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Integrating wastewater surveillance and meteorological data to monitor seasonal variability of enteric and respiratory pathogens for infectious disease control in Dhaka city

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ABSTRACT

Background: Seasonal meteorological variations influence the spread of infectious diseases. Wastewater surveillance helps understanding pathogen transmission dynamics, particularly in urban areas of climate-vulnerable countries like Bangladesh.

Methods: We analysed 54 weeks of wastewater surveillance, clinical surveillance, and meteorological data from Dhaka, Bangladesh. Samples from 11 sites were tested for *Vibrio cholerae (V. cholerae)*, SARS-CoV-2, *Salmonella enterica* subspecies *enterica* serovar Typhi (*S.* Typhi), and Group A rotavirus. Diarrhoeal Disease Surveillance data were sourced from icddr,b, and meteorological data from the Bangladesh Meteorological Department. Regression models adjusted for site and time variations were used for statistical analysis.

Results: Proportion of confirmed cholera cases among the diarrhoeal disease surveillance recruits were highest during post-monsoon (coef: 2.53; 95 % CI: 0.41 to 4.67; $p = 0.029$). *V. cholerae* log10 concentrations in wastewater were positively associated with pre-monsoon (coef: 0.93; 95 % CI: 0.26 to 1.58; $p = 0.010$), while SARS-CoV-2 peaked during monsoon (coef: 1.85; 95 % CI: 0.96 to 2.73; $p < 0.001$). *S.* Typhi and rotavirus log10 concentrations showed negative associations with pre-monsoon (coef: −0.96; 95 % CI: −1.68 to −0.27; $p = 0.011$, and −0.84; 95 % CI: −1.17 to −0.50; $p < 0.001$, respectively). Temperature positively influenced log10 concentrations of *V. cholerae* (adj. coef: 0.09; 95 % CI: 0.02 to 0.15; $p = 0.014$) and SARS-CoV-2 (adj. coef: 0.19; 95 % CI: 0.10 to 0.27; $p < 0.001$), but negatively associated with rotavirus (adj. coef: −0.06; 95 % CI: −0.10 to −0.03; $p < 0.001$). Similar associations were found between pathogen-positive samples and temperature.

Conclusion: Our study shows that seasonal, and meteorological factors (particularly temperature) influence the patterns and abundance of pathogens in wastewater and help in understanding disease transmission across different weather patterns.

Abbreviations: BMD, (Bangladesh Meteorological Department); **DDSS**, (Diarrhoeal Disease Surveillance System); **DNCC**, (Dhaka North City Corporation); **DSCC**, (Dhaka South City Corporation); **DWASA**, (Dhaka Water Supply & Sewerage Authority); **gc/L**, (Genome Copy per Liter); **GIS**, (Geographic Information System); **icddr,b**, (International Centre for Diarrhoeal Disease Research, Bangladesh); **IPCC**, (Intergovernmental Panel on Climate Change); **LMICs**, (low- and middle-income countries); **qPCR**, (Quantitative Polymerase Chain Reaction); ***S.* Typhi**, (*Salmonella enterica* subspecies *enterica* serovar Typhi); **SARS-CoV-2**, (Severe Acute Respiratory Syndrome Coronavirus 2); ***V. cholerae***, (*Vibrio cholerae*); **WS**, (Wastewater surveillance); **WHO**, (World Health Organization); **OR**, (Odds Ratio).

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1. Introduction

The Lancet Commission and World Health Organization (WHO) have identified climate change as the foremost global health hazard of the 21st century (Costello et al., 2009; Rahman et al., 2019; Watts et al., 2017). The impact of climate change on health outcomes, exacerbated by socioeconomic determinants and environmental changes, underscores the need for deeper understanding, particularly in low- and middle-income countries (LMICs) such as Bangladesh (Haines and Patz, 2004). Since 2007, Bangladesh has consistently ranked among the highest on the Intergovernmental Panel on Climate Change (IPCC) risk index due to its vulnerability to climate-related hazards (Pachauri and Reisinger, 2007). The country faces an increased risk of extreme weather events that facilitate the spread of vector-borne and waterborne diseases, including malaria, dengue, and cholera (Rahman, 2008; Gilman et al., 2007; Waits et al., 2018; Barnes, 2018; Phung et al., 2018; Kabir et al., 2014). Millions of people in the country are already facing the health impacts of climate change, with increasing incidences of these diseases (Haque et al., 2012; Shahid, 2010).

Local environmental factors, along with socioeconomic determinants, and behavioural adaptations are critical in shaping the health impacts of climate change (Bickerstaff and Walker, 2001; Mercer et al., 2012). Meteorological factors, including temperature, precipitation, and humidity, are known to influence the incidence of communicable diseases (Baharom et al., 2021; Wu et al., 2016; Al-Amin et al., 2013). Studies conducted in Bangladesh have similarly identified associations between these factors and various communicable diseases, including acute watery diarrhea, typhoid fever, and COVID-19 (Chowdhury et al., 2018; Wu et al., 2014; Islam et al., 2021a).

Wastewater surveillance (WS) is a passive, sensitive, and cost-effective tool that complements existing clinical surveillance systems (Haque et al., 2022). This method detects pathogens by analysing markers in wastewater samples, allowing for the monitoring of both the overall prevalence and spatial-temporal trends of pathogens, including emerging ones, within communities (Haque et al., 2022; Maida et al., 2023; Aborhyem et al., 2022; Pillay et al.; Panchal et al., 2021; Bivins et al., 2022). It also holds potential as an alternative method for tracking climate-sensitive diseases (Diamond et al., 2023). Integrating WS data with other metrics of disease transmission, such as reported case numbers and hospitalization rates, can provide a more comprehensive understanding of disease dynamics, thereby informing more effective public health interventions. In areas where case records are poorly documented or absent, WS can still offer valuable insights into the spread and trends of disease (Haque et al., 2022).

In this observational study, we investigated the seasonal patterns and abundance of four human pathogens: *Vibrio cholerae (V. cholerae)*, SARS-CoV-2, *Salmonella enterica* subspecies *enterica* serovar Typhi (*S.* Typhi), and Group A rotavirus in the wastewater of Dhaka, the capital of Bangladesh. We also examined the correlation between the presence or concentration of these pathogens and meteorological parameters, including temperature, humidity, and rainfall. By analysing WS data with meteorological variables, this study aims to enhance understanding of meteorological influence on the dynamics of these pathogens. Clinical surveillance data on cholera cases were also analysed to support these findings.

2. Materials and methods

2.1. WS

We conducted WS in Dhaka Municipality over a 54-week period from October 16, 2022 to November 2, 2023. We aimed to monitor temporal and spatial trends of infectious diseases causing pathogens: *V. cholerae*, SARS-CoV-2, *S.* Typhi, and Group A rotavirus. These pathogens were selected based on several criteria. These are significant contributors to the city's disease burden and benefit from established, validated detection methods in wastewater and other environmental samples, ensuring reliable surveillance data. Additionally, as vaccine-preventable diseases, monitoring their prevalence can inform the allocation of health resources and vaccine campaign strategies. The analysis of their temporal and spatial trends further supports targeted public health interventions, including vaccine distribution and outbreak response measures. Wastewater samples were collected from 11 selected sites within Dhaka municipality and analysed for those four pathogens mentioned above.

2.1.1. Sampling sites of WS

The study area was divided into two regions in accordance with the administrative divisions of Dhaka: Dhaka North City Corporation (DNCC) and Dhaka South City Corporation (DSCC), with approximately six and four million inhabitants, respectively (Byuro). A total of 11 sampling sites were selected for wastewater sampling, comprising four sites in DSCC and seven in DNCC. All DNCC sites were located at the endpoints of drains and canals, while all DSCC sites were pumping stations [Fig. 1]. The sampling points in DSCC were located in Basabo, Narinda, Saydabad, and Faridabad, while those in DNCC were situated in Mohammadpur (Baitul Aman Housing Society), Uttara, Ashkona, Chamur Khan, Badda (Podardia and Barkatpur), and Mirpur (Dhaka Dental College). Samples were collected weekly from each site over 54 weeks, except for two non-consecutive weeks when heavy rainfall prevented collection. At the Basabo site, 45 samples were collected, while 52 samples were collected from each of the other 10 sites, totaling 565 samples. Detailed sampling site selection procedures are provided in Supplementary File 1.

2.1.2. Laboratory analysis of wastewater pathogens

We investigated the presence and concentration of four pathogens (SARS-CoV-2, *S.* Typhi, *V. cholerae*, and Group A rotavirus) in wastewater samples. The pathogens were detected and quantified by one step real time RT-PCR and PCR targeting pathogen specific characteristic genes. Wastewater samples were treated with Nanotrap® Microbiome A Particles (Ceres Nanosciences; SKU# 44202), which contain magnetized porous particles designed to isolate and concentrate microorganisms from wastewater matrix. Nucleic acid extraction was carried out using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit (Thermo Fisher Scientific™; Cat# A42357) according to the procedure recommended elsewhere (Ceres). Subsequently, OneStepTM PCR Inhibitor Removal Kit (www.zymoresearch.eu) was used to remove potential PCR inhibitors, naturally present in wastewater, according to the manufacturer's instructions. The one step real time RT-qPCR was conducted in a final reaction volume of 20 μL using TaqPath™ 1-Step RT-qPCR Master Mix (Thermo Fisher Scientific™, Massachusetts, USA). 5 μL of nucleic acid (denatured nucleic acid in case of rotavirus) were used as template. Specific oligonucleotide primers and probes were employed for the amplification and detection of the specific gene targets of the pathogens [Supplementary file 2]. Bio-Rad CFX Opus 96 Real-Time PCR Detection System was used with the following thermal cycling conditions: 2 min at 25 °C for UNG Incubation, 10 min at 53 °C for Reverse Transcription (when required), 2 min at 95 °C for Polymerase Activation followed by 45 cycles. Each cycle consisted of a denaturation step of 15 s at 95 °C, and annealing/extension at 56.5 °C (58 °C for SARS-CoV-2) for 1 min. To calculate gene copies of the pathogens in raw wastewater, a standard curve for each pathogen was generated using real time PCR data (CT values) of serially diluted standard synthetic plasmid DNA.

2.2. Clinical case data from the diarrhoeal disease surveillance system (DDSS) of icddr,b

The Diarrhoeal Disease Surveillance System (DDSS) was established by icddr,b at its urban Dhaka Hospital in 1979. This system routinely selects 2 % of all patients admitted with diarrhea, regardless of age, gender, or socioeconomic background. Specifically, every fiftieth

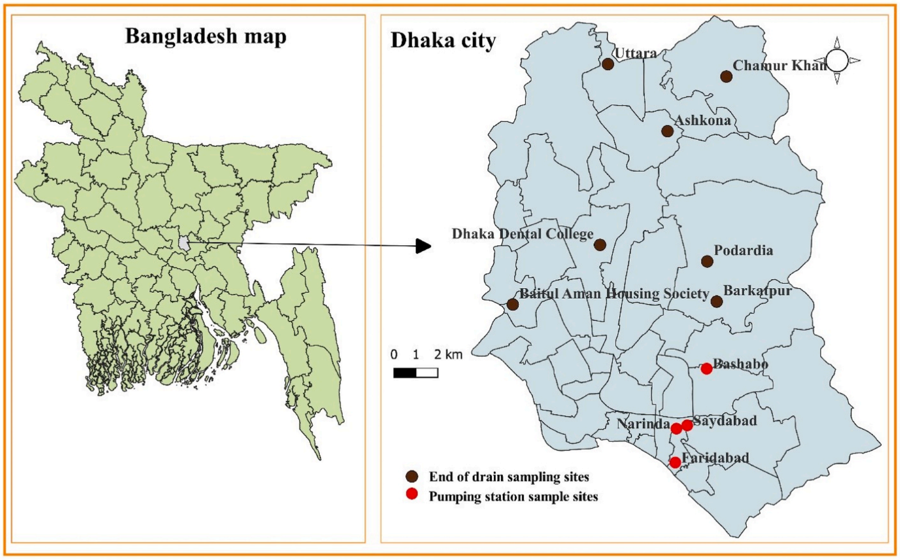

Fig. 1. Map showing sampling sites in Dhaka city (Map boundaries define study area and may not accurately represent recognized national borders).

patient is systematically enrolled in the surveillance process. Clinical, epidemiological, and diagnostic data are collected from these patients, including fecal cultures for the isolation and identification of pathogenic organisms such as *S.* Typhi, *V. cholerae*, and rotavirus (Das et al., 2014; Stoll et al., 1982). For this study, we obtained daily counts of 2 % of diarrhoeal cases and confirmed cholera cases from the DDSS data for the period corresponding to the timeframe of the WS.

2.3. Collection of meteorological data

Meteorological data for Dhaka district were sourced from the Bangladesh Meteorological Department (BMD). These data were obtained from the BMD's Dhaka Observation Station (Local ID: 11111, World Meteorological Organization ID: 41923). The dataset included daily averages, minimum and maximum temperatures (°C), relative humidity (%), and precipitation (mm) recorded between October 16, 2022, and November 2, 2023. The analysis of seasonality was conducted based on the following classifications: winter (December to February), pre-monsoon summer (March to May), monsoon or rainy season (June to September), and post-monsoon autumn (October to November) (Bazlur Rashid AS et al., 2024).

2.4. Data analysis

The concentrations of pathogens in the wastewater samples were converted to base-10 logarithmic values (log10) for analysis. To define positive samples, the qPCR cycle threshold (CT) values were used. CT value of 37 or below indicates a positive result, whereas values above 37 are considered false positives or nonspecific binding for the assay used in this study and are categorized as negative. Rainfall was categorized into two groups: "Rain," for any amount of rainfall in the prior seven days, including the sample collection day, and "No Rain," if no rainfall has occurred. We chose to binarize the rainfall data because, rainy days were rare during our study, with most days having no rain: 248 days (43.89 %) of "No Rain" vs. 317 days (56.11 %) of "Rain." For regression models, average rainfall in the prior seven days, including the sample collection day was used.

Shapiro-Wilk test was employed to assess normality of pathogen log10 concentrations. Subsequent analyses utilized non-parametric tests, as the results indicated non-normal distributions ($p < 0.05$). Descriptive statistics, such as medians and ranges, were used to evaluate the proportion of positive samples. Meteorological variables, including temperature, humidity, and rainfall, were examined across four distinct seasons: post-monsoon (PM), winter (W), pre-monsoon (Pre-M), and monsoon (M). Seasonal variations were visualized through trends in monthly averages.

The Wilcoxon Rank-Sum test was conducted to evaluate differences in meteorological variables (temperature, rainfall, and humidity) between when wastewater samples were positive and when they were negative. Box plots were used to illustrate the differences in temperature, rainfall, and humidity for each pathogen between positive and negative samples. Chi-square tests were performed to assess the association between rainfall and the proportion of positive samples in the wastewater. Scatter plots were utilized to visually represent the correlations between pathogen log10 concentrations and meteorological variables.

A hierarchical linear regression model was employed to assess the association between seasonal variation and pathogen log10 concentrations in wastewater, as well as between seasonal variation and the number of clinical cases. The analysis focused on the effects of different seasons, considering winter as the reference season due to its lowest temperature and rainfall levels. The following model was applied:

$$Y_{ij} = \beta_0 + \beta_1 X_{ij} + U_i + \epsilon_{ij}$$

In this model, Y_{ij} is the log10 concentration of the pathogen at site *i* and time *j*, $X_{i,i}$ is the seasonal category for time *j*, β_0 is the intercept, β_1 is the coefficient for the season category, U_i denotes the random intercept at the site level, and ϵ_{ij} is the residual error at site *i* and time *j*.

A hierarchical logistic regression analysis was conducted to assess the relationship between meteorological variables (average temperature, humidity, and rainfall) and the likelihood of a positive wastewater sample (classified based on CT threshold of 37). The model is expressed as follows:

$$logit\ (P(Z_{ij} = 1)) = \beta_0 + \beta_1 Temp_{i,j} + \beta_2 Humidity_{i,j} + \beta_3 Rainfall_{i,j} + U_i + \epsilon_{ij}$$

In this equation, $Z_{i,j}$ is the binary outcome at site *i* and time *j* (1 if the sample is positive, 0 if negative), $Temp_{i,j}, Humidity_{i,j}$ and $Humidity_{i,j}$ are the meteorological variables, β_0 is the intercept, β_1, β_2, and β_3 are the coefficients for each meteorological variable, U_i is the random intercept at the site level, and ϵ_{ij} is the residual error at site *i* and time *j*.

Also, a hierarchical linear regression analysis was conducted where the outcome is the log10 concentration of pathogens in wastewater samples at different sites and times, and the predictor is the number of 2

% diarrhea cases from DDSS at a given time. The model is specified as follows:

$$W_{ij} = \beta_0 + \beta_1 X_{ij} + U_i + \epsilon_{ij}$$

Here, W_i, represents the log10 concentration of Vibrio cholerae at site i and time j, $X_{i,i}$ is the number of 2 % suspected cholera cases at time j, β_0 is the intercept, β_1 is the coefficient for $X_{i,i}$, U_i denotes the random intercept at the site level, and ϵ_{ij} is the residual error at site i and time j.

A hierarchical linear regression analysis was conducted to assess the relationship between meteorological variables (average temperature, humidity, and rainfall) and the log10 concentration of the pathogens. The model is expressed as follows:

$$V_{ij} = \beta_0 + \beta_1 Temp_{i,j} + \beta_2 Humidity_{i,j} + \beta_3 Rainfall_{i,j} + U_i + \epsilon_{ij}$$

In this equation, $V_{i,j}$ is the log10 concentration of the pathogen at site i and time j, $Temp_{i,j}, Humidity_{i,j}$ and $Humidity_{i,j}$ are the meteorological variables, β_0 is the intercept, β_1, β_2, and β_3 are the coefficients for each meteorological variable, U_i is the random intercept at the site level, and ϵ_{ij} is the residual error at site i and time j.

Data management was performed using Excel and STATA, while analyses and visualizations were done using R version 4.2.2.

2.5. Ethical Considerations

Ethical approval for this study (protocol number: PR-22014) was granted by the Research Review Committee (RRC) and the Ethical Review Committee (ERC) of icddr,b. The procedures adhered to the organization's established ethical standards and regulations.

3. Results

The variations in pathogen detection and concentrations were observed across 565 wastewater samples collected through WS [Table 1]. Group A rotavirus had the highest prevalence at 530 positive samples (93.81 %) and a median log10 concentration of 8.53 gc/L (Range: 4.03, 10.86). *V. cholerae* followed with 366 samples (64.78 %) and a median log10 concentration of 6.28 gc/L (Range: 4.49, 7.89). SARS-CoV-2 was detected in 353 samples (62.48 %) with a median log10 concentration of 4.84 gc/L (Range: 3.03, 7.01). *S.* Typhi had the lowest prevalence, with 184 samples (32.57 %) and a median log10 concentration of 5.76 gc/L (Range: 4.17, 67.61). The median number of cholera cases observed in the diarrhoeal disease surveillance system (DDSS) data was 4 (Range: 1, 12) (not included in the table).

3.1. Seasonal variation of meteorological variables in Dhaka city

Fig. 2 illustrates seasonal variations in temperature, humidity, and rainfall. The monsoon season is characterized by high humidity (78 %) and substantial rainfall (11 mm), with temperatures averaging 30 °C. Post-monsoon conditions show reduced rainfall (6 mm), slightly lower temperatures (27 °C), and moderate humidity (74 %). Pre-monsoon is marked by rising temperatures (30 °C), the lowest humidity (61 %), and minimal rainfall (<1 mm). Winter experiences the coolest temperatures (22 °C) with moderate humidity (70 %) and no significant rainfall.

Table 1
Descriptive statistics of the positive samples in wastewater.

Pathogens	Number of positive samples n (%), N = 565	Median log10 gc/L (Range)
V. cholerae	366 (64.78)	6.28 (4.49, 7.89)
SARS-CoV-2	353 (62.48)	4.84 (3.03, 7.01)
***S.* Typhi**	184 (32.57)	5.76 (4.17, 67.61)
Rotavirus	530 (93.81)	8.53 (4.03, 10.86)

3.2. Seasonal variability of the positive samples and pathogen log10 concentrations in wastewater

3.2.1. Seasonal variability of the positive samples and proportion of confirmed cholera cases among 2 % diarrhoeal cases

Seasonal variations were observed across the positive detection rates of *V. cholerae*, SARS-CoV-2, *S.* Typhi, and rotavirus in wastewater [Fig. 3]. Number of *V. cholerae* positive samples increased steadily from late 2022, peaking at approximately 80 % during the 2023 monsoon, followed by minor fluctuations in the post-monsoon season. SARS-CoV-2 positive samples showed a high baseline of about 80 % in the post-monsoon season of 2022, a sharp peak at approximately 90 % in the early monsoon of 2023, and steep declines during winter and post-monsoon. *S.* Typhi exhibited a seasonal trend that tended to be inverse to *V. cholerae* and SARS-CoV-2, with peak positivity at about 60 % in early winter 2022, declining to approximately 20 % by pre-monsoon 2023, and stabilizing at around 30 % during the monsoon and post-monsoon seasons. Rotavirus positive samples remained consistently high, with values around 90 %–95 % year-round, showing only minor seasonal fluctuations.

Proportion of confirmed cholera cases among 2 % diarrhoeal cases peaked during the post-monsoon period. The proportion was lowest during the transition of winter and pre-monsoon periods.

3.2.2. Seasonal variability of pathogen log10 concentrations in wastewater

Seasonal variations were observed in pathogen log10 concentrations in wastewater, corresponding to fluctuations in the proportion of positive samples [Fig. 4]. Across pathogens, log10 concentrations were generally higher during the monsoon and post-monsoon seasons, while lower levels were observed during the winter and pre-monsoon periods.

V. cholerae log10 concentrations peaked at around 5.5 log10 gc/L during the early monsoon season (2023), with a secondary rise observed in the post-monsoon season (2023), reaching approximately 4.75 log10 gc/L. Similarly, SARS-CoV-2 log10 concentrations sharply increased to about 6.0 log10 gc/L at the onset of the monsoon season, followed by a rapid decline to approximately 2.0 log10 gc/L in the post-monsoon season. *S.* Typhi log10 concentrations were elevated during the early winter season (2022) at about 3.0 log10 gc/L, with a decline to 1.5 log10 gc/L during the pre-monsoon period in 2023, and slight increases during the monsoon season, stabilizing around 2.0 log10 gc/L. Rotavirus, on the other hand, exhibited the highest log10 concentrations overall, peaking at 8.5 log10 gc/L during the winter season and remaining consistently high throughout the winter, pre-monsoon, and monsoon seasons.

3.3. Association between seasonal variation and wastewater pathogen log10 concentration

Distinct influence of seasons on pathogen log10 concentration was observed across the four seasons using a hierarchical linear regression model, with winter as the reference season [Table 2]. Count of 2 % diarrhoeal cases showed a significant positive association with post-monsoon (coef: 2.53; 95 % CI: 0.41 to 4.67; p < 0.029). *V. cholerae* log10 concentration was significantly associated with the pre-monsoon period (coef: 0.93; 95 % CI: 0.26 to 1.58; p = 0.010). SARS-CoV-2 was significantly associated with the pre-monsoon (coef: 1.20; 95 % CI: 0.34 to 2.09; p = 0.011) and monsoon periods (coef: 1.85; 95 % CI: 0.96 to 2.73; p < 0.001). A significant negative association with *S.* Typhi was found in the pre-monsoon period (coef: −0.96; 95 % CI: −1.68 to −0.27; p = 0.011), with no significant associations in later seasons. Rotavirus log10 concentration showed a significant negative association with the pre-monsoon period (coef: −0.84; 95 % CI: −1.17 to −0.50; p < 0.001) and monsoon period (coef: −0.45; 95 % CI: −0.79 to −0.11; p = 0.013).

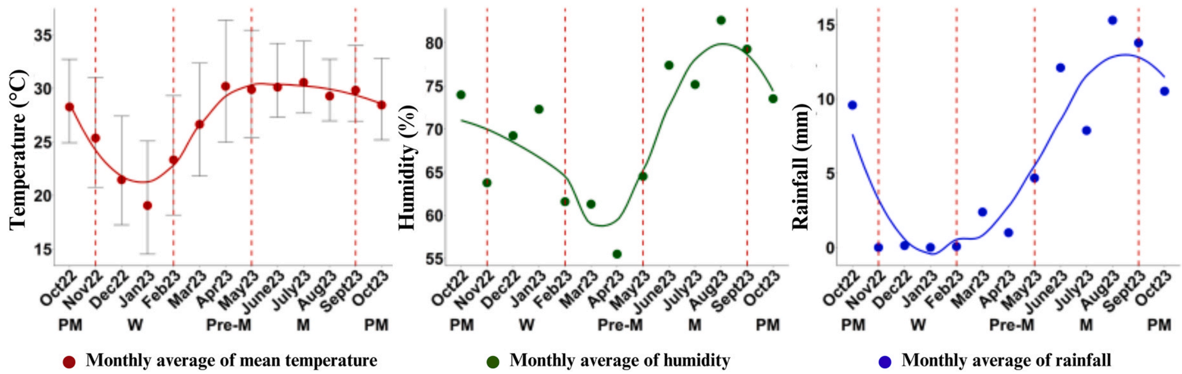

Fig. 2. Seasonal Variation in Temperature, Humidity, and Rainfall [Trendlines are generated using LOESS (locally estimated scatterplot smoothing). Error bars on each month point shows the minimum and maximum temperature of each month. Red vertical dashed lines mark specific seasons: PM: post-monsoon season W: winter, Pre-M: pre-monsoon season; M: monsoon.]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

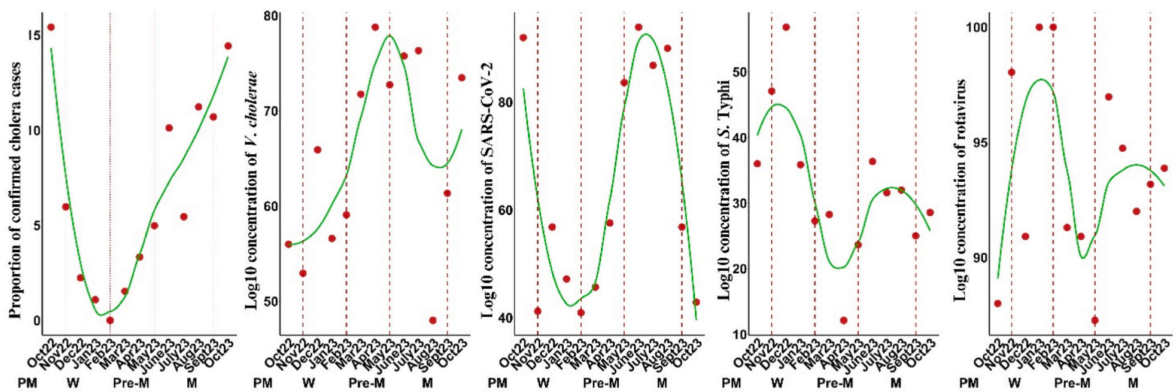

Fig. 3. Seasonal variability of positive samples in Dhaka city wastewater and the proportion of confirmed cholera cases among 2 % of diarrheal cases in DDSS [Red dots represent the monthly average. Green trendlines are generated using LOESS (locally estimated scatterplot smoothing). Red vertical dashed lines mark specific seasons: PM: post-monsoon season W: winter, Pre-M: pre-monsoon season; M: monsoon]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

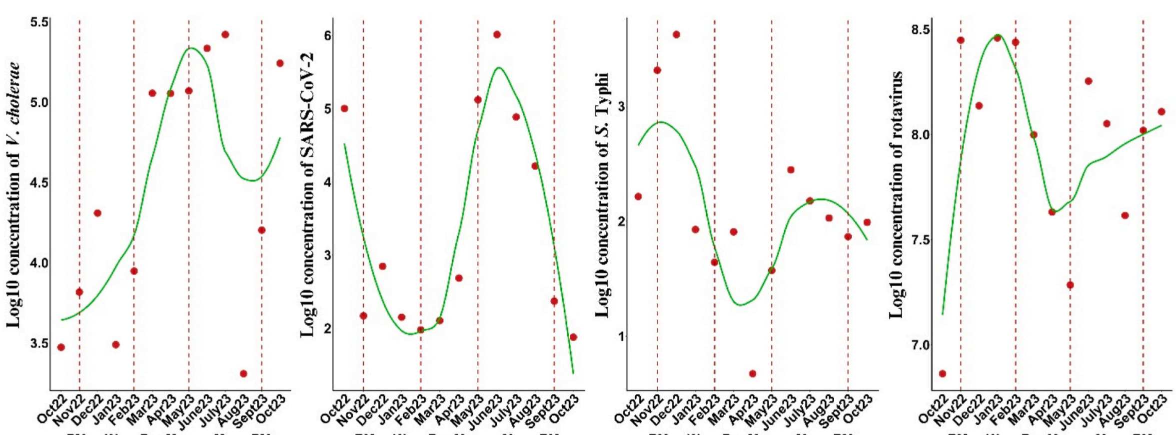

Fig. 4. Seasonal variability in the log10 concentrations of pathogens in Dhaka city wastewater [Red dots represent the monthly average. Green trendlines are generated using LOESS (locally estimated scatterplot smoothing). Red vertical dashed lines mark specific seasons: PM: post-monsoon season W: winter, Pre-M: pre-monsoon season; M: monsoon]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.4. Influence of temperature on pathogen detection, pathogen log10 concentration, and cholera case numbers

3.4.1. Influence of temperature on pathogen detection

The boxplots illustrate the association between positive and negative sample groups of targeted pathogens in wastewater and daily mean temperatures [Fig. 5 A]. For *V. cholerae*, higher mean temperatures were significantly associated with the detection of this pathogen ($p \leq 0.05$). For SARS-CoV-2, positive samples were significantly associated with higher mean temperatures ($p \leq 0.001$). Positive *S.* Typhi samples

Table 2
Association between wastewater pathogen log10 concentrations and 2 % diarrhoeal cases with seasonal variation.

Pathogens	Pre-monsoon		Monsoon		Post-monsoon		Winter
	β **(95 % CI)**	***p-value***	*β* **(95 % CI)**	***p-value***	***B*** **(95 % CI)**	***p-value***	***Ref.***
2 % diarrhoeal cases	−0.36 (1–1.12, 0.38)	*0.307*	0.96 (−0.75, 2.68)	*0.285*	2.53 (0.41, 4.67)	***0.029***	
V. cholerae	0.93 (0.26, 1.58)	***0.010***	0.30 (−0.59, 1.19)	*0.508*	0.16 (−0.62, 0.95)	*0.682*	
SARS-CoV-2	1.20 (0.34, 2.09)	***0.011***	1.85 (0.96, 2.73)	***<0.001***	0.19 (−0.67, 1.07)	*0.665*	
***S.* Typhi**	−0.96 (−1.68, −0.27)	***0.011***	−0.45 (−1.13, 0.23)	*0.200*	0.11 (−0.690.93)	*0.786*	
Rotavirus	−0.84 (−1.17, −0.50)	***<0.001***	−0.45 (−0.79, −0.11)	***0.013***	−0.33 (−0.80, 0.14)	*0.176*	

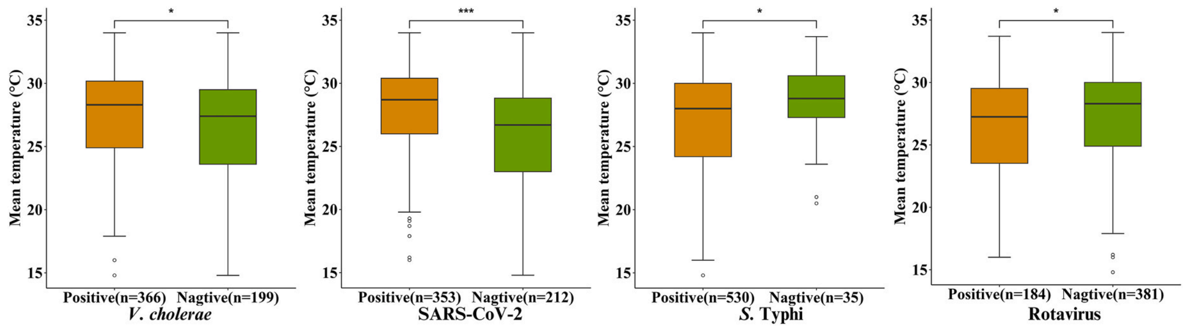

Fig. 5. A: Box plots of the mean temperatures (°C) associated with positive and negative samples [Results are presented as median (IQR); analysis by Wilcoxon Rank-Sum test. ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05].

exhibited a significant association with lower mean ($p \leq 0.05$) temperatures. Similarly, Rotavirus detection was inversely associated with temperatures; positive samples were significantly associated with lower mean ($p \leq 0.05$) temperatures. Boxplots illustrating association between positive and negative sample groups of targeted pathogens in wastewater with daily minimum and maximum temperatures are shared as supplementary file 3.

3.4.2. Influence of temperature on pathogen log10 concentrations and 2 % diarrhoeal cases

To examine the relationship of maximum temperature with pathogen log10 concentrations and cholera case numbers, linear regression analysis was performed [Fig. 6]. Significant but weak positive correlations were observed between maximum temperature and 2 % Diarrhoeal case numbers (coef: 0.11; $p < 0.001$; $R^2 = 0.05$), as well as with log10 *V. cholerae* log10 concentration (coef: 0.03; $p = 0.001$; $R^2 = 0.03$), log10 SARS-CoV-2 log10 concentration (coef: 0.05; $p < 0.001$; $R^2 = 0.05$), and log10 *S.* Typhi log10 concentration (coef: 0.04; $p = 0.004$; $R^2 = 0.05$). A weak negative association was observed between maximum temperature and rotavirus log10 concentration (coef: −0.02; $p = 0.052$; $R^2 = 0.01$), but this relationship was not statistically significant. Similar weak associations were observed between mean and minimum temperatures and the log10 concentrations of these pathogens [see Supplementary file 4].

3.5. Influence of rainfall on pathogen detection, pathogen log10 concentration, and cholera case numbers

3.5.1. Influence of rainfall on pathogen detection

The distribution of positive and negative samples for *V. cholerae*, SARS-CoV-2, *S.* Typhi, and rotavirus was analysed in relation to rainfall [Table 3]. The results show that a significantly higher proportion of SARS-CoV-2 positive samples occurred during rainy periods (76.39 %) compared to non-rainy periods (57.72 %) ($p < 0.001$). No significant statistical differences were observed in the proportion of positive samples for *V. cholerae*, *S.* Typhi, and rotavirus between rain and no rain, with p-values of 0.204, 0.551, and 0.442, respectively.

3.5.2. Influence of rainfall on pathogen log10 concentrations and cholera case numbers

The relationship between rainfall and pathogen log10 concentrations, as well as cholera case numbers, are presented in boxplots and analysed using Wilcoxon Rank-Sum test [Fig. 7]. Higher rainfall was significantly associated with lower rotavirus positive samples ($p \leq 0.001$), while no significant statistical differences were observed for other pathogens.

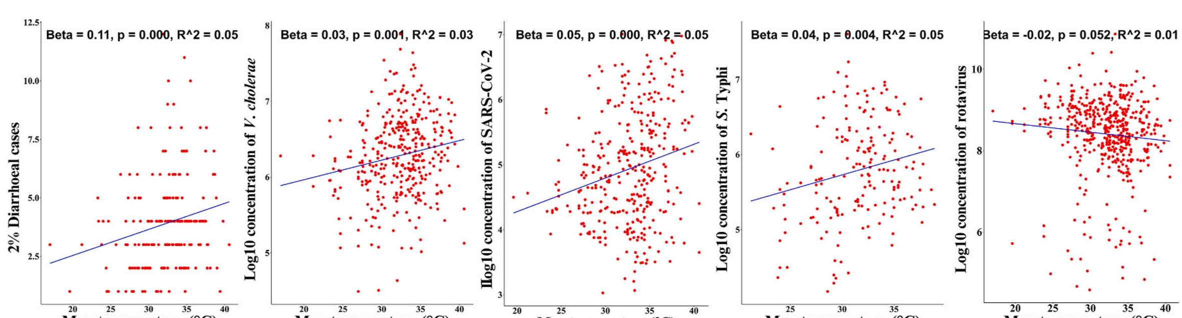

Fig. 6. A: Scatter plot illustrating the association between pathogen log10 concentrations and maximum temperature.

Table 3
The proportion of Positive and Negative Samples with Rainfall.

	V. cholerae			SARS-CoV-2			*S.* Typhi			Rotavirus		
	Rain (n, %)	No rain (n, %)	*p-value* [a]	Rain (n,%)	No rain (n, %)	*p-value* [a]	Rain (n,%)	No rain (n, %)	*p-value* [a]	Rain (n,%)	No rain (n, %)	*p-value* [a]
Positive	87 (60.42)	279 (66.42)	0.204	110 (76.39)	243 (57.72)	$<$0.001	44 (30.56)	140 (33.25)	0.551	137 (95.14)	393 (93.35)	0.442
Negative	57 (39.58)	142 (33.73)		34 (23.61)	178 (42.28)		100 (69.44)	281 (66.75)		7 (4.86)	28 (6.65)	

[a] Chi^2 test.

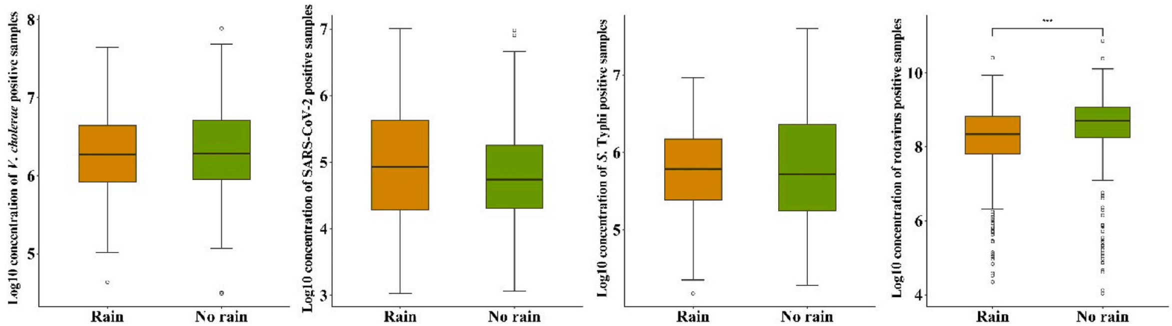

Fig. 7. Box plots illustrating the association between pathogen log10 concentrations and rainfall [Results are presented as median (IQR); analysis by Wilcoxon Rank-Sum test. ***p $\leq$ 0.001].

3.6. Influence of humidity on pathogen detection, pathogen log10 concentration, and cholera case numbers

3.6.1. Influence of humidity on pathogen detection in wastewater

The boxplots in Fig. 8 illustrate the association between humidity levels and the positive versus negative sample groups for our targeted pathogens. A statistically significant positive association was found between humidity and SARS-CoV-2 detection in samples (p $\leq$ 0.01). No substantial associations were observed between humidity levels and *V. cholerae*, rotavirus, or *S.* Typhi.

3.6.2. Influence of humidity on pathogen log10 concentration and 2 % diarrhoeal case numbers

The influence of humidity on pathogen log10 concentrations and 2 % diarrhoeal case numbers is presented in Fig. 9 using linear regression models. Humidity showed a significant but weak positive correlation with 2 % diarrhoeal case numbers (coef: 0.02; p $<$ 0.001; $R^2 = 0.02$). In contrast, log10 V. cholerae, SARS-CoV-2, and S. Typhi log10 concentrations exhibited weak but statistically significant negative correlations with humidity (coef: −0.01; p = 0.008; $R^2 = 0.02$; coef: −0.01; p = 0.038; $R^2 = 0.01$; and coef: −0.01; p = 0.014; $R^2 = 0.03$, respectively).

3.7. Association between pathogen detection in wastewater and meteorological variables

Table 4 presents the hierarchical logistic regression analysis of the relationships between pathogen detection in wastewater and various meteorological variables. For *V. cholerae*, there was a weak positive association with temperature in the unadjusted model (unadj. OR: 1.12, 95 % CI: 1.02 to 1.21, p = 0.011). This relationship remained significant after adjustment, with a slightly stronger positive association (adj. OR: 1.10, 95 % CI: 1.01 to 1.20, p = 0.023). For SARS-CoV-2, temperature exhibited a moderate positive association in both the unadjusted model (unadj. OR: 1.20, 95 % CI: 1.10 to 1.30, p $<$ 0.001) and the adjusted model (adj. OR: 1.21, 95 % CI: 1.12 to 1.31, p $<$ 0.001). Humidity showed weak positive associations with SARS-CoV-2 after adjusting the model (unadj. OR: 1.02, 95 % CI: 1.01 to 1.04, p = 0.178; adj. OR: 1.03, 95 % CI: 1.01 to 1.06, p = 0.012) For *S.* Typhi, no significant association was found with any meteorological variable. The proportion of rotavirus-positive samples was moderately and inversely associated with temperature in both the unadjusted model (unadj. OR: 0.85, 95 % CI: 0.73 to 0.98, p = 0.023) and the adjusted model (adj. OR: 0.83, 95 % CI: 0.72 to 0.96, p = 0.014).

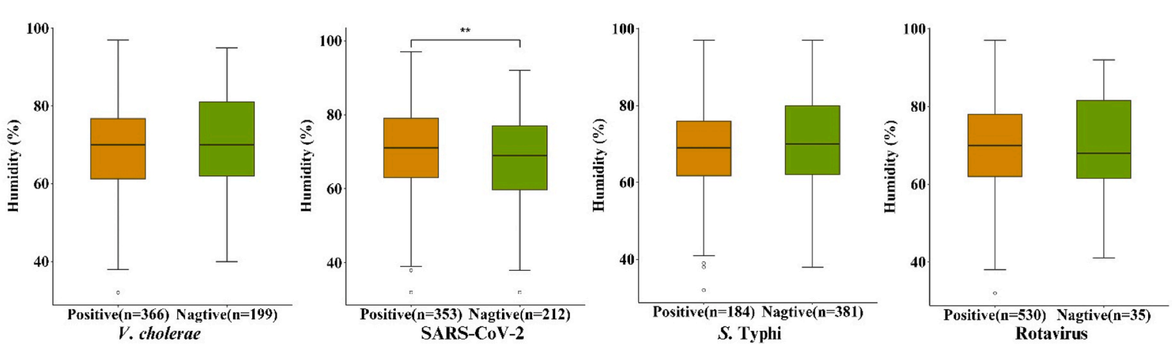

Fig. 8. Boxplots of humidity (%) and the proportion of positive versus negative samples in wastewater [Results are presented as median (IQR); analysis by Wilcoxon Rank-Sum test. **p $\leq$ 0.01].

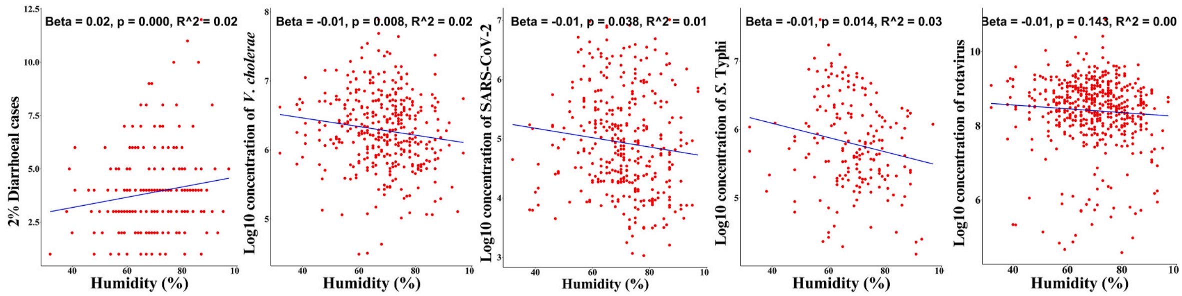

Fig. 9. Scatter plot illustrating the association between pathogen log10 concentrations and humidity.

Table 4
The association between meteorological variables and proportion of positive samples.

Pathogens	Temperature				Humidity				Rainfall			
	Unadjusted Odds Ratio (95 % CI)	P -value	Adjusted Odds Ratio (95 % CI)	P -value	Unadjusted Odds Ratio (95 % CI)	P -value	Adjusted Odds Ratio (95 % CI)	P -value	Unadjusted Odds Ratio (95 % CI)	P -value	Adjusted Odds Ratio (95 % CI)	P -value
V. cholerae	1.12 (1.02, 1.21)	***0.011***	1.10 (1.01, 1.20)	***0.023***	0.99 (0.95, 1.01)	*0.115*	0.98 (0.95,1.01)	*0.162*	1.01 (0.99, 1.02)	*0.567*	1.00 (0.98, 1.02)	*0.666*
SARS-CoV-2	1.20 (1.10, 1.30)	***<0.001***	1.21 (1.12, 1.31)	***<0.001***	1.02 (1.01, 1.04)	*0.178*	1.03 (1.01, 1.06)	***0.012***	1.01 (0.99, 1.03)	*0.532*	0.99 (0.98, 1.01)	*0.675*
***S.* Typhi**	0.94 (0.88, 1.01)	*0.108*	0.95 (0.88, 1.02)	*0.145*	0.99 (0.97, 1.01)	*0.284*	0.99 (0.97, 1.01)	*0.318*	0.99 (0.98, 1.00)	*0.353*	0.99 (0.97, 1.01)	*0.215*
Rotavirus	0.85 (0.73, 0.98)	***0.023***	0.83 (0.72, 0.96)	***0.014***	1.01 (0.97, 1.05)	*0.622*	1.00 (0.96, 1.04)	*0.989*	1.03 (0.98, 1.09)	*0.267*	1.04 (0.98, 1.11)	*0.195*

3.8. Association between meteorological variables with pathogen log10 concentrations in wastewater and 2 % diarrhoeal cases

Table 5 presents linear regression model results between meteorological variables with pathogen log10 concentrations in wastewater and 2 % diarrhoeal cases. Except *V. cholerae, all other pathogen had significant association ($p < 0.05$).*

3.9. Association between meteorological variables with pathogen log10 concentrations in wastewater and 2 % diarrhoeal cases

Table 6 presents the hierarchical linear regression analysis results between wastewater pathogen log10 concentrations and meteorological variables. Number of 2 % diarrhoeal cases had strongest positive relationship with temperature (unadj. coef: 0.26; 95 % CI: 0.20 to 0.33; p < 0.001; adj. coef: 0.29; 95 % CI: 0.20 to 0.37; p < 0.001). Humidity showed a slight negative association before adjustment, but this was not significant after adjustment (unadj. coef: −0.03; 95 % CI: −0.03 to −0.01; p < 0.001; adj. coef: 0.01; 95 % CI: −0.01 to 0.02; p = 433). Rainfall had a significant but very weak negative association with 25 % of diarrheal case numbers (unadj. coef: −0.03; 95 % CI: −0.06 to 0.040; p = 0.062; adj. coef: −0.03; 95 % CI: −0.05 to 0.00; p < 0.046). *V. cholerae* log10 concentrations had a moderate positive association with temperature (unadj. coef: 0.09; 95 % CI: 0.03 to 0.16; p = 0.006; adj. coef: 0.09; 95 % CI: 0.02 to 0.15; p = 0.014). SARS-CoV-2 log10 concentrations had strong positive association with temperature (unadj. coef: 0.17; 95 % CI: 0.09 to 0.25; p < 0.001; adj. coef: 0.19; 95 % CI: 0.10 to 0.27; p < 0.001).

4. Discussion

4.1. Seasonal and meteorological influences on V. cholerae and cholera cases

In this study, the proportion of confirmed cholera cases among 2 % diarrheal cases from DDSS peaked during the post-monsoon period and was lowest during the winter–pre-monsoon transition. The proportion increased steadily during the pre-monsoon and monsoon seasons. These findings align with established seasonal patterns in Bangladesh, where cholera peaks in the pre-monsoon and post-monsoon periods and declines during winter (Islam et al., 2015, 2023; Glass et al., 1982; Alam et al., 2011; Khan et al., 2019; Longini et al., 2002; Cash et al., 2014; Akanda et al., 2009; Sack et al., 2003; Koelle et al., 2005). The seasonal abundance of *V. cholerae* in environmental water also follows similar bimodal pattern, as reported in a study conducted in Matlab, Bangladesh (Islam et al., 2015). This pattern aligns with the pathogen detection rates and concentrations in wastewater observed in our study, which showed significantly higher concentrations during the pre-monsoon season. It has been suggested that increased rainfall during the monsoon season may lead to a marked reduction in environmental pathogen concentration (Pascual et al., 2002a).

Higher temperatures likely contribute to the increased detection and concentration of *V. cholerae* in wastewater, reflecting the seasonal trends observed in the current study. Elevated temperatures, often reaches closer to the microbial optimum growth temperature of 37 °C, facilitate the growth and proliferation of pathogens by creating more conducive environmental conditions (Lipp et al., 2002). This correlation is evident in the significant increase in environmental *V. cholerae* counts and the subsequent rise in cholera cases observed in various countries, including

Table 5
The Association Between wastewater pathogen log10 concentrations and 2 % diarrhoeal cases.

	V. cholerae		SARS-CoV-2		*S.* Typhi		Rotavirus	
	β (95 % CI)	*p-value*	β (95 % CI)	*p-value*	β (95 % CI)	*p-value*	β (95 % CI)	*p-value*
2 % diarrhoeal cases	−0.05 (−0.14, 0.038)	*0.248*	0.12 (0.02, 0.24)	***0.024***	0.12 (0.03, 0.20)	***0.008***	0.06 (0.01, 0.11)	***0.025***

Table 6
Relationship between wastewater pathogen log10 concentrations and 2 % diarrhoeal cases with meteorological variables.

Pathogens	Temperature				Humidity				Rainfall			
	Unadjusted β (95 % CI)	*P-value*	Adjusted β (95 % CI)	*P-value*	Unadjusted β (95 % CI)	*P-value*	Adjusted β (95 % CI)	*P-value*	Unadjusted β (95 % CI)	*P-value*	Adjusted β (95 % CI)	*P-value*
2 % diarrhoeal cases	0.26 (0.20, 0.33)	***<0.001***	0.29 (0.20, 0.37)	***<0.001***	−0.03 (−0.03, −0.01)	***<0.001***	0.01 (−0.01, 0.02)	*0.433*	−0.03 (−0.06, 0.00)	*0.062*	−0.03 (−0.05, −0.00)	***0.046***
V. cholerae	0.09 (0.03, 0.16)	***0.006***	0.09 (0.02,0.15)	***0.014***	−0.02 (−0.04, 0.00)	*0.071*	−0.02 (−0.03, 0.01)	*0.122*	0.00 (−0.01, 0.02)	*0.546*	0.00 (−0.01, 0.02)	*0.700*
SARS-CoV-2	0.17 (0.09, 0.25)	***<0.001***	0.19 (0.10, 0.27)	***<0.001***	0.00 (−0.01, 0.03)	*0.764*	0.02 (−0.003, 0.04)	*0.095*	−0.00 (−0.02, 0.02)	*0.905*	−0.01 (−0.02, 0.01)	*0.5716*
***S.* Typhi**	−0.04 (−0.10, 0.03)	*0.227*	−0.04 (−0.10, 0.03)	*0.252*	−0.01 (−0.04, 0.01)	*0.167*	−0.01 (−0.03, 0.01)	*0.183*	−0.01 (−0.03, 0.00)	*0.085*	−0.01 (−0.02, 0.007)	*0.321*
Rotavirus	−0.07 (−0.09, −0.02)	***0.002***	−0.06 (−0.10, −0.03)	***<0.001***	−0.00 (−0.01, 0.01)	*0.791*	−0.01 (−0.02, 0.01)	*0.391*	0.00 (−0.006, 0.01)	*0.401*	0.01 (−0.001, 0.01)	*0.125*

Bangladesh (Huq et al., 2005). Several studies in Bangladesh have also found an association between higher temperatures and increased cholera cases (Koelle et al., 2005; Ali et al., 2013).

Previous studies suggest an ambiguous effect of rainfall on clinical cholera cases (Huq et al., 2005; Ali et al., 2013; Hashizume et al., 2008). Some research indicates that high rainfall may be inversely related to cholera cases, as increased river discharge can dilute pathogens and reduce water salinity (Huq et al., 2005; Pascual et al., 2002b; Louis Valérie et al., 2003). Another study posits that this relationship may be a seasonal coincidence, driven by unmeasured environmental or behavioral factors that are prevalent during the monsoon season (Hashizume et al., 2008). It also suggests that low rainfall might explain increased cholera cases through changes in hygiene behaviors and increased multiple uses of surface water due to scarcity. In our study, the detection and concentration of *V. cholerae* had no significant association with rainfall. A previous study suggested that rainfall may dilute the concentration of pathogens in wastewater (Hashizume et al., 2008). Environmental surveillance in Haiti's natural reservoirs revealed a positive correlation between rainfall and the isolation frequency of the pathogen (Alam et al., 2015). This suggests that reservoirs, unlike flowing wastewater, may provide the stable environmental conditions necessary for the multiplication of the pathogen, which may explain the discrepancies observed in our study.

Several studies have indicated that high humidity, which usually corresponds with increased rainfall, is associated with higher cholera cases (Ruiz-Moreno et al., 2007). Similar to rainfall, humidity exhibited no significant relationship with the isolation and quantification of *V. cholerae* in wastewater samples in our study.

4.2. Seasonal and meteorological influences on SARS-CoV-2

A multitude of studies has demonstrated a strong link between COVID-19 clinical cases and SARS-CoV-2 viral abundance in wastewater, a bimodal pattern in both case rates and viral abundance over the year (Smith et al., 2024; Anastopoulou et al., 2024; Tisza et al., 2023; Gitter et al., 2024; Giron-Guzman et al., 2024; Boehm et al., 2023; Hill et al., 2023; Barua et al., 2022; Sanchez Jimenez et al., 2023; Zhao et al., 2022; Triggiano et al., 2023). However, some studies suggest that wastewater concentrations are higher during winter than during the monsoon peak (Smith et al., 2024; Anastopoulou et al., 2024; Tisza et al., 2023; Gitter et al., 2024; Giron-Guzman et al., 2024; Boehm et al., 2023; Hill et al., 2023; Barua et al., 2022), while others report the opposite, the latter is aligned with the findings of the current study (Sanchez Jimenez et al., 2023; Zhao et al., 2022). Variations in clinical case numbers across different areas and times, along with differing containment efforts against COVID-19 surges, likely contribute to these discrepancies. Unlike most Western and European countries where these studies were conducted, Bangladesh experienced the majority of its COVID-19 surges during the monsoon seasons (COVID-19 Dynamic Dashboard for Bangladesh, 2024). Our wastewater findings closely align with the trends in confirmed case numbers and test positivity rates reported by the government of Bangladesh during the same period (COVID-19 Dynamic Dashboard for Bangladesh, 2024). A study from Bangladesh found that the number of positive test cases was higher during the July–August (monsoon) and January–February (winter) seasons, further supporting our findings (Kabir, 2023).

Our study revealed that temperature had moderately significant positive correlations with the detection and concentration of SARS-CoV-2 in wastewater, while humidity and rainfall had weak correlations. A study conducted in Nagpur, India, indicated that humidity might limit the abundance of SARS-CoV-2 in wastewater, while no relationship was found with temperature (Acheampong et al., 2024). Another study from Greece identified a significant negative correlation between daily average temperature and viral load (Anastopoulou et al., 2024). Similarly, a study in southern Italy showed an inverse relationship between SARS-CoV-2 concentrations in wastewater and median atmospheric temperature, suggesting greater viral persistence in colder conditions. The same study reported that rainfall had no significant impact on SARS-CoV-2 load (Triggiano et al., 2023). The rationale behind this is that rising temperatures may inhibit the survival of enveloped viruses, such as SARS-CoV-2, by destabilizing viral proteins and enhancing the activity of extracellular enzymes (Carducci et al., 2020; Ye et al., 2016).

Although global data indicate a negative correlation between daily new cases of COVID-19 and temperature as well as relative humidity (Wu et al., 2020; Wang et al., 2021), these variables exhibited a significant positive correlation in Bangladesh (Islam et al., 2021b; Hridoy et al., 2021). Other studies have indicated a strong positive correlation between humidity, rainfall, and the number of cases in Dhaka and across Bangladesh (Kabir, 2023; Islam et al., 2021c). However, some researchers claim that temperature and humidity have minimal impact on COVID-19 transmission in the country (Hasan and Siddik, 2020). Previous research has demonstrated that the biological and physical properties of wastewater; such as water temperature, sunlight/UV light, organic matter, total dissolved solids, turbidity, acidity/alkalinity, and nitrate substances, can influence the viral load of RNA viruses (Gundy et al., 2008; John and Rose, 2005). These factors, combined with social and behavioral influences, complicate the interpretation of the impact of meteorological parameters on COVID-19 transmission.

4.3. Seasonal and meteorological influences on S. Typhi

A study conducted in Honolulu, Hawaii, found a seasonal trend similar to that observed in our study, with consistently higher concentrations of *S.* Typhi in wastewater during the monsoon season and the highest peak occurring in the winter (Yan et al., 2018). Another environmental surveillance study from Vellore, India, reported the highest number of positive samples around the transition period from post-monsoon to winter (Uzzell et al., 2024). the same study indicated that Blantyre, Malawi, had a lower rate of positive samples compared to Vellore, with the highest number occurring during the monsoon season. Studies conducted in Dhaka and Sylhet, Bangladesh, indicate that the highest incidence of typhoid cases occurs during the monsoon season, which aligns with our findings from wastewater analysis (Chowdhury et al., 2018; Dewan et al., 2013; Saha et al., 2001).

Although non-significant, negative correlation between temperature and both the presence and concentration levels of *S.* Typhi in wastewater in our study necessitates further investigation, as it contradicts the well-documented association between higher temperatures and increased typhoid incidence in Bangladesh (Chowdhury et al., 2018; Dewan et al., 2013; Zhang et al., 2008). Also, rainfall and humidity were not correlated with the presence and concentration levels of S. Typhi in our study. While rainfall may increase typhoid incidence by facilitating the transmission of waterborne pathogens, frequent monsoon rains in South Asia exacerbate this issue by contaminating surface water, particularly in densely populated urban areas (Chowdhury et al., 2018; Dewan et al., 2013). However, in Australia, rainfall has been associated with a lower incidence of enteric fever (Zhang et al., 2008), while in Sylhet, Bangladesh, humidity showed no significant influence on the incidence of enteric fever (Chowdhury et al., 2018).

4.4. Seasonal and meteorological influences on rotavirus

Similar to the results of the current study, research from Spain, the United States, and China has reported an increase in positive samples and higher concentrations of rotavirus in wastewater during the cooler months of the year (Giron-Guzman et al., 2024; Boehm et al., 2024; Zhou et al., 2016; He et al., 2011; Li et al., 2011). Large cities, including Dhaka, and countries in Southeast Asia and tropical regions have also experienced rotavirus outbreaks during winter seasons (Hasan et al., 2018; Broor et al., 2003; Chao et al., 2019; Levy et al., 2009; Jagai et al., 2012; Ansari et al., 1991), although the seasonality of rotavirus in tropical regions is less well established (Levy et al., 2009). Studies from Japan and Sweden have shown that rotavirus detection in wastewater consistently increases during the winter months preceding years with pandemic restrictions (Wang et al., 2023; Ando et al., 2023). This seasonal pattern was abruptly disrupted, likely due to measures implemented to control the spread of SARS-CoV-2 (Wang et al., 2023; Ando et al., 2023). On the other hand, studies from Kenya, Argentina, and Norway indicate the absence of a distinct seasonal pattern, with rotavirus circulating continuously throughout the year (Kiulia et al., 2021; Barril et al., 2015; Myrmel et al., 2006; Prez et al., 2015).

A study conducted in Argentina observed an inverse relationship between rotavirus concentration and mean temperature, while relative humidity and precipitation did not demonstrate any significant relationship with rotavirus concentration in wastewater (Barril et al., 2015). Similarly, a study in Poland found a negative correlation between rotavirus concentration and water temperature in surface water (Stobnicka-Kupiec and Gorny, 2022). Consistent with these findings, our study indicates that temperature has a weak to moderate significant negative association with rotavirus presence and concentration in wastewater. In our study, rainfall and humidity did not exhibit any significant association with rotavirus detection. However, although higher temperatures have been reported to correlate significantly with decreased rotavirus cases, conflicting evidence exists regarding the impact of humidity and rainfall on rotavirus case prevalence in Southeast Asian countries (Chao et al., 2019; Levy et al., 2009; Jagai et al., 2012).

4.5. Strengths and limitations

Our study utilizes a comprehensive dataset that integrates WS, meteorological data, and clinical surveillance data to examine the impact of weather on pathogen presence and concentrations in wastewater. However, several limitations exist. While the findings provide valuable insights for Dhaka and similar urban tropical regions, their generalizability to areas with different climatic, infrastructural, or socioeconomic conditions may be limited. Climatic effects on pathogen dynamics can vary widely across geographic regions, influenced by local environmental factors, health systems, and infrastructure. As such, while the study identifies correlations between climatic variables and pathogen concentrations in Dhaka, these correlations may not apply universally, especially in regions with different weather patterns or urban structures.

The study primarily captures the interaction of weather and pathogens in an urban tropical context and may not fully account for other factors such as local behavior patterns, public health infrastructure, water management practices, sewage flows, and social determinants that vary in different locations. Data on environmental and behavioral factors were not available for this study. The one-year dataset also limits the ability to assess long-term trends and capture rare, but potentially significant, events. Therefore, the applicability of these findings in different contexts should be considered with caution, and future studies should explore similar datasets in diverse regions to test the robustness of these findings. Extending the temporal scope of research could also provide a better understanding of long-term trends, rare events, and how these factors interact with local socio-behavioral conditions.

There is also potential for data quality issues due to the design of the WS system. The data were collected weekly using grab samples, which are subject to temporal variations in pathogen concentrations in wastewater over different times of the day and days of the week. The drainage system in Dhaka might have contributed to these issues. A vast majority of Dhaka's drainage system is unstructured, consisting of open sewage canals, drains, reservoirs etc. which are often impacted by rainfall and other discharges from households, businesses, and industries. In this study, we did not use any human fecal markers or human-specific markers to standardize pathogen concentrations for these analyses.

The meteorological data used in this study are based on a single observatory station, which limits the assessment of intra-city heterogeneity. Data from a single station can be influenced by various external factors, leading to discrepancies between recorded measurements and actual meteorological variations at sampling sites. These factors include changes in instruments, observers, observation methods and practices, the station's geographical location, and modifications in the surrounding environment (Colston et al., 2018; WMO, 2008). Clinical case numbers were derived from DDSS, where only every 50th patient is tested. This represents a subset of suspected cases rather than all individuals with acute watery diarrhea, potentially leading to selection bias or underestimation.

5. Conclusion

In wastewater, the target pathogens of our study exhibited distinct seasonal variations. While temperature, humidity, and rainfall influenced these variations, the effects of humidity and rainfall were marginal in most instances. These findings highlight the importance of understanding meteorological variables in predicting pathogen dynamics, which is essential for developing targeted public health strategies. Given the seasonal nature of pathogen concentrations, public health authorities can enhance preparedness by focusing resources during high-risk periods. Early-warning systems can be implemented to track meteorological patterns and correlate them with pathogen levels, triggering timely interventions such as vaccination campaigns, improvements in water sanitation, and vector control measures.

Strengthening public health infrastructure to manage seasonal surges in pathogen transmission is critical. Education campaigns can be tailored to at-risk populations during peak seasons to promote hygiene practices and vaccination uptake. Integrating WS with climatic data will allow for evidence-based decision-making, fostering more resilient health systems capable of responding proactively to disease outbreaks. This approach not only improves public health preparedness but also enhances community resilience to the health impacts of climate change.

CRediT authorship contribution statement

Farjana Jahan: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Formal analysis, Conceptualization. **Mizanul Islam Nasim:** Writing – original draft, Methodology, Formal analysis. **Yuke Wang:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Sk Md Kamrul Bashar:** Writing – original draft, Visualization, Formal analysis, Data curation. **Rezaul Hasan:** Writing – review & editing, Supervision, Project administration, Investigation. **Afroza Jannat Suchana:** Investigation, Formal analysis, Data curation. **Nuhu Amin:** Writing – review & editing, Supervision, Methodology. **Rehnuma Haque:** Writing – review & editing. **Md Abul Hares:** Writing – review & editing, Formal analysis, Data curation. **Akash Saha:** Writing – review & editing, Investigation, Data curation. **Mohammad Enayet Hossain:** Writing – review & editing, Investigation, Data curation. **Mohammed Ziaur Rahman:** Writing – review & editing, Methodology, Investigation. **Megan Diamond:** Writing – review & editing, Supervision. **Suraja Raj:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Stephen Patrick Hilton:** Writing – review & editing, Supervision, Methodology. **Pengbo Liu:** Writing – review & editing, Supervision, Resources, Methodology. **Christine Moe:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Mahbubur Rahman:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Data availability

The WS data used and analysed in this study are available from the corresponding author upon reasonable request. Access to meteorological and clinical surveillance data requires prior approval from BMD and icddr,b, respectively.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2025.114591.

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