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Pepper mild mottle virus as an indicator and a tracer of fecal pollution in water environments: Comparative evaluation with wastewater-tracer pharmaceuticals in Hanoi, Vietnam

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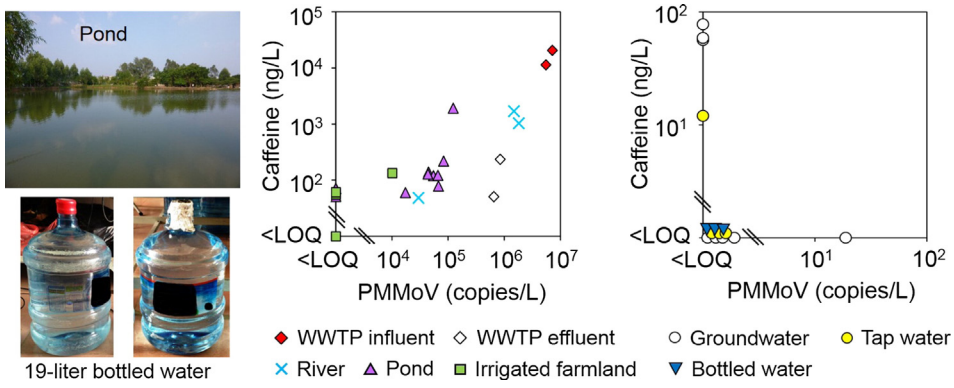
HIGHLIGHTS

- PMMoV and PPCPs were prevalent in surface water and wastewater in Hanoi.
- The detection rate of PMMoV was higher than human enteric viruses in surface water.
- The detection rate of PMMoV was low in groundwater, tap water and bottled water.
- PMMoV was a sensitive fecal indicator of pathogenic viruses in surface water.
- PMMoV was useful as a conservative tracer of fecal pollution in surface water.

GRAPHICAL ABSTRACT



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ABSTRACT

We analyzed pepper mild mottle virus (PMMoV) in 36 samples taken from surface water, wastewater, groundwater, tap water and bottled water in Hanoi, Vietnam. We then compared the occurrence and fates of PMMoV with pharmaceuticals and personal care products (PPCPs), which are known wastewater tracers. PMMoV was detected in 94% of the surface water samples (ponds, water from irrigated farmlands and rivers) and in all the wastewater samples. The PMMoV concentration ranged from 5.5×10^6–7.2×10^6 copies/L in wastewater treatment plant (WWTP) influents, 6.5×10^5–8.5×10^5 copies/L in WWTP effluents and 1.0×10^4–1.8×10^6 copies/L in surface water. Among the sixty PPCPs analyzed, caffeine and carbamazepine had high detection rates in surface water (100% and 88%, respectively). In surface water, the concentration ratio of PMMoV to caffeine remained unchanged than that in WWTP influents, suggesting that the persistence of PMMoV in surface water was

Abbreviations: PMMoV, pepper mild mottle virus; PPCPs, pharmaceuticals and personal care products; HEV, human enteric viruses; AdV, human adenovirus; PyV, human polyomavirus; AiV1, aichi virus 1; NoV GI, norovirus genogroup I; NoV GII, norovirus genogroup II; EV, enterovirus; *E. coli*, *Escherichia coli*; TC, total coliforms; WWTP, wastewater treatment plant; BQL, below the quantification limit; BDL, below the detection limit; LOQ, limit of quantification; LOD, limit of detection.

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comparable to that of caffeine. The persistence and the large concentration ratio of PMMoV in WWTP influents to the method detection limit would account for its ubiquitous detection in surface water. In comparison, human enteric viruses (HEV) were less frequently detected (18–59%) than PMMoV in surface water, probably because of their faster decay. Together with the reported high human feces-specificity, our results suggested that PMMoV is useful as a sensitive *fecal indicator* for evaluating the potential occurrence of pathogenic viruses in surface water. Moreover, PMMoV can be useful as a moderately conservative *fecal tracer* for specifically tracking fecal pollution of surface water. PMMoV was detected in 38% of the groundwater samples at low concentrations (up to 19 copies/L). PMMoV was not detected in the tap water and bottled water samples. In groundwater, tap water and bottled water samples, the occurrence of PPCPs and HEV disagreed with that of PMMoV, suggesting that PMMoV is not suitable as an indicator or a tracer in those waters.

1. Introduction

Fecal pollution of aquatic environments is a major concern for public health worldwide, as it exposes humans to pathogens. Microbial and chemical indicators are useful for monitoring fecal pollution, identifying pollution sources, controlling and preventing pollution events, evaluating the performance of water and wastewater treatment and ensuring water safety. *Fecal indicators* are used to estimate the occurrence of fecal-borne pathogens in water. Historically, bacteria such as *Escherichia coli* (*E. coli*) are commonly used as fecal indicators, but it is widely recognized that the occurrence of such bacterial indicators is inconsistent with pathogenic viruses (Baggi et al., 2001). Therefore, for the evaluation of fecal-borne viruses, various viruses have been proposed as fecal indicators, including human enteric viruses (HEV), such as human adenovirus (AdV) and human polyomavirus (PyV) (Hamza et al., 2009; Pina et al., 1998).

Recently, pepper mild mottle virus (PMMoV) has been proposed as a potential fecal indicator in water (Hamza et al., 2011; Kitajima et al., 2014; Rosario et al., 2009). PMMoV is a pathogen of peppers (*Capsicum* spp.) and its primary source in human feces is considered to be food products containing peppers (Colson et al., 2010; Zhang et al., 2006). PMMoV was the most abundant virus in human stool (Zhang et al., 2006) and contained in human feces at 10^5–10^{10} copies/g (Hamza et al., 2011; Zhang et al., 2006). Raw wastewater in Germany, Singapore, the US and Bolivia contained PMMoV at 10^6–10^{10} copies/L (Hamza et al., 2011; Kitajima et al., 2014; Rosario et al., 2009; Symonds et al., 2014). In animal feces, PMMoV has been found only in some samples of limited animal species (chickens, cows, geese and seagulls) at much lower concentrations (Hamza et al., 2011; Rosario et al., 2009). Studies in the US, Singapore, Germany and Japan show that PMMoV is detected more frequently and in greater abundance, with lesser temporal variation, in wastewater, surface water and marine water than HEV including AdV and PyV (Hamza et al., 2011; Haramoto et al., 2013; Kitajima et al., 2014; Rosario et al., 2009). PMMoV is more persistent in surface and marine waters than HEV (Hamza et al., 2011; Rosario et al., 2009). In Japan, PMMoV was detected in only 15% of graywater (domestic wastewater excluding toilet wastewater) samples investigated, with an average concentration of 10^3 copies/L, showing that toilet wastewater (i.e. blackwater) is the predominant source of PMMoV in the environmental waters (Ng, 2013). The above-mentioned PMMoV data illustrate that PMMoV is potentially useful as a fecal indicator in environmental waters worldwide, but limited data is available on its geographic distribution and co-occurrence with pathogens in various water bodies. Especially, such data does not exist in developing nations. Furthermore, the occurrence and fates of PMMoV in stagnant surface waters (e.g. ponds, lakes), groundwater, tap water and drinking water have never been investigated.

In addition to being used as a fecal indicator, the reported PMMoV data suggest the possibility that PMMoV is useful as a fecal tracer, as it may be a more specific and suitable tracer for sensitively identifying and tracking fecal pollution of water than the known chemical wastewater tracers. *Wastewater tracers*, or *wastewater markers*, have been proposed and used as wastewater-specific indicators, which can sensitively trace even a low level of wastewater pollution (Glassmeyer et al., 2005). In contrast to fecal indicators, wastewater tracers primarily aim at evaluating the wastewater pollution of water, regardless of the occurrence of pathogens. Useful wastewater tracers are synthetic chemicals having frequent and abundant occurrence in contaminated waters, such as pharmaceuticals and personal care products (PPCPs) (Glassmeyer et al., 2005; Seiler et al., 1999). In particular, caffeine and carbamazepine are well-known wastewater tracers. As carbamazepine is a highly persistent compound, it is an indicator of accumulative wastewater pollution of surface water and groundwater (Gasser et al., 2010; Nakada et al., 2008). Caffeine undergoes fast and substantial degradation in biological wastewater treatment processes, but it is moderately persistent in surface and coastal waters (Benotti and Brownawell, 2007; Bradley et al., 2007; Buerge et al., 2003). Although wastewater-tracer PPCPs can indicate microbial pollution of water (Sauvé et al., 2012; Sidhu et al., 2013), not all wastewater tracer PPCPs are derived from fecal materials. For example, caffeine is largely derived from wastewater from sinks (Seiler et al., 1999). Therefore, a detection of caffeine may not always indicate a fecal pollution of water in areas where graywater is disposed separately from blackwater. In comparison, PMMoV, which is reported to be predominantly derived from human feces, may enable a more specific evaluation of fecal pollution than PPCPs. Although the notion that PMMoV can be used as a tracer of fecal pollution was first presented by Rosario et al. (2009), the occurrence, fates and utility as a fecal tracer of PMMoV in various environmental waters have never been evaluated through comparison with wastewater-tracer PPCPs. The use of both microbial and chemical indicators is beneficial for evaluating indicator behavior, such as source-specificity, fate and persistence (Glassmeyer et al., 2005; Jeanneau et al., 2012).

In Hanoi, Vietnam, the water environment is widely polluted by wastewater because of insufficient collection and treatment of domestic wastewater with insufficient drainage of stormwater. So far, the literature on the occurrence of PPCPs in Vietnamese waters has been limited to antibiotics and several pharmaceuticals (e.g. Hoa et al., 2011; Tran et al., 2014b); hence, the occurrence of a diverse range of PPCPs, including caffeine and carbamazepine, has not been studied. The occurrence of PMMoV in Vietnamese waters is also unknown.

Hence, the objectives of this study were 1) to investigate the occurrence of PMMoV and PPCPs in surface water, wastewater, groundwater, tap water and bottled water in Hanoi; 2) to compare the fate of PMMoV in those waters with PPCPs; and 3) to evaluate the utility of PMMoV as a fecal indicator and a fecal tracer in water environments. Thirty-six samples in total were analyzed, while PPCPs were analyzed in five more samples. Groundwater, tap water and bottled water samples with volumes of up to 100 L were tested so as to detect low levels of PMMoV (detection limit: ~1 copy/L). We measured sixty PPCPs to investigate water pollution by a diverse range of PPCPs. We also compared the occurrence of PMMoV with those of HEV (AdV; aichi virus 1, AiV1; norovirus genogroup I, NoV