Application of the Human Viral Surrogate Pepper Mild Mottle Virus for Wastewater Fecal Pollution Management

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Abstract: Global water scarcity has led to significant dependence on reclaimed or recycled water for potable uses. Effluents arising from human and animal gut microbiomes highly influence water quality. Wastewater pollution is, therefore, frequently monitored using bacterial indicators (BI). However, threats to public health arise from the frequent incidence of wastewater-mediated viral infections–undetected by BI. Moreover, the enteric viromes contaminating wastewater are characterized by high abundance, genetic diversity and persistence in various water environments. Furthermore, humans usually suffer a minimum of a single acute diarrheal episode over their lifetime arising from extraneously acquired enteric microbiomes. A wide range of management methods are employed—in particular, microbial source tracking (MST) approaches to confront infections arising from exposure to contaminated wastewater. This review elaborates the viral contamination of treated wastewater and associated public health issues. Latterly, we discuss the various management strategies of wastewater pollution using conventional fecal indicators, viral indicators and human viral surrogates, with particular interest in the pepper mild mottle virus (PMMoV). Globally, PMMoV has been detected in rivers, aquifers, irrigation systems, and coastal and marine waters at high prevalence rates and concentrations greater than 10^5 genome copies per liter (gc/L). PMMoV was also found in almost all untreated wastewater environments. PMMoV concentrations in wastewater vary from 10^3 to 10^7 gc/L. These values are more than the maximum recorded viral indicator concentrations in wastewater for other proposed indicators. Limited variability in the daily concentrations of PMMoV in fecal wastewater has been studied, with an estimated average concentration of 10^5 gc/L with insignificant seasonal variability. The information summarized in this article offers fundamental knowledge for decision making in terms of defining the suitability criteria of candidate fecal indicators, risk assessment application and efficient wastewater management.

Keywords: wastewater; indicators; management; pepper mild mottle virus; fecal contamination; waterborne viruses

1. Introduction

The wastewater virome is a distinct subset of the microbiome owing to frequent infection of humans and domestic/companion animals [1–4]. For instance, a typical healthy human is estimated to harbor more than 10 virus-mediated chronic infections and occasionally more [5]. In particular, the human gut virome composition is intensively studied because of the continuous introduction of newly pathogenic agents and altered pathogenesis patterns, along with changes in immune responses owing to selection pressure imposed on the existent virome [6,7]. Gastroenteritis displays the potential pathogenesis of acquired gut virome [3,8]. For instance, rotavirus A, noroviruses and astroviruses are considered as the major causes of acute gastroenteritis worldwide and mainly result in infantile acute diarrhea [9–11].

Exposure to wastewater represents a common transmission route of enteric viruses via recreation water, surface water usage, wastewater/greywater-mediated irrigation and toilet flushing [12–16]. Therefore, wastewater reuse guidelines were suggested for safe use of wastewater. Moreover, fecal contamination indicators were proposed to ensure compliance with these guidelines. Coliform members represent the frequently used fecal indicators; however, other indicators have also been proposed involving bacteriophages, enterococci and sulfite-reducing bacteria [17–20]. These have not, however, met the expected sensitivity of enteric viruses' detection [21]. On the contrary, microbial source tracking (MST) tools provided higher specificity. Currently, MST professionals adopt a toolbox approach, i.e., implementing numerous MST markers, such as using pepper mild mottle virus (PMMoV) together with cross-assembly phage crAssphage [22–24].

However, MST protocols are commonly hampered by the low viral concentrations in water, thus demanding efficient water concentration methods to enable enteric viruses' detection [25]. Establishment of concentration methods for enteric viruses is mainly aimed at viral recovery from small water volumes [26]. Currently, recent methods including skimmed milk flocculation, monolithic adsorption filtration columns and the VirWaTest method depicted great success in enteric viruses' recovery from different water sources [27–30].

2. Viral Contamination of Treated Wastewater

Viruses are obligatory intracellular parasites of both eukaryotic and prokaryotic cells, and considered as the smallest microorganisms capable of replication [31]. Since they are unable to metabolize, they cannot engage in any energy-dependent processes like growth, respiration or reproduction on their own [32]. The main component of viruses is nucleic acid (DNA or RNA), which is shielded by a protein capsid that, in some cases, is surrounded by a lipid envelope [33]. Despite their apparent simplicity, viruses can nevertheless penetrate host cells using a number of physical and chemical mechanisms that are part of both the virus and the cell's structure. Moreover, viruses can also manipulate cellular processes to produce progeny viruses using various routes of entry [34]. Of particular interest is that enteric viruses are transmitted via the fecal–oral pathway. Enteric viruses are considered as the most persistent fecal microorganisms even during treatment approaches of contaminated raw water owing to their unique characteristics [35]. These viruses have an icosahedral structure, are between 20 and 100 nm in size, and primarily show a negative charge at neutral pH [36]. They are principal causes of viral gastroenteritis, hepatitis and poliomyelitis, displaying their adverse effect on public health. In addition, some enteric viruses, including polyomaviruses, have been linked to cancer [37]. Moreover, enteric viruses are highly efficient at surviving outside the gut for long periods, thus are easily disseminated through water resources [38].

Inappropriate wastewater treatment has caused viral contamination of shellfish, fresh produce and recreational waterways [39]. Many developing nations struggle with this ongoing problem because they lack the resources for effective wastewater treatment [40]. It is not surprising that, in current US frameworks, viruses demand substantially bigger reductions than bacteria or protozoan parasites when wastewater is reused for potable reasons. Target log_{10} removal value (LRV) attributions are computed using a risk-modelling methodology, assuming the worst-case scenario (very high viral concentrations, as would be the situation during a big epidemic), and a final risk of less than one illness in 10,000 exposures, per year [41]. Currently, states are choosing between one of these three LRV programs that were previously developed [42]. Although Texas' requirements seem to be less stringent than those of California or the National Water Research Institute (NWRI), it should be emphasized that Texas only counts LRVs from treated effluent toward final product water, excluding wastewater treatment reduction [43]. The wastewater treatment plant must exhibit its ability to eliminate pathogens to the levels required by the state prior to earning an LRV attribution, typically through a pilot demonstration [44].

Validating virus elimination is necessary for LRV attribution in reuse schemes. However, pathogenic virus concentrations in sewage and treated effluent vary, and frequently are not at levels that might effectively verify an 8–12 LRV [45]. It is frequently not practical or safe to spike pathogenic viruses at each phase to confirm overall LRV. Non-pathogenic viruses are frequently used as a process indicator [46].

According to conservative estimates, a good process indicator should: (i) be present in higher concentrations than human pathogenic viruses throughout treatment; (ii) be eliminated less effectively than human pathogenic viruses; (iii) correlate favorably with human pathogenic viruses; and (iv) be simple to detect and applicable to a variety of treatment processes [47].

3. Human Health Risk of Virus-Associated Water Pollution

On an annual basis, there are over 4 billion instances of waterborne diarrheal illnesses, which cause 2 million deaths, with under-five year olds the majority [48]. Enteric viral infections account for a sizable fraction of these diseases [49]. The most crucial way for enteric viruses to spread is through direct contact with infected individuals. Enteric viruses are spreading via the fecal–oral pathway as shown in Figure 1 [50]. However, the majority of enteric viruses remain persistent in areas where residential wastewater discharges exist and are frequently linked to waterborne epidemics [50]. Although typical wastewater treatment techniques can be comparatively inefficient at eliminating enteric viruses, wastewater is frequently treated before being released into the environment [51]. Fecal matter pollutes the environment and drinking water sources in poor countries since many locations lack suitable sanitary infrastructure and wastewater treatment facilities [52]. Additionally, significant amounts of untreated wastewater may be released by combined sewer overflows (CSOs) during periods of high rainfall as well as through dry water overflows, such as those caused by snowmelt, tidal infiltration, system failures and obstructions [53–55]. Consequently, people who come into direct or indirect contact with contaminated waters are prone to the risk of contracting viral infections as a result of these events, which allow enteric pathogens to contaminate the environment directly [56]. Enteric viruses are extremely contagious in ambient waters and can stick to particles in the water column or accumulate in sediment [57]. They might subsequently be consumed by aquatic organisms, such as bivalve shellfish harvested for human consumption [58]. Additionally, wastewater is regularly used for irrigation in areas with a shortage of freshwater; as a result, enteric viruses may directly contaminate fruit and salad vegetables, and result in foodborne outbreaks [54]. The typical duration of gastroenteritis caused by enteric viruses is 2–5 days [59]. In certain circumstances, the infection goes asymptomatic or causes symptoms in the skin, neurological system or respiratory system [60]. The Picornaviridae, Caliciviridae, Reoviridae, and Adenoviridae families make up the majority of those responsible for gastroenteritis (Table 1). For instance, noroviruses (family Caliciviridae) account for a sizable portion of gastroenteritis infections worldwide, causing 685 million cases and roughly 200,000 fatalities [61], with a total direct cost to the healthcare system of USD 4.2 billion and associated societal costs of USD 60.3 billion annually [61]. The main etiological agents of gastroenteritis in newborns and young children are rotaviruses (family Reoviridae) and group F mastadenoviruses (AdVs; family Adenoviridae) [62]. The three most frequent viral pathogens linked to waterborne and water-associated foodborne outbreaks are noroviruses, hepatitis A virus (family Picornaviridae) and AdVs [63]. Infection can cause significant illness, such as acute hepatitis [64].
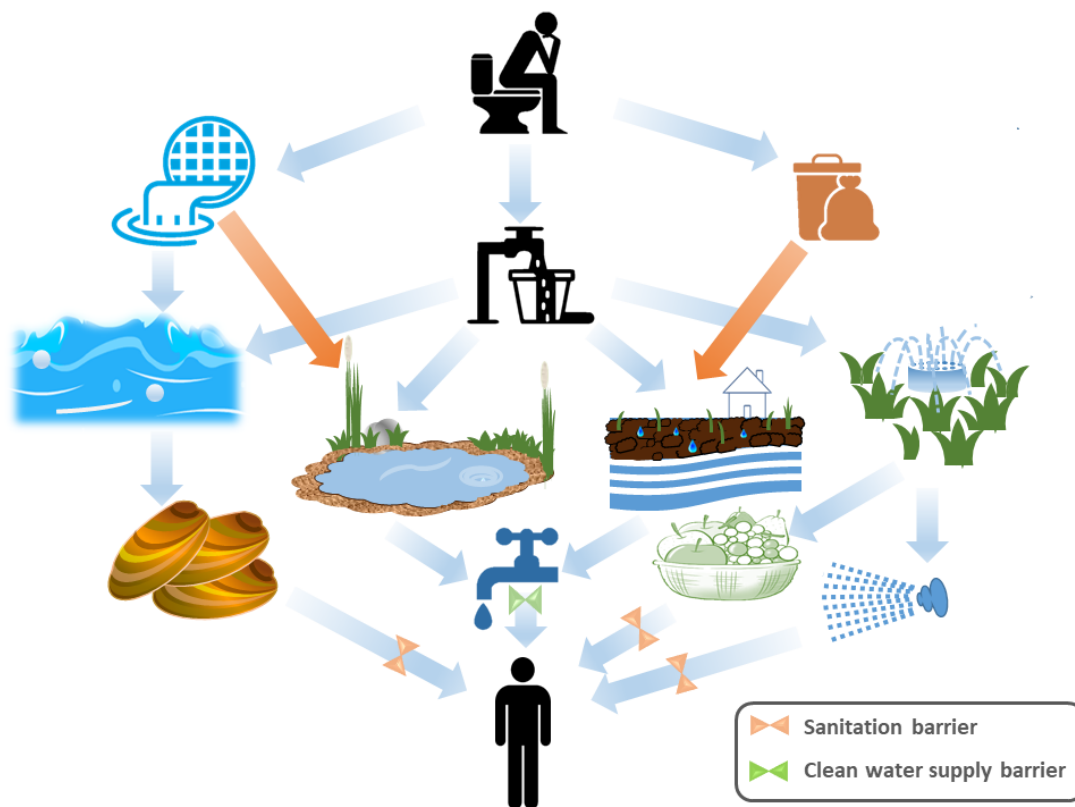
Figure 1. Diagrammatic representation of the fecal–oral route for transmission of enteric viruses. Human excreta go through land runoff and sewage that contaminate oceans, rivers, lakes and ground water. Moreover, sewage can contaminate irrigation water. The contaminated oceans, rivers and lakes influence filter feeders (shellfish) and recreation water, whereas the direct water supply would be affected by the improperly decontaminated ground water and rivers. Crops and irrigation-based aerosols are also contaminated by inadequately treated irrigation water. On the other hand, the human excreta give rise to solid wastes that affect the groundwater, leading to unclean water supply. Absence of a sanitation barrier and a properly clean water supply barrier lead to enteric virus infection of a new human host.

Several studies have linked the pollution of wastewater with rotaviruses, enteroviruses, sapoviruses, astroviruses, Aichi virus (AiV) and hepatitis E virus [65–68]. For instance, in Maharashtra state, India in 2017, contaminated drinking water wells were the source of a rotavirus B outbreak with a 22.8% attack rate [66]. A number of viral gastroenteritis outbreaks connected to sewage-contaminated water that contained enteroviruses such as AdV, norovirus, sapovirus, astrovirus and rotavirus have also been reported [67]. Hepatitis E virus was linked to the greatest viral waterborne outbreak in Kanpur, India, which affected almost 80,000 individuals [68].

Environmental waterways have recently been found to include both recently discovered viruses and well-known viruses that weren't previously connected to wastewater (Table 1). Infected people's feces and urine have only lately been found to include human polyomaviruses (PyVs) and papillomaviruses, which were initially identified in the 1970s and 1950s, respectively [69]. High concentrations of several PyVs, such as BKPyV, WUPyV, KIPyV, MCPyV and JCPyV, have been found in wastewater, river and ocean, silt, swimming pools, and tap water (up to 10^8 genome copies (gc)/l) [70,71]. Although the method of transmission of these viruses is not yet known because healthy persons frequently show no symptoms, aquatic infections are most likely [72]. On the other hand, the first description of Bocaviruses (family Parvoviridae), which cause gastroenteritis and respiratory tract infections, was made in 2005 [73]. Since then, human bocaviruses have been discovered in wastewater at quantities of 10^3–10^5 genome copies (gc)/l in both untreated and treated

wastewater [74]. Additionally, sewage and contaminated river waters have been shown to contain the gastroenteritis-causing torque teno virus (family Anelloviridae). Similar to bocaviruses, the torque teno virus has much lower concentrations (up to 10^6 gc/l) than other, more prevalent enteric viruses (10^4–10^9 gc/l) [75]. Additionally, human picobirnaviruses (family Picobirnaviridae) have been found in contaminated rivers and wastewater with concentrations ranging from 10^3 to 10^6 gc/l [76]. Wastewater has also been shown to contain the entire or partial genomes of circoviruses (family Circoviridae), cardioviruses (family Picornaviridae), and enveloped viruses (coronaviruses, influenza virus) [77]. Human infections from aquatic corona- and influenza viruses (such as SARS-CoV-2) are uncommon since enveloped viruses break down quickly in water [78].

Table 1. Human pathogenic viruses detected in the aquatic environment.

Virus	Size of Viral Particle	Zoonotic Transmission	Aquatic Environment	References
Mastadenovirus A–F	70–90 nm	No	Wastewater	[79,80]
Torque teno virus	30 nm	Yes	River	[79–81]
Astrovirus	28–30 nm	Potentially	Sewage water	[79,82,83]
Norovirus GI, GII	35–40 nm	No	River	[79,84]
Sapovirus GI, GII		No	Wastewater and river	[75,79]
Human-associated circovirus	15–25 nm	No	Sewage	[85,86]
Hepatitis E virus type 1–4	27–34 nm	Yes	Tap and bottled water	[87,88]
Assorted papillomaviruses	55 nm	No	Wastewater	[89,90]
Human bocavirus type 1–4	22 nm	No	Recycled water and sewage	[79,91]
Aichivirus A–B	30–32 nm	No	Sewage and surface water	[92]
Cosavirus A		No	River and waste water	[85,93]
Coxsackievirus B		No	Sewage water	[93,94]
Enterovirus A–D		No	Groundwater	[93,95]
Poliovirus type 1–3		No	Wastewater	[93,96]
Hepatitis A virus	40–45 nm	No	Wastewater	[93,97]
BK polyomavirus		No	River and sewage water	[98]
JC polyomavirus		No	Wastewater	[99]
Rotavirus A	60–80 nm	Potentially	Drinking water	[79,100]

4. Management Strategies for Wastewater Pollution

4.1. Traditional Fecal Bacterial Indicators

The microbiological safety of irrigation water is monitored using indicator organisms [101]. *E. coli* is classified as specifically having fecal origin and is a member of the coliform subgroup known as the fecal coliforms [102]. The primary indicator of fecal contamination of water is frequently *E. coli* [103]. There are several problems with *E. coli* as a fecal indicator. To begin with, the presence of viral infections is not correlated with *E. coli* which is not host-specific. Moreover, *E. coli* also decays in the environment more quickly compared to other foodborne bacteria [104]. In contrast, the standard fecal indicator should show environmental survival and movement across the matrix that are equal to or greater than those of the pathogen, exist at higher concentrations than the pathogen, and provide source specificity [105]. Also, the indicator organism assay method should be accurate, specific, quick, quantitative, sensitive, widely applicable and indicative of infectivity [106].

Levels of fecal contamination in water have conventionally been assessed using fecal indicator bacteria (FIB; including coliform bacteria, Enterococcus, *E. coli* and *Streptococcus* spp.) [107]. In this context, bacterial pathogens, like fecal coliforms, can survive for up to 15 days on the surface of food and up to 30 days in water and sewage [108]. However, bacteria have been demonstrated to be substantially less persistent in the environment and significantly less resistant to wastewater treatment than enteric viruses [31]. Consequently, FIB are subpar predictors of the risk of viral infection, which implies that current water-quality monitoring programs based only on FIB are insufficient [109].

4.2. Viral Indicators

Human enteric viruses come in about 100 different varieties and the number is growing due to newly discovered and emerging strains [110]. Surrogates and indicators are frequently employed to study the fate and transport of pathogenic strains in the environment owing to the high diversity of viral pathogens [111]. An indicator may be useful for evaluating pathogen abundance, persistence, adsorption and transit in the aquatic environment, as well as for making a general assessment of the effectiveness of wastewater and drinking water treatment [44]. Therefore, a good viral indicator should ideally have comparable inactivation and retention of the target pathogens and should be present year-round in wastewater and habitats impacted by wastewater [112]. This would allow for ongoing monitoring and provide information on the degree of pollution and the probability that pathogens are present [113]. Table 2 lists some enteric viruses that are connected to wastewater and may be utilized as indicators, but not all of these viruses meet the criteria. High concentrations of influenza, corona-, circo- and papillomaviruses have been found in wastewater but not in contaminated areas, which may be because of how quickly they degrade in water [114].

Additionally, several enteric viruses (such as the astrovirus, rotavirus, torque teno virus and hepatitis E virus) may be zoonotic; as a result, their occurrence in the environment may be caused by things other than human waste, such as agricultural operations [115]. Although the hepatitis A and E viruses are widespread in less developed countries, they only sometimes cause epidemics in more developed areas [116]. Furthermore, in temperate regions, enteroviruses, noroviruses and sapoviruses all exhibit distinct seasonality, peaking either in the summer (for enteroviruses) or the winter (for noroviruses and sapoviruses) [52]. Consequently, these viruses are not constantly present in contaminated environments and wastewater throughout the year [117]. On the other hand, it has been proposed that human adenovirus, polyomaviruses and Aichi viruses can serve as accurate fecal markers because they are frequently found in sewage and other contaminated areas without any discernible seasonality [118].

Table 2. Survival of enteric viruses in various water environments.

Organism	Habitat	Temperature	Duration (Days)	Log Reduction	Reference
Adenovirus	Groundwater	4	132	1.00	[119]
		20	36	1.00	
Adenovirus 40	Seawater	15	28	1.40	[120]
		15	85	2.00	
	Drinking water	4	60	0.49	
		4	92	2.00	
Adenovirus 41	Seawater	15	28	1.60	[120]
		15	77	2.00	
	Drinking water	4	60	1.00	
		4	304	2.00	
Rotavirus	Fresh water	20	10	2.00	[121]
		4	32	2.00	
	Seawater	37	7	5.00	[122]
	Soil	37	7	1.70	[123]
	Drinking water	20	64	2.00	[124]
	Groundwater	25	1266	1.79	[125]
Norovirus	Mineral water	25	80	1.30	[126]
		4	80	0.89	
	Tap water	25	80	0.80	
		4	80	3.00	
Hepatitis A virus	Seawater	20	28	4.00	[127]
	Artificial seawater	25	11	1.00	[128]
		24	19	1.00	
	Drinking water	4	60	1.60	[120]
		4	56	2.00	
	Bottled water	21	21	1.99	[129]
Astrovirus	Tap water	20	30	2.00	[130]
		4	60	2.00	

4.3. Human Viral Surrogates

New possible human virus surrogates were also discovered in wastewater thanks to advancements in genome-based approaches, with the candidates pepper mild mottle virus (PMMoV) and crAssphage emerging as particularly intriguing ones [46]. Despite being morphologically and physiologically dissimilar from human enteric viruses, these possible human viral surrogates are present in large amounts in municipal wastewater [57].

4.3.1. Coliphages

Wastewater is frequently contaminated with bacteriophages that attack microorganisms related to the human gut [131]. Coliphages, which are present in human feces, are bacterial viruses that attack *E. coli* [132]. Furthermore, coliphages can be counted as plaque-forming units (PFU) on agar containing the host bacteria using culture-based procedures, which are simple and affordable to use [133]. This method offers a rough estimate of the presence and quantity of infectious coliphage viruses [134]. Moreover, this technique helped overcome the drawbacks of PCR, which estimates genetic material regardless of infectivity [135]. In general, coliphages are predicted to be persistent in environmental waters and respond to treatment similarly to human enteric viruses, although thorough analyses of environmental data have revealed a variety of patterns [136]. The discovery of an infectious coliphage in recycled water suggests that there may be an infectious human virus present in the same wastewater or that the treatment process failed to eliminate the infective virus [137]. It is common practice to evaluate wastewater pollution using somatic coliphages (phages that infect *E. coli*) and F-specific RNA bacteriophages (FRNAP; phages that infect bacteria through the F-pili) [138]. Moreover, it is worth mentioning that the European Union introduced in the revised drinking water directive (2020/2184) analyses of somatic coliphages from raw waters with an established reference value of not more than 50 PFU/100 mL [139].

4.3.2. CrAssphage

Bacteriophages that infect Bacteroides species may also be a sign of contaminated wastewater [140]. These phages include the recently identified class of viruses known as crAss-like phages [141]. Wastewater contains a significant amount of CrAssphage, which is excreted by 50–70% of humans [142]. Importantly, crAssphage can be particularly linked to people and is a sign of human waste that can be distinguished from animal waste [143]. The genome of CrAssphage (metagenome-assembled genome), co-evolved with humans and is a member of the typical gut virome [57].

4.3.3. Pepper Mild Mottle Virus

Pepper mild mottle viruses (PMMoV) have a rod-shaped, non-enveloped and single-stranded RNA (ssRNA) genome [46]. In high concentrations, PMMoV is found in human feces all throughout the world [144]. Additionally, PMMoV virions are resilient to a variety of environmental conditions [145]. PMMoV could be a more reliable indicator of fecal load than viruses that cause human disease because its presence is dietary in origin [32].

Due to its dietary origin in feces, pepper mild mottle virus (PMMoV; family Virgaviridae), a plant virus from the genus Tobamovirus [146], differs from other suggested surrogates for enteric viruses in terms of host, transmission route (Figure 2), higher persistence, abundance and human waste correlation–discussed later. For instance, PMMoV has been demonstrated to be connected to human waste, and it can be detected in contaminated surface water, groundwater and drinking water [54]. Quantitative PCR verified discharged amounts of 10^6 to 10^9 viral copies per gram (dry weight) of human feces, when PMMoV was found to predominate the RNA viral metagenome of human feces [147]. Consumption of peppers (*Capsicum* spp.) and food products made with peppers that are contaminated with the virus is the main cause of PMMoV in human excreta [148]. Despite the fact that PMMoV's size and shape (17–300 nm rod-shaped capsid) differ from other pathogenic viruses with icosahedral capsids, its fate and behavior in the environment may be different,

it has been suggested that PMMoV can be a useful indicator of wastewater contamination [47]. Since its discovery in human feces in 2006, high concentrations of PMMoV have been detected in food products, human feces and wastewater in Asia, Europe and the United States [147,149–152].
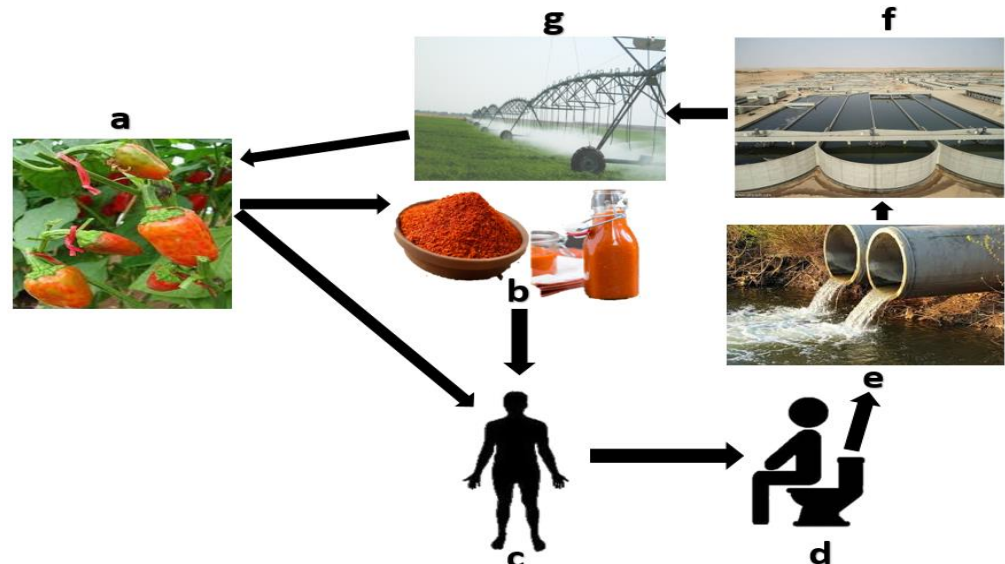

Figure 2. Transmission routes of PMMoV. PMMoV-infected pepper (**a**) is either used for preparation of powdered pepper products, in chili sauces (**b**) or directly consumed as a fresh product by humans (**c**). The human excreta (**d**) containing PMMoV are discharged into sewage water (**e**) that is prone to treatment approaches (**f**) for reuse in irrigation (**g**).

In a French study, food products tested from France, U.S., Mexico and unknown origins were positive for PMMoV and showed as much as 10^7 PMMoV copies/mL [151]. In addition, PMMoV concentrations in wastewater vary from 10^6 to 10^{10} copies/mL [144,150]. These values are more than the maximum recorded viral indicator concentrations in wastewater for other proposed indicators [150]. There is limited variability in the daily concentrations of PMMoV in fecal wastewater with an estimated average concentration of 10^8 copies/mL, when investigated over a two-week period [144], and insignificant seasonal variability was also detected [123].

Given the large quantities of enteric viruses in wastewater, PMMoV has potential as a process indicator for tracking enteric virus eradication during potable water and wastewater treatment. However, compared to enteric viruses, PMMoV is extremely resistant to the (waste) water treatment process, with as little as 1-$\log_{10}$ PMMoV elimination following a variety of wastewater treatment techniques [153,154]. The possibility exists that PMMoV detection in surface waters may exaggerate the degree of fecal pollution due to the high concentrations of PMMoV in household wastewater and its resilience during the wastewater treatment process [63]. However, prior research has discovered a number of benefits to employing PMMoV as a marker of human fecal pollution in surface waterways [144,147,149,153]. These advantages of PMMoV include that it is absent in environmental waters devoid of recognized wastewater sources [155]. Furthermore, PMMoV was reported to be regularly present in quantifiable proportions in wastewater [144]. In addition, PMMoV co-occurs with interesting enteric viruses in river and seawater exposed to point sources of wastewater pollution [152]. Moreover, PMMoV concentration is relatively high in human feces (10^5–10^7 copies/mg) [147,151] in comparison to animal feces (10^2–10^3 copies/mg in seabird, goose, chicken and cow feces; [144,152]). Interestingly, PMMoV revealed host specificity ranging from 90 to 92% and a host sensitivity of 100% [156]. As PMMoV becomes more widely used for microbial water quality assessments, it is important to understand the locations and settings in which PMMoV can be utilized as a

surrogate for enteric viruses, and the extent to which its detection suggests a significant health risk [157].

5. PMMoV Application as Fecal Indicator

5.1. Characteristics of Ideal Viral Indicator

The repetitive investigation of environmental water samples for the prevalence of pathogenic viral strains is often problematic owing to low density and sensitivity of detection methods such as cell culture and molecular-based assays [158]. The infectivity cannot be determined by molecular-based tests [159]. It has been predicted that it will be difficult to estimate the concentration of infectious enteric viruses in recycled water [133]. Indicator viruses, which co-occur with enteric viruses and are simple to count and grow, have thus been the subject of investigation [160].

An ideal viral indicator for fecal contamination of waters should have the following characteristics: (i) it should not be able to replicate in contaminated water; (ii) it should specifically relate to contamination by human feces and pathogens; (iii) it should be non-pathogenic to humans; (iv) it should have physical characteristics similar to pathogenic viruses; (v) it should be at least as resistant to inactivation as pathogenic viruses; (vi) it should be a member of the intestinal microflora of warm-blooded animals; (vii) it should be easy to detect; and (viii) it should be applicable to all types of waters [137].

Novel viruses that are prevalent in human feces and wastewater samples have recently been identified thanks to developments in metagenomics and high-throughput sequencing technology [77]. Examples of novel indicators of sewage contamination tracking and wastewater treatment procedures are crAssphage and pepper mild mottle virus (PMMoV), which were discovered using this method [161]. A fecal marker, on the other hand, is used to signal the presence of harmful viruses arising from fecal contamination, while a process indicator virus is used to evaluate the efficacy of a treatment procedure [162]. Simplicity of measurement and detection, association with human waste, high amounts of presence in wastewater, wastewater treatment resistance, determination in aquatic environments and global dispersion should exist in any candidate fecal indicator [163].

5.1.1. Ease of Detection and Quantification

Environmental samples are frequently concentrated before viruses are detected in order to identify low viral titers accurately [54]. As previously discussed [164], ultracentrifugation, ultrafiltration, adsorption/elution and flocculation are frequently utilized for the concentration of water samples. The sort of concentration technique utilized, the type of sample and the virus type all affect how effectively viruses can be recovered [165]. Therefore, indicator viruses should be those that can be readily and consistently recovered utilizing straightforward concentration methods [159]. qPCR-based assays are mostly used to identify and quantify enteric viruses, crAssphage and PMMoV [166]. Plaque assay or integrated cell culture–qPCR (ICC-qPCR) were, however, employed in a small number of investigations for AdV identification [167].

The combined methodology of cell culture and qPCR is utilized for the investigation of viral replication, enabling the detection of infectious viruses, which grew slowly and/or failed to exhibit cytopathic effects [168]. This method has decreased the time needed for infectivity analysis from one week to two days, allowing for rapid detection [167]. The greater concentrations identified using ICC-qPCR associated with the traditional culturing assays suggest that using qPCR-based quantification of cultured viruses is further sensitive and therefore more consistent in environmental settings [169]. Contrarily, any culturing-based technique for enteric virus identification has the drawback of requiring specialized staff, BSL2 or BSL3 environments, and equipment (like a CO_2 incubator), which may not be available in routine monitoring facilities [170].

5.1.2. Human Waste Association

It makes sense to employ viruses like the human-specific AdV, PyV and AiV strains as indicators of human fecal contamination [118]. The source of contamination (e.g., human vs. wildlife, livestock, etc.) can be evaluated using these viruses and their matching animal-associated strains [171]. By differentiating between human, bovine, porcine, canine and avian AdV genome sequences based on their melting temperatures, Staggemeier et al. (2015) used SYBR Green qPCR for the identification and quantification of AdVs in water and sediment samples [172].

It has been demonstrated that PMMoV correlates well with other human markers such as Bacteriodes HF183 and PyV, suggesting that it is associated with human waste [173]. PM-MoV has been found at high concentrations in domestic raw and treated fecal wastewater and also in wastewater-polluted environments [54]. In addition, qPCR assays targeting PM-MoV demonstrate great sensitivity [156]. Further research into the prevalence of PMMoV is necessary because it has been claimed that areas with higher consumption of pepper products have higher concentrations of PMMoV in feces and wastewater [155].

5.1.3. Presence in Wastewater at High Concentrations

With concentrations larger than 10^5 genome copies gc/L, these analyses revealed that PMMoV was found in practically all untreated wastewater samples. According to a recent study conducted in Costa Rica, a human-specific (HF183) Bacteroides marker has a specificity of 94 percent compared to the 100 percent PMMoV qPCR signal for domestic wastewater [174]. Additionally, Stachler et al. demonstrated that about $0.02\% \pm 0.06\%$ of the metagenomic sequence reads originating from sewage wastewater and biosolids samples from the United States and Spain were mapped to PMMoV, showing the high abundance of PMMoV in wastewater samples compared to the other viral pathogens of humans (average number of mapped sequence reads was found to be 395 ± 619 for PMMoV while mapped sequences for norovirus were found to be 102 ± 66) [143]. Collectively, these results demonstrate the value of PMMoV as a precise viral marker for domestic wastewater. High concentrations of PMMoV (up to 10^{10} gc/l) have also been found in wastewater (Figure 3). The highest reported PMMoV concentrations were detected in Florida and other US states [144], Germany (10^7–10^8 gc/l) [152], New Zealand (10^7 gc/l) [157], Vietnam and the US (Arizona; 10^6–10^7 gc/l) [150,154].
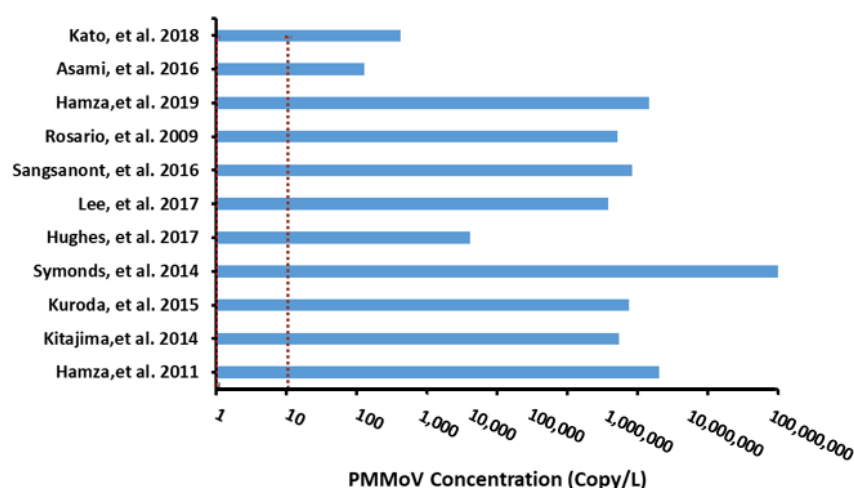

Figure 3. PMMoV abundance in wastewater treatment plants (WWTPs) [144,145,150,152,154,175–180]. PMMoV persistence in WWTPs' effluents: red dashed line denotes LOD of PMMoV (0.66–1.3 copies. L^{-1}) in large scale samples whereas brown dashed line refers to LOQ for the same sample size (7.0–14 copies. L^{-1}) [150].

5.1.4. Resistance to Wastewater Treatment

Traditional wastewater treatment techniques have been demonstrated to be only moderately effective against enteric viruses [181]. Comparative studies have been carried out to examine enteric virus resistance and potential indicators during wastewater treatment since removal performance differs between locations and the type of treatment technique [182]. According to the available data, PMMoV is stable during secondary treatment and chlorination, resulting in a <2 log reduction [148]. Haramoto et al. investigated drinking water sources in Japan from seven distinct geographic regions and discovered that PMMoV was detected in 140/184 total samples (76%) [153]. There were no appreciable seasonal changes in PMMoV detection frequency or concentration among the months of autumn, winter and summer, according to this study's evaluation of potential seasonal fluctuation [136].

Asami et al. tracked the presence of PMMoV before and after treatment activities in Bangkok, Thailand. The levels of PMMoV were found to be 102.88 $\pm$ 0.35, 102.39 $\pm$ 0.55 and 101.06 $\pm$ 0.53 GC/L, respectively, in raw water (canal water), post-coagulation sedimentation (CS) and post rapid sand filtering (RSF) [179]. In this investigation, PMMoV was discovered more frequently than any other viruses (i.e., NoVs, Aichi virus 1, enteric HAdV, enterovirus, HPyVs). Sangsanont et al. investigated the prevalence of PMMoV in drinking water samples in Hanoi, Vietnam, recording relative PMMoV concentrations ranging from 1.9×10^5 to 2.7×10^6 GC/L [178]. Shirasaki et al. examined the removal of PMMoV by membrane filtration (MF) with and without coagulation, as well as by ultrafiltration (UF), and compared it to that of other enteric viruses [183]. They discovered that the removal of PMMoV for all filtration processes was highly correlated with the enteric viruses examined, with comparable $\log_{10}$ reductions. Kato et al. investigated the efficiency of step-wise removal of PMMoV in sampling campaigns at two full-scale drinking water treatment plants in Japan, finding that reductions of PMMoV by coagulation sedimentation (plant A: ~2.38 $\log_{10}$; plant B ~2.62 $\log_{10}$), were significantly higher than reductions of turbidity and indicator bacteria [180].

5.1.5. Persistence in the Aquatic Environment
PMMoV Occurrence in Freshwater Environments

Around the world, PMMoV has been detected in rivers, aquifers, irrigation systems, and coastal and marine waters at high prevalence rates (Figure 4) and significant concentrations (Figure 5). The influence of effluent, the rainy vs dry seasons, and the relationship with other indicators of water quality and pollution have all been considered in regard to the various circumstances surrounding PMMoV prevalence in these water types [184]. The PMMoV, torque teno virus, human adenoviruses, human picobirnaviruses and human polyomaviruses were among the viruses that were detected in samples taken from the Ruhr and Rhine rivers in Germany [152]. The study found that PMMoV was detected in 100% (*n* = 108) of the analyzed samples while PMMoV concentrations were found in the range of 3.0×10^3 to 1.1×10^6 gc/L. This range is in accordance with the range described by Kuroda et al. for river water samples from Vietnam (*n* = 3), which ranged from 3.0×10^4 to 1.8×10^6 gc/L [150]. The samples for this study were collected from diverse locations along river systems, including a site that collects wastewater from a separate place, 500 m downstream from a wastewater treatment facility and upstream from an urban area. The PMMoV prevalence in pond and irrigation waters was also assessed and recorded as 91% and 100% at concentrations of up 1.2×10^5 gc/L and 1.0×10^4 gc/L, respectively. Another study, conducted in 2017, examined irrigation waters from various sources utilized to irrigate fresh food in the Kathmandu Valley [185]. Six groundwater samples and 35 samples of surface water were taken. Six rivers, two ponds, a canal and six groundwater wells were sampled. Ninety-six percent of the samples from rivers (27/28), 100% of the samples from canals (2/2), 60% of the samples from ponds (3/5) and 83% of the samples from groundwater (5/6) tested positive for PMMoV. In groundwater samples taken from controlled aquifer recharge locations in Colorado, California and Arizona in the United States, Betancourt et al. concluded that PMMoV was more frequently found than human

enteric viruses (HAdV, enterovirus and Aichi virus 1) [149]. Only 3/8 (37.5%) of groundwater samples from Vietnam in another investigation were positive for PMMoV. The third sample exhibited a concentration of just 19 GC/L, while two of the samples could not be quantified [150].
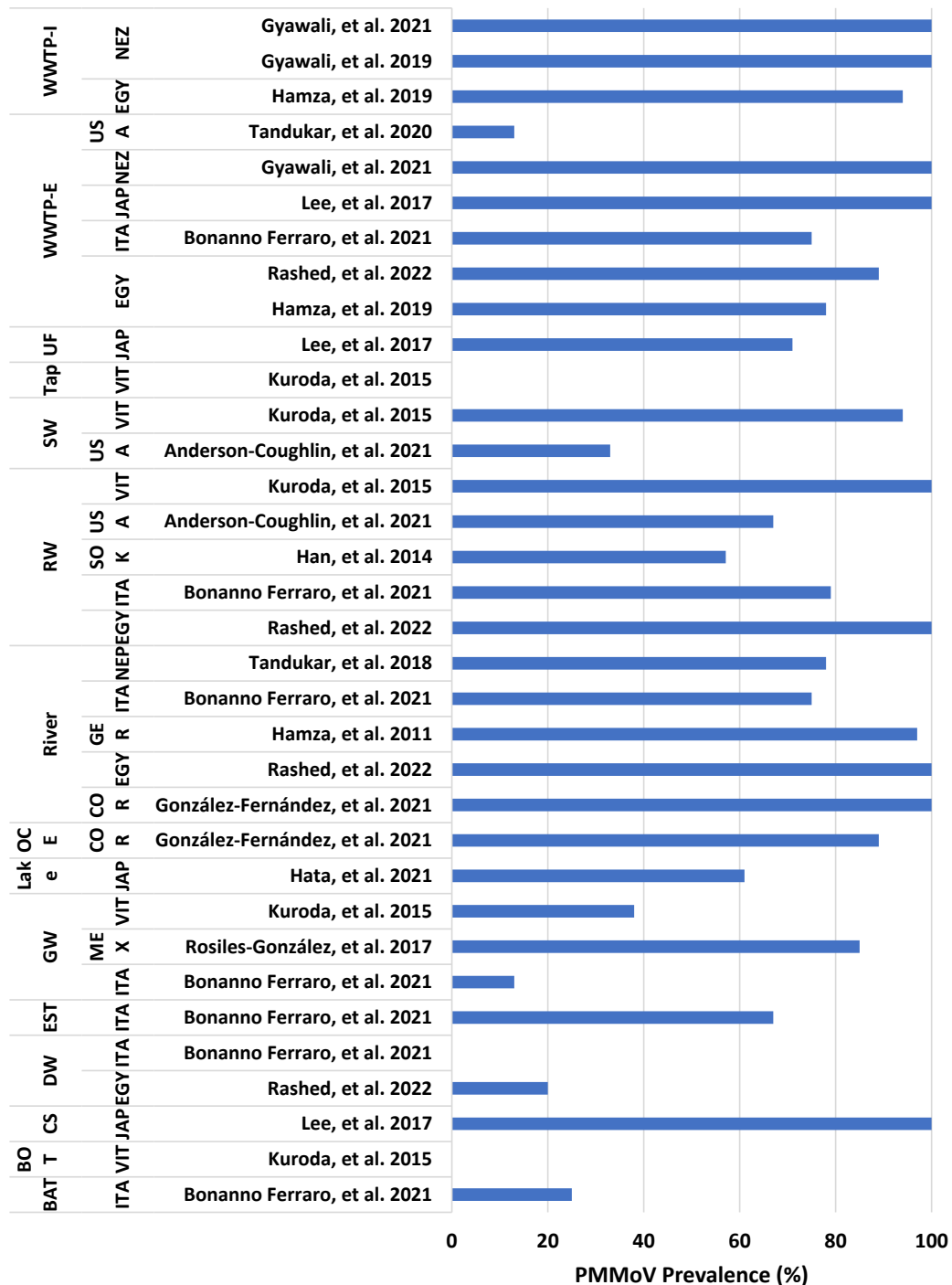

Figure 4. PMMoV prevalence in various water sources [136,145,150,152,157,161,177–192]. WTTP-I: wastewater treatment plant influents, WTTP-E: wastewater treatment plant effluents, UF: ultrafiltration treated wastewater, SW: surface water, RW: raw sewage water, OCE: ocean, GW: groundwater, EST: estuarine water, DW: drinking water, CS: coagulation–sedimentation-treated wastewater, BOT: bottled water, BAT: bathing water. Countries included New Zealand (NEZ), Egypt (EGY), United States of America (USA), Japan (JAP), Italy (ITA), Vietnam (VIT), South Korea (SOK), Nepal (NEP), Germany (GER), Costa Rica (COR) and Mexico (MEX).
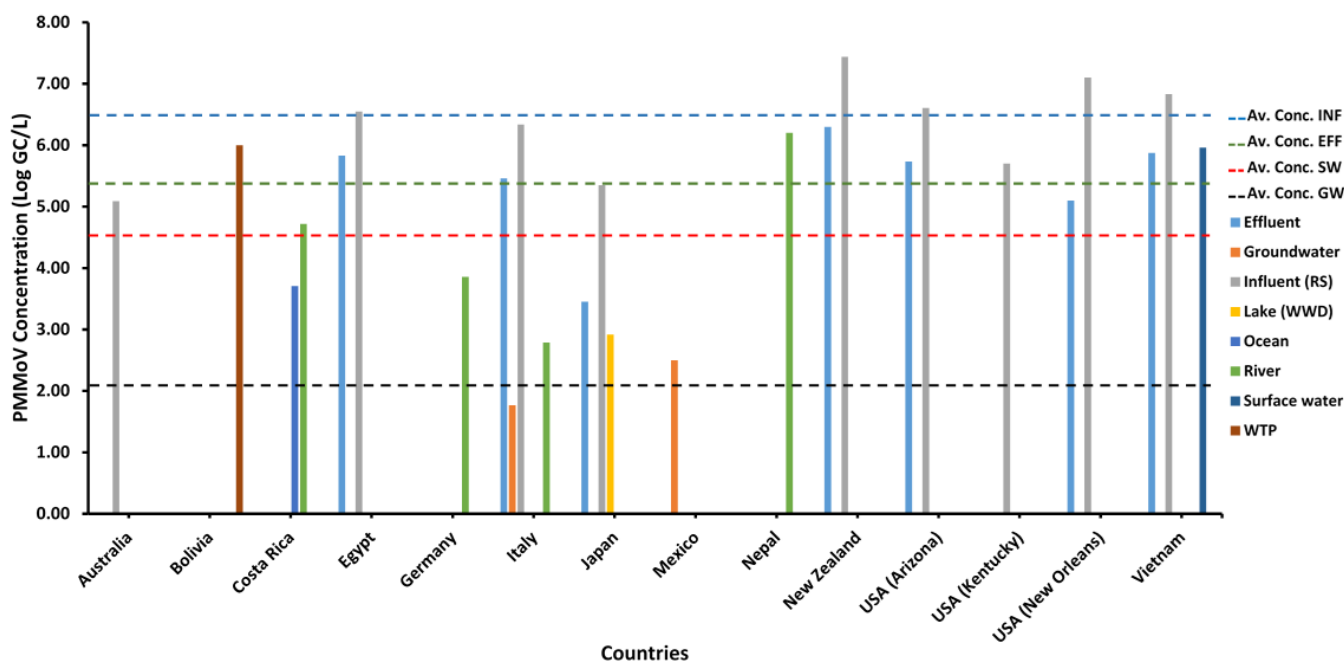
Figure 5. PMMoV concentration in different water environments [136,145,150,152,154,157,161, 175,177,184,186,187,192–194]. WTP: wastewater treatment pond, Av. Conc. INF denotes average concentration of PMMoV in wastewater influents (including raw sewage (RS) and untreated wastewater) expressed in log genome copies (GC)/L, EFF: wastewater effluents, SW: surface water, GW: groundwater.

Because of the heterogeneity in occurrence and concentrations between places, it is likely that the presence of PMMoV in groundwater strongly depends on the soil and aquifer characteristics for a given area [44]. Another study examined the persistence of PMMoV in effluent wastewater discharge in Arizona and Colorado and detected the presence of torque teno virus, HAdV and HPyV in addition to PMMoV [149]. The viral concentrations were investigated at 1, 3, 6, 10 and 21 days after inoculation. When compared to the other viruses examined, PMMoV appeared to be the most stable over time, displaying just a 1.1 $\log_{10}$ reduction after 21 days at 25 °C as opposed to more significant declines for torque teno virus (3.0 $\log_{10}$), HAdV (3.7 $\log_{10}$) and HPyV (4.2 $\log_{10}$). This study demonstrates the high thermal stability of PMMoV virus particles [149].

PMMoV Occurrence in Marine Environment

The viral concentrations of PMMoV ranged from 4.09×10^5 to 6.00×10^7 GC/L in marine water samples that tested positive (4/7) which were collected from the Gulf Stream (USA). The presence of numerous microbial source tracking (MST) markers also linked with the prevalence of PMMoV in coastal waters near Florida. Five coastal regions were used to gather a total of 30 samples. PMMoV was positive in 60% (18/30) of the collected samples. These samples' concentrations ranged from below the quantifiable level to 8.73×10^5 GC/L [25].

Additionally, the Gulf of Nicoya's coastal waters near Costa Rica were assessed. Eight samples were taken for this investigation from four key areas in Costa Rica where shellfish are produced. PMMoV and other MST markers were not found in any of the coastal samples [174]. However, minimal concentrations of FIB and E. coli were detected, suggesting that wastewater discharge in these locations has little effect. This is one of the few studies that has been published that has not been able to find PMMoV in any samples of environmental water. Only 33.3% (4/12) of the water samples, collected from southeast Queensland, Australia, tested positive for PMMoV according to the study's findings. These samples had concentrations that ranged from 3.6×10^4 to 8.6×10^4 GC/L [176].

Rosario et al. evaluated the durability of the PMMoV detectability and found that it was still detectable by qPCR after 7 days of incubation. This led the researchers to estimate that the half-life of PMMoV in saltwater at 31 to 33 °C is 1.54 days [144].

5.1.6. Global Distribution and Temporal Stability

In treated and untreated wastewater over a year, PMMoV and AiV displayed steady titers [45]; however, peak AiV levels were found in Japanese wastewater in the winter and spring [154]. Likewise, crAssphage, AdV and PyV did not exhibit any seasonal variations in concentrations in river, seawater, and treated and untreated wastewater samples [54]. However, higher AdV concentrations were found in treated wastewater collected in Wales throughout the summer compared to the winter and spring, which was most likely brought on by dry conditions and a temporary rise in population brought on by summertime tourist [195]. Additionally, in Norway during January through March, untreated wastewater had greater AdV contents than during April through December [74]. In Egyptian wastewater collected during the spring and summer, AdV prevalence was lower than it was in the autumn and winter [196]. Similarly, AdVs were found in river water samples taken from Japan and Germany in the summer and autumn at low concentrations, respectively [197,198], perhaps as a result of the dry weather. In contrast, PMMoV revealed no seasonality in river water [144,153]. These results collectively imply that the markers are detectable and measurable year-round, allowing for continual assessment of wastewater contamination.

5.2. Suitability of PMMoV as a Viral Indicator of Human Fecal Pollution
5.2.1. Advantages of PMMoV as a Viral Indicator of Fecal Pollution

The remarkable benefit of PMMoV as a fecal indicator is that it can be more regularly detected in quantifiable and higher concentrations without substantial seasonal changes in environmental incidence than any human virus (Table 3) [31]. Consequently, PMMoV can exist anywhere human enteric viruses do, and PMMoV qPCR results are a sensitive biological marker for the detection of viral infections in a specific environmental water sample [165]. The use of PMMoV as a fecal indicator can be expanded to other treatment procedures such as membrane filtration, though more research is required to affirm whether PMMoV reductions caused by various disinfection techniques are comparable to those caused by enteric viruses in the latter case [150].

As a viral tracer of fecal pollution, PMMoV was found to have more advantages than chemical markers [199]. This is partially due to the fact that PMMoV ought to behave more like enteric viruses than chemical indicators [155]. According to Kuroda et al., the effectiveness of PMMoV as a fecal indicator in surface water was demonstrated to be similar to that of caffeine, a commonly used chemical marker for human feces contamination in water bodies [150]. In particular, the quantity of PMMoV in untreated wastewater, concentration dynamic range, persistence and ubiquity in surface water were comparable to or greater than those of caffeine. Due to its great abundance, stability in aquatic habitats and absence of seasonal fluctuations, PMMoV has the potential to be used as a microbial source tracking (MST) marker [174]. In fact, PMMoV has gained significant attention as a new MST technique and has been used as a viral marker in MST research examining human fecal/sewage pollution in coastal waters [200].

Table 3. Criteria for PMMoV as an ideal human fecal indicator.

Selection Criteria	Applicability of PMMoV	Reason	Reference
Could be detected in all water types	Applicable	High prevalence in all types of water	[144]
Simple methodology of testing	Applicable	Can be examined alongside the other viral pathogens	[150]
Comparatively more durable than the most enteric pathogens	Applicable	Enduring compared to human enteric viruses	[199]
The incidence of indicator is associated with enteric viruses	Applicable	Greater frequency than the majority of enteric pathogens	[69]
There is a relation between the indicator prevalence and the level of fecal contamination.	Applicable	However, it can be too persistent to detect new contamination.	[152]
No aquatic growth	Applicable	Without its host plant, there is no replication	[201]
Member of the microflora of warm-blooded animals	Applicable	Highly abundant in human feces	[151]

5.2.2. Limitations of Utilizing PMMoV as a Viral Indicator of Fecal Pollution

Compared to human viruses, there are changes in morphology and surface charge. The morphology of PMMoV (rod shaped) differs noticeably from that of human enteric viruses (icosahedral shaped) [183]. Under some conditions, this may result in variations in the way the environment behaves, removal/reduction rates during treatment operations and recovery efficiency for virus concentration techniques [202]. It is necessary to conduct more research to ascertain the contribution of each of these elements to the notable differences in viral capture and removal behaviors between PMMoV and enteric viruses of interest [155].

Due to its unpredictable incidence and behavior when compared to human viruses, PMMoV has been shown to have limitations as a viral indicator in a number of investigations [203]. Due to the extremely low detection rates and concentrations of PMMoV in groundwater, tap water and bottled water, as well as the fact that their occurrence did not coincide with that of pharmaceuticals, personal care products or enteric viruses, Kuroda et al. came to the conclusion that PMMoV is not suitable as a fecal indicator or tracer in these sources of water [150]. Hamza et al. asserted that due to PMMoV's exceptionally high environmental stability, it may not be appropriate for identifying fresh fecal pollution in water bodies, which is likely to be associated with infections [145]. Additionally, despite the discovery of FIB, a research carried out in Bolivia revealed that PMMoV was not found in any surface water samples [204]. However, a recent study reported PMMoV abundance in surface water, with intact viral capsid [199]. Therefore, it was proposed that PMMoV limitation could be solved relying on the detection of intact PMMoV virus particles and that the absence of intact PMMoV could assure the viral safety of surface-water-dependent tap water [199].

5.3. Application in Risk Assessment

In risk-based studies of human contact with ecosystems affected by wastewater, novel indicator viruses like PMMoV offer substantial potential benefits [205]. The ability to detect wastewater contamination in the environment and assess the ensuing risk to human health is increased by greater representation of pathogenic viruses and high quantities in wastewater [206]. A tool called the Quantitative Microbial Risk Assessment (QMRA) makes it possible to estimate the risk to human health and the related uncertainty [207]. The most precise technique to assess risk is to directly measure infectious pathogens; however, it may not be possible to measure every pathogen in a given environment due to low concentration, low prevalence or a variety of potential pathogen targets [208]. Using a ratio to calculate pathogen concentrations based on indicator concentrations is one way to deal with issue. This technique presupposes contamination from a single source (usually wastewater), as well as identical pathogen and indicator fate and travel [209]. The present World Health Organization advice to convert between FIB concentrations and viral pathogen

concentrations is the foundation for this strategy [210]. In recent QMRAs, adenovirus and PMMoV have been used as viral indicators to represent viral pathogens using a ratio method [211]. This method was employed by Crank et al. using PMMoV in a QMRA that demonstrated the potential to lower the existing US EPA Recreational Water Criteria of around 32 illnesses per 1000 swimmers to approximately 1 ailment per 1000 swimmers (based on fecal indicator bacteria detection limits) [211]. However, the fecal indicator to pathogen ratio, which is typically only indicative of a fresh sewage contamination incident, is a major drawback of this approach [212]. Since viral infections account for the majority of gastrointestinal disorders, the WHO acknowledges the importance of a risk-based approach in understanding and preventing human disease [213]. To go forward, better methodologies for quantification and risk characterization are required [152].

5.4. Correlation with Other Fecal Indicators

Following fresh sewage pollution episodes, PMMoV correlates well with bacterial fecal markers such as Bacteroides HF183 in sewage wastewaters, and *E. coli* and enterococci in sewage waters [214]. In surface waters affected by sewage, PMMoV was present more frequently and in higher concentrations than adenovirus, polyomavirus and norovirus. In addition, in sewage-contaminated waters, PMMoV was of higher correlation to enteric viruses than adenoviruses, enterovirus, Aichi virus and polyomavirus (Figure 6) [153]. The PMMoV decrease levels during wastewater treatment (0.7–0.9 $\log_{10}$ reduction) were also lower than those of other viruses, indicating resistance to wastewater treatment [145]. This suggests that PMMoV would be more useful as a cautious indication of fecal contamination. Compared to bacterial markers, PMMoV have a longer environmental persistence [205]. Adenovirus, polyomavirus and torque teno virus are among the other viral markers whose decay is faster than that of PMMoV [54]. It is likely that changes in their genomes are what account for the differential in crAssphage and PMMoV persistence (dsDNA vs. ssRNA, respectively) [203].
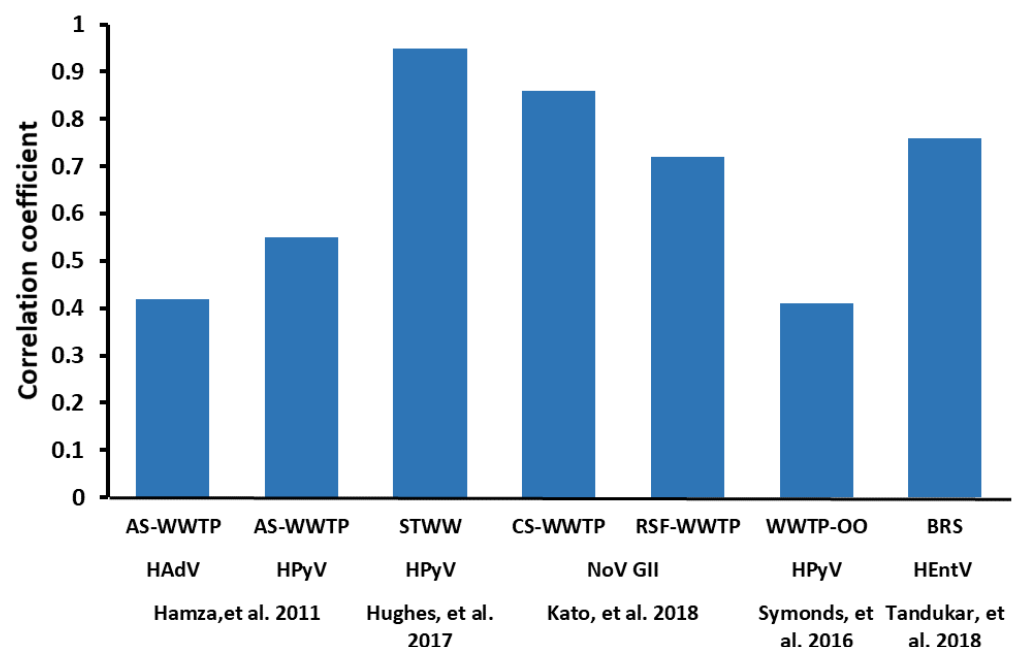

Figure 6. PMMoV correlation to a wide spectrum of water viral pathogens in both WWTPs as well as rivers and oceans [25,152,176,180,186]. AS-WWTP: active sludge-based WWTP, STWW: secondary treated wastewater, CS-WWTP: coagulation–sedimentation-based treatment plant, RSF-WWTP: rapid sand filtration (RSF)-mediated WWTP, WWTP-OO: WWTP ocean outfall, BRS: Bagmati river stream, HPyV: human polyomavirus, HEntV: refers to 8 human enteric viruses cumulatively, including Aichi virus 1, enteroviruses, human cosaviruses, HAdVs, NoV GI and NoV GII, rotaviruses group A and saliviruses.

5.5. Removal in Wastewater and Wastewater Treatment Plant

In wastewater treatment facilities in Germany and the United States, qPCR measurements of PMMoV reduction ranged from <1 to 3.7 $\log_{10}$ [155]. In Thailand, coagulation and filtration were used to remove 1–2 $\log_{10}$ of contaminants from drinking water, while reverse osmosis was used to remove PMMoV to levels below LOD [175].

Hamza et al. published the first study on PMMoV reduction efficacy by wastewater treatment in 2011. In a wastewater treatment facility in Germany that used a traditional activated sludge technique, this study revealed PMMoV reductions ranging from 1.7 to 3.7 $\log_{10}$ ($n = 12$) [152]. Consequently, Kitajima et al. demonstrated that the reduction efficiencies of PMMoV by activated sludge and trickling filter were $0.76 \pm 0.53 \log_{10}$ ($n = 12$) and $0.99 \pm 0.64 \log_{10}$ ($n = 12$), respectively [47]. In contrast, viral removal by two wastewater treatment pond systems was investigated by Symonds et al. in Bolivia [175]. They found that neither system showed any discernible decrease in PMMoV and enteric viruses (NoV genogroup I [GI] and rotavirus). For additional wastewater treatment, Rachmadi et al. evaluated the attenuation of PMMoV by two surface flow wetlands in Arizona, United States, and found that there was little to no removal (≤ 1 log) [69]. Based on controlled laboratory tests, they also looked at the durability of PMMoV qPCR signal in wetland water and found that it was unaffected by a temperature range of 4 to 37 °C for 21 days. Collectively, PMMoV is more enduring in wastewater reclamation systems than human enteric viruses [54]. These findings are in line with a laboratory-scale investigation that found no appreciable reduction in PMMoV over a 21-day period at temperatures ranging from 4 to 37 °C. These findings highlight that PMMoV could be more stable in river water than enteric viruses like HAdV [152]. This might be due to that PMMoV having a more durable capsid structure than human enteric viruses [183]. Despite the fact that enteric viruses have a round-shaped virion with a diameter of 30–90 nm while PMMoV has an extremely stable rod-shaped virion with a length of more than 300 nm, PMMoV's behavior in the environment is not always comparable to that of enteric viruses [154]. However, PMMoV appears to be useful as a conservative "viral tracer" in wastewater reclamation systems because of its low average reduction rate [150] (Figure 7).
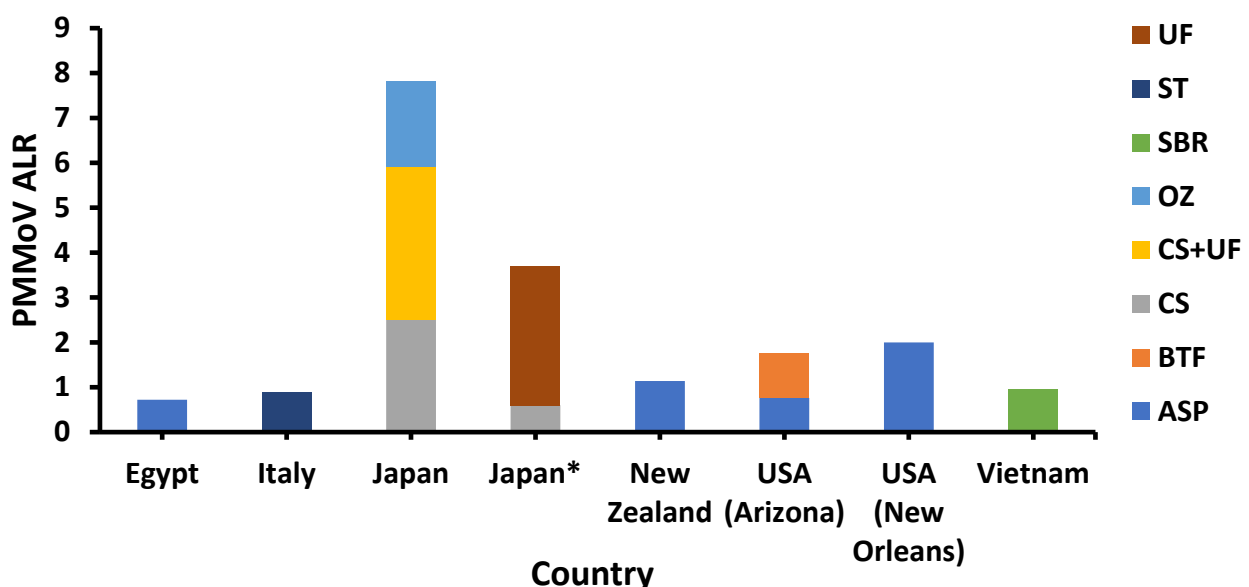

Figure 7. Average log reduction (ALR) rate of PMMoV by different wastewater treatment approaches in various countries [150,154,161,177,180,187,188,191]. UF: ultrafiltration, ST: secondary treatment, SBR: sequential batch reactor, OZ: ozone treatment, CS: coagulation sedimentation, BTF: biological trickling filter (Biotower), ASP: activated sludge process. *: refers to a different study conducted in Japan [177].

6. Conclusions

Disinfection treatment procedures must lower virus concentrations in wastewater before direct water reuse or release into environmental waters in order to safeguard public health as well as the microbial safety of drinking water. Because many pathogenic viruses are found in concentrations that are too low to quantify and because human enteric viruses that are dangerous to public health, like norovirus, lack culture-based methods or are challenging to culture, it is frequently challenging to estimate the log reduction of viruses achieved by a particular treatment technique. As a result, testing for viral reductions frequently involves molecular techniques like quantitative reverse transcription PCR. Unlike other viruses, PMMoV's high concentrations in wastewater allow for the quantification of virus gene copy removal at full-scale treatment plants, making it an ideal virus process indicator to assess drinking water, wastewater and water reclamation treatment technologies and facilities. Additionally, PMMoV decrease levels after wastewater treatment and water treatment for drinking are often comparable to those of human enteric viruses. In contrast to other viral indicators (bacteriophages MS2 and X174), PMMoV gene copy removal more frequently has a significant, positive correlation with the gene copy removal of human enteric viruses during the treatment of drinking water (at plants with ozonation, coagulation–sedimentation, rapid sand filtration and biological activated carbon treatments).

PMMoV is useful for quantifying virus reductions at smaller scales of innovative water treatment technologies, such as point-of-use household drinking water treatment systems, in addition to having practical applications for measuring virus reduction at full-scale treatment facilities. The incorporation of culture-based analyses (which would require plant growth chambers) and/or selective pretreatment for infectious particles could improve future virus reduction analyses to evaluate treatment efficacy, as PMMoV is currently quantified using molecular methods that cannot determine virus infectivity.

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