteria from porous media have a width-length ratio >0.6 in a laboratory study (Weiss et al., 1995). Comparing to *Escherichia coli*, *Sphingopyxis alaskensis*, *Vibrio cholerae*, *Legionella pneumophila* and *Brevundimonas diminuta*, the slender spirillum-shaped *Hylemonella gracilis* showed a superior ability to pass through sterile membrane filters (Wang et al., 2008). Due to the increase of surface heterogeneity and hydrophobicity during the stretching process, the migration ability of rod-shaped bacteria is weaker than that of spherical ones (Seymour et al., 2013). Flagellum structure is reported to inhibit bacterial migration ability in porous media by promoting their motility and attachment efficiencies (Haznedaroglu et al., 2010).

Gram-negative bacteria with flagella are more difficult to migrate than non-movable ones (McClaine and Ford, 2002). In addition, flagella can promote the formation of biofilms and suppress bacterial migration (Abu-Lail and Camesano, 2003; Tong et al., 2010a; Du et al., 2020). Accordingly, in groundwater environment, common pathogenic bacteria with rod-shape and flagella-free are easy to migrate, e.g., *Streptococcus faecalis* and *Staphylococcus aureus*, whereas bacilli (*Escherichia coli*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, *Mycobacterium leprosy*, *Bacillus cholerae*, *Mycobacterium typhoid*, etc.) are opposite (Nola et al., 2010).

For viruses, their main morphological shapes are categorized into spherical (e.g., *phiX174*, PM2, PRD1, *phi6*, MS2), filamentous (e.g., M13), polymorphic (e.g., MVL2) and tailed (e.g., T4, T7, AG3) (Dion et al., 2020). Similar to bacteria, spherical viruses are easier to migrate than polymorphic ones because of the directional effect that spherical viruses have a higher retention in porous media (Liu et al., 2010). Rod-like tailed phage reduces the migration by change the surface charge distribution during the transportation in porous media (Aronino et al., 2009). Spherical viruses are much easier to transport than non-spherical ones. For example, diffusivity of Q β ($16.6 \times 10^{12} \text{ m}^2/\text{s}$) is higher than P22 ($3.8 \times 10^{12} \text{ m}^2/\text{s}$) (Baltus et al., 2017). Tailless viruses are reported to migrate more easily than tailed ones, and the migration abilities of viruses with different tail structure rank as the order of icosahedral Leviviridae [tailless, $K_d = (2.6 \pm 1.2) \times 10^{-2}/\text{h}$] > Podoviridae [nonconstrictile short tail, $K_d = (1.8 \pm 1.7) \times 10^{-2}/\text{h}$] > Siphoviridae [nonconstrictile long tail, $K_d = (1.6 \pm 0.6) \times 10^{-2}/\text{h}$] (Ghanem et al., 2016). For instance, only 26% of tailed T4 viruses pass through the porous media comparing to tailless phage like *phiX174* (48%) and MS2 (45%) (Aronino et al., 2009). In addition, viruses with spike proteins on surfaces, coronaviruses SARS-CoV-1 and SARS-CoV-2 as examples, have less mobility as they have higher surface roughness and can attach via receptor-specific interactions (Shen and Bradford, 2021; Shen et al., 2014). As common pathogenic viruses in groundwater (enterovirus, hepatitis virus, norovirus, rotavirus, and adenovirus) are spherical in shape, they are more likely to migrate in subsurface environment. Particularly for SARS-CoV-2 which are enveloped, spherical and tailless (Kumar et al., 2020), they are hypothetically to have a strong migration capability in porous media and pose significant risks.

2.3. Surface charge

Surface charge, mostly represented by zeta potential or isoelectric point, is also a key factor influencing pathogen migration by affecting the adsorption of pathogens in porous media (Pang et al., 2009). Normally, sand grains, clay minerals and bacterial pathogens like *Escherichia coli* O157:H7, *Yersinia enterocolitica* and *Enterococcus faecalis* are negatively charged in most pH range, and the electrostatic repulsion therefore inhibits pathogenic attachment and promotes their migration (Jacobs et al., 2007; Schinner et al., 2010). For instance, *Escherichia coli* with a lower surface charge (–44.7 mV, sticking efficiency = 0.02) are easier to migrate than those with higher surface charge (–22.1 mV, sticking efficiency = 0.4) (Lutterodt et al., 2009).

Surface charge density of pathogens can also influence their interactions with porous media and affect the migration behaviour (Baygents et al., 1998). A thinner double layer charge on the cell surface can increase isoelectric point and reduce the repulsive force between biocolloids and medium particles, benefiting pathogenic attachment on porous media and suppressing their migration (Zhang et al., 2018). For most RNA viruses, surface charge distribution is uneven (Jin and Flury, 2002). For example, caudophages have negatively charged head/tail tubes and positively charged filament (Penrod et al., 1996). Such uneven surface charge significantly affects the isoelectric point of tailed caudophages and

mitigates their transport in groundwater, further explaining their weaker migration ability than spherical viruses.

2.4. Surface hydrophobicity

Pathogenic surface hydrophobicity is a crucial factor in pathogen migration process that hydrophobic pathogens normally have weaker migration abilities comparing with hydrophilic ones (Lutterodt et al., 2009; Liu et al., 2020). Short-term irreversible adsorption occurs between pathogen and medium surface through hydrophobic interaction (Gordesli and Abu-Lail, 2012; Zhao et al., 2014). Close to the medium surface, the hydrophobic interaction is detrimental to pathogen migration by eliminating the water between the interacting surfaces (Rodrigues et al., 2006; Zeraik and Nitschke, 2010), and thus pathogens with a weaker hydrophobicity migrate faster (Zhong et al., 2015). As most pathogenic outer membrane proteins are hydrophilic, pathogens with a higher content of outer membrane proteins have a stronger hydrophilicity and migration capability (Nikaido, 2003; Liu et al., 2020), whereas the hydrophobic functional groups on pathogenic surface could inhibit their migration (Alizadeh-Pasdar and Li-Chan, 2000). As the majority of subsurface media are hydrophilic (Cheng et al., 2012), we mainly consider their positive impacts on hydrophobic pathogen migration. For instance, with the decreasing aquifer saturation from 100% to 40%, the migration rates of hydrophobic pathogens decrease more significantly from 82.5% to 32.5% than hydrophilic pathogens (from 93% to 43.5%) (Gargiulo et al., 2008).

Generally, unenveloped and enveloped viruses are hydrophilic and hydrophobic (lipophilic), respectively (Feng et al., 2019). Owing to the dominance of both hydrophobic effects and electrostatic force amidst sorbent surface and capsid protein, enveloped viruses, such as mouse hepatitis virus and *Pseudomonas* phage ϕ 6, exhibit high adsorption potential on porous media with poorer migration ability (Ye et al., 2016). RNA-phages are more easily attached to media surface with their increasing hydrophobicity (GA 2300 ng/cm² > Q β > MS2 2000 ng/cm²) (Dika et al., 2013). Thus, Phage GA migrates slower in aquifer than phage MS2 (Armanious et al., 2016).

2.5. Membrane structure and composition

Cell surface macromolecules including extracellular polymeric substances (EPS), outer membrane proteins and lipopolysaccharides affect the adsorption of pathogens and their migration behavior in porous media by altering cell surface charge and hydrophobicity (Tufenkji, 2007). Bacteria can be categorized as Gram-positive and Gram-negative ones according to the structure and composition of cell wall. Gram-positive bacteria have a large amount of peptidoglycan (about 40%–90% of dry cell weight) and some teichoic acids, exhibiting more negative charge and higher migration capability. In contrast, Gram-negative bacteria contain

11%–22% lipid, thin peptidoglycan layer (about 5% to 20% of the dry cell weight) and no teichoic acid on cell walls (Kang et al., 2006), commonly behaving relatively smaller migration ability. For example, *Staphylococcus aureus* showed a stronger migration ability (76.5% of retention) than *Escherichia coli* (99% of retention) (Weidhaas et al., 2014).

Bacterial EPS are reported to improve the adsorption efficiency (Liu et al., 2007; Tsuneda et al., 2003). The breakthrough concentration of *Escherichia coli* mutants with EPS production (55%) is less than the mutants not producing EPS (80%), indicating that EPS can promote microbial migration by inhibit the attachment (Tong et al., 2010b). In other studies, the outer membrane protein AG43 also promotes bacterial attachment and inhibits their migration by encouraging biofilm formation (Henderson, 1997; Klemm et al., 2003; Lutterodt et al., 2009). Lipopolysaccharides on cell surface can decelerate bacterial migration by promoting their adsorption on media (Abu-Lail and Camesano, 2003; Walker et al., 2004).

Different from pathogenic bacteria, viruses have unique structure and are categorized into enveloped, unenveloped (Blanco et al., 2019) and vesicle ones (Zhang et al., 2021d). Previous studies mainly focus on the migration of non-enveloped viruses. Enveloped viruses have similar lipid bilayer membrane structure like bacteria, except for viral glycoproteins which are more sensitive to environmental changes (Wolfe et al., 2017). As viral envelope is hydrophobic and more prone to adsorb on organic particles or solid surfaces, their migration rates are relatively low (Gundy et al., 2009; Paul et al., 2021).

2.6. Evaluation of pathogen migration capacity by physiological properties

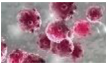
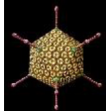
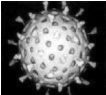
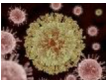
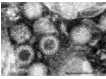
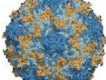
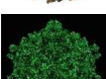

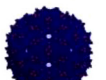
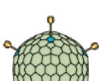

Pathogen size, morphological features, surface charge, surface hydrophobicity and membrane structure/composition are the four key influential variables affecting pathogen migration abilities in subsurface environment. Nevertheless, most previous studies only focus on limited factors or pathogens, and there is lack of assessment algorithm to evaluate the migration ability of a variety of pathogens in subsurface environment (Dika et al., 2015; Blanco et al., 2019; Paul et al., 2021). From the effects of physiological properties on pathogen migration given in the literature, pathogen size is the most important variable, followed by surface charge represented by isoelectric point, surface hydrophobicity and membrane structure/composition represented by contact angle, and morphological features of width-length ratio and flagella (Haznedaroglu et al., 2010; Ghanem et al., 2016; Pang et al., 2021). Based on the importance of each factor reported by previous literatures, we propose an assessment algorithm considering the five indices to quantitatively evaluate the migration ability of pathogens, including pathogen size (40%), isoelectric point (20%), contact angle (20%), width-length ratio (15%) and flagella (5%) (Table 1). It is worth noting that the importance of these factors

Table 1
Criteria for estimating the migration ability of pathogens.

| Weighted term | Parameter | Weight | Classification and scores | | | | |
|------------------------|------------------------|--------|---------------------------|------------------|------------------|------------------|-----------------|
| Pathogen size | Size (μm) | 40% | <0.06 | 0.06–0.30 | 0.30–1.00 | 1.00–3.00 | >3.00 |
| | | | 1.0 | 0.8 | 0.6 | 0.4 | 0.2 |
| Surface charge | Isoelectric point | 20% | <4.0 | 4.0–5.0 | 5.0–6.0 | >6.0 | – |
| | | | 1.0 | 0.7 | 0.4 | 0.1 | – |
| Surface hydrophobicity | Contact angle | 20% | <25° | 25°–35° | 35°–45° | 45°–65° | >65° |
| | | | 1.0 | 0.9 | 0.7 | 0.5 | 0.1 |
| Morphological features | Width-length ratio | 15% | >0.8 | 0.8–0.6 | 0.6–0.4 | 0.4–0.2 | <0.2 |
| | | | 1.0 | 0.8 | 0.6 | 0.4 | 0.2 |
| Membrane structure | Flagella | 5% | No | Yes | | | |
| | | | 1.0 | 0.5 | | | |

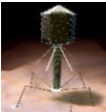






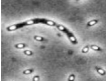
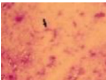



The bold fonts indicate criteria for classification, and normal fonts indicate scores.

Table 2
Estimated migration abilities of different pathogens based on their size, morphological features, surface charge, surface hydrophobicity and membrane structure¹.

| Pathogens | Morphology ² | Flagella | Size (nm ²) | pH _{pzc} | Contact angle ³ | Reference | Estimated migration ability | | | |
|-----------------------|-------------------------|---|-------------------------|-------------------|----------------------------|---------------|--|---|----------------------|------|
| Non-enveloped viruses | Norovirus |  | Spherical (1) | No | 38 | 5.0 | 42° | (Redman et al., 1997; Collins et al., 2006) | 0.88 | |
| | Adenovirus |  | Spherical (1) | No | 70–90 | 7.2 | 68° | (Flint, 2004; Baker et al., 2021; Dang and Tarabara, 2021) | 0.56 | |
| | Rotavirus |  | Spherical (1) | No | 80 | 3.9 | - | (Collins et al., 2006; Jimenez-Zaragoza et al., 2018) | 0.86 | |
| | Rhinovirus |  | Spherical (1) | No | 28–30 | 6.4 | - | (Flint et al., 2004) | (Flint et al., 2004) | 0.72 |
| | Reovirus |  | Spherical (1) | No | 70–80 | 3.9 | - | | 0.82 | |
| | Poliovirus |  | Spherical (1) | No | 28–30 | 3.8–8.2 (6.0) | PRD1 > MS2(33°) ≈ Echovirus > Coxsackievirus > Poliovirus > T2 ≈ T4 > ΦX174(26°) | (Schijven and Hassanizadeh, 2000; Flint et al., 2004; Collins et al., 2006) | 0.86 | |
| | Echovirus |  | Spherical (1) | No | 28–30 | 5.1–6.4 (5.8) | | (Flint et al., 2004) | 0.86 | |
| | Coxsackievirus |  | Spherical (1) | No | 28–30 | 4.8–6.1 (5.4) | - | (Flint et al., 2004) | 0.86 | |
| Phage viruses | MS2 |  | Spherical (1) | No | 26–27 | 3.9 | | (Shields and Farrah, 1983; Penrod et al., 1996; Schijven and Hassanizadeh, 2000; Gitis et al., 2002; Pang et al., 2009; Chrysikopoulos and Syngouna, 2012a) | 0.98 | |
| | PRD-1 |  | Spherical (1) | No | 62 | 3–4.2 | | (Schijven and Hassanizadeh, 2000; Abudalo et al., 2005; Collins et al., 2006;) | 0.86 | |
| | Φx174 |  | Spherical (1) | No | 25–27 | 6.0 | | (Schijven and Hassanizadeh, 2000; Collins et al., 2006; Zhang et al., 2010; Chrysikopoulos and Syngouna, 2012b) | 0.86 | |

(continued on next page)

Table 2 (continued)

| Pathogens | Morphology ² | Flagella | Size (nm ²) | pH _{PZC} | Contact angle ³ | Reference | Estimated migration ability | |
|---|---|---------------------------|-------------------------|--------------------------|----------------------------|-------------------------------------|--|------|
| T2 phage |  | Tailed (0.524) | No | Head 109, tail 99 | 4.2 | (Deblois and Wesley, 1977) | 0.72 | |
| T4 phage |  | Tailed (0.479) | No | Head 84–100, tail 100 | 4–5 | (Carrascosa and Kellenberger, 1978) | 0.72 | |
| Phage λ |  | Tailed (0.265) | No | Head 54, tail 150 | 3.9–5.0 | – | (Penrod et al., 1996) | 0.61 |
| <i>E. coli</i> phage |  | Tailed (0.331) | No | Head 53, tail 107 | 4.37 | 66.8° | (Yu, 2016; Qin, 2020) | 0.53 |
| Pathogenic viruses (Enveloped virus) |  | Spherical (1) | No | 60–140 | 5.9 | >60° | (Pandey, 2020; Zhu et al., 2020) | 0.70 |
| Bacteria | | | | | | | | |
| <i>Enterococcus faecalis</i> |  | Spherical or chain (1) | No | 1290–1380 | 4.6 | 19° | (Fernando et al., 1991; Millsap et al., 1994; Signoretto et al., 2000; Lu Ting, 2012) | 0.70 |
| <i>Streptococcus</i> |  | Spherical or chain (1) | No | 600–1000 | 5.3 | 21° | (Millsap et al., 1994; Kamezawa et al., 1997) | 0.72 |
| <i>Bacillus subtilis</i> |  | Oval to cylindrical (0.3) | Yes | (700–800) × (2000–3000) | 3.5 | 32° | (Ahimou et al., 2001; Ren et al., 2018; Wu, 2021; Rong, 2008) | 0.63 |
| <i>Clostridium perfringens</i> |  | Fusiform (0.657) | Yes | (300–2000) × (1500–2000) | 4.0 | – | (Ando and Tsuzuki, 1984) | 0.61 |
| <i>Staphylococcus aureus</i> |  | Spherical (1) | No | 800 | 4.0 | 19.5° | (Rawlinson et al., 2011; Zmantar et al., 2011) | 0.84 |
| <i>Escherichia coli</i> |  | Rod (0.35) | Yes | 2000 × 700 | 2.0–4.3 | 22° | (Gilbert et al., 1991; Lytle et al., 1999; Oh et al., 2007; Pang et al., 2009; Wu, 2021) | 0.65 |
| <i>Pseudomonas putida</i> |  | Rod (0.231) | Yes | (500–1000) × (1500–5000) | 3.5 | 24° | (Abu-Lail and Camesano, 2003; Rong, 2008) | 0.57 |

Notes:

¹Detailed calculation and estimated data for each physiological character are listed in Supplementary data Table S1.

²The number in brackets is the width-length ratio of pathogenic in the column of morphology.

³“–” indicates not retrieved from the literature.

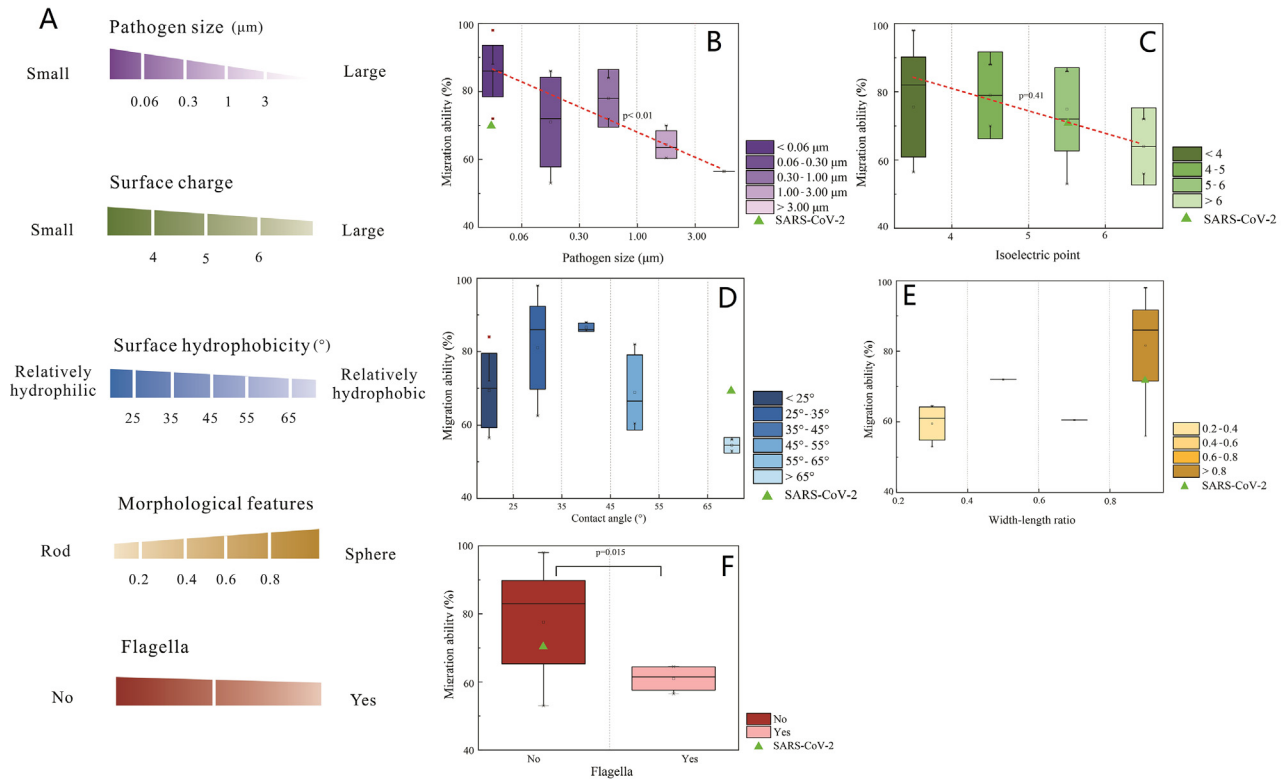


Fig. 1. (A) Impacts of pathogenic physiological characteristics on the estimated migration abilities of pathogens. (B) The estimated migration ability vs pathogen size. (C) The estimated migration ability vs surface charge (isoelectric point). (D) The estimated migration ability vs surface hydrophobicity (contact angle). (E) The estimated migration ability vs morphological features (width-length ratio). (F) The estimated migration ability vs membrane structure (flagella).

depends on the geochemical and hydrological conditions in subsurface system, and this assessment algorithm is only suitable for most scenarios.

For the classification of pathogen size, viruses (0.023–0.08 μm) generally have stronger migration ability than bacteria (0.5–3 μm) (Walshe et al., 2010), and a significant stronger migration ability is observed for viruses with size <0.06 μm (Cao et al., 2010; Chrysikopoulos et al., 2010; Walshe et al., 2010). Accordingly, the size classification includes <0.06, 0.06–0.30, 0.30–1.00, 1.00–3.00 and >3.00 μm. Isoelectric point has been widely studied in migration of many pathogens, and the four classification groups are set as <4.0, 4.0–5.0, 5.0–6.0 and >6.0 based on the normal isoelectric point range of pathogens. The contact angle is directly proportional to the hydrophobicity and inversely proportional to migration ability. As the hydrophobic interaction between surfaces is effective at contact angle >65° and some published contact angles of pathogens are as follows: PRD1 > MS2 (33° ≈ Echovirus > Coxsackie virus > Poliovirus > T2 ≈ T4 > ΦX174 ≈ 26°) (Dika et al., 2015; Shields, 1986), the classification of contact angle is <25°, 25°–35°, 35°–45°, 45°–65° and >65°. The width-length ratio representing pathogenic morphological characteristics increases from 0.0 (rod-shaped) to 1.0 (spherical-shaped), calculated as the ratio of head width to total length of tail phage and classified into five categories of >0.8, 0.8–0.6, 0.6–0.4, 0.4–0.2 and <0.2.

Based on the assessment algorithm and classification, the migration abilities of many well-known pathogens are calculated and listed in Table 2. For instance, *Escherichia coli* has a low score of 0.65, indicating its relatively poor migration ability, owing to the large size (about 2000 nm × 700 nm) and non-spherical shape (rod-shaped) (Pang et al., 2009). In contrast, *Staphylococcus aureus* has a higher score of 0.84, indicating a stronger migration ability, owing to the smaller size (about 800 nm) and spherical shape

(Rawlinson et al., 2011). The estimated average migration abilities of Gram-positive bacteria (0.7, including *Enterococcus faecalis*, *Streptococcus*, *Bacillus subtilis*, *Clostridium perfringens* and *Staphylococcus aureus*) are stronger than those of Gram-negative bacteria (0.61, including *Escherichia coli*, *Pseudomonas putida*). For viruses, *Norovirus* has a small size (about 0.035 μm), non-membrane and spherical shape (Collins et al., 2006), resulting in a relatively higher score of 0.88. Generally, most viruses except for some tailed phages have a higher score (0.86–0.98) than bacterial pathogens, suggesting higher migration abilities of viruses in subsurface environment, consistent with some previous laboratory studies (Bradford et al., 2006a; da Silva et al., 2011).

From the calculated migration abilities in Table 2, the correlations between pathogen migration and individual physiological characteristics are illustrated in Fig. 1. From the general trend in Fig. 1A, pathogenic migration abilities dramatically decrease with pathogen size, but only slightly decrease with surface charge, surface hydrophobicity and morphological structure. Regression curve in Fig. 1B also evidences that pathogen size is inversely proportional to migration ability. As for surface charge (Fig. 1C), surface hydrophobicity (Fig. 1D) and morphological structure (Fig. 1E), the correlations are not significant and suggest that pathogen size is the most key factor influencing pathogen migration in subsurface environment. It might be explained by limited experimental data till now on these physiological parameters, and only pathogen size is investigated in most previous studies. It is worth highlighting that, although the weight of flagella is only 5% in the assessment algorithm, pathogens with and without flagella exhibit significant difference (Fig. 1F), hinting that flagellum is also a key factor affecting pathogen behavior in aquifers.

As an enveloped (Ke et al., 2020), spherical and pleomorphic virus (Zhu et al., 2020), SARS-CoV-2 virus has a size of

60–140 nm. SARS-CoV-2 consists of four major structural proteins: spike protein (S), membrane protein (M), nucleocapsid protein (N) and envelope protein (E) (Sarkar and Saha, 2020). Among them, spike protein promotes host attachment and viral cell membrane fusion for infection (Wu et al., 2020), and adheres to other materials (Pandey, 2020). Although the interfacial dynamics of SARS-CoV-2 pseudoviruses were investigated (Liu et al., 2021b), its migration ability is still not clear. Here, we speculate that the adhesion between SARS-CoV-2 and porous media is mainly attributing to spike protein. Although the isoelectric point and hydrophobicity of SARS-CoV-2 remain unclear (Scheller et al., 2020), it can be estimated by the ProtParam tool of ExPASy (Gasteiger et al., 2003) or of the same value of 5.90 as spike protein (Pandey, 2020). In addition, as both spike protein and capsule lipid bilayer are hydrophobic (Pandey, 2020), SARS-CoV-2 is highly possible hydrophobic. Based on this information, the migration ability of SARS-CoV-2 is scored as 0.70 in the proposed assessment algorithm, indicating that SARS-CoV-2 has a relatively strong migration ability in subsurface environment.

3. Environmental geochemical and hydrological variables

Although pathogen migration behavior is intrinsically determined by their physiological characteristics, their migration process in subsurface environment is also influenced by geochemical variables of porous media and hydrological variables in groundwater. Considering the key variables that associate with pathogenic physiological characteristics, those environmental geochemical and hydrological variables include soil structure, groundwater chemical composition, pH, ionic strength, temperature, moisture, and hydrodynamic condition.

3.1. Soil texture

Soil texture determines soil pore channels which directly affects pathogen migration abilities in subsurface environment, and the key factors include grain size, surface roughness and heterogeneity (Sasidharan et al., 2017a). Pathogen migration in porous media follows filtration mechanisms, e.g., strain and deposition. Bigger grain size increases pathogen migration (Bai et al., 2016; Mohanty and Boehm, 2014), evidenced by their faster migration in quartz sands with a larger size (0.71 mm) than smaller one (0.15 mm) (Bradford et al., 2006a). For grain size <50 μm , pathogen migration is suppressed by straining (Torkzaban et al., 2015).

Surface roughness is proportional to the specific surface area of porous media. Higher surface roughness and specific surface area increases the active attachment sites to capture pathogens and inhibits their migration (Morales et al., 2009; Krishna Darbha et al., 2012; Torkzaban and Bradford, 2016). For instance, crude biochar has rougher surface with the specific surface area 5 orders of magnitude larger than sand, resulting in a significantly stronger adsorption of *Escherichia coli* through biochar (log removal of 2.32) than sand (log removal of 0.29) (Mohanty et al., 2014). In addition, the adsorption of *Escherichia coli* are equal to or higher on rough beads than smooth beads in a simulated groundwater migration system (Shellenberger and Logan, 2002). Moreover, nanoscale surface roughness can alter the interaction energies by decreasing the primary minimum depth and decrease pathogen attachment on medium surface (Shen et al., 2018; Rasmuson et al., 2019), although secondary energy minimum and wells might be increased under some unfavorable chemical conditions, e.g., low ionic strength (Shen et al., 2012). Taking these two mechanisms together, surface roughness can promote pathogen deposition and inhibit their migration.

The heterogeneity of porous media can affect pathogen migration by altering the collision efficiencies and deposition capacities,

because of the higher attachment at secondary minima at concave locations or detachment from primary minima at nanoscale convex asperities (Shen et al., 2020). Fine-textured and poor-structured soils intercept bacteria through mechanical filtration for their small pore sizes and low hydraulic conductivity (Morales et al., 2015). Accordingly, an increasing virus migration is observed with the standard deviations of hydraulic conductivity in heterogeneous aquifers in a migration model (Torkzaban et al., 2019).

3.2. Groundwater chemical composition

Groundwater chemical composition determines pathogen migration through hydrophobic and hydrophilic interactions in groundwater, thus linked to pathogen surface hydrophobicity. A wide variety of inorganic and organic materials exist as colloids in groundwater, including mineral precipitates like iron and manganese oxides, rock and mineral fragments and macromolecular components of natural organic matters (McCarthy and McKay, 2004; Foppen et al., 2008; Walshe et al., 2010), exhibiting strong interactions and changing pathogenic migration behavior in groundwater (Foppen et al., 2008; Yang et al., 2012b; Cai et al., 2013). The presence of phosphate can alter bacterial EPS and increase the repulsion between pathogens and media. For instance, phosphate is reported to promote the migration of *Escherichia coli* O157:H7 in quartz sand (Wang et al., 2011). Another study finds that silicate could increase bacterial surface charge and weaken the electrostatic repulsion between bacteria and media, thereby reducing the migration ability of *Escherichia coli* (Dong et al., 2014). Colloid-facilitated transport is an important mechanism of pathogen migration, and it is believed that colloids could increase the migration velocity and distances of pathogens (Babich and Stotzky, 1980; Jin et al., 2000). Nevertheless, organic and inorganic colloids exhibit entirely different transport mechanisms (Katzourakis and Chrysikopoulos, 2014; Bellou et al., 2015; Syngouna and Chrysikopoulos, 2016). Organic colloids would form stable aggregates with pathogens via interparticle interaction and expedite their migration owing to the rough surface with functional groups and long-chain macromolecular structure (Akbour et al., 2002). In contrast, the inorganic colloids are prone to agglomerate causing blockage and inhibiting pathogen migration (Qin et al., 2020). In porous media, the migration of *Escherichia coli* phage vB_EcoM-ep3 with silica colloids (deposition rate of $7.23 \times 10^{-2} \text{ s}^{-1}$) is faster than with fusiform humic acid colloids (deposition rate of $5.14 \times 10^{-3} \text{ s}^{-1}$) (Qin, 2020). Another study reports 27.73% increase of viral migration rate in the presence of humic acid colloids (Yu, 2016).

Natural dissolved organic matters also influence the deposition and migration behavior of pathogens (Foppen et al., 2008; Yang et al., 2012a; Zhao et al., 2014). As they can be adsorbed on hydrophobic organic compounds and increase solubility (Foppen et al., 2008), natural dissolved organic matters might also interact with pathogens with high hydrophobicity and accelerate their migration. For instance, the penetration rate of *E. coli* increases about 20% in the presence dissolved humic acids (Foppen et al., 2008). In addition, dissolved organic matters can also inhibit microbial attachment on porous media via competitive adsorption by occupying the active sites on medium and increasing the steric hindrance of deposition, encouraging pathogen transportation (Foppen et al., 2006). Some evidence can be found from previous studies that bacterial migration is accelerated with the declined adsorption of hematite onto cell surfaces in the presence of humic acids (Foppen et al., 2008; Yang et al., 2016). Mineral-associated organic matters are reported to significantly promote the migration of phage MS-2 in phosphate-buffered saline (Zhuang and Jin, 2003a). Generally, pathogens migrate faster in organic-rich aquifers which require extensive attentions.

3.3. pH

pH together with pathogenic isoelectronic point determines pathogen migration in subsurface environment by changing the adsorption–desorption and diffusion process. Pathogen adsorption on porous media is enhanced at lower pH, which decreases their migration rates (Zhang et al., 2018). At higher pH value, pathogenic surface is more negatively charged and is harder to be adsorbed by aquifer media. Some experimental evidence shows that MS2 bacteriophage has a higher migration rate in slightly alkaline groundwater (1.13 m/s, pH = 8.1) than acidic groundwater (0.90 m/s, pH = 6.1) (Schulze-Makuch et al., 2003), and the diffusion coefficients of viruses increase with pH value (Langlet et al., 2008). Elevated solution pH is reported to increase bacterial migration (Kim et al., 2009), whereas the declined pH enhances virus attachment to aquifer media and colloids, resulting in more retarded virus migration with lower peak-concentration, longer peak-concentration time and higher apparent collision efficiency (Walshe et al., 2010; Zhang et al., 2018). More bacteriophages MS2 and Φ X174 are attached on the solid–water interface under low pH condition and their migration is slowed (Torkzaban et al., 2006). In addition, higher pH can also enhance pathogen migration by encouraging the deprotonation of functional groups on cell surface and increasing the electrostatic repulsion (Schinner et al., 2010; Zhang et al., 2018). It is also worth noting that, when pH is close to the isoelectric point of pathogens, viruses might aggregate due to the weak electrostatic repulsion interactions, possibly causing blockage and immobile (Schinner et al., 2010; He et al., 2014). Generally, pathogen migration in subsurface environment is faster under neutral or weak alkali conditions, deserving more attentions.

3.4. Ionic strength

Ionic strength is reported to affect pathogenic attachment efficiency and then their migration ability (Zhuang and Jin, 2003b), explained as the change of pathogenic surface charge and membrane composition by ionic strength (Degrève et al., 2012). Ionic strength has a remarkable influence on the electrokinetic properties of either pathogens or grains (Kim and Walker, 2009) by decreasing the repulsive energy barrier and increasing secondary minima (Li et al., 2020). As viruses have lower attachment efficiencies on the negatively charged quartz sand at lower ionic strength (Gomez-Suarez et al., 2001), higher ionic strength increases pathogenic attachment efficiency (Han et al., 2006; Alexis et al., 2007; Rong et al., 2007; Kim and Walker, 2009). The electrostatic repulsion is reported to reduce as ionic strength increases, promoting the adhesion of bacterial proteins (Cui et al., 2015; Miao et al., 2015), and the migration rate of human adenovirus decreases with the increasing ionic strength from 0.001 mol/L to 0.1 mol/L (Wong et al., 2014). In addition, ionic valence is also a key factor affecting pathogen attachment and polyvalent cations are more likely to slow pathogen migration (Kim et al., 2009; Schinner et al., 2010). For instance, bacteria have higher adhesion efficiencies in CaCl₂ solution than KCl solution (Chen and Walker, 2007). Generally, pathogens have relatively high migration rates at the ionic strength of groundwater (about 0.02–0.04 mol/L).

Ionic strength can also change pathogen migration by altering the molecular structure of membrane proteins. By studying two noroviruses (GI.1 and GII.4) with the same isoelectric point but different membrane structure and composition, ionic strength shows distinct effects on their attachment on media (positive correlation with GII.4 but negative correlation with GI.1) (da Silva et al., 2011), explained by the significant shifts from β -strand to α -helix and slight losses of ordered secondary structure in GI.1 and barely change in GII.4 (Samandoulgou et al., 2015).

3.5. Temperature

A rise in temperature can decrease the energy barrier and water viscosity, thus increasing pathogen attachment and inhibiting their migration (Gharabaghi et al., 2015; Sasidharan et al., 2017b). Many studies have reported that temperature influences viral attachment to solid surfaces and affects their transport in subsurface environment (Gallardo-Moreno et al., 2003; Bradford et al., 2006b; Castro and Tufenkji, 2007; Kim and Walker, 2009; Chrysikopoulos and Aravantinou, 2014). The attachment rate coefficient of viruses PRD1 and Φ X174 increases up to 109% when temperature increases from 4 °C to 20 °C (Sasidharan et al., 2017b). Additionally, pathogenic physiological properties also change with temperature, e.g., EPS formation, protein folding and hydrophobicity, resulting in an increasing attachment of pathogens on some adsorbents with temperature (Bales et al., 1991; Bellamy et al., 1985). As groundwater temperature normally ranges of 10–22 °C in the thermostatic zone and deep phreatic water rises 1 °C for every 33 m increase of depth (Yates et al., 1985; Gunawardhana et al., 2009; Kar et al., 2010; Vanderzalm et al., 2010), pathogen migration in deep groundwater is relatively slow, but it is non-neglectable fast in shallow groundwater and poses significant risks for drinking water safety.

3.6. Moisture

Pathogen transport in subsurface environment is governed by advection, dispersion, and inactivation across multiple interfaces. As pathogens mainly enter the aquifer through the vadose zone, their vertical migration in the vadose zone is critical and moisture is a key factor (Flury and Aramrak, 2017; Kamrani et al., 2018; Sirivithayapakorn and Keller, 2003). Pathogen migration in soils of the vadose zone is inhibited due to the increasing of attachment as the moisture decreases (Gargiulo et al., 2008; Kim et al., 2008). As pathogens and the air–water interface are both negatively charged (Graciaa et al., 1995; Marinova et al., 1996), pathogens are usually electrostatically repelled from the air–water interface and attach to surfaces by thin water films and capillary menisci at the air–water–solid interface. Accordingly, viral attachment on the solid–water interface is more dominant than air–water interface, and it is controlled by solution chemistry, particle surface charge, and hydrophobicity (Wan and Tokunaga, 2002). In the vadose zone with lower saturation conditions, pathogens can be retained significantly by their attachment to the solid–water interface due to electrostatic interactions. As moisture decreases, viral attachment to solid–water and air–water–solid interface are enhanced (Flury and Aramrak, 2017), thus slowing pathogen vertical migration. For instance, the retention of viruses MS2 and Φ 174 increases as moisture decreases in laboratory test (Torkzaban et al., 2006). However, the presence of colloids in the vadose zone can enhance pathogen irreversible attachment to solid–water interface and adsorption to air–water–solid interface, mitigating the effects of moisture (Chu et al., 2001). For example, silica colloids are reported to inhibit the migration of *Escherichia coli* phage in saturated zone but oppositely promote their migration in the vadose zone when moisture decreases (Qin et al., 2020). Therefore, the effects of moisture on migration or remigration ability of pathogens during the intermittent water infiltration deserve attentions.

3.7. Hydrodynamic condition

Besides geochemical variables, pathogen migration in subsurface environment is also related to the hydrogeological conditions, such as flow rate (Yan et al., 2020). Lower flow rates and interruptions with higher hydraulic residence time can retard *Escherichia coli* and slow their migration (Sasidharan et al., 2017a). Conversely, a higher flow rate is reported to promote pathogen migration

(Predelus et al., 2017). The positive correlation between pathogen migration ability and flow rate can be firstly explained by the increasing higher hydraulic residence time of pathogens in porous media (Meinders et al., 1994; Xu et al., 2005). Secondly, the deposition kinetics are determined by the adhesive strength, which is higher and benefits pathogen deposition on solid surface at higher flow rates (Ko and Elimelech, 2000; Xu and Logan, 2006; Sasidharan et al., 2017a). Last but not least, lower flow rate means weaker hydrodynamic forces acting on pathogens which satisfy the torque balance on the surface of aquifer media (Bradford et al., 2011; Sasidharan et al., 2017a).

3.8. Subsurface environment with high risks of pathogen migration

Considering these key environmental geochemical and hydrological variables, the risks of pathogen migration in subsurface can also be evaluated by our proposed assessment algorithm but with different indices, following a similar rating and weighting

Table 3
Scoring criteria of migration ability of pathogen with different environmental conditions.

| Scoring item | Weight | Classification and scores | | | |
|------------------------|--------|---------------------------|-----------------|-------------------|------------------|
| Surface roughness | 25% | Roughness | Smooth | | |
| | | 1.0 | 5.0 | | |
| Moisture | 25% | >75% | 25%–75% | <25% | |
| | | 1.0 | 0.7 | 0.1 | |
| Flow rate (m/d) | 25% | >4 | 2–4 | 1–2 | <1 |
| | | 1.0 | 0.7 | 0.4 | 0.2 |
| Ionic strength (mol/L) | 15% | >0.1 | 0.01–0.1 | 0.001–0.01 | <0.001 |
| | | 1.0 | 0.8 | 0.5 | 0.2 |
| pH | 10% | >9.0 | 7.0–9.0 | <7.0 | |
| | | 1.0 | 0.7 | 0.3 | |

The bold fonts indicate criteria for classification, and normal fonts indicate scores.

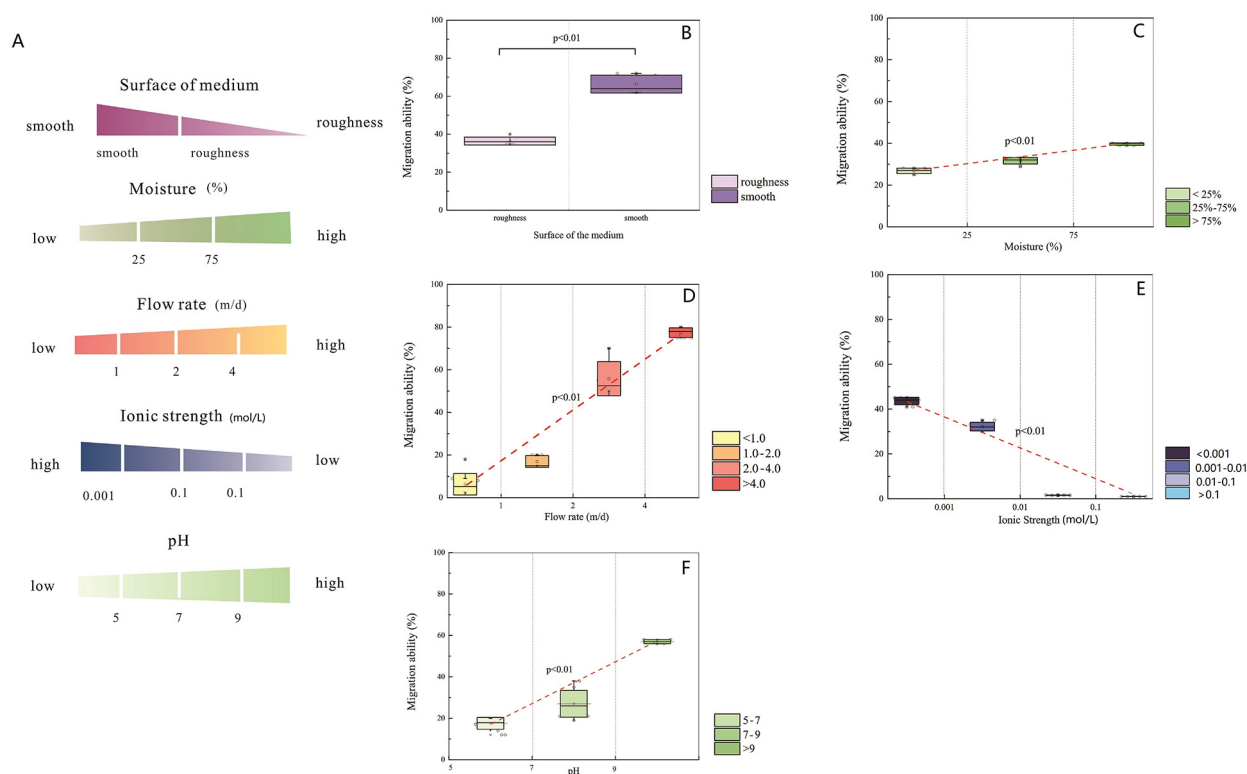


Fig. 2. (A) Impacts of geochemical and hydrological variables in subsurface environment on the estimated migration abilities of pathogens. (B) The estimated migration ability vs medium surface. (C) The estimated migration ability vs moisture. (D) The estimated migration ability vs flow rate. (E) The estimated migration ability vs ionic strength. (F) The estimated migration ability vs pH. Data are from (Walsh et al., 2010; Wong et al., 2014; Lv et al., 2016; Sasidharan et al., 2016; Yu, 2016; Li, 2018; Zhang et al., 2018).

into four groups of <0.001, 0.001–0.01, 0.01–0.1 and >0.1 mol/L. Generally, the pH value in groundwater ranges from 5.0 to 9.0 (Schulze-Makuch et al., 2003), which are then evenly divided into three categories of <7.0, 7.0–9.0 and >9.0.

Taking bacteriophage MS2 as a typical and representative pathogen, we evaluate its migration abilities under different environmental geochemical and hydrological conditions (Fig. 2). All variables show significant influence on the migration of bacteriophage MS2 (Fig. 2A). The migration ability is significantly higher in media with smooth surfaces than rough ones ($p < 0.01$, Fig. 2B). For other variables, remarkable positive correlations are found between MS2 migration ability and moisture (Fig. 2C), flow rate (Fig. 2D) and pH (Fig. 2F), whereas ionic strength exhibits a negative relationship with migration ability (Fig. 2E). Accordingly, the porous media in subsurface environment with smooth surface, high moisture, high flow rate, high pH and low ionic strength are of significantly high biosafety risks regarding pathogen migration, e.g., coarse sandy, karst and fissure aquifers. As SARS-CoV-2 has a considerable migration ability, their spreading potential should deserve careful attentions in these areas with biosafety fragility.

4. Conclusion and prospective

The outbreak of COVID-19 and other emerging diseases has aroused world-wide concerns about pathogen migration and spread in natural environment. As the main source of drinking water, groundwater is threatened by pathogen vertical migration from medical waste disposal, agricultural activities, as well as landfills, and might behave as a secondary source for the concealed pathogen spread, urgently requiring explicit studies on the influential factors on pathogen migration in subsurface system. In this work, we comprehensively review physiological characteristics, geochemical properties and hydrological variables influencing pathogen migration in subsurface environment. Five pathogenic physiological characteristics including pathogen size, isoelectric point, contact angle, width-length ratio and flagella intrinsically determine pathogen migration ability; the deterministic geochemical properties and hydrological variables include moisture, flow rate, surface roughness, ionic strength and pH. It is obvious that pathogen migration is complicated and affected by many intrinsic and extrinsic factors in subsurface environment, challenging appropriate evaluation of their migration abilities, particularly for those pathogens without sufficient information. Here, we propose an assessment algorithm and two rating/weighting schemes for pathogenic physiological characteristics and geochemical/hydrological variables to evaluate the migration abilities and risks of pathogens in subsurface system. In addition, we use this method to predict the migration abilities of SARS-CoV-2 viruses and subsurface systems with biosafety fragility. Although these algorithms and schemes are still an exploratory attempt owing to the limited works studying pathogen migration currently and different investigated parameters across literatures, our work highlights and points out the prior variables to explore in future experimental and field studies. The non-neglectable biosafety risks in subsurface system call for our attentions on both receiving pathogens by above-ground natural human disturbance (rainfall infiltration, leaching, artificial groundwater recharge, etc.) via vertical migration as a sink and spreading pathogens to nearby groundwater resources via horizontal migration as a source.

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

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.gsf.2021.101346>.

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