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Physicochemical Factors Influencing *E. coli* Contamination in Kathmandu Valley Ponds: Public Health and Environmental Implications

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Abstract
Ponds in Kathmandu Valley reflect its rich history with water resources, but increasing pollution threatens public health and the environment. This study aimed to assess the physicochemical and microbial quality of pond water and further analyze the factors influencing *E. coli* contamination. In 2023, water samples from 27 out of a total of 35 ponds were examined for physicochemical parameters (temperature, pH, TSS, turbidity, iron (Fe^{2+}), nitrite (NO_2^-), phosphate (PO_4^{3-}), ammonia (NH_3), DO, BOD, and COD) and microbial parameters (total coliforms and *E. coli*). Results revealed that all ponds exceeded WHO limits for TSS and turbidity for drinking water. Furthermore 67% surpassed the iron limit, while 96% exceeded USEPA's BOD and COD levels for supporting aquatic life. Coliforms were present in all ponds, with *E. coli* detected in 67%, indicating the water was unfit for drinking under EU guidelines. Logistic regression revealed a significant association of COD and temperature (*P*-values 0.001 and 0.023 respectively) with *E. coli* presence. A 3D visualization of the data further supports the association and illustrates these relationships, COD having a greater impact. These findings underscore public health risks and environmental concerns, urging sewage and runoff management and recommending expanded seasonal studies to establish comprehensive water quality guidelines.

Keywords
Kathmandu Valley, *E. coli* contamination, physicochemical parameters, pollution, pond water

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Introduction

Pollution of freshwater bodies is a major threat to both public health and aquatic life, and one of the leading causes of the global water crisis.[1,2] The key water pollutants are of physical, chemical and microbiological origin. Microbial pollutants include several pathogenic bacteria, viruses, fungi and parasites. The sources of these pathogens are sewage, industrial effluents, human and animal waste, etc. *Vibrio, Salmonella, Enterobacter, Shigella, Campylobacter, Escherichia*, and *Legionella* are some of the important genera of bacterial pathogens which create public health emergencies.[3] While physicochemical parameters are essential for water quality assessment, the presence of *Escherichia coli* (*E. coli*) is a primary indicator of fecal contamination and potential pathogens.[4,5] The Kathmandu Valley faces recurring outbreaks of *E. coli*-related diarrhea, cholera, dysentery, and other waterborne infections, particularly during the monsoon season. This is due to the region's densely populated urban areas, inadequate sanitation infrastructure, and reliance on contaminated water sources, making *E. coli* a key indicator of the water quality crisis and a critical focus for safeguarding public health.

Temperature, pH, total suspended solids (TSS), Turbidity, iron, phosphate, ammonia, nitrite, biological oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO)—both individually and in their complex interplay influence *E. coli* growth and water contamination levels. Higher BOD and COD levels reduce

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DO concentration, favoring *E. coli* growth.[6,7] Nutrients like ammonia, phosphate and nitrite influence microorganism growth. *E. coli* harnesses ammonia and nitrites as vital nitrogen sources for protein synthesis and cellular functions, while phosphate, crucial for ATP, DNA, RNA, and cell membranes, drives energy metabolism, genetic replication, and cellular division, significantly accelerating bacterial growth. Ammonia also raises pH creating alkaline conditions unfavorable *for E. coli.* Warmer temperatures lower DO, increase nutrient solubility and favor the growth of the bacterium.[8,9] Higher temperatures increase the kinetic energy of water molecules, causing them to move more rapidly, which reduces the water's ability to hold oxygen, leading to lower DO. Additionally, higher temperatures increase the solubility of the nutrients by accelerating the rate of chemical reaction. Contaminated water poses a real threat to human health and aquatic life, causing severe illnesses like *E. coli* diarrhea and cholera while disrupting ecosystems. WHO recommends 0 CFU of *E. coli* per 100 ml in drinking water, while recreational water above 200 CFU per 100 ml is associated with gastrointestinal illnesses. *E. coli* presence also indicates organic pollution, which causes oxygen depletion. A BOD level above 3 to 6 mg/L stresses organisms.

Kathmandu Valley, encompassing Kathmandu, Bhaktapur and Lalitpur districts, has ponds, rivers and supply water as key water sources. Rapid population growth, expansion of built-up areas, and increased demand for water resources have stressed existing infrastructure. The valley has around 35 ponds (Pokhari) that provide water for livestock, irrigation, recreation, and household needs. These ponds also hold many historical, religious, and cultural values.[10] However, in recent years, pollution has emerged as a pressing concern, primarily attributed to unplanned urbanization and other human activities, and presents a risk to human health and aquatic life.[11] *E. coli* contamination has been detected in ponds such as Khashi Pokhari and Taudaha Pokhari,[12] as well as in the Bagmati River in Kathmandu.[13] Shallow-dug wells were identified as having the highest risk of *E. coli* infection in a study on groundwater sources,[14] while a separate investigation reported 169 cholera cases in the valley—higher than in other regions of the country.[15] The study of pond water pollution in Kathmandu Valley is notably underexplored, revealing a significant gap in research. Furthermore, research on the influence of physicochemical parameters on *E. coli* presence remains relatively uncharted. The valley is vulnerable to waterborne infection outbreaks, particularly during the rainy season, posing significant public health concerns. Additionally, the increasing pollution of the ponds is indeed threatening aquatic life which justifies the urgency of the research.

This study first focuses on evaluating the physicochemical parameters—temperature, pH, TSS, turbidity, BOD, COD, DO, iron ($Fe^{2}+$), nitrite (NO_2^{-}) ammonia (NH_3), phosphate (PO_4^{3-})—and microbial parameters (coliforms and *E. coli*) of the pond water in Kathmandu Valley to understand whether it is safe specifically for public health or environmental well-being. It then further delves into the impact of physicochemical parameters on *E. coli* contamination, which has not been done before in pollution assessment. Furthermore, our study stands out by leveraging advanced statistical modeling tools in Python, which enhances the precision and robustness of our findings. Thus, the study will offer critical insights into public health risks and threats to aquatic life associated with polluted water, empowering local authorities to take timely and decisive action to mitigate the impact. The limitations of the study include the lack of spatial data, biases due to anthropogenic influences at the sampling sites, and the need for more environmental data.

Methods

Study Area and Period

The research study was conducted in Kathmandu Valley, situated at a latitude of 27° 20′ 0″ N and 85° 30′ 0″ E (Figure 1), from May 2023 to August 2023. The study focused on the rainy season due to the higher incidence of waterborne infections.

Sample Size

A total of 27 water samples from 35 different ponds in the Kathmandu Valley were analyzed. With more than 75% of the total sample, the sample size was sufficient representation and was large enough to capture the main characteristics of the data maintaining statistical reliability. Furthermore, this sample size was enough to maintain homogeneity and to ensure that findings and conclusions drawn from the analysis were more reliable. The exclusion of 8 ponds was to avoid redundancy, as they shared similar characteristics—pollution sources, geographical location, water sources, and land use—with the selected ponds, and their inclusion would not have added new insights.

Sample Collection

All necessary permissions were obtained from local authorities and water samples were collected during the rainy season, as surface runoff and potential contamination are at their peak due to monsoon rainfall. Surface water samples (from 0.2 to 0.5 m depth), which are typical for assessing immediate runoff effects and pollutants that remain near the surface, were collected using grab sampling technique. The sampling points were determined based on the knowledge of local authorities about pollution sources, like agricultural runoff and sewage discharge. For microbial and physicochemical analysis, pond water was collected in sterile bottles of 1000 ml. Additionally, water from the same site was collected in a BOD bottle of 300 ml for the determination of BOD. The temperature and pH were measured onsite and the water samples were transported to the laboratory within 6 hours in a cold box. Then, samples were processed for the pollution assessment in aseptic conditions.

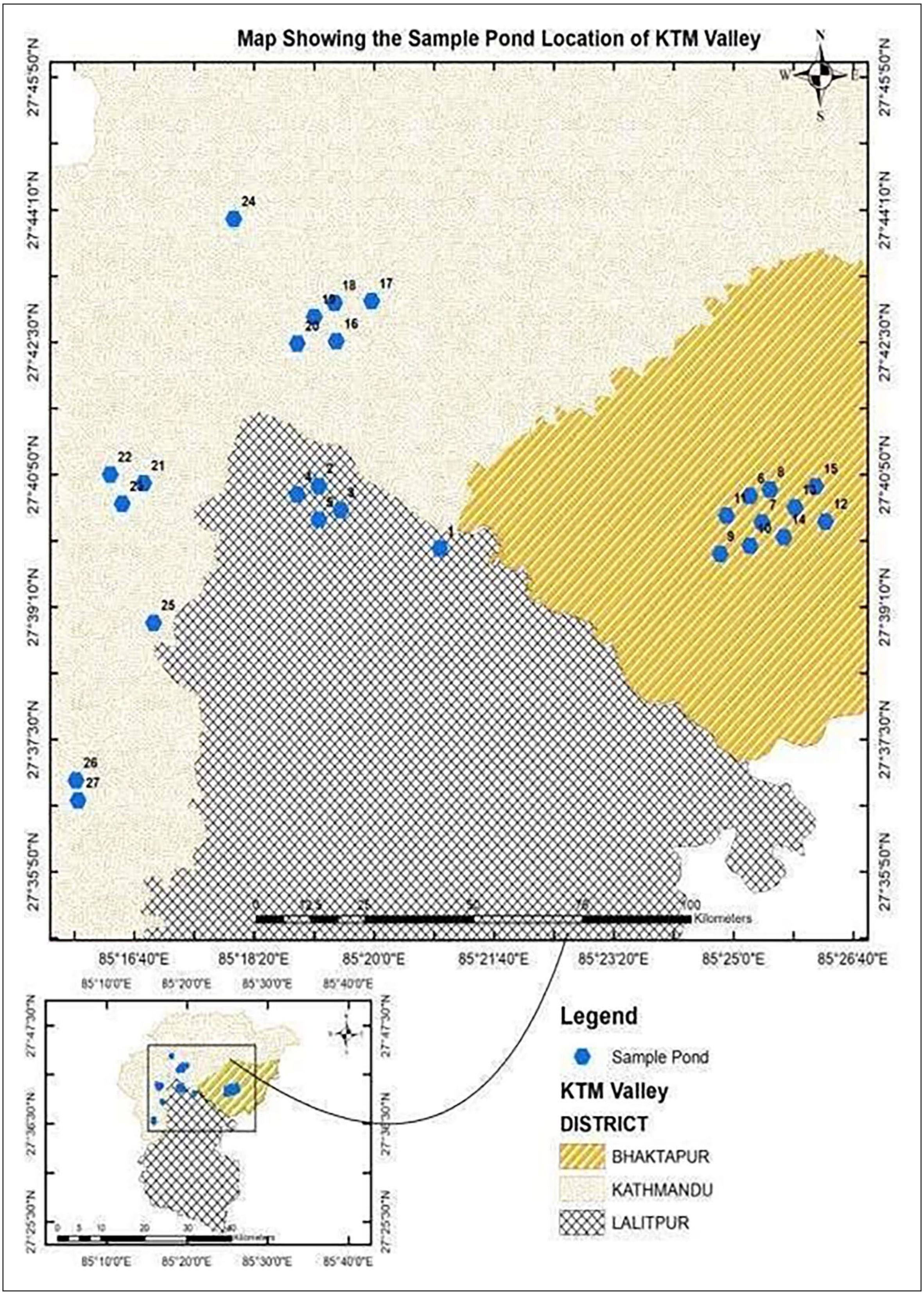

Figure 1. Location map of studied ponds in Kathmandu Valley. Name of the studied ponds according to the numbers in the map. (1) Boje Pokhari, (2) Pimbahal Pokhari, (3) Bhandarkhal Pokhari Ltp, (4) Situnga Pokhari, (5) Nhu Pokhari, (6) Na Pokhari, (7) Tekha Pokhari, (8) Bhandarkhal Pokhari, (9) Siddha Pokhari, (10) Bhajya Pokhari, (11) Rani Pokhari- Bkt, (12) Chyamasingh Pokhari, (13) Mangal Kunda Pokhari, (14) Kwathandu Pokhari, (15) Kamal Pokhari-Bkt, (16) Kamal Pokhari, (17) Gahana Pokhari, (18) Naag Pokhari, (19) Hiti Pokhari, (20) Rani Pokhari- Ktm, (21) Khasi Pokhari-1, (22) Khasi Pokhari-2, (23) Dee Pokhari, (24) Bais Dhara Pokhari, (25) Taudaha Pokhari, (26) Shesh Narayan Pokhari, (27) Pharphing Pokhari.

Abbreviations: Ltp, Lalitpur district; Bkt, Bhaktapur district; Ktm, Kathmandu district.

The same name of the ponds from different districts was added short name of the district to make it clear.

Analysis of Different Parameters

Temperature, pH, and Turbidity

Temperature, pH, and turbidity were measured using instruments calibrated according to the manufacturer's guidelines.

Temperature was determined with the help of a calibrated digital Micro 1000 IR thermometer (Hf Scientific, Inc.). The pH of the water samples was measured with the help of a calibrated pH meter (Hf Scientific, Inc.). The pH meter was calibrated daily using a three-point standard buffer solution (pH 4.0, 7.0, and 10.0). The turbidity of the pond water samples was measured with the help of a calibrated Micro 1000 IR turbidimeter (Hf Scientific, Inc.)

TSS

TSS was determined by the Gravimetric method.

A 50 ml water sample was poured into a filter paper fitted in a funnel and the filter paper was oven dried at 105°C for 2 hours. The TSS was calculated from the dry weight of the filter paper before and after sample filtration and the water volume.

TSS concentration (mg/L) = mass of suspended solids (mg)/vol. of water sample (L).

DO, BOD

The DO of different pond water samples was measured on-site using a calibrated HANNA DO meter (HI 2400).

For the determination of BOD_5, DO_1 was measured on the first day of incubation and the water bottle was further incubated for 5 days to measure DO_5. Then BOD was calculated as: $BOD = DO_5 - DO_1$.

COD

It was determined by the Closed Reflux Titrimetric method.

A 10 ml pond water sample was digested with 14 ml of reagent A [a solution of silver sulfate (Ag_2SO_4) and concentrated sulfuric acid (H_2SO_4)] and 6 ml of reagent B [a solution of potassium dichromate ($K_2Cr_2O_7$), concentrated sulfuric acid and mercuric sulfate ($HgSO_4$)] in the COD digester for 3 hours. The digested water was titrated with a Ferrous Ammonium Sulfate (FAS) solution. Then, the COD was calculated using the formula given below.

$$COD\left(\frac{mg}{L}\right) = \frac{(A-B)M \times 8000}{sample\ volume\,(ml)}$$

Where, A = ml of FAS used for blank.
B = ml of FAS used for sample.
M = Molarity of FAS.

Nitrite (NO_2^-)

The concentration of nitrite in the water sample was determined by a spectrophotometric method based on the Griess Reaction. The intensity of the resulting color after the reaction which is directly proportional to the nitrite concentration was measured using a calibrated spectrophotometer (Agilent Technologies Company) at a wavelength of 540 nm. The spectrophotometer was calibrated using a blank solution before each measurement, with periodic wavelength accuracy verification using certified standards. The concentration of nitrite was calculated in mg/L by comparing the absorbance of the sample to a standard curve created from known concentrations of nitrite.

Ammonia (NH_3)

The semi-quantitative measurement of ammonia was conducted using the Nesslerization method with the aid of a color comparator (Disk Comparison Method). In this method, ammonia reacts with an alkaline solution of potassium tetraiodomercurate (II), forming a yellow-brown complex. The intensity of the color was proportional to the concentration of ammonia present. A color comparator was used to match the color produced in the sample to a series of standard solutions with known ammonia concentrations to determine the approximate concentration in the sample.

Phosphate (PO_4^{3-})

The stannous chloride method was used to determine phosphate concentration.

A reagent (mixture of ammonium molybdate, sulfuric acid, and ascorbic acid) was added to the water sample and mixed slowly. A colored compound was formed. To increase the color intensity stannous chloride was added. Now the absorbance was measured using a calibrated spectrophotometer (Agilent Technologies Company). The absorbance was compared to a standard curve generated from known phosphate concentrations to determine the concentration of phosphates in the sample.

Iron (Fe^{2+})

Iron concentration was determined by the Thioglycolate method. Firstly, a few drops of 1 N HCl were added to a 50 ml water sample and boiled for 5 minutes. After the solution was cooled, thioglycolate and ammonia were added and a pink color was observed in the presence of iron in the water sample. The concentration of iron was noted with the help of a Photo Colorimeter (Esico Company, model-7312).

MPN (Most Probable Number) Method for Total Coliform Count

The water sample was inoculated into MacConkey broth (HiMedia Laboratories Pvt Ltd). 3 sets of test tubes were used: (1) 50 ml of DSM (Double Strength Media), (2) 10 ml of DSM, and (3) 5 ml of SSM (Single Strength Media). Five test tubes were taken in each set. All the test tubes contained an inverted Durham tube to trap gas production. Then, a 50 ml water sample was added to a 50 ml DSM tube, a 10 ml water sample to each 10 ml DSM tube, and a 1 ml water sample to each 5 ml SSM tube in aseptic conditions. All the tubes were incubated at 37°C for 24 hours.

The tubes were observed for a pink color with turbidity and the formation of gas. The tubes showing these changes were taken as positive and the result was interpreted with the help of an MPN chart similar to the process done by Rubini et al.[16] The tubes with positive results were further cultured for *E. coli* confirmation.

E. coli *Confirmation*

A loopful of inoculum from the MPN-positive tube was taken and cultured on 2 sets of M-Endo agar plates (HiMedia Laboratories Pvt. Ltd.). One set was incubated at 37°C and another set was incubated at 44°C for 24 hours. Colonies with a green metallic sheen were observed othe plate which was incubated at 44°C. The colony characteristics were observed, and additional morphological features were noted after Gram staining. Biochemical test characteristics were determined using the following: Catalase (positive), Oxidase (negative), Oxidative-Fermentative (OF) test, IMViC (Indole positive, Methyl Red positive, Voges-Proskauer negative, Citrate negative), TSI test (A/A, gas production, no H_2S production), SIM test (H_2S negative, Indole positive, motile), and Urease (negative).

Data Analysis

All the data gathered for the microbial, chemical, and physical analyses were entered and organized in the MS Excel program. Further analysis was performed using SPSS, version 26, and Python 3.12. Figure 1 was generated using Arc GIS, Figures 2 and 3 were created using an Excel file, and Figure 4 was created using Python 3.12.

Results and Discussion

Physicochemical Parameters

The temperature, pH, turbidity and TSS of the studied pond water were found in the range of 24.4°C to 25.5°C, 7.36 to 10, 16.26 to 611.46 NTU, and 5.7 to 450 mg/L respectively. The average temperature for Kathmandu, Bhaktapur, and Lalitpur in this season is normally 24°C to 26°C.[17] The World Health Organization[18] recommends pH between 6.5 and 8.5 and turbidity below 5 mg/L for drinking water. Although the WHO does not provide particular numerical values for temperature and TSS, a temperature of water between 12°C and 25°C is considered good for health, and TSS is related to turbidity and should be kept low. So, 70% of the ponds had a pH within the limit of the drinking water quality standard. The pH between 7.5 and 7.8, found for some of the ponds in this study, was close to the pH recorded for the natural ponds in Lebanon, that is, pH 6.5 to 7.6.[19] The higher pH values found in Situnga Pokhari and Siddha Pokhari in this study might be because of ash deposition and ammonia contamination by human activities nearby. People wash dishes near these ponds using ash from burned wood. Another reason for high water pH might be due to the excessive growth of blue-green algae.[20]

Water from all the ponds in this study did not have the required concentration of TSS and turbidity for drinking purposes. Nandal et al[21] noted TSS between 1.33 to 15.33 mg/L (smaller values compared to this study) in pond water in Haryana, India, and Sugiarti and Aisyah[22] recorded TSS between 61 to 685.5 mg/L (larger values than this study) in Banten Bay, Indonesia.

Further, Siddha Pokhari in this study had the highest TSS and turbidity values, making it the most polluted. Several activities such as ongoing construction work, solid waste disposal and domesticated birds might have helped to increase the TSS in the ponds. High TSS and turbidity make water treatment processes like chlorination ineffective. However, all the pond water was suitable for irrigation as the TSS value was below the FAO recommendation that is, $<$450 mg/L.[23] High turbidity and TSS values, along with extreme pH levels, can negatively impact aquatic life and water quality, highlighting the need for regular monitoring and restoration projects to maintain suitable conditions for both ecosystems and human use.

In this study, DO, BOD and COD values of pond water were 8 to 19, 8 to 17.26, and 40 to 1184 mg/L respectively. The U.S. Environmental Protection Agency[24] recommends a minimum of 5 mg/L DO concentration, below 5 mg/L BOD concentration and less than 250 mg/L COD concentration for surface waters. These limits are set to ensure that surface waters can support aquatic life and can be further treated for drinking purposes. All the ponds in this study, except Boje Pokhari, had higher DO concentrations. A study found that the DO of Rupa and Begnas Lake in Pokhara was 5.09 and 6.46 mg/L, respectively.[25] Higher DO values observed in this study might be attributed to the abundant growth of algae in the ponds, which release oxygen during photosynthesis. A study of the Cipager River in Indonesia reported maximum BOD and COD values of 1.17 and 138.6 mg/L, respectively.[26] These values are smaller compared to this study, as running water contains less COD and BOD. As mentioned in the study by Anyanwu and Solomon[27], BOD below 10 mg/L is optimum for aquaculture. Therefore, Boje Pokhari, Pimbahal Pokhari, Bhandarkhal Pokhari Ltp, and Situnga Pokhari were found to be suitable for aquaculture.

Many of these ponds are considered sacred in Hinduism and are believed to have a purifying quality. People perform rituals in these ponds that involve bathing and offering flowers, fruits, and food grains. These activities introduce organic matters. Thus, these organic matters increased the microbial activity and BOD concentration. The decomposition of organic as well as inorganic substances also occurs through the chemical oxidation process as well. Consequently, oxygen is depleted leading to high COD. So, elevated BOD, COD, and low DO levels indicate organic pollution and oxygen depletion, requiring improved wastewater treatment and public awareness campaigns to protect aquatic ecosystems.

The study found that the concentrations of ammonia, nitrite, phosphate, and iron in the pond water were in the range of 0 to 2, 0 to 3, 0 to 3.5, and 0.1 to 3 mg/L, respectively. The WHO[18] recommends an ammonia level below

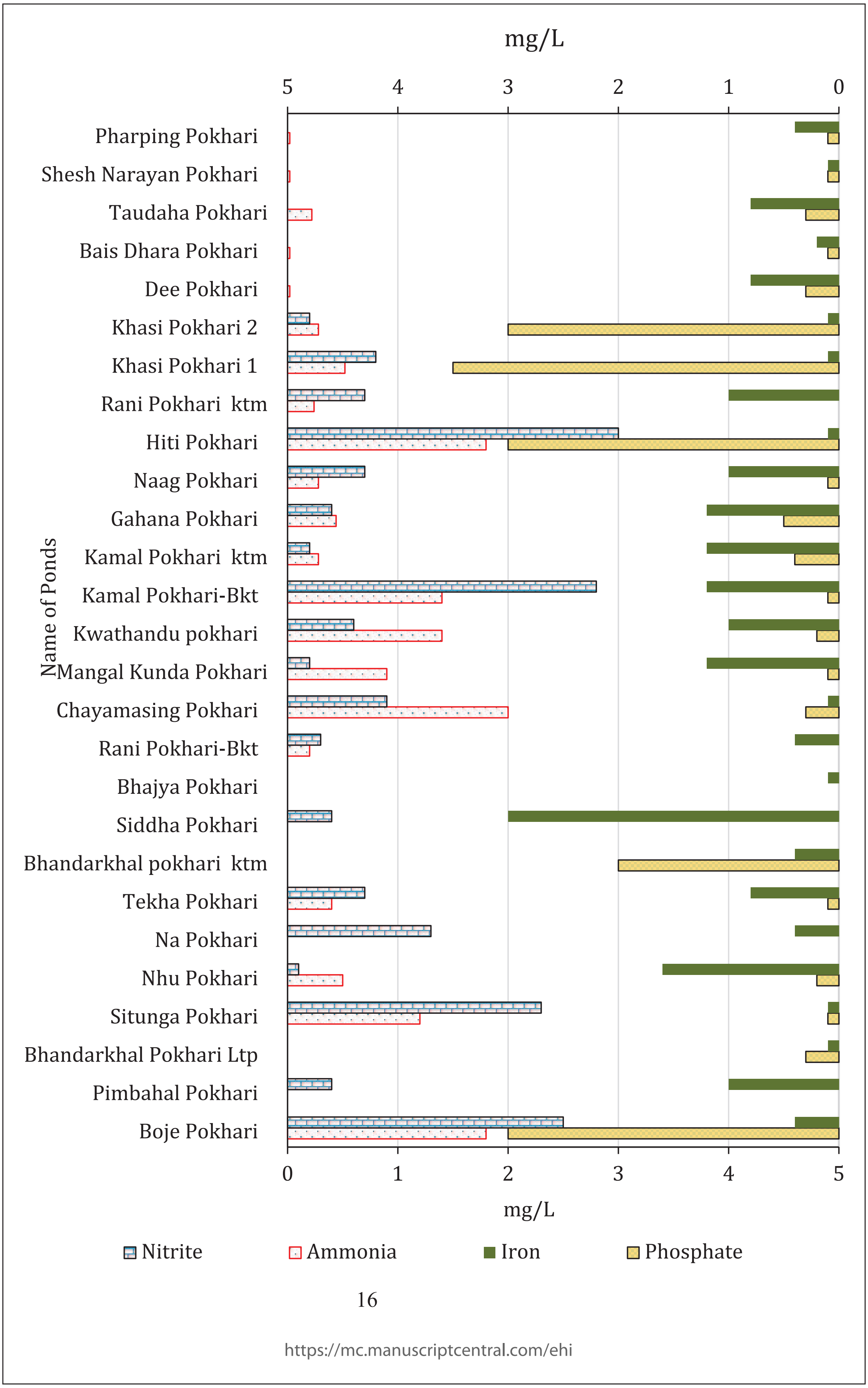

Figure 2. Chemical parameters of pond water.

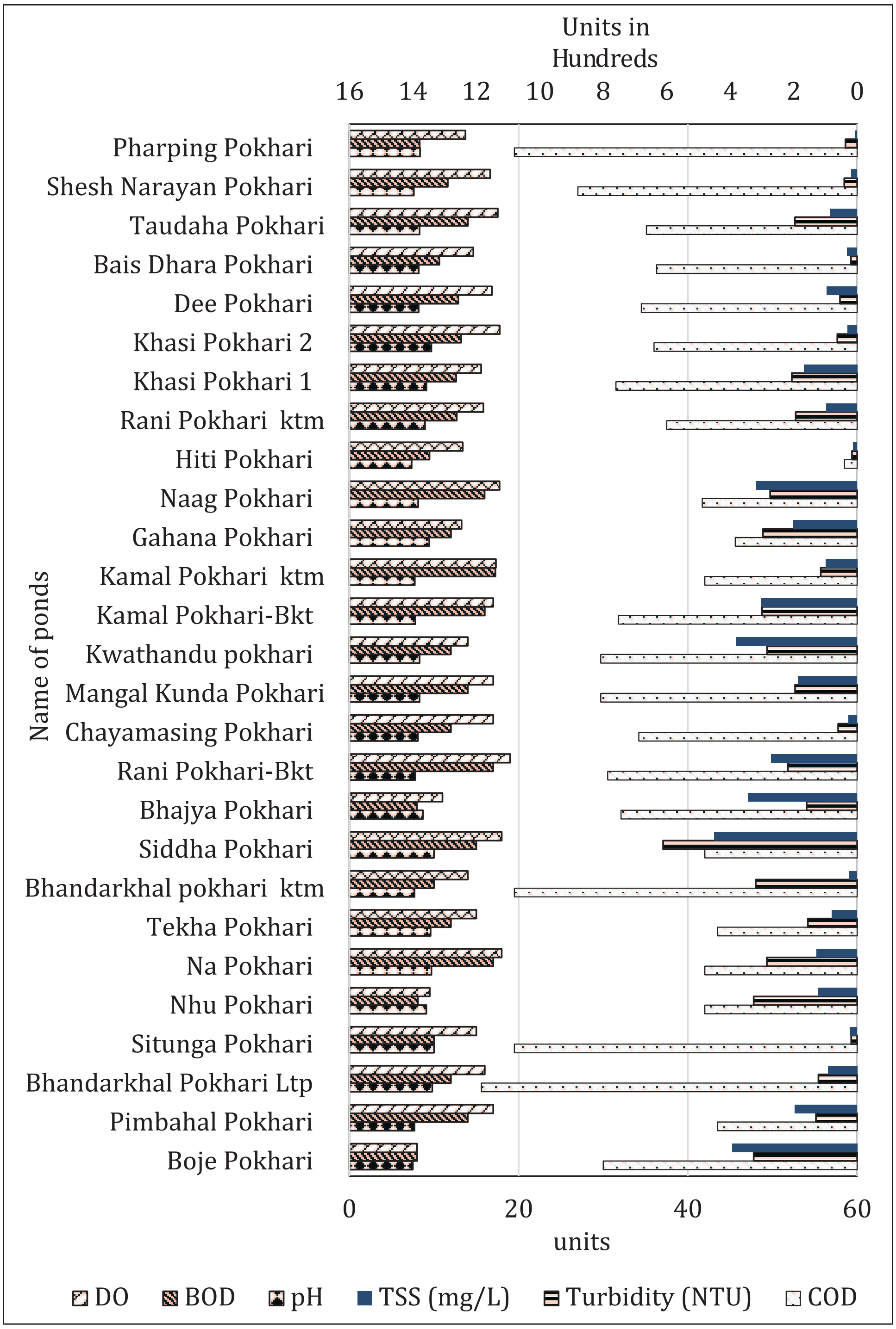

Figure 3. Physicochemical parameters of pond water.

1.5 mg/L (to prevent odor issues), nitrite below 0.2 mg/L, and iron below 0.3 mg/L for drinking water. Phosphate levels are not explicitly mentioned by the WHO, but a 0.1 mg/L limit is suggested to manage eutrophication. Almost all the ponds had a nitrite concentration within the limit of the drinking water standard, whereas only 33% of the ponds had an iron concentration within the standard value. It was found that 44% of ponds exceeded the phosphate concentration limit to manage eutrophication and 91% ponds had an ammonia concentration within the limit to prevent odor issues in drinking water.

The ammonia concentration in Taudaha Pokhari and Shesh Narayan Pokhari was the same as the findings for the Erlong Lake in China, that is, 1.8 mg/L.[28] In a study conducted in Kazakhstan on water quality contamination by heavy metals in rivers, the concentration of iron was found to be 0.8 mg/L in the Nura River.[29] The finding was similar to the iron concentrations in Kamal Pokhari-Bkt, Kamal Pokhari-Ktm, and Gahana Pokhari. In a study on drinking water quality assessment conducted in Punjab, Pakistan, the nitrite concentration was found to be 0.006 mg/L.[30] The finding was smaller value than the results of this study.

A study found that the phosphate concentration was 2.144 ± 0.513 mg/L in the dry season at Nike Lake located in Southeast, Nigeria.[31] The results were similar to this study. Cattle freely roaming around the ponds, excreting feces and urine, along with people feeding fish as recreational activity, pose the risk of pond water contamination by chemicals such as nitrite, ammonia, phosphorus, and pathogens from fecal matter. Runoff from surrounding soil, and natural geological deposits in the ponds are the reasons for the high concentration of iron in the water. Elevated levels of ammonia in drinking water may cause respiratory issues, while high nitrite concentration can lead to methemoglobinemia, also known as blue baby syndrome.[32] High phosphate levels can lead to eutrophication in water bodies, which is not directly linked to any human diseases.[33] A high concentration of iron in drinking water may cause gastrointestinal diseases like diarrhea, stomach discomfort, vomiting, and nausea.[34] Individuals with hemochromatosis are at risk of iron overload leading to organ damage. So, elevated concentration of ammonia, phosphate, nitrite, and iron require regulations on agricultural runoff and sustainable farming practices to minimize nutrient pollution.

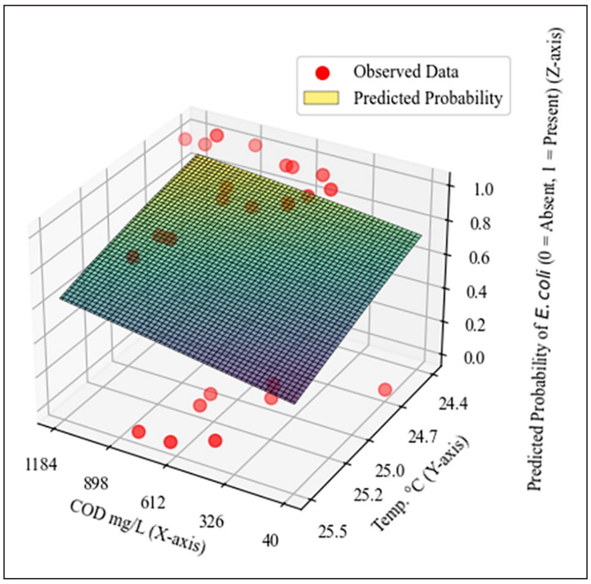

Figure 4. 3D surface plot of predicted *E. coli* probability based on COD and temp. The surface represents predicted probabilities from logistic regression, and points indicate observed data.

Microbiological Parameters

The total coliform count in all pond water samples exceeded 180 MPN per 100 ml. Furthermore, *E. coli* was detected in 67% of ponds indicating fecal contamination (Table 1). The European Union[35] mandates that water samples contain 0 coliform per 100 ml, and *E. coli* should be absent in 100 ml of water for drinking water. So, the pond water was. not suitable for drinking purpose. Fecal contamination indicated by the presence of *E. coli* poses a clear threat for several fatal, water borne diseases. In a study carried out in Wegeda Town, Northwest Ethiopia for drinking water quality assessment, the total coliform count was found in the range of 5 to 27 MPN/100 ml.[36] Although these values are lower compared to this study, the water was not suitable for consumption.

Manandhar and Luthi[37] found that 64% of ponds in Lalitpur were contaminated with fecal coliform, the results were similar to this study. The high coliform count and presence of *E. coli* in the studied pond water might be due to contamination by human waste and sewage leakage, which were observed during the study. Coliform contamination is common in open water sources, especially in stagnant water. Water temperature and turbidity also favor coliform growth. Contamination of drinking water by coliform and pathogenic organisms like *E. coli* may lead to gastrointestinal and urinary tract infections that include severe diarrhea, hemolytic uremic syndrome, and kidney failure. Furthermore, as the bacterium is an indicator of fecal pollution, water from these ponds may contain pathogens that

Table 1. E. coli detection in different pond water. SN—serial number corresponds to the map in Figure 1.

S. No.	Pond's name	Total coliforms per 100 ml	*E. coli*	S. No.	Pond's name	Total coliforms per 100 ml	*E. coli*
1	Boje Pokhari	>180 MPN	Present	15	Kamal Pokhari Bkt	>180 MPN	Present
2	Pimbahal Pokhari		Present	16	Kamal Pokhari Ktm		Present
3	Bhandarkhal Pokhari Ltp		Absent	17	Gahana Pokhari		Present
4	Situnga Pokhari		Absent	18	Naag Pokhari		Present
5	Nhu Pokhari		Present	19	Hiti Pokhari		Present
6	Na Pokhari		Present	20	Rani Pokhari Ktm		Present
7	Tekha Pokhari		Absent	21	Khasi Pokhari 1		Present
8	Bhandarkhal Pokhari Ktm		Absent	22	Khasi Pokhari 2		Present
9	Siddha Pokhari		Present	23	Dee pokhari		Present
10	Bhajya Pokhari		Absent	24	Bais Dhara Pokhari		Present
11	Rani Pokhari Bkt		Absent	25	Taudaha Pokhari		Present
12	Chayamasing Pokhari		Present	26	Shesh Narayan Pokhari		Absent
13	Mangal Kunda Pokhari		Absent	27	Pharping Pokhari		Absent
14	Kwathandu Pokhari		Present				

cause deadly diseases like cholera, dysentery, typhoid etc. The findings of this study necessitate community education on the risks of untreated water and improved sanitation infrastructure to prevent contamination.

Physicochemical Parameter Influence on E. coli *Contamination*

From the logistic regression analysis, the study found that turbidity, TSS, pH, nitrite, ammonia, phosphate, DO, and BOD were not associated with the presence of *E. coli*, whereas COD and temperature were noted to be associated significantly with *P*-values 0.001 and 0.023, respectively, and corresponding 95% confidence intervals. Iron, with a *P*-value of 0.081 and 95% confidence intervals was close to the significant threshold. To better understand the association of COD and temperature with the *E. coli* through visualization, a 3D scatter plot with a logistic curve was created (Figure 4). The plot clearly indicated that as COD and temperature increase, the probability of *E. coli* presence also increases, visually captured by the transition from blue (low probability) to yellow (high probability) color on the grid surface. Furthermore, the impact of COD is more than that of temperature on *E. coli* presence. Higher COD means increased organic pollution. The organic compounds provide a favorable environment for *E. coli* by supplying carbon sources necessary for bacterial metabolism. Additionally, high COD levels can lead to oxygen depletion, favoring *E. coli* growth.

The study of well waters from a rural area of southern Changchun City, China noted that higher levels of ammonia may favor an extended survival of *E. coli* O157:H7, and increased pH may decrease the survival time of the bacterium.[38] In this study these parameters did not show any impact on *E. coli* presence. The narrow pH range was not enough to cover extreme cases, so the results should be interpreted cautiously. Although few parameters in this study showed an association with *E. coli* contamination, physicochemical parameters do impact *E. coli* growth. It was due to the interaction between multiple parameters that a significant impact of an individual parameter was somewhat diluted and not observed.

Public Health and Environmental Implications

The study's findings—that all ponds in Kathmandu Valley were contaminated with coliforms, and in 67% *E. coli* was present—clearly shows there is an active threat to public health in the community of the valley. Such pathogenic indicators present in the water sources pose a serious health risk and can cause diarrhea, cholera, and other water-borne diseases due to direct and indirect transmission.[39] Globally, waterborne diseases caused by *E. coli*, such as diarrhea, cholera, and dysentery, are major contributors to the burden of disease, particularly in low- and middle-income countries (LMICs).

This study reveals that all ponds exceed WHO drinking water limits for TSS and turbidity, while 96% of ponds surpass USEPA standards for BOD and COD levels required to support aquatic life. These elevated TSS, BOD, and COD levels indicate severe organic pollution, which threatens aquatic life by depleting dissolved oxygen.[40] The strong association between temperature and COD levels with *E. coli* contamination in this study highlights a high risk of infection outbreaks during the rainy season, when warmer temperatures prevail. Increased COD levels, largely due to urban runoff and sewage leakage, further amplify the contamination risk. The observed high temperatures, which promote *E. coli* survival and growth, align with global climate change trends. Kathmandu's water contamination reflects a broader global challenge.

The likelihood of exposure to *E. coli* is elevated due to the widespread contamination of pond water used for several purposes. Given the local sanitation infrastructure challenges, the severity of the health impact could be significant, particularly for vulnerable populations like children and the elderly. This study underscores the urgent need for public health intervention and regular assessment of pond water quality. It informs policies to regulate pollution from agricultural and urban runoff, helping to prevent *E. coli* outbreaks and other waterborne diseases. Implementing community-level education on safe water practices and reinforcing infrastructure to reduce contamination risks would support both public health and environmental sustainability in the valley.

Conclusion

The study found that all ponds exceeded concentrations of TSS and turbidity recommended by WHO for drinking water, while 30% surpassed the pH levels for potable water. Additionally, 67% of ponds had iron concentration beyond the recommended limit by WHO for drinking water, and 96% had BOD and COD levels above the USEPA limits for supporting aquatic life. All the ponds were found to be contaminated with coliforms, and 67% tested positive for *E. coli*, making the water unfit for drinking purposes as per EU directives. A significant association of COD and temperature (*P*-values 0.001 and 0.023 respectively) was observed with *E. coli* presence, COD exerting a stronger influence. A 3D visualization of the data further supports the association and illustrates these relationships. This suggests that organic pollution, exacerbated by warmer temperatures, provides favorable conditions for *E. coli* growth. Iron was close to the significant threshold indicating its potential role in microbial contamination and overall water quality. Coliform and fecal coliform (*E. coli*) contamination of the ponds clearly indicated that the consumption of water from these ponds may cause several life-threatening water-borne diseases. Polluted pond water is clearly a threat to the survival of aquatic life. These findings underscore the urgent need for targeted interventions to mitigate contamination risks. Water treatment strategies should prioritize reducing COD to control organic pollution and limit microbial growth. Regulatory enforcement must focus on managing industrial, agricultural, and urban runoff. Public awareness campaigns should educate communities on the risks of using contaminated pond water for drinking, irrigation, and recreation, emphasizing the link between pollution sources and microbial contamination. To strengthen these findings, future research should incorporate larger sample sizes, seasonal variations, and additional environmental parameters for a more comprehensive water quality assessment. The use of geospatial tools for hotspot mapping is recommended to identify high-risk areas and support data-driven policymaking for sustainable water management.

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Statements and Declarations

Author Contributions

Concept development, data analysis, manuscript drafting, and critical revision: NP; Sample collection, data generation, data interpretation, and manuscript review: PN; Sample collection, data generation, data interpretation, and manuscript review: AK; Sample collection, data generation, data interpretation, and manuscript review: RT; Supervision, critical manuscript review, and substantial intellectual contributions: SKP.

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Data Availability Statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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