Applied Environmental Research (2026) 48(3), 025
https://doi.org/10.35762/AER.2026025

Applied Environmental Research
- RESEARCH FOR SUSTAINABLE PLANET -

Research Article

Seasonal Wastewater Monitoring and Quantitative Microbial Risk Assessment of *Escherichia coli* in a University Wastewater System, Pathum Thani, Central Thailand

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Abstract

In this study, the physicochemical properties of wastewater were characterized, and a quantitative microbial risk assessment (QMRA) of *E. coli* was conducted in the wastewater treatment system of the Faculty of Science and Technology, Valaya Alongkorn Rajabhat University, under the Royal Patronage, Pathum Thani, Thailand (VRU-SciTech). This study was conducted because the current water-quality assessment does not include the QMRA or evaluate the occupational and incidental public risk of infection. Over a one-year monitoring period, this study quantified wastewater generation (~6.09 L $person^{-1}$ day^{-1}; ~3.21 × 10^3 m^3 $year^{-1}$) and analyzed its physicochemical parameters and *E. coli* concentrations. High concentrations of nitrate, orthophosphate, BOD_5, and COD were observed during the summer. Using a β-Poisson dose–response model, the probability of infection from a single unintentional ingestion was quantified from the influent and effluents of VRU-WWTP and Chiang Rak Noi Canal. The QMRA exposure scenarios represent occupational exposure (e.g., wastewater operators and maintenance staff) and incidental environmental contact (e.g., students, staff, and canal users). The infection risk of the 1 mL sample ranged from 4.92 to 88.08%, and that of the 100 mL sample ranged from 64.68 to 98.65%. These results indicate significant health risks associated with repeated contact with *E. coli*-contaminated water. The estimated annual infection probabilities exceeded the commonly referenced tolerable risk levels proposed by the World Health Organization (WHO). There is an urgent need for improved wastewater management and microbial risk-based monitoring to reduce human health risks.

ARTICLE HISTORY

Received: 18 Nov. 2025
Revised: 27 Feb. 2026
Accepted: 28 Feb. 2026
Published: 17 Mar. 2026

KEYWORDS

Wastewater monitoring; Quantitative microbial risk assessment; *Escherichia coli*; Seasonal variation; University wastewater

Introduction

Ensuring the safety and sustainability of water resources through effective wastewater monitoring remains a global challenge, particularly in developing countries (Drechsel et al., 2024). Despite advances in treatment technologies, insufficiently treated wastewater continues to pose significant health hazards, contributing to disease outbreaks worldwide (Lin et al., 2022). It has been estimated that unsafe water, sanitation, and hygiene (WASH) led to 1.6 million deaths in 2016 and 1.7 million deaths in 2019 (Wolf et al., 2023). In Thailand, the general water quality index (WQI) is typically used as an indicator of overall river conditions and is categorized as excellent, good, fair, or poor, without specifying its suitability for use (PCD, 2018). For most wastewater treatment plants (WWTPs) in Thailand, water quality monitoring focuses primarily on physicochemical parameters such as BOD5, COD, pH, turbidity, ammonia, and heavy metals (Olabode et al., 2020).

However, microorganisms can serve as bioindicators of an ecosystem's health and can signal the presence of water-related diseases. Pathogenic infection from bacteria, protozoa, and viruses can lead to diarrheal diseases, which can result in severe health consequences (Shayo et al., 2023). Moreover, the pathogenic microbes in wastewater can adhere to solid particles, protecting them from disinfectants such as chlorine or UV radiation and posing a significant public health concern (Chahal et al., 2016). WWTP workers and communities with occupational or incidental contact with contaminated water are at particular risk of infection. For instance, workers exposed to pathogenic *E. coli* through weekly unintentional ingestion of approximately 1 mL of contaminated water for more than one year experienced estimated annual infection probabilities (Pns) of 1.2% for treated effluent and approximately 100% for untreated influent samples (Mbanga et al., 2020). Similarly, the community health risks associated with recreational or domestic use of river water were greater for upstream sites than for downstream sites. Therefore, water quality assessment plays a vital role in identifying pollution sources and preventing waterborne diseases by providing evidence of the human health risks associated with different water uses (Girardi et al., 2019; Katukiza et al., 2014).

Valaya Alongkorn Rajabhat University (VRU) under the Royal Patronage is a public university in Pathum Thani Province, Thailand. The university provides education to diverse groups across eight faculties and one demonstration school, serving more than 10,979 individuals annually. It has been estimated that the VRU consumes more than 1,029 m^3 of water per month, with more than 70% of treated wastewater being recycled for garden irrigation, floor cleaning, and shelter washing. The effluent is monitored monthly for physicochemical parameters such as pH, turbidity, DO, BOD_5, COD, EC, TKN, and TDS. Despite this, there are no biological monitoring data that could indicate pathogenic contamination, which poses a major risk for waterborne infections (Abia et al, 2016; Zhu et al., 2018) [11, 12].

Fecal coliform bacteria (FCB), particularly *Escherichia coli*, are widely used as major indicators of fecal contamination in water sources (Mbanga et al., 2020; Voltezou et al., 2025). In Thailand, microbial indicators such as total coliform bacteria and fecal coliform bacteria are primarily regulated under surface water quality standards rather than effluent standards for buildings (Kongprajug et al., 2024). Although universities are classified as regulated buildings under the Notification of the Ministry of Natural Resources and Environment on Effluent Standards for buildings of certain types and sizes (B.E. 2567), *E. coli* and fecal coliform bacteria are not included as mandatory compliance parameters in this regulation. Bacterial contamination assessments in community wastewater often rely on fecal indicator bacteria such as *E. coli* and *enterococci* (Karkman et al., 2018; McMinn et al., 2024).

Quantitative microbial risk assessment (QMRA) has been applied to estimate the disease burden caused by pathogens and to assess community health risks resulting from exposure to polluted water (Mbanga et al., 2020; Voltezou et al., 2025). While the Ministry of Public Health recommends that *E. coli* concentrations in treated wastewater be less than 1,000 MPN per 100 mL for sanitation safety, this guideline is not directly specified under building effluent standards for universities (Kanchanapiya and Tantisattayakul, 2022; Yuthawong and Laungprasert, 2019). For surface water, the National Environment Board (1994) specifies that total coliform bacteria (TCB) should not exceed 20,000 MPN per 100 mL and that the amount of fecal coliform bacteria (FCB) should not exceed 4,000 MPN per 100 mL. Therefore, biological monitoring is needed for effective water management and disease prevention.

The objectives of this study were to characterize the seasonal variation in the physicochemical properties of wastewater generated from the VRU-SciTech facility and to assess microbial human health risks by quantifying *Escherichia coli* from the VRU-SciTech facility and its receiving water body, the Chiang Rak Noi Canal. This study addresses this research gap by integrating seasonal wastewater monitoring with QMRA to evaluate occupational and incidental exposure risks in a university wastewater system. The results of this study provide evidence for improving wastewater management strategies and strengthening occupational health protection through reducing water-borne disease infection risk.

Materials and methods

1) Study site and sample collection

Wastewater quality monitoring was conducted at the Faculty of Science and Technology, VRU, under the Royal Patronage, Pathum Thani Province, covering a total operational area of 4,197 m^2. Three representative sampling points were randomly selected, and the water quality of the composite wastewater was determined each month (Figure 1). Wastewater samples were collected for one year (August 2024–July 2025) using grab sampling techniques following the guidelines of the Pollution Control Department, Ministry of Natural Resources and Environment, Thailand (PCD, 2020).

2) Physicochemical analysis

The key physicochemical parameters of the collected wastewater samples were analyzed to determine wastewater quality. The potential of hydrogen was measured with a pH meter (pH 150; EUTECH), and electrical conductivity was measured with a conductivity meter (CyberScan CON 610; EUTECH). Chemical oxygen demand (COD), biological oxygen demand over 5 days (BOD_5), and nitrate and orthophosphate levels were measured following a standardized procedure according to the APHA guidelines (2020). In brief, COD was measured by the closed reflux method, BOD_5 by azide modification, nitrate by the brucine method, and phosphate by the ascorbic acid method.

3) Microbial analysis for *Escherichia coli*

For fecal indicator analysis, water samples were collected every fourth month between August 2024 and July 2025 from multiple sampling points, including wastewater influent (IF) originating from the Faculty of Science and Technology Building, VRU (VRU-SciTech), effluent (EF) from the university's wastewater treatment plant system (VRU-WWTP), and surface water samples from the receiving Chiang Rak Noi Canal, both upstream (BW) and downstream (AW) of the effluent discharge point (Figure 1).

E. coli contamination was quantified using 3 M™ Petrifilm™ *E. coli*/Coliform Count Plates (3 M Company, United States). In brief, water samples were spread onto the selective media and incubated at 35°C for 48 hours. Blue and red–blue colonies with gas bubbles were counted and expressed as CFU mL^{-1}. Triplicate analyses, sterile blanks, and aseptic handling procedures were applied as quality control measures.

4) *E. coli* exposure risk assessment

The potential health risks associated with *E. coli* contamination in various water sources were assessed using the beta–Poisson dose–response model for *E. coli* (Buchanan et al., 2000; Mbanga et al., 2020). The exposure scenarios represent occupational exposure (1 mL) and incidental public contact (100 mL). The detection limits of the Petrifilm analysis were considered. For samples with *E. coli* levels below the method detection limit (LOD), values were substituted with LOD/2 in the Monte Carlo simulation. The LOD and limit of quantification (LOQ) were 1 CFU mL^{-1} and 15 CFU mL^{-1}, respectively. The probability of infection (Pi), annual probability of infection (Pn), and ingested dose (N) were estimated as shown in Eq. 1.

$$\mathrm{Pi} = 1 - \left(1 + \frac{N}{\beta}\right)^{-\alpha} \qquad \text{(Eq. 1)}$$

where N is the ingested dose (number of microbes) and α and β are infectivity constants specific to the pathogen. In this study, the parameters α=0.395 and β = 2.473 were selected on the basis of the established literature (Buchanan et al., 2000; Mbanga et al., 2020).

The annual probability of infection (Pn) for repeated exposures over n events is calculated as shown in Eq. 2.

$$\mathrm{Pn} = 1 - (1 - \mathrm{Pi})^{n} \qquad \text{(Eq. 2)}$$

The ingested dose estimation (N) was calculated as shown in Eq. 3.

$$N = V \times C \times f \qquad \text{(Eq. 3)}$$

where N = the ingested dose (number of pathogens), V = the volume of water ingested, C = the concentration of pathogens in water (CFU mL^{-1}), and f = the fraction of pathogenic organisms within the total microbial population (8% or 0.08) (Abia et al., 2016; Mbanga et al., 2020). Two ingestion volumes were evaluated: 1 mL to represent unintentional ingestion during occupational activities (e.g., wastewater operators and maintenance staff) and 100 mL to represent incidental environmental contact (e.g., students, staff, and canal users).

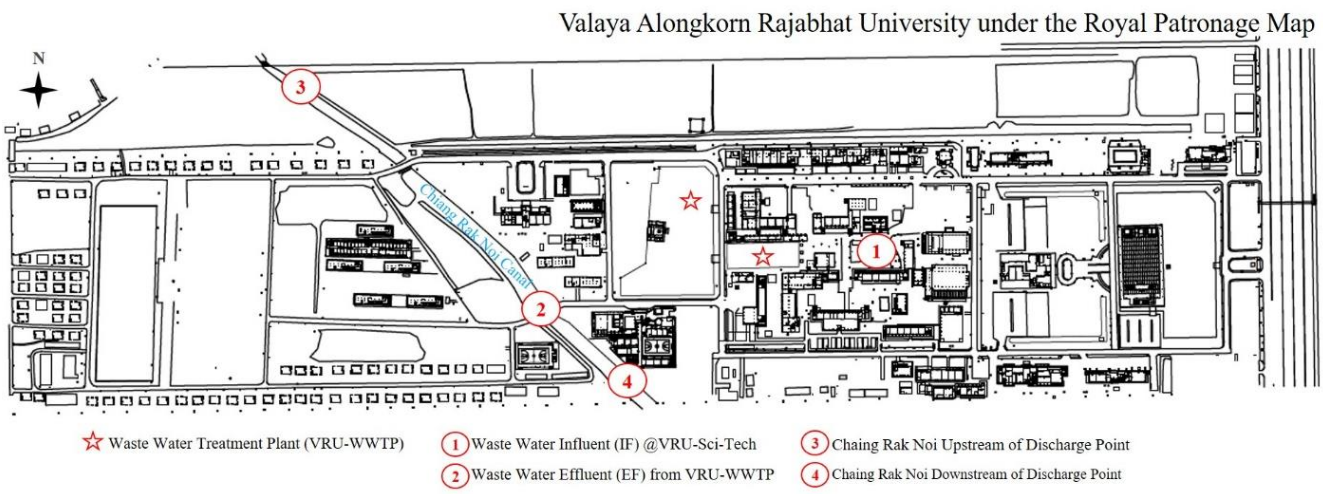

Figure 1 Map of the sampling sites.

5) Ethical approval

The research protocol was approved by the Human Research Ethics Committee of Valaya Alongkorn Rajabhat University under the Royal Patronage, Pathum Thani Province (COA No. 0076/67; REC No. 0001/2568). Biosafety precautions, including personal protective equipment and controlled laboratory handling, were implemented during sampling and analysis to minimize occupational exposure risks.

6) Data and statistical analysis

The collected data were analyzed using analysis of variance (ANOVA) to determine whether there were statistically significant differences in the mean values of the selected wastewater quality parameters. Pearson's correlation analysis was conducted to assess the relationships among all pairs of measured variables at a 95% confidence level ($p < 0.05$). A Pearson's correlation matrix of the environmental variables and microbial characteristics was constructed with the PAST (Paleontological Statistics (PAST) software package 4.07). All other statistical analyses were carried out using Statistix version 8.0 (FL, USA)

Results and discussion

1) Physicochemical parameters

The results of wastewater monitoring at the VRU-SciTech, VRU, under the Royal Patronage are shown in Figure 2. Wastewater volumes exhibited marked seasonal variation, with the highest volumes observed in the wet season months (August–October 2024 and June–July 2025), reaching a peak in June at 353,568 L month^{-1}, while lower volumes occurred in the dry season (December 2024–April 2025), ranging between 131,264 and 323,567 L month^{-1}. These fluctuations suggest the direct influence of rainfall patterns on effluent discharge rates. The total annual volume of wastewater generated was 3.21 × 10^{3} m^{3} year^{-1}, with a monthly average of 267.7 m^{3}, based on an estimated 1,465 users per month, each generating an average of 6.09 L person^{-1} day^{-1}. The electrical conductivity (EC) ranged from approximately 884.33 to 1,318.33 µS cm^{-1}, with the highest value occurring on November 2024 (1318.33 µS cm^{-1}), which was inversely related to the wastewater volume. The pH remained neutral to slightly alkaline (7.73–8.21), showing consistent stability across seasons.

The BOD_5 concentration in the wastewater ranged from 81.67 to 176.67 mg L^{-1}, while the COD concentrtion ranged from 133.33 to 396.21 mg L^{-1}. The nitrate (NO_3^-) concentration ranged from 1.34–6.15 mg N L^{-1}, and the orthophosphate concentration ranged from 0.47 to 3.95 mg P L^{-1}. In general, the concentrations of COD, BOD_5, and phosphate were higher in the dry season (November-Decemcer) than in the wet season. In contrast, the nitrate concentration was highest in the wet season (June–July), likely because of increased surface runoff and fertilizer application during rainfall events. These results indicate that episodic nutrient influx driven by university activities and seasonal variation shapes the physicochemical profile of wastewater (Hammoumi et al., 2024; Teklehaimanot et al., 2015). In addition to rainfall, fluctuations in wastewater volume may also be influenced by the academic calendar, with higher occupancy during semester periods and reduced water use during academic breaks.

The seasonal variation in organic and nutrient loads significantly differed between the BOD_5/COD ratios. A BOD_5/COD ratio above 0.4 indicates a high degree of degradability (Mamun et al., 2022). The average BOD_5/COD ratios during the dry season were 0.67 and 0.46 during the wet season in this study. A BOD_5/COD ratio higher than 0.4 in both the dry and wet seasons indicates that the university wastewater was composed mostly of biodegradable compounds that are treatable by a conventional wastewater treatment system. However, this finding also indicates that more substrates are present in wastewater and may serve as substrates for pathogenic microorganisms such as *E. coli* (Pokharel et al., 2025). Furthermore, the average OP concentration during the dry season was 2.24 mg P L^{-1}, which was 51% higher than that during the wet season. In general, elevated water temperature during the summer promotes microbial activity, which results in increased oxygen consumption (Dey et al., 2021). The peak concentrations of BOD_5 and COD and OP coincided with the summer period in Thailand, as predicted.

Interestingly, the nitrate concentration was higher during the wet season, presumably because of activities on the campus. This finding indicates that the surface runoff rate was higher during the wet season. These findings indicate that careful management of fertilizer application and surface nitrate sources on campuses is needed during the wet season to mitigate nutrient pollution. Overall, the monitoring data provided concerning data that represented optimal conditions for microbial growth, which may lead to significant public health concerns with improper management (Oates et al., 2025). Therefore, adaptive management strategies are needed to mitigate the environmental risks associated with university wastewater discharge.

2) *E. coli* concentration and quantitative microbial risk assessment

The *E. coli* concentration and its QMRA are shown in Table 1. Annual average *E. coli* concentrations were used to represent long-term exposure potential for the QMRA rather than short-term temporal variation. The highest concentration was detected in the influents of VRU-WWTP (6.73 × 10^{3} CFU mL^{-1}), whereas the EF of VRU-WWTP significantly decreased to 4 CFU mL^{-1}. In the Chiang Rak Noi Canal, the concentrations of *E. coli* contamination were 1.74 × 10^3 and 1.55 × 10^3 CFU mL^{-1} in BW and AW, respectively. The Pi was computed to determine the risk of *E. coli* infection. For a single unintentional ingestion event (1 mL), the Pi was high for the IF samples (88.08% ± 0.35%), whereas the EF risk decreased to 4.92% ± 0.90%. BW and AW in the canal had moderate risks (78.9% ± 3.85% and 77.67% ± 4.76%, respectively). The Pn ranged from 91.79–100%, indicating significant *E. coli* exposure risk in all the collected samples.

The annual infection probability was very high across all locations. This increase in annual infection risk reflects the cumulative effect of repeated exposure events combined with the nonlinear nature of the β-Poisson dose–response model. Even the effluents of VRU-WWTP, which contained relatively low numbers of *E. coli*, may still pose significant infection risks under repeated exposure scenarios. Uncertainty analysis derived from 10,000 Monte Carlo simulations indicated an increase in microbial infection risk (Figure 3). This finding is consistent with earlier QMRA studies, which reported that minimal concentrations of *E. coli* can lead to significant health risks over long-term exposure [8, 13]. The concentration of VRU-WWTP EF was 4 CFU mL^{-1}, indicating residual microbial contamination despite treatment. Considering that this water is effluent from wastewater treatment systems, the microbial risk at the VRU is a public health concern for workers who may contact this contaminated water. In addition, all the tested water samples, including the water from the Chiang Rak Noi Canal, showed that the Pn exceeded the WHO-referenced tolerable risk level (10^{-3} infections $person^{-1}$ $year^{-1}$) (Abia et al., 2016; Halvorson et al., 2011; Zhu et al., 2018), indicating unacceptable microbial risk. Any detectable level of *E. coli* indicates fecal contamination and a potential health risk (Soller et al, 2010). These pathogenic microorganisms can persist in polluted water for prolonged periods, leading to infection among exposed populations (Sosah et al., 2025).

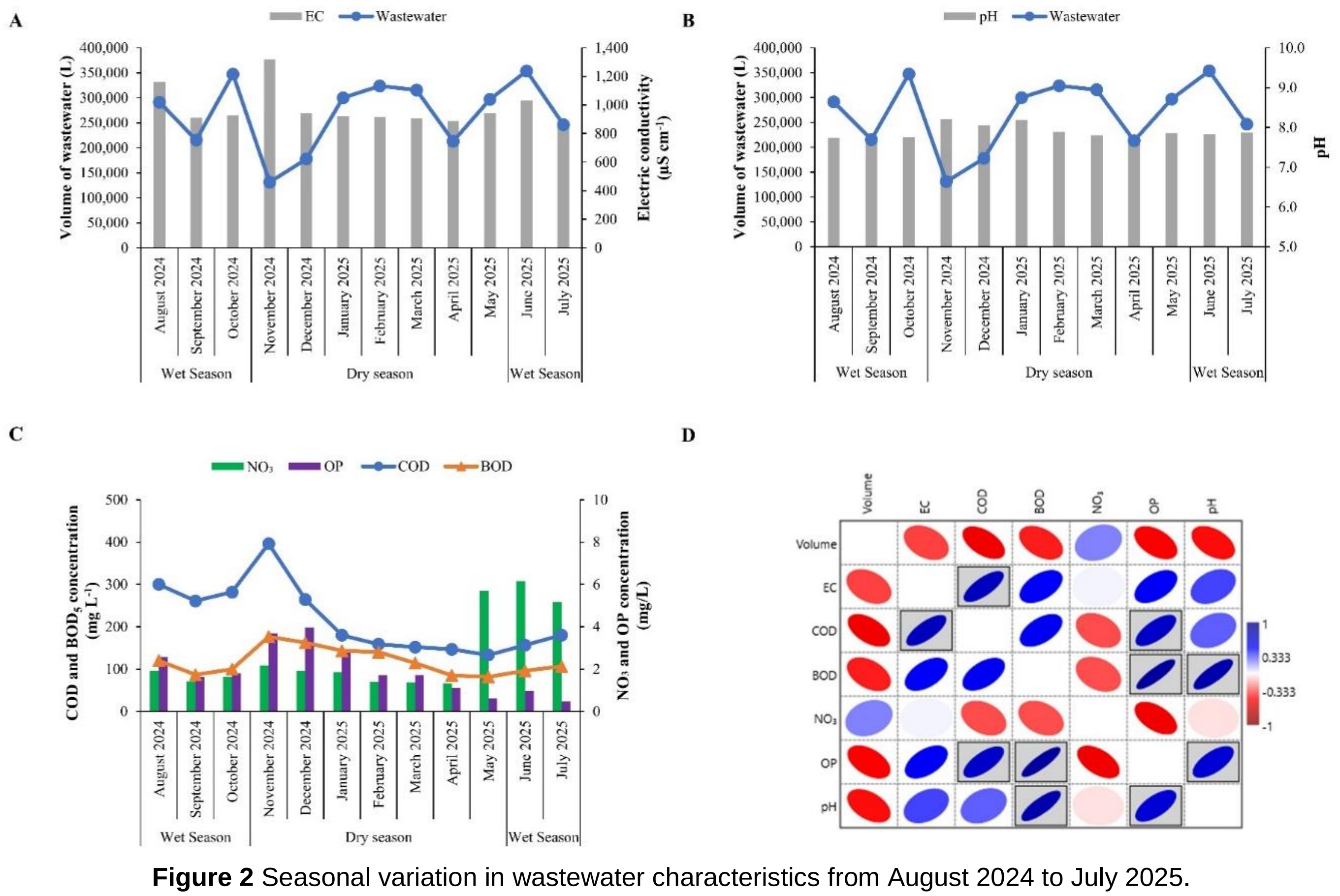

Figure 2 Seasonal variation in wastewater characteristics from August 2024 to July 2025. (A) Monthly wastewater volume (m^3 $month^{-1}$) and electrical conductivity (µS cm^{-1}). (B) Monthly wastewater volume (m^3 $month^{-1}$) and pH values. (C) Concentrations of chemical oxygen demand (COD, mg L^{-1}), biochemical oxygen demand (BOD, mg L^{-1}), nitrate (NO_3^-, mg N L^{-1}), and orthophosphate (OP, mg P L^{-1}), demonstrating temporal patterns among organic and nutrient loads. (D) Correlation matrix of monitored variables, with color intensity and ellipse orientation indicating the direction and strength of associations, respectively. Boxes indicate significant correlations ($p < 0.05$).

Table 1 Microbial contamination and infection risk associated with wastewater

Parameters	VRU-WWTP		Chiang Rak Noi Canal	
	IF	EF	BW	AW
E. coli (CFU mL^{-1})	6.73×10^3	4.00	1.74×10^3	1.55×10^3
1 mL Pi (%)	88.08 (0.35)	4.92 (0.90)	78.90 (3.85)	77.67 (4.76)
100 mL Pi (%)	98.65 (0.04)	64.68 (1.91)	97.59 (0.45)	97.44 (0.56)
Pn (%)	100	91.79	100	100

Remark: IF = Influent wastewater from the Faculty of Science and Technology Building (VRU-SciTech); EF = effluent from VRU-WWTP; BW and AW = before and after the effluent discharge point in the Chiang Rak Noi Canal.

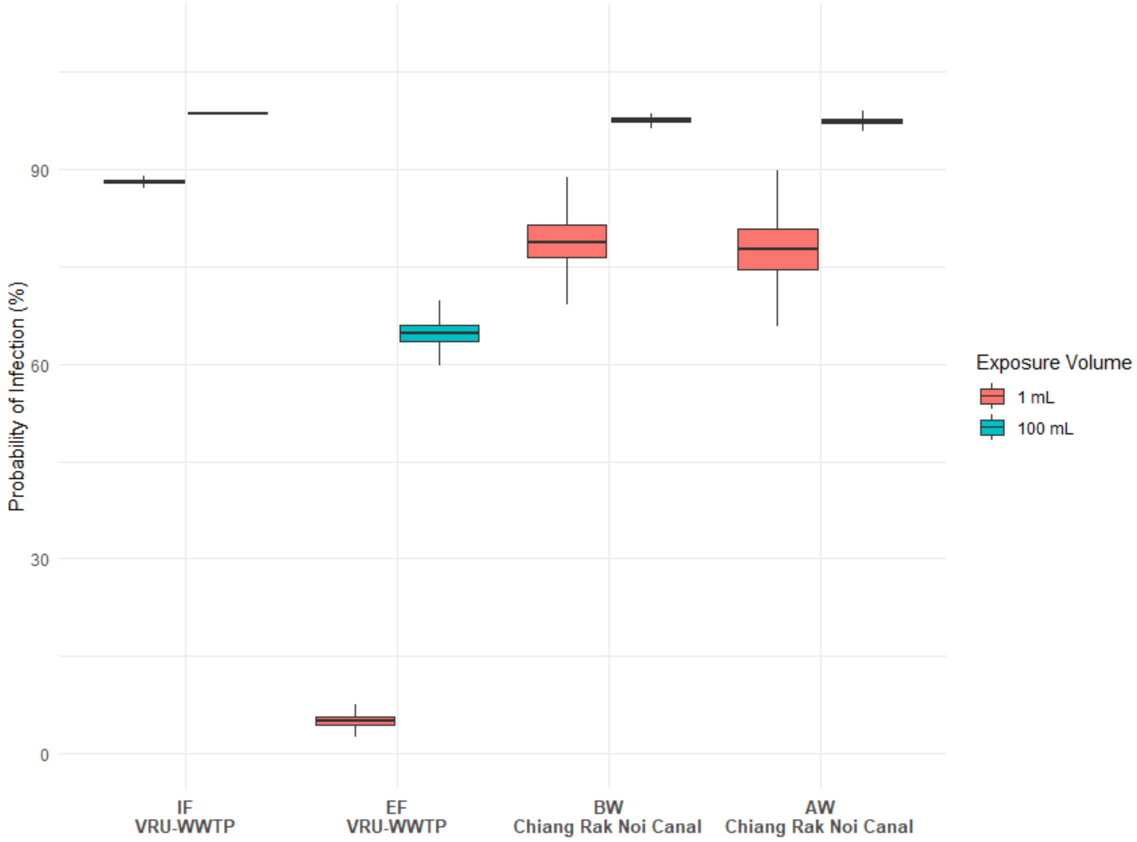

Figure 3 Monte Carlo simulation plot showing the probability of infection (Pi) per exposure to *E. coli.*

The Chiang Rak Noi Canal serves as a receiving body for wastewater from five surrounding communities, thirteen industrial factories, agricultural areas covering more than 42%, and other sources such as restaurants, markets, and local wastewater treatment plants (Chiang Rak Noi Subdistrict Administrative Organization, 2024). A high level of *E. coli* contamination reflects the improper management of water systems, which poses serious environmental and public health challenges (Buchanan et al., 2000; Girardi et al., 2019; Mbanga et al., 2020; Voltezou et al., 2025). Evaluating both low-volume (1 mL) and high-volume (100 mL) ingestion scenarios enables differentiation between occupational exposure and incidental environmental contact, strengthening health risk interpretation beyond routine microbial monitoring alone.

Taken together, the results from the present study demonstrated that VRU-WWTP effluent and canal waters present extreme and unacceptable microbial risk. Although the VRU-WWTP includes a disinfection step (e.g., UV treatment), residual E. coli detected in the effluent indicates incomplete microbial inactivation and potential exposure risk under repeated contact scenarios. Thus, there is an urgent need to strengthen wastewater management, improve sanitation, and enforce stricter enforcement of effluent discharge standards.

Conclusions

The wastewater generated from the Faculty of Science and Technology building was estimated at 267,739 liters per month, which is equivalent to 6.09 L of $person^{-1}$ day^{-1}. This wastewater was classified as domestic wastewater with elevated concentrations of nitrate, orthophosphate, and *E. coli*, contributing to increased BOD_5 and COD levels. The contamination was most pronounced during the summer season, which was consistent with higher temperatures and reduced dilution. Both wastewater influents and effluent from the VRU-WWTP and surface water samples upstream and downstream of the discharge point of the effluent of the Chiang Rak Noi Canal exhibited concerning concentrations of E. coli. The occupational risk of infection (Pi) with *E. coli* from single unintentional consumption of 1 mL of water samples was 88.08% for influents, 4.92% for effluent from WWTP, and 77.67–78.90% for canal water. The annual probability of infection (Pn) with pathogenic *E. coli* exceeded the safe thresholds recommended by the WHO, indicating a substantial health risk for students, staff, and nearby communities.

To address the identified risks and knowledge gaps, future research should expand pathogen monitoring beyond fecal indicators to include viral, protozoan, and antibiotic-resistant organisms to better capture the full spectrum of microbial risks. In addition, site-specific exposure behaviors and occupational practices should be incorporated to refine the QMRA assumptions and reduce uncertainty in risk estimates. Further evaluation of the seasonal treatment efficiency and disinfection performance at university-scale WWTPs is also warranted, particularly under high-temperature and low-dilution conditions. Collectively, these research priorities support the integration of QMRA into routine university wastewater monitoring and the implementation of targeted microbial risk management strategies for occupational groups (e.g., wastewater operators) as well as risk mitigation measures for incidental public contact (e.g., students, staff, and canal users).

Acknowledgements

This research was supported by the Department of Environmental Technology and Management, Faculty of Environment, Kasetsart University, and the Faculty of Science, Phranakhon Rajabhat University, Thailand, where the instruments and analytical facilities used for measurements and analyses were provided.

Data availability statement

Information and data used in the study will be disclosed upon request.

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Conflicts of interest

The authors declare that there are no conflicts of interest in competing financial or personal relationships that could have appeared to influence the work reported in this work.

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