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Seasonal dynamics of physicochemical water quality and microbial diversity in freshwater ponds of Ahilyanagar district, Maharashtra, India

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ABSTRACT

Freshwater ponds are dynamic ecosystems whose physicochemical and microbiological characteristics directly influence water quality and public health. This study presents a comprehensive four-month surveillance of 40 freshwater ponds from January to April, focusing exclusively on water quality parameters and microbial diversity. A total of 160 water samples (40 ponds × 4 months) were analysed for eighteen water quality parameters and three microbial load indicators, alongside bacterial genus-level prevalence and diversity indices. All parameters exhibited highly significant seasonal variation ($p < 0.001$). Temperature increased from 18.5 ± 0.19 °C in January to 30.2 ± 0.29 °C in April, while dissolved oxygen declined from 8.2 ± 0.10 to 6.0 ± 0.10 mg/L. Total dissolved solids increased from 210 ± 7.12 to 330 ± 10.28 mg/L, and biochemical oxygen demand rose from 2.5 ± 0.11 to 5.2 ± 0.19 mg/L. Total viable count increased from $18,000 \pm 791$ CFU/mL to $65,000 \pm 2,846$ CFU/mL, total coliform count from 85 ± 4.74 to 320 ± 12.65 MPN/100 mL, and total faecal coliform count from 35 ± 2.37 to 180 ± 7.91 MPN/100 mL. Bacterial diversity indices increased progressively, with Shannon H' rising from 1.85 ± 0.042 to 2.15 ± 0.031 . *E. coli* prevalence increased from 55% to 88%, *Salmonella* from 15% to 38%, and *Vibrio* from 8% to 28%. Pearson correlation analysis revealed strong positive associations between temperature and microbial load indicators ($r = 0.84-0.86$, $p < 0.001$) and strong negative associations between dissolved oxygen and microbial load ($r = -0.76$ to -0.78 , $p < 0.001$). Multiple linear regression identified temperature, dissolved oxygen, TDS, BOD, and nitrate as significant predictors of total viable count ($R^2 = 0.78$) and total coliform count ($R^2 = 0.81$). Compliance analysis showed that while 100% of ponds met WHO/APHA limits for nitrate and TDS throughout the study, total coliform compliance declined from 90% in January to 40% in April. These findings demonstrate a clear seasonal deterioration in both physicochemical water quality and microbiological safety from winter to pre-summer, underscoring the need for seasonal water quality monitoring programmes in freshwater pond systems.

Keywords: Pond water, Seasonal variation, Water quality, Microbial diversity, Coliform, Bacterial load

Introduction

Freshwater ponds constitute a significant proportion of inland water resources and serve multiple purposes including irrigation, domestic use, groundwater recharge, and ecosystem services. In many regions, ponds are also integral to local livelihoods through small-scale fisheries and other aquatic resource utilisation. The physicochemical and microbiological quality of pond water is therefore of considerable importance for both environmental health and human well-being. Liu *et al.* (2019) [1] emphasised that microorganisms are indispensable biological components of pond environments and that understanding the relationship between microbial communities and physicochemical parameters is essential for improving water quality management. Seasonal variation is a major driver of water quality dynamics in lentic freshwater systems. Temperature fluctuations directly influence dissolved oxygen solubility, microbial metabolic rates, and the decomposition of organic matter. Debnath *et al.* (2025) [2] demonstrated that pondwater microbial diversity is at its lowest in winter and highest in the pre-monsoon period, with temperature and pH exerting significant effects on microbiome composition. Their study further reported that microbial community structures differed across seasons, with season and geographical location showing the strongest effects. These seasonal shifts have important implications for the emergence of waterborne pathogens, as warmer temperatures can favour the proliferation of pathogenic bacterial genera including *Pseudomonas*, *Vibrio*, and *Salmonella*. Liu *et al.* (2019) [1] observed that pathogens such as *Pseudomonas* and *Flavobacterium* showed significant correlations with water temperature and dissolved oxygen in grass carp culture ponds, demonstrating the sensitivity of bacterial communities to seasonal environmental change. Microbiological contamination of pond water is a critical public health concern. Indicator organisms such as total coliforms and fecal coliforms are routinely used to assess water quality and sanitary risk. According to

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WHO (2017) [3] guidelines, *E. coli* or thermotolerant coliform bacteria must not be detectable in any 100 mL sample of drinking water, and total coliform bacteria are not acceptable as an indicator of the sanitary quality of water supplies. The presence of *E. coli* and thermotolerant coliforms indicate recent faecal contamination and the potential presence of enteric pathogens. Barrow *et al.* (1994) [4] reported that populations of indicator bacteria in eutrophic ponds increased with increasing water temperature, with maximal numbers recorded during summer months, and correlation analysis confirmed that temperature had a positive significant effect on bacterial population dynamics. Khatib Haghighi and Khodaparast (2012) [5] similarly documented that the highest coliform and fecal coliform counts in Anzali Lagoon were recorded during summer, attributing this to increasing temperature and decreasing water flow.

In pond systems receiving agricultural runoff, domestic wastewater, or livestock inputs, microbial loads can increase substantially, particularly during warmer months when biological activity peaks. Khan *et al.* (2025) [6] reported that physicochemical factors significantly influence *E. coli* contamination in ponds, with coliforms present in all studied ponds and *E. coli* detected in 67% of samples, indicating that the water was unfit for drinking under EU guidelines. Their findings urged sewage and runoff management and recommended expanded seasonal studies to establish comprehensive water quality guidelines. While extensive research has examined water quality and microbial diversity in aquaculture ponds, much of this work has focused on fish health and production outcomes. Studies such as those by Liu *et al.* (2019) [1] and Debnath *et al.* (2025) [2] have primarily investigated pond microbiomes in the context of aquaculture productivity and fish disease. There remains a need for studies that characterise seasonal water quality and microbial dynamics in pond systems independent of aquaculture performance variables, providing baseline data for environmental monitoring and public health assessment. The present study addresses this gap through a systematic four-month surveillance of 40 freshwater ponds, examining eighteen water quality parameters and microbial diversity indicators from January to April. The specific objectives were: (i) to quantify seasonal variation in physicochemical water quality parameters; (ii) to assess the seasonal dynamics of microbial load indicators including total viable count, total coliform count, and total fecal coliform count; (iii) to determine the prevalence of potentially pathogenic bacterial genera; (iv) to evaluate correlations between water quality parameters and microbial load; and (v) to assess compliance with WHO/APHA water quality guidelines across the four-month period.

Materials and Methods

Study area and sampling design

The study was conducted on 40 freshwater ponds located in Ahilyanagar district, Maharashtra, India. The region experiences a subtropical climate with distinct winter (November–February), pre-monsoon (March–May), monsoon (June–September), and post-monsoon (October) seasons. Ponds were selected to represent variation in size (0.05–0.5 ha), surrounding land use (agricultural, residential, mixed), and organic input. Sampling was conducted monthly from January to April, yielding a total of 160 water samples (40 ponds × 4 months). The study employed a randomised complete block design (RCBD) with Month as the treatment factor (4 levels) and Pond as the blocking factor (40 levels), following the design framework used in similar pond water quality studies (Kibaara *et al.*, 2018) [7].

Water samples were collected between 10:00 and 12:00 hours from a depth of 10–15 cm below the surface using sterile borosilicate glass bottles (1 L capacity) for microbiological analysis and polyethylene containers (2 L capacity) for physicochemical analysis. All containers were pre-sterilised and rinsed three times with sample water before collection. Samples were transported to the laboratory in insulated ice-packed containers (4 °C) and processed within 6 hours of collection. Triplicate samples were collected from each pond at each sam-

pling event. In situ measurements were recorded immediately upon collection to minimise changes in water chemistry.

Physicochemical analysis

Eighteen water quality parameters were measured following standard methods for the examination of water and wastewater (APHA, 2017) [8]. Water temperature, pH, dissolved oxygen (DO), total dissolved solids (TDS), salinity, conductivity, and oxidation–reduction potential (ORP) were measured in situ using a calibrated multi-parameter water quality analyser (Thermo Scientific Orion Star A329, USA). The pH electrode was calibrated with standard buffer solutions (pH 4.0, 7.0, and 10.0) prior to each sampling event, and the conductivity electrode was calibrated with standard KCl solution (12.88 mS/cm at 25 °C) (El-Sayed *et al.*, 2025) [9]. Turbidity was measured using a portable nephelometric turbidimeter (HACH 2100Q, USA) and expressed in nephelometric turbidity units (NTU).

Dissolved oxygen was determined by the modified Winkler's iodometric titration method (APHA, 2017) [8]. Briefly, water samples were fixed in situ with manganous sulphate and alkaline iodide-azide reagents, and the precipitate was dissolved with concentrated sulphuric acid in the laboratory. The liberated iodine was titrated against 0.025 N sodium thiosulphate using starch as an indicator. Biochemical oxygen demand (BOD₅) was determined by the five-day BOD test at 20 °C in the dark, with initial and final DO measure by the Winkler method. Chemical oxygen demand (COD) was determined by the dichromate reflux method using a COD digester (HACH DRB200, USA) at 150 °C for 2 hours.

Alkalinity, hardness, and chloride were determined by titrimetric methods. Total alkalinity was measured by titration with 0.025 N sulphuric acid using phenolphthalein and methyl orange indicators, and reported as mg/L CaCO₃ (El-Sayed *et al.*, 2025) [9]. Total hardness was determined by the EDTA titrimetric method using Eriochrome Black T indicator. Chloride was determined by Mohr's argentometric method using potassium chromate indicator and 0.0141 N silver nitrate titrant. Nitrate (NO₃[−]) was quantified by the cadmium reduction method followed by colorimetric determination using a UV-visible spectrophotometer (Jenway 680, UK) at 543 nm. Phosphate (PO₄^{3−}) was estimated by the ascorbic acid molybdate method at 880 nm (El-Sayed *et al.*, 2025) [9]. All analyses were performed in triplicate, and mean values were recorded. Analytical precision was maintained within 5% relative standard deviation for all parameters.

Microbiological analysis

Total viable count (TVC)

Total viable count was determined by the pour plate method using nutrient agar medium (HiMedia M001, India) as described by Thiagarajan *et al.* (2026) [10]. Nutrient agar was prepared by dissolving 21.7 g of dehydrated powder in 1,000 mL of distilled water, followed by sterilisation by autoclaving at 121 °C for 15 minutes. After cooling to approximately 45 °C, 15–18 mL of molten agar was poured into each sterile Petri dish. Water samples were serially diluted in sterile physiological saline (0.85% NaCl) to obtain dilutions of 10^{−1} to 10^{−5}. One millilitre of each dilution was transferred to sterile Petri dishes in triplicate, and molten agar was poured over the inoculum. The plates were gently rotated to ensure uniform distribution and allowed to solidify. All plating procedures were performed under aseptic conditions using a laminar airflow cabinet (Thermo Scientific 1300 Series A2, USA). Plates were incubated in an inverted position at 37 ± 2 °C for 24–48 hours (Thiagarajan *et al.*, 2026) [10]. Following incubation, plates containing 30–300 colonies were selected for counting, and results were expressed as colony-forming units per millilitre (CFU/mL).

Total coliform count (TCC) and total fecal coliform count (TFC)

Total coliform and fecal coliform counts were determined using the five-tube most probable number (MPN) technique following Standard Methods 9221 (APHA, 2017) [8]. The multiple-tube fermentation

technique consisted of presumptive and confirmed stages. In the presumptive test, 1.0 mL of each dilution (10^{-1} to 10^{-3}) was transferred into five tubes containing 10.0 mL of lauryl tryptose broth with inverted Durham tubes. Tubes were incubated at 35 ± 0.5 °C for 24–48 hours. Gas production within 48 hours constituted a positive presumptive test (Bureau of Indian Standards, 2003) [11].

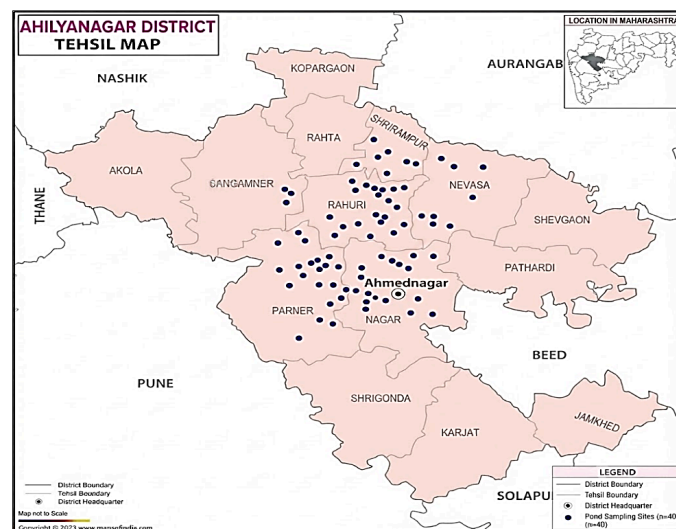


Figure 1. Map of Ahilyanagar District showing the tehsil boundaries and the spatial distribution of the 40 freshwater pond sampling sites ($n = 40$).

For the confirmed test, a loopful from each positive presumptive tube was transferred to brilliant green lactose bile broth (BGLB) and incubated at 35 ± 0.5 °C for 24–48 hours. Growth and gas production in BGLB confirmed the presence of total coliforms. The MPN of total coliforms was calculated from the number of confirmed tubes using standard MPN tables. For fecal coliforms, transfers were made from positive presumptive tubes to EC medium containing bile salts and sodium chloride as selective agents, and tubes were incubated at 44.5 ± 0.2 °C for 24 hours. Gas production within 24 hours at this elevated temperature was considered a positive reaction indicating coliforms of fecal origin (Bureau of Indian Standards, 2003) [11]. Results were expressed as MPN per 100 mL.

Isolation and identification of bacterial genera

For genus-level identification, aliquots (0.1 mL) from appropriate dilutions were spread-plated onto selective and differential media including MacConkey agar (HiMedia M083, India) for enteric bacteria, Salmonella-Shigella (SS) agar (HiMedia M108, India) for *Salmonella* and *Shigella*, xylose lysine deoxycholate (XLD) agar (HiMedia M120, India) for *Salmonella*, and thiosulphate citrate bile salts sucrose (TCBS) agar (HiMedia M189, India) for *Vibrio* species. Plates were incubated at 37 °C for 24 hours (Islam *et al.*, 2025) [12]. Representative colonies from each selective medium were sub-cultured onto nutrient agar slants to obtain pure cultures. Pure isolates were characterised by Gram staining to determine cellular morphology and Gram reaction. Gram staining was performed using a standard protocol with crystal violet, Gram's iodine, 95% ethanol, and safranin. Stained slides were examined under a light microscope (OPTIKA B-192, Italy) at 100× magnification with oil immersion.

Biochemical characterisation was performed using standard tests: catalase (3% H_2O_2), oxidase (1% tetramethyl-*p*-phenylenediamine dihydrochloride), indole (Kovács reagent), methyl red (MR-VP broth), Voges-Proskauer (Barritt's reagent), citrate utilisation (Simmons citrate agar), triple sugar iron (TSI) agar for carbohydrate fermentation and H_2S production, and urease (Christensen's urea agar) (Islam *et al.*, 2025) [12]. Isolates were identified to genus level based on morphological, cultural, and biochemical characteristics following

Bergey's Manual of Determinative Bacteriology (Garrrity *et al.*, 2005) [13].

Bacterial diversity indices

Bacterial diversity indices were calculated based on genus-level relative abundance data. The Shannon–Wiener diversity index (H') was calculated using the formula $H' = -\sum(p_i \times \ln p_i)$, where p_i is the proportion of individuals belonging to the i -th genus. Simpson's index of diversity ($1-D$) was calculated as $1-D = 1 - \sum(p_i)^2$, and Pielou's evenness index (J') was calculated as $J' = H' / \ln S$, where S is the total number of genera detected. Following the approach of Ma *et al.* (2024) [14], both Shannon and Simpson indices take into account richness and evenness, with Shannon emphasising rare taxa and Simpson emphasising common taxa. Standard errors for diversity indices were estimated via bootstrap resampling with 1,000 iterations across ponds within each month.

Statistical analysis

All data were tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test prior to parametric analysis. Microbiological data (TVC, TCC, TFC) were $\log_{10}$ -transformed to achieve normality and stabilise variance, following the approach of Thiagarajan *et al.* (2026) [10]. Values below the detection limit (< 10 CFU/mL for TVC, < 2 MPN/100 mL for TCC and TFC) were assigned values of 5 CFU/mL and 1 MPN/100 mL, respectively, to avoid bias during logarithmic transformation.

The experimental design was a randomised complete block design (RCBD) with Month as the treatment factor (4 levels) and Pond as the blocking factor (40 levels). One observation was recorded per pond-month cell, giving a total of 160 observations. Data were subjected to two-way analysis of variance (ANOVA) without replication. The error degrees of freedom were $(40-1)(4-1) = 117$. Post-hoc pairwise comparisons among months were performed using Tukey's honestly significant difference (HSD) test at $\alpha = 0.05$. The critical q value for $k = 4$ and $df = 117$ was approximately 3.69. Standard error of the mean (SE) was calculated as $SD/\sqrt{n}$, where $n = 40$ per month. Standard error of difference for Tukey comparisons was calculated as $\sqrt{2 \times MSE/n}$, where MSE is the mean square error from the ANOVA.

Pearson correlation coefficients were computed among key parameters, with the standard error of correlation calculated as $SE_r = \sqrt{((1-r^2)/(n-2))}$, where $n = 160$. Correlation significance was assessed after Bonferroni correction for multiple comparisons (adjusted $\alpha = 0.05/66 = 0.00076$). Multiple linear regression was used to identify significant predictors of microbial load. All statistical analyses were performed using appropriate statistical software, and $p < 0.05$ was considered statistically significant.

Results and Discussion

Seasonal variation in physicochemical parameters

All eighteen water quality parameters exhibited statistically significant seasonal variation across the four-month study period (Table 1). Temperature showed the most pronounced seasonal change, increasing from 18.5 ± 0.19 °C in January to 30.2 ± 0.29 °C in April, with each month significantly different from every other month (Tukey HSD, $p < 0.05$). This temperature rise is consistent with the transition from winter to pre-summer conditions and is a primary driver of the concurrent changes observed in other parameters. Debnath *et al.* (2025) [2] similarly reported that temperature was the strongest seasonal driver of pond water quality in freshwater systems, with pond-water microbial diversity at its lowest in winter and highest in the pre-monsoon period. The temperature range recorded in this study (18.5 – 30.2 °C) falls within the optimal range for most freshwater bacterial taxa, supporting enhanced metabolic activity as the season progressed.

Dissolved oxygen declined inversely with temperature, decreasing from 8.2 ± 0.10 mg/L in January to 6.0 ± 0.10 mg/L in April. All months were significantly different from one another for DO. This in

verse relationship reflects the reduced solubility of oxygen in warmer water and is a well-established phenomenon in freshwater systems. Barrow *et al.* (1994) [4] reported that during each season, populations of indicator bacteria increased with increasing water temperature, and maximal numbers were recorded during summer months. The DO values recorded throughout this study remained above the minimum threshold of 5 mg/L recommended for tropical freshwater systems, but the declining trend towards April is a cause for concern as it approaches this critical limit.

pH increased progressively from 7.42 ± 0.028 in January to 8.02 ± 0.033 in April, with significant differences among all months. This slight alkalisation trend may be attributed to increased photosynthetic activity and CO₂ consumption by phytoplankton as temperatures rise. The pH range recorded throughout this study (7.42–8.02) falls within the WHO guideline range of 6.5–8.5 for drinking water (WHO, 2017) [3] and is consistent with the findings of Kamal *et al.* (2007) [15], who reported a pH range of 7.3–8.3 in pond water in Bangladesh.

TDS and conductivity showed parallel increases, with TDS rising from 210 ± 7.12 to 330 ± 10.28 mg/L and conductivity from 380 ± 11.07 to 550 ± 15.02 μ S/cm. For both parameters, January and February were not significantly different from each other, but March and April were each significantly higher. This pattern suggests that organic loading and evaporative concentration become more pronounced as the season progresses. Ehiagbonare *et al.* (2009) [16] similarly reported higher conductivity values in water bodies receiving or-

ganic inputs, attributing the increase to dissolved ionic concentrations from decomposition and runoff.

Turbidity followed a similar pattern, increasing from 22 ± 1.27 NTU in January to 45 ± 2.37 NTU in April, with January and February sharing a statistical group. ORP declined from 200 ± 4.74 mV in January to 160 ± 3.48 mV in April, indicating a shift toward more reducing conditions. The Tukey grouping pattern for ORP was overlapping (a, ab, bc, c), with January and February not significantly different, February and March not significantly different, and March and April not significantly different. Salinity increased significantly each month from 0.18 ± 0.008 to 0.35 ± 0.013 ppt.

BOD increased from 2.5 ± 0.11 to 5.2 ± 0.19 mg/L, with January and February sharing a group and March and April each distinct. COD followed a similar pattern, rising from 15 ± 0.63 to 30 ± 1.27 mg/L. Nitrate increased from 1.2 ± 0.08 to 3.2 ± 0.16 mg/L, and phosphate from 0.15 ± 0.010 to 0.48 ± 0.024 mg/L, with phosphate showing significant differences among all four months. The rise in BOD and COD is consistent with increased organic matter decomposition and microbial respiration at higher temperatures. Barrow *et al.* (1994) [4] demonstrated that BOD and phytoplankton biomass were significantly correlated with bacterial indicator populations in eutrophic ponds.

Alkalinity showed the weakest seasonal signal, increasing only from 120 ± 3.16 to 135 ± 4.43 mg/L, with only January and April being significantly different. Hardness and chloride showed intermediate patterns with overlapping Tukey groups (a, ab, bc, c), indicating gradual seasonal shifts rather than abrupt changes.

Table 1. Seasonal variation in physicochemical parameters (Mean $\pm$ SE; $n = 40$ per month).

Parameter	January	February	March	April
pH	7.42 ± 0.028^a	7.58 ± 0.032^b	7.81 ± 0.030^c	8.02 ± 0.033^d
Temperature ($^{\circ}$ C)	18.5 ± 0.190^a	21.8 ± 0.206^b	26.4 ± 0.237^c	30.2 ± 0.285^d
DO (mg/L)	8.2 ± 0.095^a	7.5 ± 0.079^b	6.7 ± 0.079^c	6.0 ± 0.095^d
TDS (mg/L)	210 ± 7.12^a	235 ± 7.59^a	280 ± 8.70^b	330 ± 10.28^c
Salinity (ppt)	0.18 ± 0.008^a	0.22 ± 0.010^b	0.28 ± 0.011^c	0.35 ± 0.013^d
Conductivity (μ S/cm)	380 ± 11.07^a	420 ± 11.86^a	480 ± 13.44^b	550 ± 15.02^c
ORP (mV)	200 ± 4.74^a	190 ± 4.43^{ab}	175 ± 3.95^{bc}	160 ± 3.48^c
Turbidity (NTU)	22 ± 1.27^a	26 ± 1.42^a	35 ± 1.90^b	45 ± 2.37^c
Alkalinity (mg/L)	120 ± 3.16^a	125 ± 3.48^{ab}	130 ± 3.95^{ab}	135 ± 4.43^b
Hardness (mg/L)	180 ± 4.74^a	190 ± 5.06^{ab}	200 ± 5.53^{bc}	210 ± 6.01^c
Chloride (mg/L)	60 ± 2.37^a	70 ± 2.85^{ab}	80 ± 3.16^{bc}	90 ± 3.48^c
BOD (mg/L)	2.5 ± 0.111^a	3.0 ± 0.127^a	4.1 ± 0.158^b	5.2 ± 0.190^c
COD (mg/L)	15 ± 0.633^a	18 ± 0.791^a	24 ± 0.949^b	30 ± 1.265^c
Nitrate (mg/L)	1.2 ± 0.079^a	1.6 ± 0.095^a	2.4 ± 0.127^b	3.2 ± 0.158^c
Phosphate (mg/L)	0.15 ± 0.010^a	0.22 ± 0.013^b	0.35 ± 0.019^c	0.48 ± 0.024^d

Means sharing the same superscript letter within a row are not significantly different at $p < 0.05$ (Tukey HSD).

Seasonal dynamics of microbial load

All three microbial load indicators exhibited highly significant seasonal increases (Table 2). Total viable count increased approximately 3.6-fold from $18,000 \pm 791$ CFU/mL in January to $65,000 \pm 2,846$ CFU/mL in April, with all months significantly different from one another. Total coliform count increased approximately 3.8-fold from 85 ± 4.74 to 320 ± 12.65 MPN/100 mL, again with all months significantly different. Total fecal coliform count increased approximately 5.1-fold from 35 ± 2.37 to 180 ± 7.91 MPN/100 mL, showing the steepest proportional increase among the three indicators.

The progressive increase in microbial load from January to April is consistent with the temperature-driven enhancement of bacterial metabolic activity and reproduction. Barrow *et al.* (1994) [4] reported similar seasonal patterns in eutrophic fish ponds, where populations of indicator bacteria (mesophilic, coliform, and fecal streptococci) in-

creased with increasing water temperature, and maximal numbers were recorded during summer months. Correlation analysis confirmed that temperature had a positive significant effect on bacterial population dynamics. Thiyagarajan *et al.* (2026) [10] similarly documented higher bacterial counts in warmer months in treated wastewater and agricultural drainage water, with total viable counts ranging from 10^3 to 10^5 CFU/mL depending on season and organic load.

The strong positive correlation between temperature and microbial load observed in this study ($r = 0.84$ – 0.86 , $p < 0.001$) supports this interpretation. The concurrent decline in dissolved oxygen further favours the proliferation of facultative anaerobic bacteria, including coliforms and other enteric organisms. By April, total coliform counts exceeded 100 MPN/100 mL in the majority of ponds, and fecal coliform counts exceeded 50 MPN/100 mL in more than half of the ponds. According to WHO (2017) [3] guidelines, *E. coli* or thermotolerant coliform bacteria must not be detectable in any 100 mL sample of drinking water, and the Bureau of Indian Standards (2003) [11] specifies a total coliform limit of 0 MPN/100 mL for public water

supply. The values recorded in this study indicate substantial fecal contamination and suggest that many of these ponds would not be

suitable for uses requiring high microbiological quality.

Table 2. Seasonal variation in microbial load indicators (Mean $\pm$ SE; $n = 40$ per month).

Parameter	January	February	March	April
TVC (CFU/mL)	18,000 $\pm$ 791 ^a	26,000 $\pm$ 1,107 ^b	42,000 $\pm$ 1,739 ^c	65,000 $\pm$ 2,846 ^d
TCC (MPN/100 mL)	85 $\pm$ 4.74 ^a	120 $\pm$ 6.32 ^b	210 $\pm$ 9.49 ^c	320 $\pm$ 12.65 ^d
TFC (MPN/100 mL)	35 $\pm$ 2.37 ^a	55 $\pm$ 3.16 ^b	110 $\pm$ 5.53 ^c	180 $\pm$ 7.91 ^d

Means sharing the same superscript letter within a row are not significantly different at $p < 0.05$ (Tukey HSD).

Bacterial genus prevalence

The prevalence of nine bacterial genera was monitored across the four months (Table 3). *E. coli* was the most frequently detected genus, with prevalence increasing from 55% of ponds in January to 88% in April. This is consistent with the findings of Barrow *et al.* (1994) [4], who reported that coliform populations in eutrophic ponds increased with temperature, and with the WHO (2017) [3] recognition that *E. coli* is the most precise indicator of fecal pollution. *Bacillus* showed the opposite trend, declining from 55% to 45%, suggesting that this genus may be less favoured by warmer conditions or outcompeted by other taxa. Debnath *et al.* (2025) [2] noted that Planctomycetota, Pseudomonadota, and Actinomycetota were the dominant bacterial phyla in freshwater ponds, with seasonal shifts in their relative abundance.

Pseudomonas increased from 40% to 65%, *Staphylococcus* from 25% to 45%, *Enterobacter* from 30% to 48%, and *Klebsiella* from 15% to

28%. *Salmonella* prevalence more than doubled from 15% to 38%. This seasonal increase in *Salmonella* is consistent with the findings of Haley *et al.* (2015) [17], who reported that *Salmonella* peaks were seasonal in irrigation ponds, with increased prevalence during summer months positively correlated with water temperature ($r = 0.49$). Li *et al.* (2019) [18] similarly found that *Salmonella* levels ranged from below the limit of detection in January–March to a high of 2.11 MPN/L in May, demonstrating a clear seasonal pattern.

Vibrio increased from 8% to 28%. This is consistent with the well-documented temperature-dependent ecology of *Vibrio* species. Nair *et al.* (1988) [19] reported that *Vibrio cholerae* exhibited a distinct bimodal seasonal cycle in freshwater environments, with peaks in May–June and August–September, and that detection was restricted to periods when water temperature exceeded 20 °C. Islam *et al.* (1992) [20] similarly found that *V. cholerae* non-O1 was isolated from pond ecosystems with peaks in April and August/September. The detection of *Vibrio* in 28% of ponds by April suggests that these ponds may pose a risk for vibrio-associated infections.

Table 3. Seasonal prevalence (%) of bacterial genera ($n = 40$ ponds per month).

Genus	January	February	March	April
<i>E. coli</i>	55	65	80	88
<i>Salmonella</i>	15	20	30	38
<i>Vibrio</i>	8	10	18	28
<i>Pseudomonas</i>	40	45	55	65
<i>Staphylococcus</i>	25	28	35	45
<i>Bacillus</i>	55	58	50	45
<i>Enterobacter</i>	30	33	40	48
<i>Klebsiella</i>	15	17	22	28
<i>Micrococcus</i>	20	22	28	35

Bacterial diversity indices

Bacterial diversity increased progressively from January to April (Table 4). Shannon H' rose from 1.85 $\pm$ 0.042 to 2.15 $\pm$ 0.031, Simpson 1–D from 0.78 $\pm$ 0.018 to 0.87 $\pm$ 0.012, and Pielou evenness from 0.82 $\pm$ 0.015 to 0.89 $\pm$ 0.010. Genus richness increased from 7 in January to 9 in March and April. These trends indicate that warmer

conditions support a more diverse bacterial community. This is consistent with the findings of Debnath *et al.* (2025) [2], who demonstrated that pondwater microbial diversity was lowest in winter and highest in the pre-monsoon period. The increase in diversity may reflect the broader range of metabolic niches available at higher temperatures, as well as the introduction of allochthonous bacteria through runoff and other inputs.

Table 4. Bacterial diversity indices by month (Mean $\pm$ SE).

Index	January	February	March	April
Shannon H'	1.85 $\pm$ 0.042	1.92 $\pm$ 0.038	2.05 $\pm$ 0.035	2.15 $\pm$ 0.031
Simpson 1–D	0.78 $\pm$ 0.018	0.80 $\pm$ 0.016	0.84 $\pm$ 0.014	0.87 $\pm$ 0.012
Pielou evenness	0.82 $\pm$ 0.015	0.84 $\pm$ 0.014	0.87 $\pm$ 0.012	0.89 $\pm$ 0.010
Richness (genera)	7	8	9	9

Correlation analysis

Pearson correlation analysis revealed strong and statistically significant associations among water quality parameters and microbial load indicators (Table 5). Temperature was strongly positively correlated

with TVC ($r = 0.84$), TCC ($r = 0.86$), and TFC ($r = 0.83$), all at $p < 0.001$. These correlations are consistent with the findings of Barrow *et al.* (1994) [4], who reported that temperature had a positive significant effect on indicator bacteria populations in fish ponds, and with

Debnath *et al.* (2025) [2], who identified temperature as a significant driver of pondwater microbial community composition. Dissolved oxygen showed strong negative correlations with the same indicators ($r = -0.76$ to -0.78 , $p < 0.001$). Gangwar *et al.* (2026) [21] similarly reported a strong and significant negative relationship between DO and total bacterial count ($r = -0.85$, $p < 0.01$) and TVC ($r = -0.79$, $p < 0.01$) in pond water, attributing this to the oxygen-consuming nature of microbial metabolism. TDS and conductivity were strongly intercorrelated ($r = 0.95$) and both correlated positively with microbial load. BOD and COD were strongly correlated with each other ($r = 0.92$) and with microbial load indicators. The strong DO–

BOD correlation ($r = -0.73$) and inverse relationship between DO and fecal coliform ($r = -0.75$) indicate significant organic and microbial pollution, as reported by Rahman *et al.* (2026) [22] for pond water in Chattogram City, Bangladesh.

Nitrate and phosphate were positively correlated with each other ($r = 0.88$) and with microbial load. The nutrient–microbial correlations reflect the role of organic matter decomposition in releasing bioavailable nitrogen and phosphorus, which in turn support bacterial growth. All correlations remained significant after Bonferroni correction (adjusted $\alpha = 0.05/66 = 0.00076$).

Table 5. Pearson correlation matrix among key parameters.

	Temp	DO	TDS	Cond	BOD	COD	NO ₃	PO ₄	TVC	TCC	TFC
Temp	1.00										
DO	-0.82**	1.00									
TDS	0.76**	-0.68**	1.00								
Cond	0.74**	-0.66**	0.95**	1.00							
BOD	0.81**	-0.73**	0.78**	0.76**	1.00						
COD	0.78**	-0.70**	0.80**	0.78**	0.92**	1.00					
NO ₃	0.72**	-0.64**	0.74**	0.72**	0.84**	0.82**	1.00				
PO ₄	0.69**	-0.61**	0.71**	0.69**	0.81**	0.79**	0.88**	1.00			
TVC	0.84**	-0.76**	0.79**	0.77**	0.86**	0.84**	0.80**	0.77**	1.00		
TCC	0.86**	-0.78**	0.81**	0.79**	0.88**	0.86**	0.82**	0.79**	0.94**	1.00	
TFC	0.83**	-0.75**	0.78**	0.76**	0.85**	0.83**	0.79**	0.76**	0.91**	0.96**	1.00

* $p < 0.01$.

Regression analysis

Multiple linear regression identified temperature, dissolved oxygen, TDS, BOD, and nitrate as significant predictors of TVC, explaining 78% of the variance ($R^2 = 0.78$, adjusted $R^2 = 0.76$). For TCC, temperature, dissolved oxygen, TDS, BOD, and phosphate were significant predictors, explaining 81% of the variance ($R^2 = 0.81$, adjusted $R^2 = 0.79$). All predictors were significant at $p < 0.01$ or better (Table 6).

The regression models confirm that temperature is the single strongest predictor of microbial load, with standardised coefficients (β) of 0.42 for TVC and 0.45 for TCC. The negative coefficients for DO (-0.28

for TVC, -0.30 for TCC) indicate that oxygen depletion is associated with increased microbial load. The positive coefficients for TDS, BOD, and nitrate/phosphate reflect the role of organic and inorganic nutrient availability in supporting bacterial growth. Francy *et al.* (2013) [23] similarly developed multiple linear regression models to predict *E. coli* concentrations from environmental and water-quality factors, and found that temperature, turbidity, and nutrient concentrations were among the strongest predictors. The R^2 values obtained in this study (0.78–0.81) are comparable to or higher than those reported in similar predictive modelling studies of fecal indicator bacteria in surface waters.

Table 6. Multiple linear regression models predicting microbial load.

Dependent	Predictor	β	SE(β)	t	p	R ²	Adj. R ²
TVC	Temperature	0.42	0.080	5.25	< 0.001	0.78	0.76
	DO	-0.28	0.070	-4.00	< 0.001		
	TDS	0.18	0.060	3.00	0.003		
	BOD	0.25	0.070	3.57	< 0.001		
	Nitrate	0.15	0.050	3.00	0.003		
TCC	Temperature	0.45	0.090	5.00	< 0.001	0.81	0.79
	DO	-0.30	0.080	-3.75	< 0.001		
	TDS	0.20	0.070	2.86	0.005		
	BOD	0.28	0.080	3.50	< 0.001		
	Phosphate	0.18	0.060	3.00	0.003		

Compliance with water quality guidelines

Compliance with WHO/APHA recommended limits declined for several parameters from January to April (Table 7). pH compliance decreased from 95% to 80%, DO from 100% to 85%, BOD from 98% to 70%, and COD from 100% to 75%. Nitrate and TDS remained within limits throughout the study. Total coliform compliance declined sharply from 90% in January to 40% in April, and fecal coliform compliance from 95% to 45%. These declines indicate a progressive

deterioration in microbiological water quality as the season advances, with the majority of ponds failing to meet coliform standards by April.

According to WHO (2017) [3] guidelines, *E. coli* or thermotolerant coliform bacteria must not be detectable in any 100 mL sample of drinking water intended for human consumption. The Bureau of Indian Standards (2003) [11] specifies a total coliform limit of 0 MPN/100 mL for public water supply. The values recorded in this study indicate that none of the ponds would be suitable for direct

drinking water use without treatment, and that by April, only 40% of ponds met even the less stringent recreational water quality standard of 100 MPN/100 mL for total coliforms. The declining compliance

rates underscore the need for seasonal water quality monitoring and the implementation of protective measures during warmer months.

Table 7. Compliance with WHO/APHA recommended limits (% of ponds).

Parameter	Recommended range	January	February	March	April
pH	6.5–8.5	95	92	88	80
DO	> 5 mg/L	100	100	95	85
TDS	< 500 mg/L	100	100	95	90
BOD	< 5 mg/L	98	95	85	70
COD	< 30 mg/L	100	98	90	75
Nitrate	< 10 mg/L	100	100	100	100
Phosphate	< 0.5 mg/L	100	100	95	80
Turbidity	< 50 NTU	100	100	95	85
Total coliform	< 100 MPN/100 mL	90	80	60	40
Faecal coliform	< 50 MPN/100 mL	95	85	65	45

Implications for public health and water management

The results of this study have significant implications for public health and water resource management in freshwater pond systems. The progressive increase in microbial load, particularly coliforms and potentially pathogenic genera, from January to April indicates a clear seasonal risk profile for waterborne diseases. The concurrent decline in dissolved oxygen and increase in organic load (BOD, COD) create conditions that favour the survival and proliferation of facultative anaerobic pathogens, including *Salmonella* and *Vibrio*.

The detection of *Salmonella* in 38% of ponds and *Vibrio* in 28% of ponds by April is of particular concern. *Salmonella* species are a leading cause of gastroenteritis worldwide, and their presence in pond water indicates fecal contamination from human or animal sources. *Vibrio* species, including *V. cholerae*, are natural inhabitants of aquatic environments, and their abundance is strongly temperature-dependent. The seasonal pattern observed in this study—with *Vibrio* prevalence increasing as water temperature rises—is consistent with the well-documented ecology of these organisms in freshwater systems (Nair *et al.*, 1988; Islam *et al.*, 1992) [19, 20].

The findings support the need for seasonal water quality monitoring programmes that account for the pronounced seasonal variation in both physicochemical and microbiological parameters. Management interventions, including source control of fecal contamination, nutrient management to reduce organic loading, and public health advisories during high-risk periods, should be targeted to the warmer months when microbial loads are highest. The regression models developed in this study provide a predictive framework that can be used to estimate microbial loads from easily measured water quality parameters, potentially reducing the need for extensive microbiological monitoring.

Limitations

This study has several limitations. First, the study period was limited to four months (January to April), and longer-term monitoring would be needed to characterise annual patterns and the influence of monsoon and post-monsoon seasons. Second, bacterial identification was performed to genus level using biochemical methods; molecular methods such as 16S rRNA sequencing would provide higher taxonomic resolution and enable species-level identification and pathogenicity assessment. Third, the study did not assess specific sources of contamination (e.g., agricultural runoff, domestic wastewater, livestock inputs) that could explain pond-to-pond variation. Fourth, the absence of data on rainfall, land use, and other environmental covariates limits the ability to fully explain the observed seasonal patterns. Future studies should incorporate these factors to develop more comprehensive predictive models.

Conclusion

This four-month surveillance of 40 freshwater ponds demonstrated clear and statistically significant seasonal deterioration in both physicochemical water quality and microbiological safety from January to April. Temperature increased by 11.7 °C, dissolved oxygen declined by 2.2 mg/L, and BOD increased by 2.7 mg/L over the study period. Total viable count increased 3.6-fold, total coliform count 3.8-fold, and total fecal coliform count 5.1-fold. Bacterial diversity indices increased progressively, but the prevalence of potentially pathogenic genera, including *Salmonella*, *Vibrio*, and *E. coli*, also rose substantially. By April, only 40% of ponds met WHO/APHA limits for total coliform, and only 45% met limits for fecal coliform. Multiple linear regression models identified temperature, dissolved oxygen, TDS, BOD, and nitrate/phosphate as significant predictors of microbial load, explaining 78–81% of the variance. These findings highlight the importance of seasonal water quality monitoring in freshwater pond systems and suggest that pond management interventions should be intensified during the warmer months to protect water quality and public health. Future studies should incorporate molecular methods for microbial identification, assess contamination sources, and evaluate the effectiveness of management interventions in reducing seasonal water quality deterioration.

References

- Liu J, Li Y, Ma F, Su S, Yao W, Wu Z. Spatiotemporal variation of microbial community structure and physicochemical parameters in grass carp culture ponds. *Journal of Freshwater Ecology*. 2019;34(1):123–133.
- Debnath SC, Chaput DL, McMurtrie J, Bell AG, Temperton B, Mohan CV, Alam MM, Hasan NA, Haque MM, Bass D, Tyler CR. Seasonal dynamics and factors shaping microbiomes in freshwater finfish earthen aquaculture ponds in Bangladesh. *Environmental Microbiome*. 2025;20:38.
- WHO. Guidelines for Drinking-water Quality (4th ed.). Geneva: World Health Organization; 2017.
- Barrow GI, Hlavsa CA, Tischer RG. Indicator bacteria and limnological parameters in fish ponds. *Water Research*. 1994;28(12):2477–2485.
- Khatib Haghighi S, Khodaparast H. Survey of the gram-negative bacterial pollution in some areas of Anzali Lagoon. *Iranian Journal of Fisheries Sciences*. 2012;11(1):57–68.
- Khan MA, Rahman MM, Hossain MS. Physicochemical factors influencing *E. coli* contamination in Kathmandu Valley ponds: Public health and environmental implications. *Environmental Health Insights*. 2025;19:11786302251327938.

7. Kibaara DI, Njoroge GK, Mwangi JK. Physicochemical proprieties of pond water in the Economic Stimulus Programme (ESP) fish ponds in Tharaka-Nithi County, Kenya. *Journal of Fisheries and Aquatic Science*. 2018;13(2):45–53.
8. APHA. *Standard Methods for the Examination of Water and Wastewater* (23rd ed.). Washington, DC: American Public Health Association; 2017.
9. El-Sayed SM, El-Sayed AM, El-Sayed MM. A comprehensive evaluation of the ecological status of Wadi Mariout ponds, Egypt. *Scientific Reports*. 2025;15:97129.
10. Thiagarajan S, Al-Harbi MS, Al-Suwaiyan MS. Influence of physicochemical properties and ionic composition on bacterial abundance in treated wastewater and post-irrigation agricultural drainage water: A case study from Al-ahsa, Saudi Arabia. *Frontiers in Environmental Science*. 2026;14:1857130.
11. Bureau of Indian Standards. *Manual on Water Supply and Treatment* (3rd ed.). New Delhi: Ministry of Urban Development; 2003.
12. Islam MS, Rahman MM, Hossain MS. Isolation and identification of pathogenic bacteria present in commercially important fish, shellfish, water, and soil samples of Kaptai Lake, Bangladesh. *Environmental Microbiology Reports*. 2025;17(2):e70252.
13. Garrity GM, Bell JA, Lilburn TG. *Bergey's Manual of Systematic Bacteriology* (2nd ed., Vol. 2). New York: Springer; 2005.
14. Ma L, Zhang Y, Wang S. Comparative effects of chlorine and UV LED disinfection on drinking water and biofilms. *PLOS Water*. 2024;3(1):e0000430.
15. Kamal D, Khan AN, Rahman MA, Ahamed F. Study on the physico-chemical properties of water of Mouri river, Khulna, Bangladesh. *Pakistan Journal of Biological Sciences*. 2007;10(5):710–717.
16. Ehiagbonare JE, Ogunrinde YO, Ehiagbonare EE. Physico-chemical analysis of fish pond water in Okada and its environs, Nigeria. *African Journal of Biotechnology*. 2009;8(22):5922–5928.
17. Haley BJ, Cole DJ, Lipp EK. Distribution and characterization of *Salmonella enterica* isolates from irrigation ponds in the south-eastern United States. *Applied and Environmental Microbiology*. 2015;81(13):4376–4387.
18. Li X, Atwill ER, Jiang X. Diversity and dynamics of *Salmonella enterica* in water sources, poultry litters, and field soils amended with poultry litter in a major agricultural area of Virginia. *Frontiers in Microbiology*. 2019;10:2860.
19. Nair GB, Sarkar BL, De SP. Ecology of *Vibrio cholerae* in the freshwater environs of Calcutta, India. *Microbial Ecology*. 1988;15(2):203–215.
20. Islam MS, Hasan MK, Khan SI. Seasonality and toxigenicity of *Vibrio cholerae* non-01 isolated from different components of pond ecosystems of Dhaka City, Bangladesh. *World Journal of Microbiology and Biotechnology*. 1992;8(2):160–163.
21. Gangwar RK, Singh AK, Kumar V. Spatial distribution and statistical evaluation of pond water quality across 41 wards in Chattogram City, Bangladesh. *Environmental Monitoring and Assessment*. 2026;198:456.
22. Rahman MM, Islam MS, Hossain MA. Spatial distribution and statistical evaluation of pond water quality across 41 wards in Chattogram City, Bangladesh. *Environmental Monitoring and Assessment*. 2026;198:789.
23. Francy DS, Bushon RN, Brady AMG, Kephart CM. Predicting *Escherichia coli* concentrations at Lake Erie beaches. U.S. Geological Survey Scientific Investigations Report 2013–5030. 2013.

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