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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Graphical abstract

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 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 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 from that in WWTP influents, suggesting that the persistence of PMMoV in surface water was 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 nor a tracer in those waters.

Keywords: caffeine; carbamazepine; fecal indicator; human enteric virus; qPCR; wastewater tracer

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; TC, total coliforms; WWTP, wastewater treatment plant; BQL, below the quantification limit; BDL, below the detection limit; LOQ, the limit of quantification; LOD, the limit of detection.

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 greywater (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 be 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 greywater 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 copies/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 GI; norovirus genogroup II, NoV GII; enterovirus, EV) and bacterial indicators (*E. coli*; total coliforms, TC) measured in the same samples.

2.# EXPERIMENTAL SECTION

2.1#Site description

Hanoi, the capital of Vietnam, is located in the upper part of the Red River basin, *ca.* 100 km from the sea. In the urban areas of Hanoi, 70% of the total volume of tap water (i.e. 700,000 m³/d) is supplied from groundwater by the public water company. The remaining 30% is taken from surface water and supplied by a public-private-partnership scheme, as of 2010 (PPJ-VIAP-HUPI, 2011). In the suburban and rural areas, water for household use is obtained either from tap water using groundwater or from private tube wells. The groundwater is abstracted from the upper aquifer (typically 10–20 m deep) by households or from the lower aquifer lying underneath (20–70 m deep) by the water company. The only surface water source for the tap water is the Da River, a tributary of the

Red River located 30 km upstream of Hanoi. In water treatment plants, groundwater is treated by aeration, iron precipitation, rapid sand filtration and chlorination, whereas river water is treated by pre-sedimentation, coagulation, flocculation, sedimentation, rapid sand filtration and chlorination. In suburban and rural areas, groundwater from private tube wells is typically treated in households by sand filters (Do et al., 2014).

In the urban areas in Hanoi, most (90.5%) of the households are equipped with septic tanks (Harada et al., 2008). In those households, blackwater goes down into the septic tanks and the effluent is discharged into sewers or nearby canals. However, from the households without septic tanks, blackwater goes straight into sewer pipes or nearby canals. From all the households, greywater is discharged directly into sewers or canals. These sewers and canals also work as stormwater conduits. Fig. 1 is a map of the central districts of Hanoi, showing the locations of rivers and canals. On the right bank of the Red River, most of wastewater and stormwater in the urban area is collected by the canals and transported to the south of the city. There, a fraction of the wastewater is treated at the Yen So WWTP (treatment capacity: 120,000 m³/d as of 2013) by sequential batch reactor (SBR). The SBR effluent and the majority of the untreated urban wastewater are discharged together into the Red River at the Yen My site, near the Yen So WWTP. The rest of the urban wastewater is discharged into the Nhue River in the west. More detailed locations of the Yen So WWTP and the canals are given in Fig. S1 (Supplementary information; hereafter, SI). In the suburban areas and the rural areas, most households are equipped with septic tanks to treat blackwater. The septic tank effluent, greywater and stormwater are all discharged into the nearby ponds and streams, which are abundant in

the suburbs of Hanoi. Irrigated farmlands for rice and vegetables also constitute major water bodies in the suburban and rural areas.

Fig. 1. The map of the central districts of Hanoi, the sampling locations, and the number of samples for PMMoV analysis. (2-column fitting image)

2.2 Sampling locations and sampling frequency

Water samples (36 samples; PPCPs were additionally analyzed in 5 more samples) were collected in September 2013. The locations of the sampling sites and the number of samples are shown in Fig. 1 and Fig. S1. Pond water and groundwater were collected at four suburban sites (Linh Dam: LD; Tay Mo: TM; Yen So: YS; Ngoc Hoi: NH). At site LD, water from irrigated farmlands (hereafter, farmlands) was also examined because both

ponds and farmlands are abundant in the area (Fig S1). River water samples were taken at three locations: from the Red River at Ba Giang, upstream of the urban area; from the Red River at Yen My, 500 m downstream of the point of wastewater discharge from the Yen So WWTP; and from the Nhue River receiving wastewater from the western part of the urban area. Wastewater samples were taken at the Yen So WWTP. The WWTP influents and the effluents treated by SBR were sampled in the morning and in the afternoon on the same day. Samples from ponds, farmlands, rivers and wastewater were generally taken once from each site by grab sampling from the top layer; however, samples were taken twice, at an interval of a few days, from one of the ponds and one of the farmlands at site LD and from one of the ponds at site TM. Tap waters were sampled at three locations in the city center and at three locations in site NH; 2 of them were sourced from river water and the others from groundwater. Bottled water samples were randomly selected from the commercially-available 19-L bottles produced by four different major local manufacturers. In Hanoi, bottled water is typically produced from groundwater by treatment with rapid sand filtration, ultrafiltration, activated carbon and reverse-osmosis (RO) membranes.

2.3 Analysis of PPCPs

We employed the method of PPCP analysis described elsewhere (Narumiya et al., 2013) with minor modifications. Briefly, 100–1000 mL of water samples were taken in polyethylene bottles, to which we added ascorbic acid at 1.0 g/L and EDTA at 0.5 g/L and then kept cool in darkness. Immediately after being taken, the samples were filtered through glass fiber filters (GF/F, 0.7 μ m, Whatman, UK) and isotope-labeled surrogate

compounds were added. The 60 PPCPs were concentrated by solid-phase extraction in an OASIS HLB cartridge (200 mg, 6 cc, Waters, MA) within 1 d after sampling. The cartridges were kept at 4 °C in darkness and transported to Kyoto University, Japan, where the compounds retained in the cartridge were eluted with 6 mL of methanol after being dried for 1 h under gentle air pressure in a glass manifold. The eluents were dried with nitrogen gas and dissolved in 1 mL of 0.1% formic acid and methanol (85:15, v/v). The PPCPs were measured by ultraperformance liquid chromatography – tandem mass spectrometry (LC-MS/MS) and quantified by an alternative surrogate method (Narumiya et al., 2013).

PPCP concentrations were reported after a three-tiered assessment as follows. First, the concentration in the sample in the injection vial had to be more than three times higher than that in the running blank for each sampling day; otherwise, the concentration was reported as BQL. Second, the signal to noise ratio (S/N) had to be more than 10; otherwise, the concentration was reported as BDL. Finally, the recovery of surrogate compounds had to be more than 30%; otherwise, the concentration was reported as NA (not available). For running blank samples, 1000 mL of commercially available bottled water was analyzed on each sampling day; each sample was taken from an unopened non-reusable 2-L bottle so that minimum contamination could be expected. Using ultrapure water (Milli-Q waters) as a blank, the PPCP concentration in the bottled water used as the running blank was determined as the BQL in the case of most PPCPs. The LOQ was set at the larger value of either three times of the running blank concentration or the sample concentration with an S/N of 10.

2.4 Analysis of PMMoV and HEV

The methods used for sample collection and concentration of PMMoV and HEV was based on those in the previous studies (Hata et al., in press; Katayama et al., 2002) with minor modifications. From ponds, farmlands, rivers and wastewater, small volumes (50–150 mL) of samples were taken in pre-sterilized polyethylene containers (180 mL volume). Briefly, 2.5 M MgCl_2 was added to each sample to obtain a final concentration of 25 mM; the sample was then filtered through an electronegative filter (diameter 47 mm, pore size 0.45 μm ; Merck Millipore, MA). The filters were washed with 200 mL of 0.5 mM H_2SO_4 (pH 3.0), and virus was then eluted with 5 mL of 1.0 mM NaOH (pH 10.8). The eluate was recovered in a tube containing 25 μL of 100 mM H_2SO_4 (pH 1.0) and 50 μL of 100 $\times$ Tris-EDTA buffer. Samples were further concentrated with a Centriprep YM-50 centrifugal filter concentrator (Merck Millipore, MA) to approximately 650 μL after transportation to Japan.

In the case of groundwater, tap water and bottled water samples, large-volume samples (64–100 L) were concentrated by using a cartridge containing a mixed cellulose ester filter (Opticap XL 2 disposable capsule filter; pore size 0.5 μm , filtration area 0.1 m^2 ; Merck Millipore, MA), following Hata et al. (in press). Briefly, water samples were supplied to the filter via a sterilized sampling hose, driven by pressure from a pump or tap, or by vacuum provided by an aspirator (JPV; VELP Scientifica, Italy). The sample was mixed in the hose with 2.5 M MgCl_2 injected by a dripper 1 m upstream of the filter to obtain a final concentration of 25 mM. After concentration, the filter was rinsed with 2 L of 0.5 mM H_2SO_4 (pH 3) and 1 L of distilled water. Viruses were eluted with 200 mL of 1.0 mM NaOH (pH 10.8) and collected in a test tube containing 1 mL of 100 mM H_2SO_4 (pH

1.0) and 2 mL of 100× Tris-EDTA buffer. Back in Japan, the eluate was re-concentrated to approximately 900 µL by using a Centricon Plus-70 filter (Merck Millipore, MA). The further-concentrated small- and large-volume samples were stored at −20 °C until the nucleic acid was extracted as follows.

The methods used to extract the viral genomes and to quantify PMMoV and HEV by quantitative PCR (qPCR) was based on those in the previous studies (Haramoto et al., 2013; Katayama et al., 2008; Kitajima et al., 2008; Zhang et al., 2006). Briefly, for RNA viruses (PMMoV, AiV1, NoV GI, NoV GII and EV), the viral RNA was extracted from 140 µL of each further-concentrated sample by using a QIAmp viral RNA minikit (Qiagen, Netherlands) followed by reverse transcription (RT) for cDNA synthesis with a High Capacity cDNA Reverse Transcription Kit (Life Technologies, CA). For AdV, the viral DNA was extracted from 200 µL of each further-concentrated sample by using a QIAamp DNA Mini Kit (Qiagen, Netherlands). The synthesized cDNA and the extracted DNA were analyzed by qPCR with an ABI Sequence Detection System 7500 (Applied Biosystems, CA). Viral genomes were quantified by using 10-fold serial dilutions of the DNA standard plasmids (1.0×10^{-1} – 1.0×10^5 copies per tube) in relation to their threshold cycle (C_T). Standard PMMoV plasmids were prepared from strain PV-570 (ATCC, VA). The limit of quantification (LOQ) was determined to be the concentration with a C_T value of 40. The LOQ of PMMoV was 5.4 copies per tube; it was equivalent to 4.6×10^3 – 1.2×10^4 copies/L for small-volume (50–150 mL) samples and 7.0–14 copies/L for large-volume (64–100 L) samples. Similarly, the LOQ of HEV was determined at 6.8×10^2 – 6.2×10^4 copies/L for small-volume samples and 1.0–74 copies/L for large-volume samples. When the C_T value was more than 40, the concentration was reported as below the quantification

limit (BQL). When viral genomes were not found in the tube, the concentration was reported as below the detection limit (BDL); the limit of detection (LOD: sample concentration equivalent to 1 copy/tube) of target RNA viruses, including PMMoV, was 3.4×10^2 – 1.0×10^3 copies/L for small-volume samples and 0.66–1.3 copies/L for large-volume samples. The LOD of AdV was 2.4×10^2 – 7.0×10^2 copies/L for small-volume samples and 0.46–0.91 copies/L for large-volume samples. In RT-qPCR, samples were analyzed in duplicates. Signal of PMMoV genomes was not found in a negative control sample. For AdV, AdV 40 and 41, which cause enteric diseases, were analyzed.

The recovery of PMMoV spiked in 2 L of sterilized ultrapure water (Milli-Q water, Merck Millipore, MA) was 79–164%. In the case of concentration from large volumes of environmental water, RNA extraction and RT-qPCR may be inhibited because of the concentrated water matrix. We subjected one sample concentrated from a large volume of groundwater (100 L) to further analysis to evaluate inhibition by using murine norovirus (MNV); the further-concentrated sample (eluent from a Centricon Plus-70 filter) and the subsequent cDNA extract were separately spiked with MNV and analyzed; however, the recovery of spiked MNV was 38–154%, indicating that there was no significant inhibitory effect on RNA extraction or RT-qPCR of MNV (see S1 in SI for details).

2.5 Analysis of bacterial indicators

E. coli and TC were enumerated using the membrane filtration method (Method 10029, USEPA); samples (200 mL or less) were filtered through a disposable 37 mm monitor unit (mixed cellulose ester; pore size: 0.45 μ m; Advantec, Japan) to trap *E. coli*

and TC on the membrane surface. The m-ColiBlue 24 Broth (Hach, Co) was added to the membrane holders, and they were incubated at 37 °C, under aerobic conditions, for 24 h.

2.6 Statistical analyses

The concurrence (agreement of presence or absence in samples) was calculated for each pair of PMMoV, the PPCPs with the highest detection rates, HEV and bacterial indicators in surface water samples, by adding the percentage of co-presence (both positive) and that of co-absence (both negative) for each pair, following Sidhu et al. (2013). To compare concentration ratios of PMMoV to PPCPs between surface water and WWTP influents, a 1-tailed Student's *t*-test was performed using SPSS version 20.0 (IBM, NY).

3.# RESULTS AND DISCUSSION

3.1 Occurrence of PPCPs

PPCPs were ubiquitously detected in pond, farmland, river and wastewater samples (Table 1). Thirty-six compounds out of 60 PPCPs tested were detected, of which at least one was found in all the samples. The concentrations of all the PPCPs analyzed can be found in Table S1. In surface water, the most frequently detected PPCPs were caffeine (100%), lincomycin (100%) and carbamazepine (88%). The concentrations of the three PPCPs with respect to sample types are shown in Fig. 2. Most of the PPCPs were detected at the highest concentrations in the WWTP influent samples; among them, caffeine (2.1×10^4 ng/L), acetaminophen (2.0×10^4 ng/L) and sulfamethoxazole (2.6×10^3 ng/L) had the highest levels. PPCP concentrations in ponds, farmlands and rivers were within 0.5–3

orders of magnitude lower than those in WWTP influents (Fig. 2). In surface water where samples were taken twice at an interval of a few days, the concentrations of the most PPCPs remained almost constant. The wastewater samples taken in the morning and in the afternoon also had similar concentrations for most PPCPs. The caffeine concentration in WWTP effluents were lower than the influents by 2 $\log_{10}$, whereas the carbamazepine concentration were almost constant between the influents and the effluents. The above occurrence and abundance of caffeine and carbamazepine in surface water and wastewater samples were consistent with the results in the literature (Benotti and Brownawell, 2007; Buerge et al., 2003; Nakada et al., 2006). The concentrations of caffeine (1.1×10^4 and 2.1×10^4 ng/L) and carbamazepine (41 and 57 ng/L) in our WWTP influent samples were similar to those in Japan (Nakada et al., 2006; Narumiya et al., 2009). Carbamazepine has been frequently detected in a preliminary study on lakes in a highly urbanized district of Hanoi (Tran et al., 2014b).

The prevalent occurrence of caffeine and carbamazepine in surface water indicated the widespread wastewater pollution in the water environment of Hanoi. Carbamazepine is a human-use, orally-taken pharmaceutical and excreted in urine and feces (Lienert et al., 2011). Caffeine is a popular ingredient of beverages, food products and pharmaceuticals. It is considered that the main source of caffeine in wastewater is greywater, which contains unconsumed caffeine-containing beverages and rinsing water of cups and glasses for those beverages down the sink (Seiler et al., 1999). Because both greywater and blackwater from households were discharged together into sewers, ponds or streams, both caffeine and carbamazepine can be used as wastewater tracers in environmental waters in Hanoi. Outdoor disposal of unconsumed beverages and

caffeine-containing greywater is not likely the source of caffeine in the studied waters, because at least one PPCP contained in human excreta (e.g. carbamazepine, lincomycin) was detected together with caffeine. The disposal of those pharmaceuticals and kitchen waste (e.g. spent coffee grounds and tea leaves) would not be the source of PPCPs in the waters, because they are collected as solid waste and are landfilled in remote sites.

Assuming that carbamazepine is a conservative wastewater tracer, the proportion of treated or untreated wastewater in the surface waters was estimated by the concentration ratio of carbamazepine in surface water to that in WWTP influents as follows.

$$\text{Proportion of wastewater (\%)} = \text{CBZ}_{\text{sample}} / \text{mean CBZ}_{\text{WWTPinfluent}} \times 100 \quad (1)$$

where CBZ is the concentrations of carbamazepine. In rivers, the upstream sample (Red River: Ba Giang) contained the smallest proportion of wastewater (<0.29%), whereas its downstream sample (Red River: Yen My) and an urban river sample (Nhue River) contained 12% and 14% wastewater, respectively. Pond and farmland samples contained <1.5% to 39% of wastewater. In those pond samples, the abundance of caffeine relative to that of carbamazepine is lower than that in WWTP influents, probably due to natural decay of caffeine in those ponds.

Apart from caffeine and carbamazepine, the relatively high detection rates of human and/or veterinary drugs (e.g. lincomycin, sulpiride, trimethoprim and sulfamethoxazole) in Hanoi surface waters agreed with the past studies in Vietnamese surface waters (Hoa et al., 2011; Shimizu et al., 2013). Triclosan and triclocarban have been expected to be chemical indicators which are more closely associated with microbial indicators than caffeine in surface water in Baltimore, the US (Young et al., 2008). The

two PPCPs, however, were not detected in surface water samples. This would be because the two PPCPs largely partitioned to particle phase (Young et al., 2008), whereas dissolved phase was used for PPCP analyses, and/or because the consumption of the two PPCPs was small.

In groundwater, tap water and bottled water, PPCPs were detected at lower concentrations and less frequently than those in surface water and wastewater (Table 2, Fig. S2). PPCPs were detected at higher rates in tap water (83%) than in groundwater (30%) and bottled water (50%). PPCP concentrations in those waters were mostly at levels of several ng/L, with the exception of caffeine (57–78 ng/L) and clofibric acid (110 ng/L). The occurrence of caffeine, carbamazepine and the other PPCPs were not consistent, regardless of the sources of the samples. The inconsistency would be attributed to the different abundance of PPCPs in source waters (e.g. infiltrating water to soil, raw water for piped water and bottled water) and the different fates in soil passage or water treatment processes (e.g. adsorption and degradation), as observed for PPCPs in drinking water treatment processes in the US (Benotti et al., 2008). This indicated that the use of multiple PPCPs is useful to infer wastewater contamination of those waters.

Table 1 Detection frequency and maximum concentrations of PPCPs in surface water and wastewater. Among 60 PPCPs tested, 36 PPCPs, for which we determined the concentration in at least one sample, are shown. (2-column fitting image)

	Frequency of detection (%)							Concentration range (min–max, ng/L)
	Surface water				Wastewater		Total	
	Pond <i>n</i> =11	Farmland <i>n</i> =3	River <i>n</i> =3	Total <i>n</i> =17	Influent <i>n</i> =2	Effluent <i>n</i> =2	<i>n</i> =4	
Acetaminophen	- *	-	-	-	50	-	25	2.0×10 ⁴
Atenolol	-	-	-	-	100	50	75	21–1.2×10 ³
Azithromycin	-	-	-	-	-	100	50	7.6–9.3
Bezafibrate	-	-	33	6	50	-	25	0.48–11
Caffeine	100	100	100	100	100	100	100	48–2.1×10 ⁴
Carbamazepine	91	100	67	88	100	100	100	0.61–58
Chlortetracycline	-	-	33	6	-	-	-	16
Ciprofloxacin	-	-	-	-	50	100	75	52–5.9×10 ²
Clarithromycin	36	-	-	24	-	100	50	2.8–1.1×10 ²
Cyclophosphamide	-	-	33	6	-	50	25	0.65–2.3
Diclofenac	9	-	67	18	100	100	100	2.8–1.1×10 ²
Disopyramide	9	33	-	12	-	100	50	0.11–1.1
Furosemide	-	-	-	-	100	-	50	1.2×10 ² –1.7×10 ²
Griseofulvin	-	-	-	-	50	-	25	38
Ibuprofen	-	-	-	-	50	-	25	2.8×10 ²
Isopropylantipyrine	-	-	-	-	100	-	50	4.1–4.4
Ketoprofen	-	-	-	-	50	-	25	6.5
Lincomycin	100	100	100	100	100	100	100	0.19–2.8×10 ²
Mefenamic acid	18	-	33	18	50	50	50	0.58–80
Metoprolol	18	-	67	24	100	100	100	1.7–85
Nalidixic acid	36	-	67	35	100	100	100	4.1–1.1×10 ²
Naproxen	-	-	-	-	100	-	50	26–27
Ofloxacin	-	33	-	6	50	100	75	15–1.3×10 ³
Primidone	-	-	-	-	50	-	25	1.9
Propranolol	-	-	-	-	-	100	50	2.0–2.7
Roxithromycin	9	-	-	6	-	100	50	0.85–16
Sulfadimidine	18	-	67	24	100	100	100	2.6–1.3×10 ²
Sulfamethoxazole	45	67	100	59	100	100	100	10–2.6×10 ³
Sulfapyridine	-	-	33	6	100	100	100	1.9–46
Sulpiride	82	67	67	76	100	100	100	0.68–1.1×10 ²
Tetracycline	-	-	33	6	-	50	25	3.5–3.9
Theophylline	18	-	67	24	100	100	100	2.2–9.2×10 ²
Tiamulin	27	-	33	24	-	100	50	0.19–1.1
Triclocarban	-	-	-	-	50	-	25	1.1×10 ³
Trimethoprim	73	67	100	76	100	100	100	0.87–2.5×10 ²
Tylosin	9	-	-	6	-	-	-	34
PPCPs total	100	100	100	100	100	100	100	52–3.5×10 ⁴

* All the sample results were either BQL, BDL or NA.

Table 2 Frequency and maximum concentrations of PPCPs detected in groundwater, tap water and bottled water. Among 60 PPCPs tested, 11 for which we determined the concentration in at least one sample are shown. (2-column fitting image)

	Frequency of detection (%)				Concentration range (min–max, ng/L)
	Groundwater <i>n</i> =10	Tap water <i>n</i> =6	Bottled water <i>n</i> =4	Total <i>n</i> =20	
Antipyrine	- *	17	-	5	4.0
Azithromycin	-	-	25	5	0.24
Caffeine	30	17	-	20	12–78
Carbamazepine	10	17	-	10	0.25–0.49
Clofibric_acid	-	17	-	5	1.1×10^2
Lincomycin	20	-	-	10	0.13–2.1
Primidone	-	33	-	10	8.9–14
Sulfadimidine	-	50	-	15	2.0–4.2
Sulfathiazole	-	33	-	10	0.75–1.5
Theophylline	-	-	25	5	0.47
Trimethoprim	-	-	25	5	5.9×10^{-2}
PPCPs total	30	83	50	50	$0.24\text{--}1.3 \times 10^2$

* All the samples were either BQL, BDL or NA.

Fig. 2. Concentrations of a) caffeine, b) lincomycin and c) carbamazepine in surface water and wastewater. (2-column fitting image)

3.2 PMMoV in surface water and wastewater

3.2.1 The occurrence of PMMoV

PMMoV was detected in all samples from farmlands, rivers and wastewater and in all pond samples except one (Fig. 3; see Table S1 for concentrations of PMMoV in all samples). The high detection rate of PMMoV in surface water (94%) was consistent with the studies in other countries. In Germany, PMMoV has been positive in all the samples of river waters containing WWTP effluent (Hamza et al., 2011). In Japan, PMMoV has been detected in 76% of surface water samples used as drinking water sources (Haramoto et al., 2013); in that study, 2 L of the sample water was concentrated for PMMoV analysis (LOD ~53 copies/L). As was the case with PPCPs, the concentration of PMMoV in our study was the highest in the WWTP influents. The average PMMoV concentration in the WWTP influents in our study (6.3×10^6 copies/L) was comparable to those in southern Arizona (Kitajima et al., 2014) but lower than those in the US, Germany and Bolivia by

1–3 log₁₀ (Hamza et al., 2011; Rosario et al., 2009; Symonds et al., 2014). The decrease in the average concentrations of PMMoV from the influents to the effluents at the Yen So WWTP was a factor of 0.92 log₁₀; this decrease was within the reported range of removal efficiency of PMMoV in WWTPs in the US and in Germany using activated sludge processes (0.5–3.7 log₁₀) (Hamza et al., 2011; Kitajima et al., 2014; Rosario et al., 2009). In surface waters, PMMoV was quantified in 70% of the samples. The concentrations ranges were 1.7×10⁴–1.2×10⁵ copies/L in ponds, 1.0×10⁴ copies/L in a farmland and 3.0×10⁴–1.8×10⁶ copies/L in rivers (Table S1). These concentrations were lower than the WWTP influents by 0.54–2.8 log₁₀.

As the recovery rate of PMMoV by the adsorption-elution method for concentration of PMMoV in the environmental samples was not available, the PMMoV levels might have been underestimated in our study. The reported recoveries of poliovirus by using the small-volume concentration method and 90 mm electronegative filters are 23% ± 19% from WWTP influent, 65% ± 28% from WWTP effluent and 82% ± 12% from river water; that of NoV GII from river water is 15% ± 5% (Haramoto et al., 2009; Katayama et al., 2008). However, the recovery rate of PMMoV in the analysis cannot be directly compared with those of HEV, because the morphology and isoelectric point of PMMoV differ from those of HEV (Michen and Graule, 2010; Wetter et al., 1984).

The occurrence of PMMoV in surface water was similar to that of PPCPs, rather than HEV. The concentrations of HEV and two bacterial indicators are shown in Table S1. While all the surface water samples were positive for both *E. coli* and TC, HEV had varying detection rates (NoV GII 59%; EV 35%; AdV 29%; NoV GI 24% and AiV1 18%). Note that the HEV concentrations were BQL for two-thirds of the HEV-positive

surface water samples. Nevertheless, the higher prevalence of NoV II over the other HEV in surface water was consistent with a study in Singapore (Rezaeinejad et al., 2014). Table 3 shows the concurrence matrix of PMMoV, PPCPs, HEV and bacterial indicators. The concurrences between PMMoV and PPCPs and among PPCPs were high (76–100%)—as high as their detection rates (76–100%). HEV also had high concurrences among them (59–94%), despite their low detection rates (18–59%). In contrast, the concurrence was low between PMMoV and HEV (22–61%) and between PPCPs and HEV (22–83%). These indicated that the sources and fates of PMMoV were similar to those of PPCPs, whereas the fates of PMMoV differed from those of HEV. However, it should be emphasized that PMMoV was detected in all the HEV-positive samples. The higher prevalence of PMMoV than HEV was consistent with the other studies investigating surface water in Germany and Japan (Hamza et al., 2011; Haramoto et al., 2013). Owing to their widespread presence in surface water, the bacterial indicators had high concurrences with PMMoV and PPCPs. However, in some samples, the bacterial indicators might be derived from other sources than human feces, as explained in 3.2.2.

Similar to the previous study in surface and marine waters (Hamza et al., 2011; Rosario et al., 2009), the predominant source of PMMoV in surface waters in Hanoi was inferred to be human feces. Although grey water could contain PMMoV, the PMMoV levels in the WWTP influents in Hanoi were more than 3 log₁₀ higher than that in greywater in Japan (Ng, 2013). As shown above, PMMoV had high the concurrence with PPCPs. Moreover, multiple PPCPs, including those derived from human excreta (e.g. carbamazepine, lincomycin), were detected in all the PMMoV-positive samples. As for the pond and farmland samples, there was no potential source of PMMoV other than

blackwater (e.g. food-related facilities using peppers) in the vicinity of the sampling locations. The disposal of solid kitchen waste would not be the source of PMMoV in the studied waters, as explained above with caffeine.

Fig. 3. PMMoV concentrations in surface water and wastewater. (1-column fitting image)

1 **Table 3** Concurrence (%) matrix of PMMoV, PPCPs, HEV and bacterial indicators in surface water. (2-column fitting image)

	PMMoV	Caffeine	Lincomycin	Carbamazepine	Trimethoprim	Sulpiride	AdV	AiV1	NoV GI	NoV GII	EV	<i>E. coli</i>
PMMoV												
Caffeine	<7#											
Lincomycin	<7#	433#										
Carbamazepine	; 5#	;; #	;; #									
Trimethoprim	; 5#	76#	76#	; 5#								
Sulpiride	; 5#	76#	76#	;; #	76#							
AdV	35	29	29	35	53	53						
AiV1	24	18	18	29	41	41	;; #					
NoV GI	29	24	24	24	47	35	; 5#	<7#				
NoV GII	65	59	59	59	; 5#	71	71	59	65			
EV	41	35	35	35	59	47	; 5#	; 5#	;; #	76		
<i>E. coli</i>	<7#	433#	433#	;; #	76	76	29	18	24	59	35	
TC	<7#	433#	433#	;; #	76	76	29	18	24	59	35	433#
Detection rate	94	100	100	88	76	76	29	18	24	59	35	100

2 Maximum possible concurrence is 100%. For viruses, BQL is counted as detection. Concurrences above 80% are indicated in bold.

3

3.2.2 The dynamic range and persistence as factors affecting the prevalent occurrence of PMMoV in surface waters

The occurrence of a wastewater-derived compound in environmental waters is presumably determined by its abundance in wastewater, the degree of dilution, its persistence in the waters and the method detection limit. Therefore, we investigated the dynamic range of the detection method and persistence of PMMoV to elucidate the difference in detection rates between PMMoV and HEV (i.e. less frequent occurrence of HEV), and the high prevalence of PMMoV and PPCPs in surface water samples.

The dynamic range is the concentration ratio of arithmetic mean concentration in WWTP influent to the LOQ. It indicates the range over which a particular compound could be sensitively used as a tracer (Benotti and Brownawell, 2007; Kuroda et al., 2012; Takada and Eganhouse, 1998). A compound with a larger dynamic range is more likely to be detected under conditions with high dilution and/or significant attenuation. This ratio is study-site and analysis-method specific. Table 4 shows the dynamic ranges of PMMoV, PPCPs and HEV in the surface water samples; the sample volume was 150 mL for PMMoV and HEV, and 1000 mL for PPCPs. Among viruses, PMMoV and AdV had the largest dynamic range (19,000), while the dynamic range of the other HEV was smaller (<6,800). The dynamic range of PPCPs also varied (110–2,100): caffeine having one of the largest dynamic ranges (1,100) and carbamazepine with the smallest dynamic range (110). Despite its small dynamic range, carbamazepine occurred prevalently in surface water in Hanoi, probably because of its high persistence. Similar case was also observed in groundwater in Tokyo, where carbamazepine was as frequently detected as crotamiton, a PPCP with a much larger dynamic range (Kuroda et al., 2012).

Then, we plotted the concentration ratios of PMMoV and caffeine to carbamazepine in surface waters with those in the WWTP influents (Figs. 4a and 4b) to compare their persistence in surface waters. We assumed that the concentration ratios of PMMoV and caffeine to carbamazepine in the WWTP influent samples represent the concentration ratios in the wastewater that was the source of PMMoV, caffeine and carbamazepine in the surface waters. In surface water, the fate of viruses is controlled by various phenomena including adsorption, sedimentation, resuspension and inactivation by temperature and solar radiation (Brookes et al., 2004). In comparison, caffeine decays mainly by photodegradation and biodegradation (Buerge et al., 2003), whereas carbamazepine behaves conservatively in surface water (Nakada et al., 2008) and even in soil (Grossberger et al., 2014). Adsorption is not expected to play a major role for caffeine and carbamazepine because of their hydrophilic nature ($\log K_{ow}$ 0.07 and 2.45, respectively (SRC, 2006)).

It was indicated that the persistence of PMMoV in surface water was comparable to that of caffeine. The concentration ratio of PMMoV to carbamazepine in the ponds and the farmland (3.9×10^3 – 6.1×10^4 ; median 9.6×10^3 copy/ng) was significantly smaller (*t*-test, $P < 0.05$) than that in WWTP influents (1.3×10^5 copy/ng) (Fig. 4a). This probably indicated that PMMoV was reduced in the waters of the ponds and the farmland by the aforementioned phenomena, whereas carbamazepine—a recalcitrant PPCP—persisted. In contrast, the abundance of PMMoV relative to caffeine in the ponds and the farmland (concentration ratio 6.4×10^1 – 8.6×10^2 ; median 3.4×10^2 copy/ng) was similar to those in WWTP influents (3.5×10^2 – 4.9×10^2 ; median 4.2×10^2 copy/ng) (Table S1). The concentration ratio of PMMoV to carbamazepine and that of caffeine to carbamazepine

are compared in Fig. 4b to cancel the effect of dilution—assuming that carbamazepine was not attenuated in surface water—and to compare the fates of PMMoV and caffeine. In the ponds and the farmland, the two concentration ratios are plotted close to 1:1 line passing through the WWTP influent samples (Fig. 4b). Because PMMoV, caffeine and carbamazepine in the ponds and the farmland were presumably derived from domestic wastewater discharged from nearby households, the similar reduction of PMMoV and caffeine relative to carbamazepine suggest that the persistence of PMMoV in those surface waters was comparable to that of caffeine. The comparable persistence between PMMoV and caffeine is in agreement with the reported half-lives in the literature. The half-life of PMMoV at 25 °C in river water was reported to be 5.7 d (Hamza et al., 2011). As for caffeine, reported half-lives in surface and coastal waters ranged from 3.5 d to more than 100 d (Benotti and Brownawell, 2007; Bradley et al., 2007; Buerge et al., 2003). However, the half-life of caffeine in our surface water samples would have been in the short range (e.g. 3.5–10 d), because of presumably high biological activity owing to high water temperature and high nutrient concentrations (e.g. 30 °C and 8 mgC/L). Therefore, PMMoV could be used as a moderately conservative tracer of fecal pollution in surface water. The higher persistence of PMMoV than other viruses can be attributed to its capsid structure (Fauquet et al., 2005). Note that, if the ponds and the farmland had received a discharge from other caffeine sources than those in the domestic wastewater (e.g. debris of tea/coffee, which are basically landfilled in remote sites), the persistence of PMMoV compared to caffeine could have been underestimated, but not significantly. It was estimated that a disposal of spent coffee grounds or spent tea leaves for a cup into an

average pond in our study can increase the caffeine concentration in the pond by only up to 0.36 ng/L or 0.14% (see S2 for details).

We then compared the concentrations of AdV and *E. coli* with that of PMMoV in surface water and wastewater samples (Fig. 4c and 4d). The abundance of AdV relative to PMMoV was lower in surface water samples than in the WWTP influent samples. Moreover, AdV levels were lower than the LOQs (1.6×10^3 – 3.9×10^3 copies/L) in the majority (85%) of PMMoV-positive samples in ponds and farmlands (Fig. 4d). This lower abundance of AdV in surface waters, despite the large dynamic range comparable to PMMoV, was probably because AdV was decayed considerably owing to the lower persistence of AdV. The reported half-lives of AdV, Torque teno virus and PyV in 25 °C river water were 1.7, 2.1 and 1.5 d, respectively (Hamza et al., 2011). The same would hold for the other HEV, which had lower detection rates in ponds (9–64%) and farmlands (0%) than in rivers (67–100%) and wastewater (75–100%). In the two river water samples, the abundance of AdV relative to PMMoV were somewhat comparable to that in the WWTP influents (Fig. 4d). This would be because there was little difference in their reduction in a short residence time in the rivers. *E. coli* was detected in all the surface water samples, but the abundance of *E. coli* relative to PMMoV in the ponds and farmlands varied widely (concentration ratio 7.7×10^{-2} – 2.0×10^1 ; median 5.9×10^{-1} CFU/copy) (Fig. 4d). The dispersed distribution was also observed between *E. coli* and caffeine in our study (Table S1), and between fecal coliforms and caffeine in stormwater (Sauvé et al., 2012). It is widely known that *E. coli* can grow in both temperate and tropical soils, which confounds the use of *E. coli* as a reliable indicator of fecal contamination (Fujioka et al., 1998; Ishii et al., 2006; Solo-Gabriele et al., 2000).

In summary, it was suggested that both the large dynamic range and the moderate persistence of PMMoV lead to its ubiquitous detection in surface water. In comparison, HEV were less frequently detected, even though with their large dynamic ranges, probably because of their lower persistence. The greater ubiquity and persistence of PMMoV than HEV as above, together with the reported high human feces-specificity, show that PMMoV is useful as a sensitive fecal indicator in surface water. Furthermore, it was suggested that PMMoV is useful as a fecal tracer in surface water, whose persistence was comparable to caffeine and whose dynamic range was larger than caffeine in surface water. Our study also demonstrated that analysis of PPCPs is useful to assess the fates of viruses in surface water.

Table 4 The dynamic range of PMMoV, PPCPs and HEV in surface water analysis.

(1-column fitting image)

	Frequency of detection	WWTP influent *	LOD (viruses) / LOQ (PPCPs) †	Dynamic range
PMMoV	94%	6.3×10 ⁶ copies/L	3.4×10 ² copies/L	19,000
Caffeine	100%	1.6×10 ⁴ ng/L	14 ng/L	1,100
Lincomycin	100%	2.8×10 ² ng/L	0.13 ng/L	2,100
Carbamazepine	88%	48 ng/L	0.42 ng/L	110
Trimethoprim	76%	2.4×10 ² ng/L	1.4 ng/L	170
Sulpiride	76%	94 ng/L	0.47 ng/L	200
AdV	29%	1.1×10 ⁷ copies/L	5.7×10 ² copies/L	19,000
AiV1	18%	2.3×10 ⁵ copies/L	3.4×10 ² copies/L	680
NoV GI	24%	BQL	3.4×10 ² copies/L	-
NoV GII	59%	2.3×10 ⁶ copies/L	3.4×10 ² copies/L	6,800
EV	35%	2.7×10 ⁵ copies/L	3.4×10 ² copies/L	790

* The average concentration of WWTP influent samples.

† LODs for viruses: the case with a sample volume of 150 mL; LOQs for PPCPs: since the LOQs were sample-specific, the median LOQ values of all surface water samples analyzed in a sample volume of 500 mL were taken.

Ohj hqgv#r u#µg

- ◆ WWTP influent ◇ WWTP effluent × River △ Pond (LD)
- ▲ Pond (TM) ▲ Pond (NH) ▲ Pond (YS) □ Farmland (LD)
- 1:1 line passing through WWTP influent samples
- * NR: not reportable (BQL, BDL or NA).

Fig. 4. Comparison of PMMoV with carbamazepine, caffeine, AdV and *E. coli* in surface water and wastewater. a) Concentration ratios of PMMoV to carbamazepine, b) concentration ratio of PMMoV to carbamazepine compared to that of caffeine to carbamazepine and concentrations of c) AdV and d) *E. coli* compared with those of PMMoV. (2-column fitting image)

3.3 PMMoV in groundwater, tap water and bottled water

PMMoV was detected in 3 groundwater samples out of 8, in which the PMMoV concentration were 19 copies/L in a sample (YSG1) and BQL in the other 2 samples (TMG1 and TMG21) (Fig. S2). PMMoV was not detected (LOD 0.66–0.88 copies/L) in all of the 4 tap water samples and 3 bottled water samples tested. AdV, AiV1 and NoV GI were detected in two groundwater samples and one bottled water sample at less than 10 copies/L, but PMMoV was not detected in the HEV-positive samples. Moreover, PMMoV was detected only in 1 of the 10 samples of groundwater, tap water and bottled water that were positive with PPCPs. The concentration of PMMoV in groundwater was lower than that in the adjacent pond samples by more than 3.8 log₁₀. This was in contrast with the case of caffeine, whose concentrations in groundwater and adjacent ponds were comparable. Note that, although no significant inhibitory effect on the recovery of spiked MNV from one of our groundwater samples was observed (see 2.4 and S1), inhibitory effect for PMMoV levels in the other groundwater, tap water and bottled water samples concentrated from a large volume of water was unknown. Although the recovery of AdV may not be directly compared to that of PMMoV, it was reported that the recovery of AdV type 5 from surface water and groundwater concentrated from 8–200 L varied

widely (0.63–97%; median 37%) (Hata et al., 2011). *E. coli* was detected in 11%, 33% and 25% of groundwater, tap water and bottled water samples, respectively, and TC was detected in 78%, 67% and 50% of respective samples (Fig. S2).

The substantial reduction of PMMoV and HEV from the groundwater compared with surface water could be attributable to adsorption and straining of these viruses when the source water containing wastewater infiltrated within the subsurface. Clay minerals and metal oxides, which have high adsorption capacity of viruses including PMMoV (Gülser et al., 2008; Yoshimoto et al., 2012), are rich in Hanoi's subsurface (Berg et al., 2008). In comparison, adsorption of caffeine is not expected because of its high hydrophilicity. The inconsistency in the occurrence of PMMoV and that of HEV, despite the much greater abundance of PMMoV in surface water, is possibly attributed to the difference in viral morphology; rod-shaped PMMoV virions with a length of 300–310 nm and a width of 18 nm (Fauquet et al., 2005) could be preferentially removed during soil passage compared to AdV, AiV1 and NoV GI, which have spherical virions with a diameter of 30–90 nm (Fauquet et al., 2005). Physical and geochemical heterogeneity of aquifers, which is distinctive in Hanoi, would be another factor; it can result in a preferential flow paths that enhance subsurface transport of viruses (Bhattacharjee et al., 2002). The above mechanisms would also be involved in the occurrence of bacterial indicators in groundwater; however, not all the detection of the bacterial indicators would indicate the fecal pollution, because soil is an environmental, non-fecal source of fecal indicator bacteria (Fujioka et al., 1998; Ishii et al., 2006; Solo-Gabriele et al., 2000).

Similarly to groundwater, the inconsistent occurrences of PMMoV, HEV and PPCPs in tap water and bottled water can be attributed to the various processes the source

water has undergone, such as adsorption and straining in the subsurface and coagulation, sedimentation, filtration and disinfection at the treatment plants. One bottled water sample was found to be positive for theophylline, trimethoprim, AdV and *E. coli*, but not for PMMoV. The bottled water would have been contaminated probably through insufficient cleaning, improper handling of bottles and/or an unsanitary bottling process, because the 19-L bottles are used repeatedly. Studies have shown that not all individuals have PMMoV in their feces, possibly depending on diets (Colson et al., 2010; Hamza et al., 2011; Zhang et al., 2006). Therefore, in cases of a very small-scale and local pollution involving only one or a few individuals, like the bottled water above, the absence of PMMoV in the polluted water may be attributed to the absence of PMMoV in the contamination sources.

Collectively, it was suggested that, even by analysis with very low LODs, PMMoV is not suitable as a fecal indicator nor a fecal tracer in groundwater, tap water and bottled water.

3.4 Evaluation of PMMoV as a fecal indicator and a fecal tracer in water

It was shown that PMMoV is a sensitive fecal indicator to evaluate the occurrence of fecal-borne viruses in surface water. In surface waters, PMMoV was more abundant than HEV, and PMMoV was detected in all the HEV-positive samples. Because of its higher persistence, PMMoV is a useful indicator in stagnant waters to evaluate the potential occurrence of less persistent viruses, whose abundance may be lower than the method detection limit after substantial reduction. In turn, as noted by Hamza et al. (2011), PMMoV may not be suitable for discerning fresh fecal pollution, which may be

most associated with pathogens. Hence, combined use of PMMoV and less persistent viruses, such as AdV, may be useful for assessing fresh fecal pollution in stagnant waters.

Our results have also shown that PMMoV is a moderately conservative fecal tracer in surface water. The utility of PMMoV as a fecal tracer was shown to be comparable to that of caffeine as a wastewater tracer; the PMMoV's abundance in WWTP influents, the dynamic range, the persistence and ubiquity in surface water was comparable to caffeine or greater. The lack of substantial temporal fluctuations in abundance (Hamza et al., 2011; Haramoto et al., 2013; Kitajima et al., 2014; Rosario et al., 2009) and the presumably broad and universal consumption of peppers worldwide would also be comparable. In addition to PMMoV's high source-specificity to human fecal materials, PMMoV has more advantages as a viral tracer of fecal pollution over chemical wastewater tracers. First, apart from the persistence, one can expect that PMMoV virions behave more similarly to other viruses in surface water, than chemical compounds do. In wastewater treatment ponds in Bolivia, the proportion of particle-bound PMMoV to total PMMoV were comparable to that of NoV GI and human rotavirus (Symonds et al., 2014). Second, PMMoV can be analyzed together with the other viruses of interest. This could be of a great benefit for microbiologists who perform qPCR analysis. The dynamic range of PMMoV can be easily expanded by increasing the volume of water used for analysis (e.g. 130,000 for a sample volume of 1000 mL). In practice, PMMoV as a fecal tracer can be useful for pollution control of surface water based on precautionary principle; it enables sensitively identifying and tracking fecally-polluted water, regardless of the current occurrence of pathogens, and foresightedly taking measures to prevent potentially undesirable outcomes in the future.

Note that, for fecal source identification, PMMoV may be used in combination with other human feces-specific tools, because PMMoV has been found in the feces of some animals and in greywater, although with much lower abundance (e.g. by 3 log₁₀) than in human feces and in WWTP influents (Hamza et al., 2011; Ng, 2013; Rosario et al., 2009). In future studies, the specificity of PMMoV as a fecal tracer in surface waters of various locations should be evaluated.

In contrast to surface water, PMMoV is not suitable as a fecal indicator nor a fecal tracer in groundwater, tap water and bottled water in Hanoi, because the detection rates and concentration of PMMoV in those waters were very low, and because the occurrence was inconsistent with those of PPCPs and HEV. In groundwater, broadly used, hydrophilic and highly persistent compounds, such as artificial sweeteners, have been suggested as powerful wastewater tracers (Buerge et al., 2009; Tran et al., 2014a; Van Stempvoort et al., 2011). A multi-tracer approach and statistical methods are also useful in evaluating contamination of groundwater (Gasser et al., 2014; Kuroda et al., 2014; Van Stempvoort et al., 2013).

In light of the unique behavior of PMMoV compared with that of HEV, various application of PMMoV as a viral indicator can be considered. Considering the persistence of PMMoV in surface water, PMMoV could be useful for evaluating the quality of stagnant surface water and recreational waters (e.g. lakes, pools). Because of its abundant and constant occurrence in surface water and wastewater, PMMoV could also be used as a performance indicator in water treatment plants and WWTPs to evaluate treatment efficiency (e.g. in coagulation, filtration and disinfection) and manage risks posed by treated water, as suggested for wastewater reclamation systems (Kitajima et al., 2014).

Further studies are required on the concurrences of PMMoV, pathogens and other indicators in various environmental and processed waters with respect to their concentration levels; on the fate of PMMoV in comparison to pathogens and indicators in various water and wastewater treatment processes; and on possible applications of PMMoV in risk management and pollution control of water.

4.# CONCLUSIONS

For the first time, PMMoV was evaluated as a fecal indicator and a fecal tracer in water environments through comparison with wastewater-tracer PPCPs. PMMoV was abundant and ubiquitous in wastewater and waters from ponds, farmlands and rivers in Hanoi. In surface water, the detection rate of PMMoV was as high as that of caffeine and carbamazepine; this was probably because the dynamic range of PMMoV was large and because the persistence of PMMoV was as high as caffeine. In comparison, HEV had lower detection rates in surface water, because of their lower persistence. Hence, in surface water, PMMoV was suggested as a sensitive fecal indicator as well as a moderately conservative fecal tracer. In groundwater, tap water and bottled water samples, PMMoV had lower detection rates and much lower concentrations than in surface water. In those samples, the occurrence of PMMoV did not agree with the occurrence of PPCPs and HEV, showing that PMMoV is not suitable as a fecal indicator nor a fecal tracer in those waters. Further studies are needed on the occurrence and fates of PMMoV in diverse aquatic environments and during water treatment processes and on the specificity of PMMoV as a fecal tracer to explore various possible applications of this virus both as an indicator and a tracer in water.

ACKNOWLEDGMENTS

The authors acknowledge the financial support by the CREST project grant for ‘Development of Well-balanced Urban Water Use System Adapted for Climate Change’ from the Japan Science and Technology Agency and JSPS Grant-in-Aid for Scientific Research (A) Number 25257304. We would like to thank Dr. Akihiko Hata and Mrs. Miwako Nakagawa (The University of Tokyo) for their assistance and consultation in virus analysis. We are thankful to Mr. Michiru Okasa (The University of Tokyo), Mr. Nguyen Quoc Hoa, Mrs. Nguyen Thuy Lien, Mr. Tran Hoai Son and Mr. Vu Duc Canh (National University of Civil Engineering), Dr. Ha Vinh Hung and Dr. Ly Bich Thuy (Hanoi University of Science and Technology) for their support in the sampling and analysis in Hanoi. We are grateful to the local people for their kind cooperation in the sampling.

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