



From food to sewer: Dietary inputs support the temporal and population-wide stability of pepper mild mottle virus in wastewater

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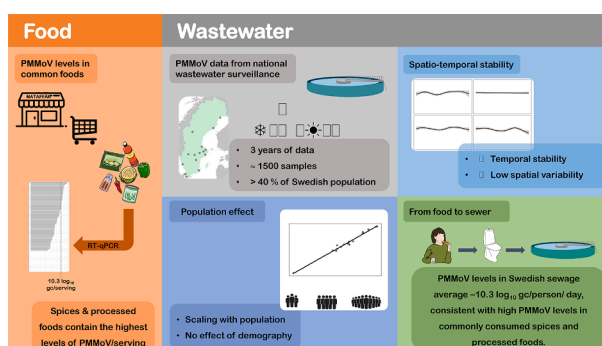
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HIGHLIGHTS

- PMMoV was consistently detected at high levels in wastewater across diverse Swedish WWTPs.
- Average PMMoV daily levels scaled almost proportionally with catchment population size.
- Only minor temporal trends were detected over days, months, and years.
- No associations were found with population structure, wastewater temperature, or weekday.
- High PMMoV levels were found in spices and processed foods like sauces, snacks, and ready meals.

GRAPHICAL ABSTRACT



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ABSTRACT

Pepper Mild Mottle Virus (PMMoV) is widely used in wastewater-based surveillance as a faecal indicator and population size marker. It is highly abundant in domestic wastewater and originates from the consumption of *Capsicum*-containing foods. Despite its widespread use, data on the variability of PMMoV concentrations in wastewater remain limited, and its primary dietary sources are not fully characterised. To evaluate the robustness of PMMoV as a human faecal marker, we analysed wastewater from multiple locations in Sweden over different timescales, ranging from daily to weekly sampling for up to nearly three years. We also investigated dietary sources by screening a broad range of foods. Wastewater consistently contained high PMMoV levels, averaging 10.34 log₁₀ genome copies per inhabitant per day. The average daily PMMoV load scaled almost proportionally with the number of inhabitants connected to each catchment area. No clear temporal trends were detected over either short or long time periods, and no substantial associations were found with potentially influencing factors such as demographic or socioeconomic characteristics of the catchment population, season, wastewater temperature or weekday. Of 60 foods tested, 45 contained PMMoV, with concentrations ranging from 5.70 to 12.21 log₁₀ genome copies per serving. The highest levels were found in spices, such as paprika powder, as well as in ready meals, spreads and snack products. These widely consumed foods likely contribute to

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the consistently high PMMoV levels observed in domestic wastewater. Taken together, our findings support the potential of PMMoV as a robust and broadly applicable human faecal marker.

1. Introduction

Wastewater-based surveillance (WBS) is a valuable tool for monitoring viral infectious diseases in communities and serves as an important complement to clinical data (Parkins et al., 2024; Polo et al., 2020). However, fluctuations in viral genome concentrations in wastewater do not always directly reflect changes in the number of infected individuals. These variations may also result from factors such as wastewater dilution due to snowmelt or rainfall, changes in the size of the contributing population, environmental conditions (e.g. pH and temperature), or variability introduced during sampling and analytical procedures (Parkins et al., 2024; Polo et al., 2020; Rosengart et al., 2024). To improve comparability across locations and over time, it is important to normalise pathogen concentrations to the human faecal content of the wastewater sample. A common approach is to use a faecal marker, which provides an estimate of the contributing population size and serves as a process control during sample preparation and analysis by accounting for matrix effects (Parkins et al., 2024). An ideal faecal marker should be abundant in human faeces, consistently excreted across populations and time, and behave similarly to the target analyte in wastewater (Dhiyebi et al., 2023; Parkins et al., 2024; Walker et al., 2020).

Pepper Mild Mottle Virus (PMMoV) is currently one of the most widely used faecal markers in viral WBS. It is a non-enveloped, single-stranded RNA virus that infects *Capsicum* plants, such as paprika and chilli, and enters the human gut through food consumption (Kitajima et al., 2018; Moore et al., 2015; Nakamura et al., 2009; Rosario et al., 2009; Zhang et al., 2006). PMMoV is highly abundant in human faeces and stable in wastewater, with concentrations in untreated samples typically ranging from 5 to 10 log₁₀ genome copies (gc)/L, making it quantifiable even in small sample volumes (Kitajima et al., 2018; Walker et al., 2020). It is also rarely present at high concentrations in animal faeces, highlighting its specificity for humans (Kitajima et al., 2018; Kuroda et al., 2015; Rosario et al., 2009).

The reliability of PMMoV as a faecal marker has, nevertheless, been questioned. In some studies, alternative normalisation approaches or even non-normalised data have correlated more strongly with clinical case numbers (Duvall et al., 2022; Feng et al., 2021; Greenwald et al., 2021). Research assessing the variability of PMMoV in wastewater across sites and over time has been conducted in North America (Dhiyebi et al., 2023; Goitom et al., 2024; Holm et al., 2022; Rosengart et al., 2024), Germany (Haeusser et al., 2023) and Saudi Arabia (Eifan et al., 2023), among others. However, comprehensive assessments from the Nordic region are lacking and few published studies include surveillance periods extending beyond one or two years (Dhiyebi et al., 2023; Goitom et al., 2024; Rosengart et al., 2024). As factors such as climate, diet and infrastructure can influence marker performance, site-specific evaluations over longer periods are essential.

Diet in particular is often pointed out as a factor influencing PMMoV levels in wastewater (Bivins et al., 2020; Pappu et al., 2026; Rosengart et al., 2024). If *Capsicum* consumption varies significantly between population groups, for example due to socioeconomic factors or cultural traditions, wastewater levels may vary accordingly, which could severely limit the reliability of PMMoV as a faecal marker. Dishes containing large amounts of paprika and chilli are not commonly found in the traditional diet of the Nordic countries but are more common in other parts of the world. However, globalisation and food trade have made such products increasingly accessible and popular worldwide. Today, processed foods containing paprika or chilli are consumed in a variety of contexts. At the same time, short-term consumption habits such as eating snacks at the weekend or certain food traditions, such as

the popular Swedish “Taco Friday” (Solér and Plazas, 2012), could still introduce day-to-day variation in PMMoV levels.

To date, research on PMMoV in food has been limited and has mainly focused on spicy items such as gochujang, chilli sauces, red chilli peppers and cayenne pepper (Ko et al., 2020; Peng et al., 2015; Zhang et al., 2006). However, *Capsicum* species, and thus PMMoV, are not limited to these products, and its presence in a wider range of commonly consumed foods remains poorly characterised. Given the central role of PMMoV in WBS, a broader assessment of its dietary sources is needed to better evaluate its suitability as a faecal marker.

In this study, we evaluated the robustness of PMMoV as a faecal marker in Sweden by analysing both wastewater and food samples. First, we assessed the regional and temporal variability of PMMoV in 18 wastewater treatment plants (WWTPs) over the course of one year, covering municipalities of different size, population structure and geographical location. To investigate the potential of PMMoV as an indicator of population size, we examined the relationship between average daily PMMoV loads and the number of inhabitants connected to each WWTP. We also investigated whether population characteristics, including socioeconomic status and the proportion of foreign-born residents in the catchment area municipalities, were associated with PMMoV levels, based on our hypothesis that socioeconomic and cultural factors could influence *Capsicum* consumption behaviour. We then analysed longer-term and seasonal dynamics using weekly samples collected over a period of nearly three years at four geographically separated WWTPs, as well as short-term variability using daily sampling at a single WWTP to examine PMMoV levels on different weekdays. Finally, to explore possible dietary sources of PMMoV, we analysed 60 food products from different categories, focusing mainly on those containing *Capsicum*-derived ingredients.

2. Material and methods

2.1. Wastewater samples

Wastewater sampling and analysis were conducted as part of the routine surveillance of human respiratory viruses by the Swedish Centre for Environmental Epidemiology (SEEC) at the Swedish University of Agricultural Sciences (SLU) (Isaksson et al., 2022).

2.1.1. Sampling sites and study period

The study included three partially overlapping monitoring series: short-term, long-term, and daily monitoring (Table 1). The short-term period was selected to capture the time when most WWTPs were included, while the long-term period was selected to capture the WWTPs with the most extended sampling series.

According to the SEEC schedule, sampling was conducted once a week on Mondays. However, this fixed, non-random sampling design could introduce bias, as PMMoV concentrations may vary between days of the week. Therefore, we also implemented a daily monitoring series in which 24-h flow-proportional composite samples were collected each day to assess potential intra-week variability.

The short-term monitoring consisted of 870 wastewater samples from 18 WWTPs (Fig. 1), collected between March 2024 and March 2025. These sites covered a total of 4516,000 inhabitants, representing 42 % of the Swedish population (Statistics Sweden, 2024a). The long-term monitoring consisted of 562 wastewater samples from four WWTPs between May 2022 and April 2025, covering approximately 511,000 inhabitants or 4.7 % of the Swedish population (Statistics Sweden, 2024a). The daily monitoring involved 35 daily 24-h flow-proportional samples from a WWTP in Uppsala over a five-week

period, from 7 January to 10 February 2025.

Holidays and logistical challenges prevented some samples from reaching the laboratory, and some additional samples ($n = 52$) lacked associated wastewater flow values, resulting in minor gaps in the weekly datasets.

2.1.2. Sampling, transport and collection of flow and temperature data

In most WWTPs, influent wastewater samples, i.e., raw, untreated wastewater entering the plants, were collected over 24 h using flow-compensated autosamplers, to ensure that contributions from both daytime and nighttime populations were captured. For Stockholm-Bromma, Stockholm-Henriksdal and Uppsala, samples were collected from several inlets and then flow-proportionally mixed into one sample, as described previously (Soares et al., 2025). In Uppsala, influent wastewater samples were collected every day instead of once a week and then flow-proportionally combined into a weekly composite sample. However, for the daily monitoring in Uppsala, the daily samples were analysed individually without mixing.

Upon collection, the samples were stored at 4 °C and transported to the laboratory in cold packs, where they were stored at 4 °C until nucleic acid extraction the following day. The total daily wastewater flow (m^3 per day) was recorded by each WWTP on the day of sampling and converted to L per day.

For WWTPs involved in long-term monitoring (Kalmar, Örebro, Umeå and Uppsala), daily average wastewater temperatures were provided by the respective WWTPs during 2024 to investigate potential associations between PMMoV levels and wastewater temperature.

2.1.3. Nucleic acid extraction

Total nucleic acids (TNA) were extracted from influent wastewater samples using the Maxwell® RSC Enviro TNA kit (Promega, AS1831) according to the manufacturer's instructions using the Maxwell® RSC instrument (Promega, AS4500) with the PureFood GMO and Authentication programme, as described previously (Isaksson et al., 2022). Each wastewater sample was divided into two subsamples. Forty ml of each subsample was used as input and the subsamples were eluted in 100 μ l of nuclease-free water. A negative extraction control consisting of 40 ml of tap water or ultrapure Milli-Q water (VWR) was included in each extraction round. All nucleic acid extracts were immediately stored at

−80 °C until RT-qPCR was performed the following day.

2.1.4. Collection of demographic and socioeconomic data

To investigate the relationship between demographic and socioeconomic factors and average PMMoV levels in wastewater, we collected the latest freely available data (CCO licence) from Statistics Sweden's statistical database (Statistics Sweden, 2024b) for each municipality served by the wastewater treatment plants included in the short-term monitoring (Table A.1). The dataset included municipal classifications (commuter city, larger city or metropolitan city), the proportion of foreign-born residents (from 2023, ranging between 10–36 %) and a socio-economic index (from 2022, ranging between 6.1–12.9). For the Stockholm WWTPs that served multiple municipalities, we calculated population-weighted averages to generate a composite estimate for each plant. However, it is important to note that the municipal boundaries often extend beyond the actual catchment areas, which means that the data does not correspond exactly to the catchment areas of the wastewater treatment plants.

2.2. Food samples

2.2.1. Sample collection

To investigate potential dietary sources of PMMoV, we collected 60 food samples of different categories, including spices and spice mixes, ready-made sauces, various processed foods such as snacks, spreads, bouillon cubes, fresh and frozen ready meals, and three *Capsicum* fruits. Most of the foods were known to contain *Capsicum* species; however, some were included without prior knowledge of their *Capsicum* content. In addition to food, we tested a tobacco sample (snus) as a possible alternative source of PMMoV, as the virus has been reported to infect *Nicotiana* plants (Zheng et al., 2023). Oats were included as a negative control to represent a food unlikely to contain PMMoV. Most samples were purchased from local retail supermarkets to reflect conditions representative of consumer exposure. Detailed descriptions of the samples and their origins are presented in Table A.2. Samples were stored at 4 °C immediately upon purchase until further processing.

2.2.2. Sample processing and nucleic acid extraction

Ribonucleic acid from food samples was extracted using the

Table 1

Sample collection dates and number of samples collected at each site. Number of valid samples included denote samples with valid quality control characteristics. An asterisk (*) next to the number of inhabitants indicates that the value is an estimate of the people connected based on a biological oxygen demand (BOD-7) value, rather than the number of inhabitants physically connected to the WWTP.

WWTP	Inhabitants	Sample collection, start date	Sample collection, end date	Number of collected samples with associated flow values	Number of valid samples	Sampling series
Gävle	89,000	03-11-2024	02-17-2025	50	49	Short-term
Göteborg	800,000	03-11-2024	03-10-2025	51	45	Short-term
Helsingborg	149,000	03-11-2024	03-10-2025	51	50	Short-term
Kalmar	66,000	05-30-2022	04-21-2025	142	138	Short-term, long-term
Karlstad	70,000	03-11-2024	03-10-2025	52	47	Short-term
Linköping	154,000	03-11-2024	03-10-2025	52	46	Short-term
Luleå	70,000	03-11-2024	03-03-2025	49	45	Short-term
Malmö	356,000	03-11-2024	03-03-2025	52	51	Short-term
Örebro	137,000	05-30-2022	04-14-2025	149	134	Short-term, long-term
Östersund	54,000	03-11-2024	03-10-2025	53	53	Short-term
Östhammar	4500*	03-11-2024	03-10-2025	47	41	Short-term
Stockholm-Bromma	375,000	03-11-2024	03-10-2025	49	48	Short-term
Stockholm-Grödinge	345,000	03-11-2024	03-10-2025	51	50	Short-term
Stockholm-Henriksdal	875,000	03-11-2024	03-10-2025	48	47	Short-term
Stockholm-Käppala	500,000	03-11-2024	03-10-2025	51	46	Short-term
Umeå	109,000	05-30-2022	04-21-2025	144	134	Short-term, long-term
Uppsala	191,000	05-30-2022	04-14-2025	182	177	Short-term, long-term, daily
Västerås	145,000	03-11-2024	03-10-2025	51	49	Short-term



Fig. 1. Locations of the WWTPs included in the study. All sites were included in the short-term monitoring, while Umeå, Uppsala, Örebro and Kalmar were included in the long-term monitoring and Uppsala in the daily monitoring.

Maxwell® RSC Plant RNA Kit (Promega, AS1500) using the Maxwell® RSC instrument (Promega, AS4500) with the Plant RNA programme. Most of the food samples were extracted in two subsamples.

For all samples except for the *Capsicum* fruits, a portion of the sample material was collected and homogenised using one of the following methods: vortex mixing, grinding with a mortar and pestle or fine chopping with two sterile scalpels (the processing method of each sample is specified in Table A.2). Then, 20–200 mg of homogenate was collected and transferred directly by an inoculation loop to a bead beating tube (PowerBead Pro Tubes, Ceramic 1.4 mm from Qiagen, 13113-50) on a scale. 600 µl of chilled 1-thioglycerol/homogenising solution (supplemented with the RNA extraction kit) was added upon

weighing the sample, whereupon bead beating (Bead Mill 24 device, fisherbrand™, 15515799) was performed for 5 min.

Most samples represented homogeneous portions of the tested items. However, due to the high complexity, for certain samples only specific parts of the food were analysed. These included, for instance, the filling of ready-made tuna and club sandwiches, the surface toppings of ready-made pan pizzas and various components of other ready-made foods. These samples are indicated in Table A.2.

For *Capsicum* fruits, different parts of the fruit were excised with a scalpel, blended and ground to a fine powder in liquid nitrogen using a sterile mortar and pestle. The powder was transferred to a Falcon tube and immediately placed on dry ice. One hundred mg of the powder was

Table 2
Primers, probes, quantification standards and internal controls.

Type	Name	Sequence (5'–3')	Length (nt)	Direction	Reference
Forward	PMMV-FP1-mod	GAGTGGTTTGACCTTAACGTTTGA	24	Plus	(Zhang et al., 2006), with modification according to (Haramoto et al., 2013)
Reverse	PMMV-RP1	TTGTGGGTTGCAATGCAAGT	20	Minus	(Zhang et al., 2006)
Probe	PMMV-Pr	FAM-CCTACCGAAGCAAATG-NFQ	16	Plus	(Zhang et al., 2006)
Quantification standard	PMMV-Std	TGCATGATCTACGTTGGTCCACATGACCTTAACGTTTGAGAGGCTACCGAAGCAAATGTCGGCACTTG	120	Double stranded	This study
Forward	IAC-F	CATTGCAACCGACAACACTAGCTCAGTCACTAGTACAGTACGCGCTGTTG	22	Plus	(Daleiden et al., 2022)
Reverse	IAC-R	TCCTCGGTACCTATTTCAGATGG	22	Minus	(Daleiden et al., 2022)
Probe	IAC-P	GCCTTAATCTAGGACGAGGTC	26	Plus	(Daleiden et al., 2022)
Template	IAC RNA template	Cy5-FTTACGTTCCGTTGGCAAAATGCAGCA-BHQ2 aUCCUCGGUACCUAUUCAGAUUGGAUACUUUACGUCCUUGGCAAAUGGCAGCACUAAGACUCUGUGGUCCUAGAUUAAGCa	80	Plus	(Daleiden et al., 2022)

then transferred to a bead beating tube (PowerBead Pro Tubes, Ceramic 1.4 mm from Qiagen, 13113-50) and placed on dry ice again. Then, 600 μ L of chilled 1-thioglycerol/homogenising solution was added to each subsample and bead beating was performed in pulses of 30 s, followed by deposition on wet ice until the samples were processed further.

For all sample types, the procedure was then continued according to the kit manufacturer's instructions. The subsamples were eluted in 50–100 μ L of nuclease-free water. At least one negative extraction control consisting of 100 μ L of nuclease-free water was included in each extraction round and processed in the same way as the samples. All nucleic acid extracts were immediately stored at -80°C until RT-qPCR.

2.3. RT-qPCR

RT-qPCR was performed on a CFX duet or a CFX 96 real-time PCR system (Bio-Rad) using the Reliance One-Step Multiplex Supermix Kit (Bio-Rad; art. nr. 12010221) with 1 mg/mL bovine serum albumin (Thermo Fisher, cat. nr. AM2618). Each reaction contained 15 μ L of master mix and 5.0 μ L of template, with 600 nM forward primer, 800 nM reverse primer and 200 nM probe (Table 2).

Starting from week 12, 2024 for the wastewater samples and for all food samples, an internal amplification control (IAC) was incorporated to assess RT-qPCR inhibition. The IAC reactions included 400 nM forward primer, 400 nM reverse primer, 200 nM probe, and 10^4 copies of synthetic RNA template (Table 2), and reactions were performed in duplex format together with the PMMoV assay. The primers, probe and synthetic RNA template were purchased from Integrated DNA Technologies (IDT).

The thermal profile was as follows: 50°C for 30 min and 95°C for 5 min, followed by 45 cycles of 95°C for 15 s and 58°C for 30 s. Results were analysed using CFX Maestro Software version 2.3 (Bio-Rad) and the cycle of quantification (Cq) values were determined using the regression method within the analysis software.

Each plate contained two wells of each subsample (extracted sample), negative extraction controls (NECs), no-template controls (NTCs) and positive controls consisting of archived wastewater TNA extracts positive for PMMoV. A gBlock standard (Table 2) was used for quantification. The standard was run as a tenfold dilution series ranging from 10^6 to 100 copies per reaction, and with two wells per dilution level. Obtained concentrations were converted to correspond to the number of gc/L for wastewater samples and to the number of gc/g for food samples, and then \log_{10} transformed before further analysis. For each sample, the average value of the two RT-qPCR wells was calculated, followed by averaging across the two subsamples, yielding the final value used in subsequent analyses.

To ensure that the primers bind to the targets of interest, the PMMoV primers and probe were evaluated *in silico*. Inclusivity, defined as the ability to detect relevant target sequences, was assessed by aligning the primer and probe sequences against all complete PMMoV genomes available in the NCBI GenBank database ($n = 79$ sequences, as of 22 November 2023). The forward primer showed a perfect match to 75 sequences, the probe to 78 sequences, and the reverse primer to 77 sequences. No mismatches of concern were identified. Exclusivity, defined as the ability of the assay to avoid detection of non-target sequences, was evaluated by separately querying the primer and probe sequences against the NCBI nucleotide collection database using blastn. Sequences assigned to the PMMoV taxonomy ID (12239) were excluded, and a permissive matching strategy was applied. No cross-reactivity of concern was observed, as the probe sequence did not produce significant matches to any non-PMMoV sequences.

2.4. Quality control

The quality control procedures included establishing a limit of blank, testing for inhibition (beginning in week 12, 2024 for the wastewater samples and for all food samples) and evaluating variability between

replicate extractions (subsamples).

Inhibition in RT-qPCR was assessed by comparing IAC Cq values. Specifically, inhibition was calculated by subtracting the average IAC Cq value in the NTCs from the IAC Cq value in each well containing a wastewater or food sample. Inhibition was considered unacceptable if the Cq shift exceeded 2 cycles (ISO, 2019), which corresponds to 75 % reduction in the target concentration at 100 % PCR efficiency. The percentage of inhibition was calculated using the following formula:

$$100 \cdot (1 - 2^{Cq_{\text{control}} - Cq_{\text{sample}}})$$

For each sample, the mean and standard deviation (SD) were calculated across subsamples, from \log_{10} transformed data. Wastewater samples with an SD greater than 0.50 between subsamples were deemed invalid. For easier interpretation, standard deviations of \log_{10} transformed data were also converted to geometric coefficients of variation (%CV) on the linear scale, by using the following formula (Figueiredo, 2022):

$$\sqrt{e^{(\log(10))^2 \cdot SD^2} - 1} \cdot 100$$

The limit of blank, i.e. the lowest concentration at which a sample was to be considered positive, was set clearly above the highest concentration obtained in the NTC and NEC wells included in the study.

In addition, for the wastewater samples, during 2024 and 2025, a control chart was established by using the positive control consisting of archived wastewater TNA extract, positive for PMMoV, to study run-to-run variability in RT-qPCR.

2.5. Data analysis

The data curation and the statistical analyses were conducted in R version 4.3.1-4.4.1 (R Core Team, 2023). Plots were generated using the ggplot2 (Wickham, 2011), ggmap (Kahle and Wickham, 2013) and ggthemes (Arnold, 2024) packages.

2.5.1. PMMoV levels in wastewater

For each WWTP, the daily PMMoV load was calculated by accounting for the daily wastewater flow, as follows:

$$\log_{10}(\text{gc} / \text{day}) = \log_{10}(\text{gc} / \text{L wastewater}) + \log_{10}(\text{L wastewater} / \text{day})$$

Similarly, the daily PMMoV load per resident was calculated by accounting for the number of inhabitants connected to each WWTP (Table 1), as follows:

$$\log_{10}(\text{gc} / \text{resident} / \text{day}) = \log_{10}(\text{gc} / \text{L wastewater}) + \log_{10}(\text{L wastewater} / \text{day}) - \log_{10}(\text{residents})$$

2.5.2. Statistical analyses for the short-term monitoring

To investigate how PMMoV levels varied within and across cities, we fitted a generalised additive model (GAM, Model 1) with \log_{10} gc/inhabitant/day as the response variable. WWTP was included as a fixed effect to assess general differences between locations, while smooth terms for decimal date were fitted separately for each WWTP to capture site-specific temporal patterns. To account for laboratory-related variability, analytical round was included as a random effect using a smooth term with a random-effect basis, as the control charts indicated substantial variation between PCR runs. The model was fitted using the gam function from the mgcv package (Wood, 2017) in R (R Core Team, 2023), with restricted maximum likelihood (REML) used for smoothness selection. Model diagnostics, including residual normality, homoscedasticity and the adequacy of basis dimension (k-index), were assessed

using gam.check and diagnostic plots. Thin plate regression splines were used for smoothing terms.

To examine the linear relationship between WWTP population size and average daily PMMoV levels, we fitted an ordinary least squares (OLS) regression model (Model 2) using the lm function in R (R Core Team, 2023). This model was further extended to test whether population structure influenced PMMoV levels by including additional explanatory variables, such as the municipal socioeconomic index and the \log_{10} -transformed proportion of foreign-born inhabitants. Model performance was compared using adjusted R^2 and the Akaike Information Criterion (AIC).

2.5.3. Statistical analyses for the long-term monitoring

To assess temporal trends in PMMoV levels over an extended period, we fitted a GAMM (Model 3) with the same structure as Model 1.

2.5.4. Statistical analyses for the daily monitoring

To examine variation over days and within weeks, we fitted a GAM (Model 4) with PMMoV (\log_{10} gc/inhabitant/day) as the response variable. Weekday was included as a fixed effect to evaluate systematic differences between days of the week, while sampling date was modelled as a smooth term to capture underlying temporal trends. The model was fitted using the gam function from mgcv (Wood, 2017) in R (R Core Team, 2023) with REML for smoothness selection. Thin plate regression splines were used for the smoothing term.

2.5.5. PMMoV levels in food samples

For the food samples, PMMoV concentrations were expressed as \log_{10} gc/g (wet weight) and then converted to estimated PMMoV loads per typical serving size. Serving size calculations were based on standard portion sizes for each food category or, when available, serving information from food packaging, online recipes, or ingredient charts. For samples where only a specific component was analysed (e.g., pizza toppings), per-serving estimates were calculated based on the typical amount of the analysed component present in a standard portion. The serving sizes used for each food item are provided in Table A.2.

3. Results

3.1. PMMoV in wastewater

3.1.1. Quality control data and raw PMMoV, flow and temperature estimates

A total of 1272 wastewater samples with associated flow data were collected from up to 18 WWTPs over three overlapping sampling series

(short-term, long-term, and daily monitoring; Table 1). Of these, 1250 samples passed quality control and were included in the final analysis.

Inhibition monitoring, initiated in March 2024, indicated generally low levels of PCR inhibition, with an average shift of 0.28 Cq units. This corresponds to an approximate 18 % reduction in target concentration, assuming 100 % PCR efficiency. No major differences in average inhibition were observed between sites, although Karlstad and Umeå had the highest numbers of severely inhibited samples (Fig. 2, A). Sixteen samples exceeded the 2 Cq threshold and were excluded from further analysis. Replicate extractions showed high repeatability, with an average SD of 0.058 \log_{10} units (corresponding to a geometric CV of 14 % on the linear scale). Five samples with an SD greater than 0.50 were discarded (Fig. 2, B).

Variability between PCR runs, assessed using a control chart, was

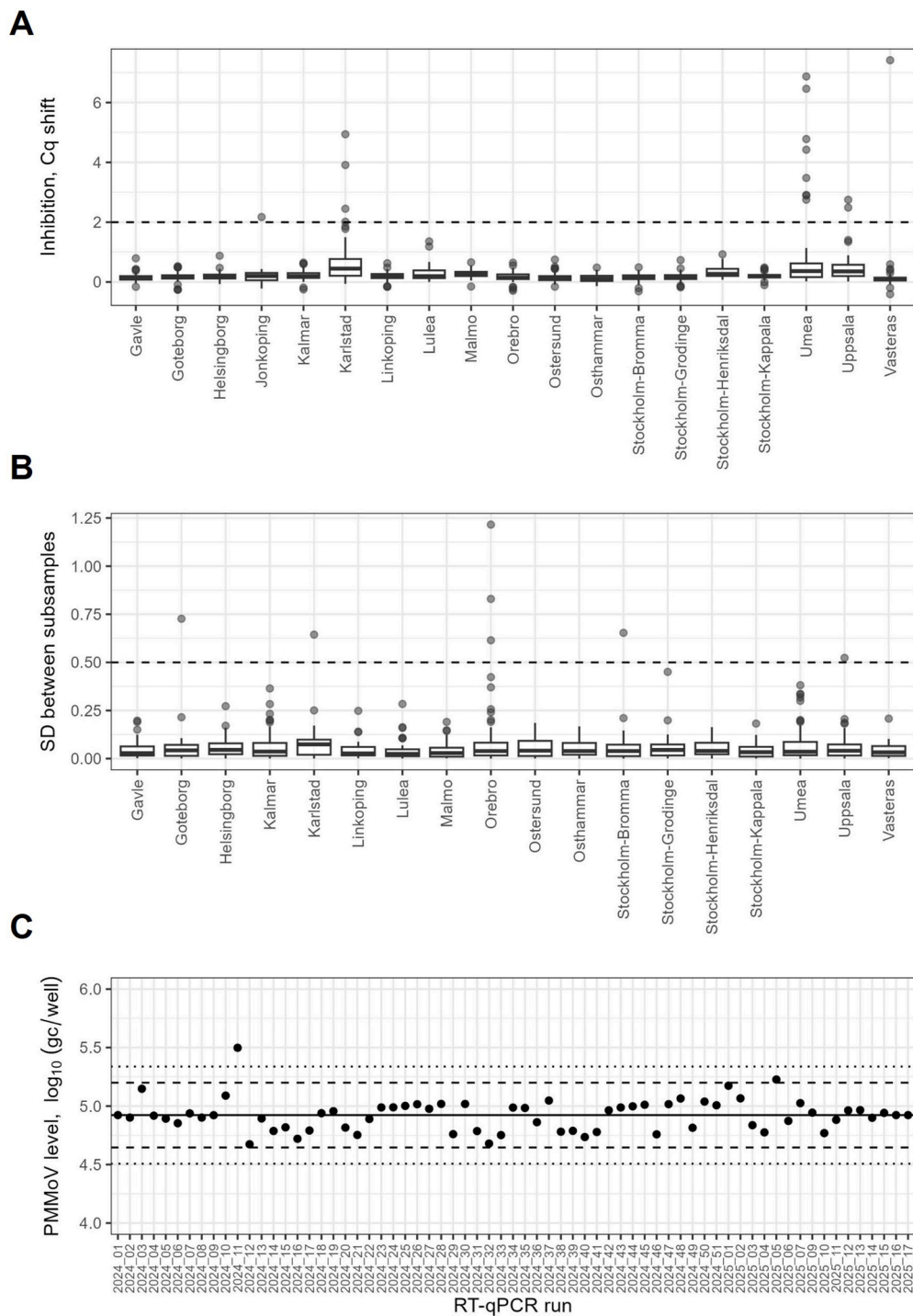


Fig. 2. Quality control data from wastewater analyses. A: RT-qPCR inhibition for the wastewater samples at each WWTP, with the dotted line indicating the threshold for acceptable inhibition. B: SD between subsamples (for \log_{10} transformed data), with the dotted line indicating the threshold for acceptable SD. C: Control chart for RT-qPCR during 2024 and 2025, based on a positive control of RNA extracts of PMMoV positive wastewater. The black dots represent the value of the positive controls, the solid black line represents the overall mean of these controls, and the dotted lines represents the mean $\pm 2 \cdot SD$ and $\pm 3 \cdot SD$, respectively.

greater than within-sample variation, with positive control values ranging from 4.67 to 5.50 \log_{10} gc/well and an SD of 0.14 \log_{10} (CV = 33 %) (Fig. 2, C).

Of 622 negative control wells (NTCs and NECs), 189 gave positive signals, mostly at low levels. The highest concentrations were 9 copies/reaction (NTCs) and 75 copies/reaction (NECs). A limit of blank of 100 copies/reaction was applied; one sample fell below this limit and was excluded.

The PMMoV concentrations for all samples included in the short-term and long-term monitoring, along with flow values and wastewater temperatures for 2024, are presented in Fig. 3. Wastewater flow was generally stable across sites and did not differ markedly when normalised per inhabitant. However, periods of increased flow were observed during the late snowmelt period (approximately April–May) in the northernmost cities (Luleå, Umeå, and Östersund). As expected, these increases in flow coincided with corresponding decreases in

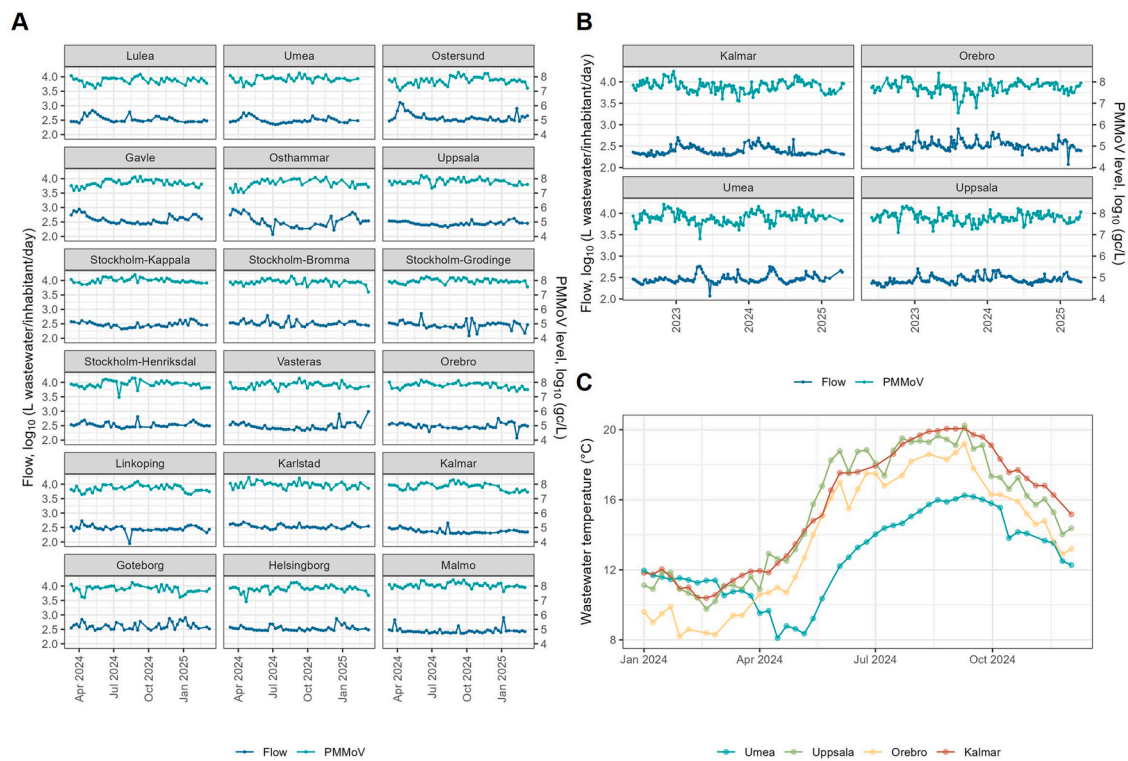


Fig. 3. A: Raw PMMoV concentrations and flow values for all valid samples from the short-term monitoring, arranged from the northernmost to the southernmost WWTP. B: Raw PMMoV concentrations and flow values for all valid samples from the long-term monitoring, arranged from the northernmost to the southernmost WWTP. C: Daily average wastewater temperatures during 2024 for all valid samples from the long-term monitoring.

PMMoV concentrations, expressed as gc/L. No meaningful associations were identified between inhibition and flow, or between inhibition and PMMoV concentration (gc/L). Pearson's correlation coefficients were low ($r = -0.065$ and $r = 0.10$, respectively).

3.1.2. Short-term monitoring: PMMoV levels across different WWTPs

During one year of weekly monitoring, PMMoV levels remained generally stable within and across most WWTPs (Fig. 4, Model 1). The overall average concentration was $10.34 \log_{10}$ gc/inhabitant/day. No substantial differences were observed between WWTPs, although some sites had somewhat higher or lower mean values compared to the reference site (Luleå). Malmö, Karlstad and Stockholm-Käppala had the highest mean values, while Linköping and Östhammar had the lowest; the difference between the highest and lowest estimated WWTP means was $0.31 \log_{10}$ units.

Overall, PMMoV levels within most WWTPs remained stable over time, though a few showed small temporal changes. Minor yet significant temporal trends were observed in five out of 18 WWTPs: Stockholm-Bromma, Örebro, Karlstad, Kalmar and Helsingborg.

Variability associated with PCR runs accounted for a substantial portion of the variation in PMMoV concentrations. The SD associated with the random effect of analytical run estimated at $0.12 \log_{10}$ units, which was consistent with the variability observed in the control chart (Fig. 2). The residual SD, representing unexplained variability, was $0.14 \log_{10}$ units. The model explained 62.1 % of the deviance (adjusted $R^2 = 0.57$), indicating that differences between cities, temporal trends and analytical rounds together accounted for a substantial portion of the observed variation in PMMoV concentrations.

A strong linear relationship was observed between the \log_{10} number of inhabitants connected to each WWTP and the average daily total \log_{10} PMMoV load (Fig. 5). The adjusted R^2 value of the model was 0.98 and the slope was 1.11. This indicates an almost proportional scaling, whereby a tenfold increase in the number of inhabitants corresponds to a slightly greater than tenfold increase in the average PMMoV load.

To assess whether population structure influenced mean PMMoV levels, we extended Model 2 by including the proportion of foreign-born residents and a socioeconomic index for each WWTP municipality (Fig. 5), as these variables could potentially reflect differences in dietary habits, including *Capsicum* consumption. Inclusion of these variables did not improve model performance, as indicated by lower adjusted R^2 values and higher AIC scores compared with the base model. This suggests that differences in population structure were not major drivers of PMMoV levels across the studied WWTPs.

3.1.3. Long-term monitoring: PMMoV levels over nearly three years

Over nearly three years of weekly monitoring at four geographically distinct WWTPs, PMMoV concentrations remained generally stable within each site (Fig. 6). The overall differences in mean PMMoV levels between WWTPs were small: Kalmar had only slightly lower average concentrations compared with the reference site (Umeå), while Uppsala and Örebro were similar to the reference.

Temporal smooths indicated limited within-site trends for Uppsala, while minor yet statistically significant temporal fluctuations were detected in Umeå, Örebro and Kalmar. Like the short-term monitoring, there was large variability between analytical rounds (SD = $0.15 \log_{10}$ units), and the residual standard deviation, representing unexplained variation, was $0.15 \log_{10}$ units. The model explained 69.5 % of the deviance (adjusted $R^2 = 0.60$).

No significant correlation was observed between PMMoV concentrations and wastewater temperature (Pearson's $r = -0.014$, $p = 0.85$), suggesting that temperature fluctuations within the observed range (8–21 °C; Fig. 3) had negligible impact on the PMMoV levels.

3.1.4. Daily monitoring: PMMoV levels at different weekdays

Daily sampling of PMMoV over five weeks at a WWTP in Uppsala indicated no pronounced association between PMMoV levels and specific weekdays (Fig. 7).

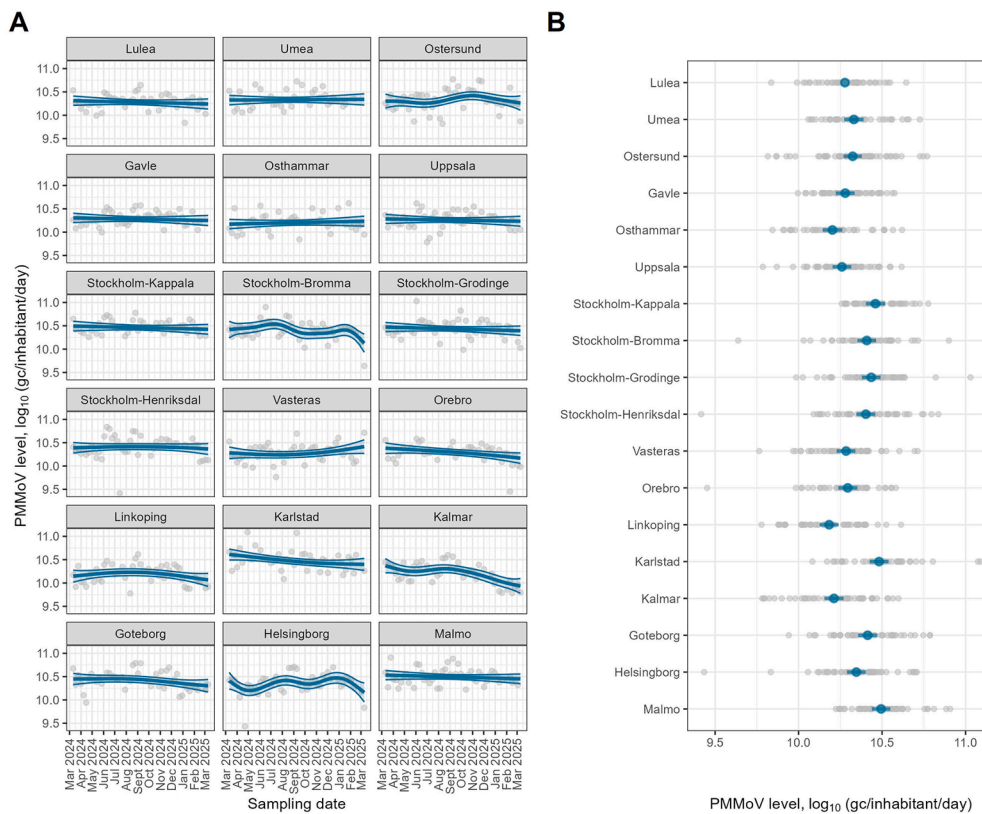


Fig. 4. One-year monitoring of PMMoV in 18 WWTPs. **A:** Time series of measured values for each WWTP, with fitted GAM smooths (Model 1) showing temporal trends during the sampling period, along with their 95 % confidence bands. Grey dots represent individual data points. **B:** Effect of each WWTP from Model 1. The blue points represent the estimated effects, while the horizontal bars indicate 95 % confidence bands. Luleå does not have a confidence band, as it was used as the reference category in the model, meaning the effects of other sites are interpreted relative to it. The WWTPs are plotted in a north to south direction. Grey dots represent individual data points.

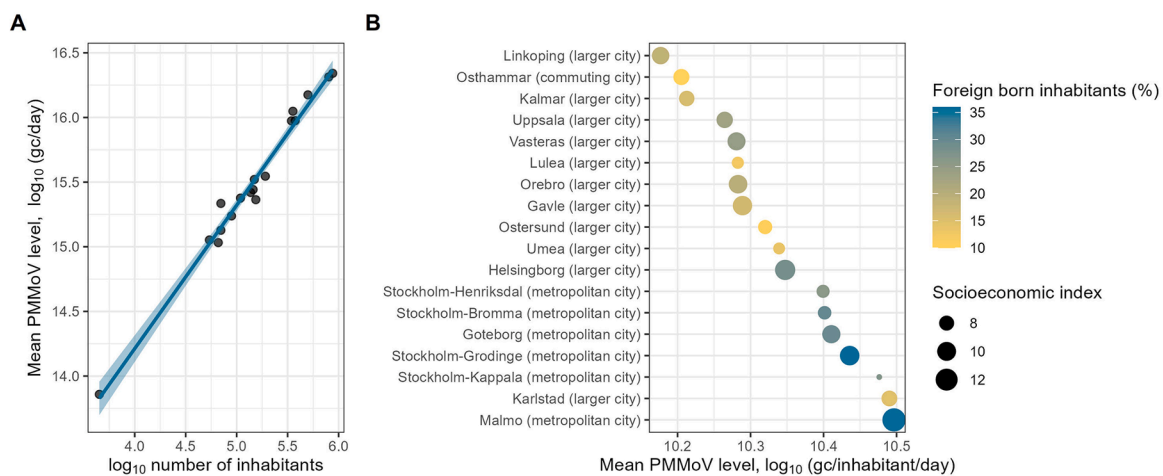


Fig. 5. **A:** Relationship between the number of inhabitants connected to each WWTP and the mean total daily PMMoV levels from the short-term monitoring. Points represent individual WWTPs, the line shows the fitted OLS regression, and the shaded area indicates the 95 % confidence interval of the fitted values. **B:** Mean per-inhabitant PMMoV levels from each WWTP, arranged from lowest to highest, alongside socioeconomic index and the percentage of foreign-born inhabitants within the municipalities included in the WWTP catchment area. The socioeconomic index represents the average of three indicators: the percentage of inhabitants with only pre-secondary education, the percentage with a low economic standard, and the percentage either receiving economic assistance or experiencing long-term unemployment. Higher index values indicate greater socioeconomic vulnerability. The official classification of each city is indicated in parentheses on the y-axis labels.

3.2. PMMoV in food

We analysed various food items to identify potential dietary sources of PMMoV. Of the 60 food products tested, 45 exhibited detectable levels of PMMoV, surpassing the limit of blank (set at 100 copies per reaction,

consistent with the threshold established in the wastewater study). None of the negative controls used in the food analyses exceeded this limit, and no food sample showed more than 2 Cq values of inhibition, with most samples showing very low levels of inhibition.

High PMMoV levels were detected across a range of food products,

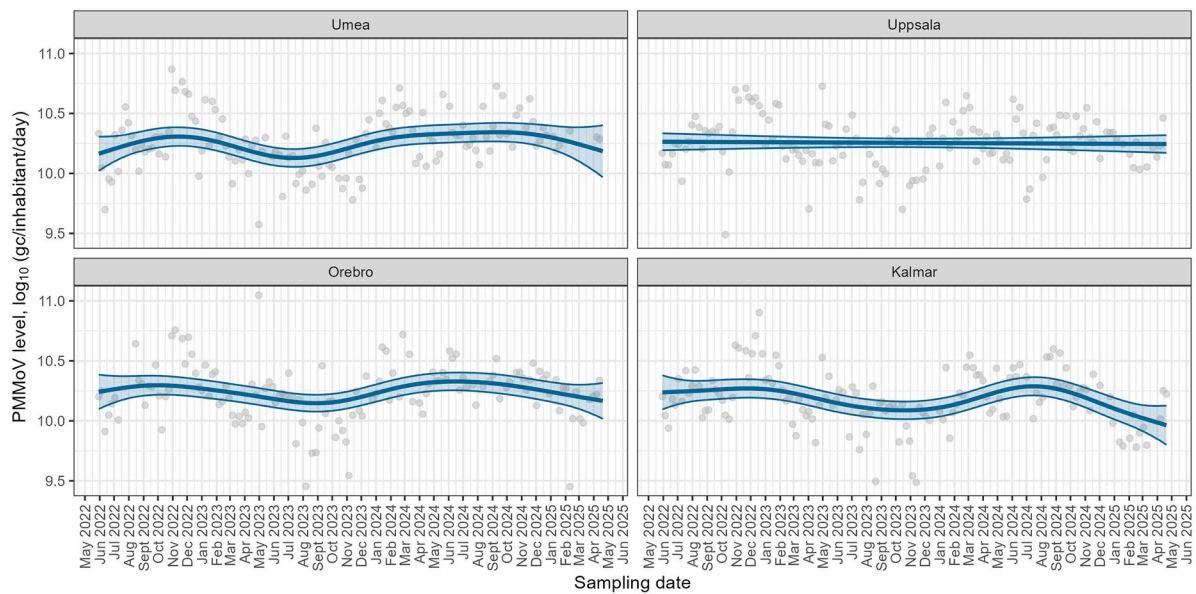


Fig. 6. Nearly three-year monitoring of PMMoV in four WWTPs, showing trends in PMMoV levels over time for each WWTP, with dots representing individual data points, solid lines indicating predicted means and shaded areas showing 95 % confidence bands from GAMM smooths (Model 3).

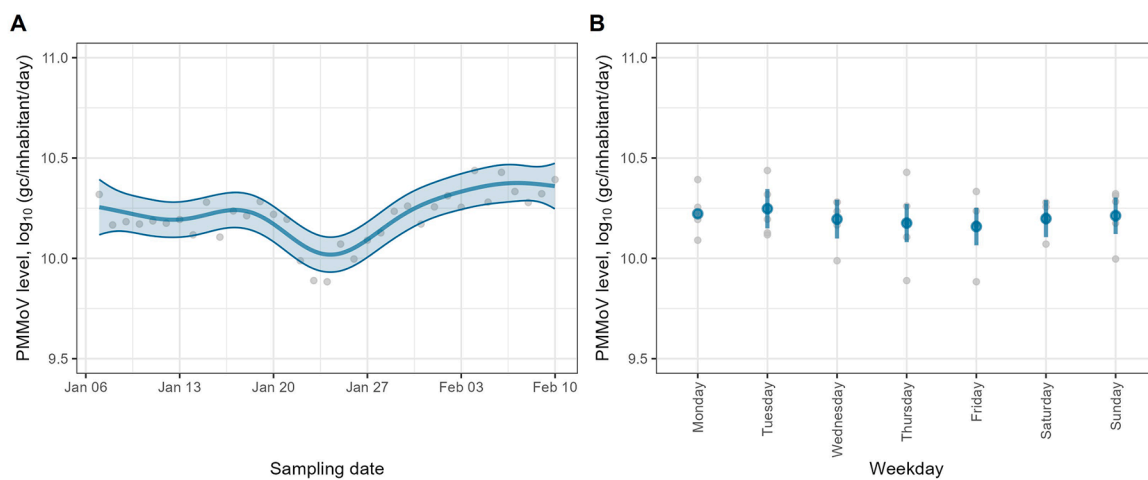


Fig. 7. Daily monitoring of PMMoV in one WWTP. A: Time series of measured PMMoV levels each day, with a fitted GAM smooth (Model 4) showing trends during the sampling period, along with 95 % confidence bands. Dots represent individual data points. B: PMMoV levels on different weekdays. The blue dots represent the estimated effects, while the bars indicate 95 % confidence bands. Monday does not have a confidence band, as it was used as the reference category, meaning the effects of other days are interpreted relative to it. Grey dots represent individual data points.

particularly in spices, spice mixes, pre-made and processed foods, pre-made sauces and various snack-related items. The highest concentrations were found in pre-made vegetarian chicken, a crisp dip seasoning blend and paprika powder, ranging from 12.21 to 11.31 \log_{10} gc/serving (Fig. 8). Notably, two of the three *Capsicum* fruits (paprika and spitzpaprika) had levels below the limit of blank, yet all tested spices and spice mixes, and most of the pre-made foods, both those sold fresh and frozen, exhibited high PMMoV levels. The estimated serving sizes and PMMoV levels expressed in copies per gram are provided in Table A.2.

Assuming that PMMoV remain largely intact as it passes through the digestive tract without significant degradation, and, conversely, that there is no substantial breakdown of plant cell walls or viral particles that could make the RNA more easily detectable in faeces than in food, our results suggest that a single serving or even markedly less of several tested foods could theoretically account for the average daily PMMoV levels detected in wastewater (10.34 \log_{10} gc/inhabitant/day). However, a potential discrepancy between PMMoV levels in food and

wastewater may stem from differences in extraction efficiencies between the methods used for their analysis. To assess this, we conducted a control extraction on three food items (Tabasco sauce, vegetable bouillon cube, and chilli powder) using the wastewater extraction method. In this analysis, the results closely matched those obtained with the plant extraction method (Table A.3).

4. Discussion

4.1. PMMoV in wastewater

4.1.1. Analytical performance

Since the reliability of PMMoV as a faecal indicator also depends on the underlying analytical performance, we began by examining the extent and sources of analytical variation in our dataset. Notably, we observed substantial variation between different RT-qPCR runs. In both the short- and the long-term monitoring, analytical run was therefore

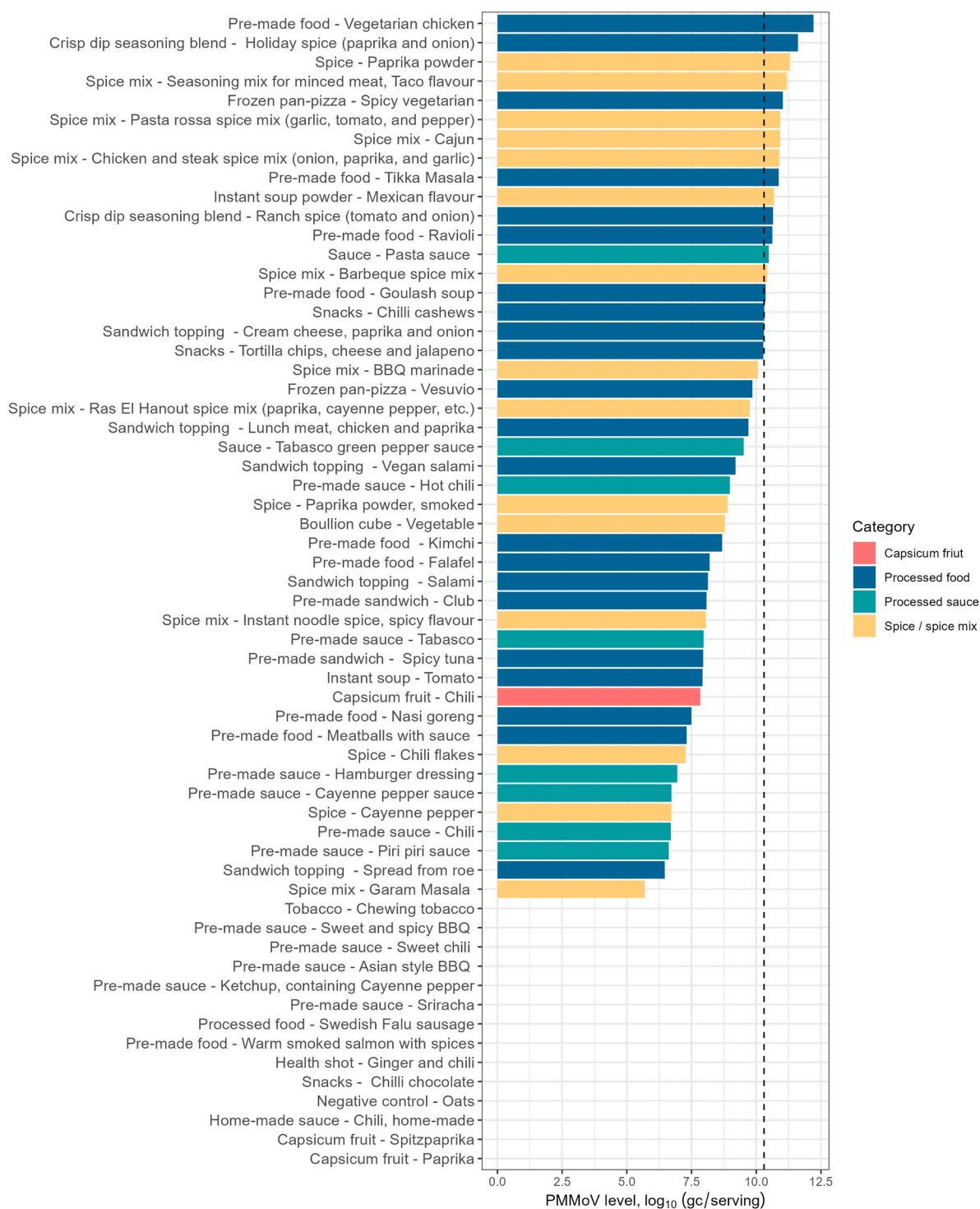


Fig. 8. PMMoV levels in various food products, presented as gc/serving. As a reference, the dotted vertical line indicates the average daily per-inhabitant PMMoV level in wastewater (10.34 log₁₀ gc/inhabitant/day).

included in the models as a random effect to account for this variability. The overall weekly variability across WWTPs may, of course, reflect not only technical variation related to the PCR run, but also other factors, such as different transport conditions, sample processing, nucleic acid extraction procedures, operator effects, or genuine general variations in PMMoV levels. Nonetheless, the standard deviations associated with the analytical run (0.13 and 0.15 log₁₀ for short-term and long-term monitoring, respectively) were very similar to those observed in the control chart (0.14 log₁₀ units, Fig. 2), which reflects purely PCR-related run-to-

run variation. This suggests that technical variation between PCR runs accounts for a large part of the total variation in PMMoV concentrations. We also tested models that included the same explanatory factors but omitted analytical run as a random effect, and these explained substantially less of the variation (adjusted R² values were 0.32 and 0.24 for short- and long-term monitoring, respectively) than the final models (adjusted R²: 0.57 and 0.60).

We explored several strategies to minimise plate-to-plate variability, including the conventional use of a quantification standard in each run

and normalisation between plates using a consistent wastewater sample positive for PMMoV RNA (positive controls shown in Fig. 2). Including a separate quantification standard in each run increased variability between runs, and applying the wastewater positive control for plate-to-plate normalisation did not decrease variability. For these data, the most effective approach was to apply a general slope and intercept derived from standard curves averaged across multiple runs. One potential option to reduce variability across runs could be to change from RT-qPCR to digital PCR (dPCR) (Hindson et al., 2013). A previous study on hepatitis A virus demonstrated a reduction in geometric CV from 35 % between RT-qPCR runs (comparable to the 33 % variation observed here) to 1.2 % with RT-dPCR (Persson et al., 2021). Therefore, dPCR-based quantification of PMMoV in wastewater warrants further investigation.

PCR inhibition is a well-recognised challenge in wastewater analysis and can cause underestimation or false-negative results. The analytical method used in this study resulted in very low levels of inhibition overall. However, two sites, Karlstad and Umeå, had the highest numbers of strongly inhibited samples. The underlying cause remains unclear, but samples from Umeå occasionally showed a darker colour, which may indicate the presence of substances that interfere with PCR amplification or the generation of fluorescent signals. The inhibited samples were distributed throughout the sampling period and were not associated with any specific time of the year.

4.1.2. Variation across WWTPs

We found no substantial variation in PMMoV levels between WWTPs and no associations with potential influencing factors such as overall socioeconomic status or population composition (proportion of foreign-born inhabitants) in the catchment municipalities, despite broad variation in these parameters among the populations of the studied WWTPs (Fig. 5, Table A.1), supporting the use of PMMoV as a robust marker. These findings are consistent with a study from Kentucky, USA, which also reported no effect of household income on PMMoV concentrations in wastewater (Holm et al., 2022). However, some limitations should be considered in this analysis. First, although the included WWTPs covered a large proportion of the Swedish population, the data were generated from only 18 WWTPs, and by selecting WWTPs with mostly large, primarily urban catchment areas, we limited the possibility of examining demographic effects at a fine scale and at rural settings and very small catchments (Pappu et al., 2026).

We also considered whether certain industrial discharges could influence PMMoV levels. For example, one of Sweden's largest crisp manufacturers, which produces paprika and chilli flavoured products, is connected to the wastewater treatment plant in Göteborg. However, the PMMoV concentrations in Göteborg did not stand out from those of the other WWTPs, suggesting that any contribution from the food industry was likely diluted due to the large population it supplies.

A large study from the United States reported longitudinal variation in PMMoV concentrations, with somewhat higher levels in western regions than in the east (Rosengart et al., 2024). The authors speculated that these patterns could potentially be influenced by dietary factors but noted that evaluating this hypothesis would require additional data on pepper consumption. In contrast, although our study covered a much smaller geographical area, we found no evidence of spatial trends in Sweden based on the latitude or longitude of the WWTPs (data not shown).

The average total daily PMMoV load scaled almost proportionally with the number of inhabitants connected to each WWTP (Fig. 5), supporting the suitability of PMMoV as a general proxy for population size in the analysed catchments. However, the slope was slightly greater than one (1.11), indicating disproportionately higher PMMoV loads in larger WWTPs. This pattern may reflect greater industrial discharges in larger cities, differences in diet or infrastructure, and most likely biases in population estimates due to contributions from temporary populations such as commuters and visitors that are not captured in official

figures. A similar scaling relationship was reported in a study from Missouri, USA, which covered a comparable range of WWTP catchments (Hsu et al., 2022).

4.1.3. Temporal variation

In general, we found no clear or consistent temporal patterns in PMMoV concentrations in either short- or long-term monitoring. Visual inspection of the raw time-series data with rolling averages also revealed no evidence of specific seasonal or month-specific patterns (Figure A.4). However, it is possible that longer-term monitoring (five years or more) would be required to detect more subtle temporal or seasonal effects.

We also observed no relationship with wastewater temperature. This apparent stability is consistent with previous studies showing that PMMoV remains stable over a wide temperature range (Burnet et al., 2023). Such robustness supports its use as a general faecal marker. However, when using PMMoV for population normalisation in WBS, its relative stability should be considered, as many viruses of interest, such as SARS-CoV-2, are less stable at higher temperatures (Burnet et al., 2023). Consequently, there is a risk that PMMoV-normalised values may underestimate infection rates during warmer periods.

Another relevant weather-related factor is snowmelt and precipitation, which typically increase wastewater flow and thus dilute PMMoV concentrations. In the northernmost cities, increased flows during the snowmelt period were accompanied by decreased PMMoV concentrations (gc/L) (Fig. 3). Outside this period, both wastewater flow and PMMoV concentrations (gc/L) remained relatively stable over time.

Some studies have shown that non-normalised SARS-CoV-2 concentrations in wastewater correlate more strongly with clinical case numbers than concentrations normalised to PMMoV (Duvall et al., 2022; Feng et al., 2021; Greenwald et al., 2021). This may be particularly true in settings where both wastewater flow and the contributing population remain relatively stable over time. In such conditions, introducing an additional normalisation target, such as PMMoV, may primarily add analytical variability rather than improve the strength of the epidemiological signal. However, in settings such as the northernmost cities in this study, where wastewater flow can vary over time, normalisation strategies are likely important to account for dilution effects and to ensure meaningful temporal comparisons, especially if wastewater flow data are not available.

Temporal fluctuations in PMMoV levels can, and ideally should, reflect changes in the number of people contributing to the wastewater system. Population mobility data from Statistics Sweden indicate that in larger cities such as Kalmar, Örebro, Uppsala, and Umeå, population levels remain relatively stable throughout the year, except in July (the main holiday season) and around Christmas (Statistics Sweden, 2023). In July, these cities typically experience a population decrease of about 10 %. However, this seasonal reduction was not clearly reflected in our PMMoV measurements. Variations between individual data points were higher, likely masking these changes due to analytical variability and environmental factors affecting PMMoV stability (Fig. 6). Future studies could investigate this in more detail by increasing sampling frequency, applying more precise quantification methods (e.g., digital PCR), and directly comparing PMMoV levels with independent mobility datasets, such as mobile phone records (Thomas et al., 2017) or other well-characterised population markers in wastewater.

Since PMMoV is ingested through food, and certain foods containing ingredients derived from *Capsicum* species, such as chilli-flavoured snacks and tacos, may be consumed more frequently on specific days of the week, we hypothesised that PMMoV concentrations in wastewater could show day-to-day variability. If present, such variability could affect the reliability of PMMoV as a faecal marker. This potential effect could not be assessed using the short-term or long-term monitoring series, as sampling in these datasets was conducted once a week, typically on Mondays. To address this limitation, we included a daily monitoring series. This series revealed no clear differences in PMMoV concentrations between weekdays (Fig. 7). Consistent with our findings, a study

from the United States also reported low daily variability in PMMoV concentrations in wastewater, with fluctuations comparable to those observed for other biological markers less directly linked to diet, such as the cryptic plasmid pBI143 and CrAssphage (Zheng et al., 2025). Taken together, these results indicate that short-term dietary patterns do not introduce substantial day-to-day variability in wastewater PMMoV concentrations,

4.1.4. Comparisons to data from other countries

In general, the PMMoV levels we detected, both in gc/L (on average $7.81 \log_{10}$) and gc/inhabitant/day ($10.34 \log_{10}$), are comparable to the results of other studies, including those in the United States [(Holm et al., 2022); approximately $6\text{--}10 \log_{10}$ gc/L], Canada [(Goitom et al., 2024); $\sim 9\text{--}11 \log_{10}$ gc/inhabitant/day and (Dhiyebi et al., 2023); $\sim 7\text{--}8 \log_{10}$ gc/L], Germany [(Haeusser et al., 2023); $\sim 5\text{--}7 \log_{10}$ gc/L] and Saudi Arabia [(Eifan et al., 2023); $\sim 5\text{--}7 \log_{10}$ gc/L].

4.2. PMMoV in food

Diet, particularly the consumption of foods containing *Capsicum* species, is likely the most important population-related factor contributing to variability in PMMoV levels. Although socioeconomic status and cultural background are often associated with dietary habits, access to detailed dietary data would be valuable to evaluate whether regional and temporal differences in *Capsicum* consumption contribute to the observed variation in PMMoV levels between WWTPs.

For PMMoV to serve as a reliable human faecal marker, its source must be foods consumed consistently throughout the year, across regions, and by diverse population groups. During the preparation of this study, we therefore considered whether *Capsicum* consumption in Sweden could be investigated in greater detail. We had access to data from Sweden's largest nationwide dietary survey (Swedish Food Agency, 2011), in which participants recorded all foods consumed over five consecutive days. However, the dataset lacks detailed information on added spices, sauces, and other minor ingredients, which substantially limited its usefulness for assessing *Capsicum* consumption. Therefore, we decided not to include it in our study.

Despite this limitation, the survey enabled us to identify the most commonly consumed dishes in Sweden. We then reviewed hundreds of recipes for these dishes, as well as ingredient lists for foods available on the Swedish market. Ingredients such as chilli pepper and paprika powder were frequently present, especially in processed foods. On this basis, it is reasonable to assume that a substantial proportion of processed foods contain ingredients derived from *Capsicum*.

To explore this further, we analysed a selection of commonly consumed foods sold in local retail supermarkets. Our results indicate that even a single serving, or in some cases considerably less, of several tested foods could theoretically account for the average daily per capita PMMoV load observed in wastewater (Fig. 8). For example, paprika powder contained some of the highest PMMoV concentrations detected ($11.25 \log_{10}$ gc/g), suggesting that a daily intake of approximately 0.1 g could correspond to the average wastewater load. Given the widespread use of paprika powder as both a flavouring and colouring agent in a wide range of processed foods, this finding may help explain the persistently high PMMoV concentrations observed in domestic wastewater.

However, this interpretation assumes that PMMoV is not extensively degraded during passage through the gastrointestinal tract and that digestion does not substantially alter viral particles or plant matrices in a way that significantly affects RNA detectability in faeces. Quantitative data on PMMoV degradation during passage through the gastrointestinal tract are limited, but one study reported the presence of PMMoV in food prior to ingestion and its subsequent detection in faecal samples from three individuals consuming these foods, using semi-quantitative RT-PCR (Zhang et al., 2006), indicating that detectable PMMoV RNA persists through the digestive process. It is also important to note that even if partial degradation occurs during digestion, the RT-qPCR assay

used in this study targets a very short RNA fragment (68 bases), which may remain amplifiable despite capsid disruption or RNA fragmentation.

The PMMoV concentrations detected in this study were comparable to or exceeded those reported previously, which have mainly focused on spicy food items. Earlier studies have reported concentrations of up to $8 \log_{10}$ gc/g or mL in products such as hot sauces and spices (Ko et al., 2020; Peng et al., 2015; Zhang et al., 2006). In contrast, very few studies have investigated milder processed or ready-to-eat foods. One exception is the study by Zhang et al. (2006), which analysed complete meals, although it did not specify the individual food components tested.

Notably, *Capsicum* fruits infected with PMMoV are more likely to be processed into spices or sauces than sold fresh, as infection often affects the fruit's appearance (Kumari et al., 2023). Furthermore, PMMoV is known to withstand challenging environments and standard food processing methods (Zhang et al., 2006). These factors suggest that spices and processed foods are more likely to contribute substantially to PMMoV levels in wastewater than fresh *Capsicum* fruits. Our results support this interpretation: the highest PMMoV concentrations were detected in spices and processed foods, whereas only one of the three fresh *Capsicum* fruits analysed contained measurable PMMoV.

This study nevertheless has some limitations. First, only a single batch of each food type was analysed, so batch-to-batch variability could not be assessed. Future studies should therefore include multiple batches to better characterise variability in PMMoV concentrations across food products. In addition, portion sizes vary considerably between individuals, introducing uncertainty into the estimated contributions shown in Fig. 8, although the concentration values per gram are given in Table A2.

Another limitation is that differences in extraction efficiency between food matrices cannot be entirely excluded. For sample preparation, we selected a plant RNA extraction kit, as PMMoV is a plant virus and many of the tested food matrices are plant-based and rich in PCR inhibitors. The selected kit is designed to efficiently lyse plant material and remove inhibitory compounds. For comparability across all food samples, including ready-made products with mixed ingredients, the same extraction kit was used for all samples, with matrix-specific preparation steps. This approach was effective, with only low levels of inhibition observed in the RT-qPCR analysis. However, a more detailed follow-up study incorporating multiple batches and recovery controls across different food matrices would be valuable to strengthen these findings.

Despite these limitations, to our knowledge, this study represents the most comprehensive investigation of PMMoV occurrence in food items to date, and the first to directly relate PMMoV concentrations in food with levels observed in wastewater.

5. Conclusions

- The apparent stability and consistently high concentrations of PMMoV in domestic wastewater across various timescales (days, months, and years) and across communities differing in size, population composition, and climatic conditions, support its suitability as a robust human faecal marker and a proxy for population size in WBS.
- By directly comparing PMMoV concentrations in wastewater and commonly consumed foods, we show that several food products contain PMMoV at per-serving levels comparable to the average daily per-inhabitant load observed in wastewater. As many of these foods are likely consumed year-round by a broad and diverse population, this finding further supports the robustness and broad applicability of PMMoV as a human faecal indicator.
- Taken together, this study is the most comprehensive investigation of PMMoV in food products to date and provides important insights into the possible dietary sources responsible for the high and stable PMMoV concentrations in domestic wastewater.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used the tools InstaText Editor and ChatGPT solely for editing and proofreading. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

CRedit authorship contribution statement

Sofia Persson: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Javier Edo Varg:** Writing – review & editing, Investigation, Data curation. **Sarah Coker:** Writing – review & editing, Investigation, Data curation. **Fanny Persson:** Writing – review & editing. **Filip Petrini:** Writing – review & editing, Investigation. **Israa Dafalla:** Writing – review & editing, Investigation. **Lauren Davies:** Writing – review & editing, Investigation. **Polina Vaulina:** Writing – review & editing, Investigation. **Monika Vilemova:** Writing – review & editing, Investigation. **Nahla Mohamed:** Writing – review & editing, Methodology, Investigation. **Maja Malmberg:** Writing – review & editing, Project administration. **Anna J. Székely:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.watres.2026.125629](https://doi.org/10.1016/j.watres.2026.125629).

Data availability

Data will be made available on request.

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