Physicochemical and Microbiological Quality of Holy Waters in the Kathmandu Valley

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Abstract

Holy water sources are regarded by many people as pure and sacred. People use these waters for various devotional activities, like bathing, sprinkling, drinking and others. As people come in direct contact with these waters, such waters should be of good quality in order to protect the devotee's health. As these sources are open or are flowing, they are vulnerable to contamination from environmental pollutants and human and animal activities. This study was carried out to evaluate the physicochemical and microbiological quality of holy waters used in Kathmandu Valley. The samples were collected aseptically in sterile and clean glass bottles and were analyzed using the standard procedures outlined in Standard Methods for the Examination of Water and Wastewater (2017) published by the American Public Health Association. All the physicochemical parameters tested were within the limits (pH 6.56, TDS 187.25 mg/l, conductivity 259.88 µS/cm, chloride 23.4 mg/l, hardness 33.25 mg/l, iron 0.03 mg/l, ammonia 0.16 mg/l) of the requirements of National Drinking Water Quality Standards, 2005 except for nitrate (31.75 mg/l). In contrast, microbiological analysis showed high coliform counts (5.114 $\log_{10}$ CFU/ml). Moreover, the presence of multidrug resistant *E. coli* in 50% of the samples suggests high risk for the devotees from various water borne diseases. Overall, the findings indicate that although these holy water sources hold great religious and cultural significance, their sanitary condition requires attention. Proper sanitation, regular monitoring, and improved hygiene practices are necessary to ensure the safety of people using these waters.

Keywords: holy water, religious water, *E. coli,* water quality, MDR

Introduction

Kathmandu, the capital city of Nepal, is well known for its rich religious and cultural heritage. The city is home to a remarkable number of Hindu temples, Buddhist shrines, monasteries, stupas, and other sacred monuments, many of which continue to serve as active places of worship. This unique coexistence of historical monuments and living religious traditions makes Kathmandu an important religious and cultural landscape (UNESCO World Heritage Centre, 1979; Nepal Tourism Board, 2025). People here are religious from the distant past. Religious people believe some natural water bodies as sacred that hold spiritual, ritual, and cultural significance. People also make ponds, spouts and lakes which is considered of similar values.

People use waters from these sources for religious offerings and purification of self from sins and impurities. The Bagmati River is the holiest river in Nepal and is central to Hindu cremation rituals at Pashupatinath (Nyaupane, 2020). Manga Hiti, Godavari kunda, Mata tirtha Kunda are a few of many religiously significant water sources (Tiwari, 2001).

Waters from these sources are used for sprinkling, bathing, swimming and even drinking, on all days or during certain occasions of the year.

Records exist where religious waters have been the source of infection. Holy water was first identified as a potential source of pathogenic microorganisms in 1992, when *Pseudomonas aeruginosa* was isolated from infected wounds of a hospital patient in Germany following exposure to holy water (Greaves & Porter, 1992). Investigations in Buddhist temples in Thailand revealed microbiological contamination of holy water, including the presence of *Escherichia coli* (Phatthararangrong et al., 1998). The holy water fonts from the churches of Seville, Spain had a strong bacterial contamination along with coliforms and other pathogens (Jurado, 2002). The study of springs of Austria reported the occurrence of *Pseudomonas aeruginosa* in holy water and also identified *Campylobacter* contamination (Kirschner et al., 2012). Studies from Slovenia reported high heterotrophic bacterial counts and the presence of opportunistic pathogens such as *Pseudomonas aeruginosa*, *Acinetobacter* spp., and enteric bacteria in holy water samples (Turk et al., 2022).

Subsequent reports linked nosocomial infections caused by *Acinetobacter baumannii* to contact with holy water (Michel et al., 2013). A landmark study by Kirschner et al. (2012) identified significant contamination in holy water fonts and springs in Vienna, Austria, with microbial loads reaching up to 10^7 colony-forming units (CFU) per ml, along with the presence of fecal indicator bacteria such as enterococci and *E. coli*.

In Bagmati river alone 40% of sampling sites contained *E. coli* (Ghimire et al., 2024). Multiple stone spouts in the valley are contaminated with *E. coli*. Coliforms were present in all ponds, with *E. coli* detected in 67% of 35 ponds in the valley (Pokharel et al., 2025).

The physicochemical characteristic of water strongly influences microbial growth, persistence, and transmission. Parameters such as pH, temperature, turbidity, total dissolved solids (TDS), electrical conductivity, dissolved oxygen (DO), nitrate, chloride, and biological oxygen demand (BOD) are commonly used to assess water quality and pollution status. Variations in these parameters may indicate organic contamination, sewage intrusion, eutrophication, and environmental degradation, all of which can promote the survival of pathogenic microorganisms.

There are many publications regarding contaminations of rivers, ponds, spouts etc. but not in relation to religious nature. Furthermore, routine monitoring and risk assessment of these sacred water sources remain inadequate despite their frequent human exposure. Therefore, the present study aims to evaluate the physicochemical and microbiological quality of holy waters of Kathmandu Valley and to assess their potential public health implications. The findings of this study may contribute to improved awareness regarding the hygienic status of sacred water sources and support the development of appropriate monitoring and management strategies for the protection of public health.

Methods and methodology

This descriptive study on water quality is based on the samples collected from different religious water sources in the Kathmandu Valley which includes rivers such as Bagmati at Pashupati (PP), and Gokarna (GK); stone spout Alkohiti (AH); and ponds from Banglamukhi (BM), Baisdhara (BD), Sapantirtha (ST), Kageshwori (KK), Matatirtha (MT), Indradaha (ID), and Machhegaun (MG). Samples from Pashupati and Baishdhara were collected in July, Banglamukhi, Sapantirtha, and Kageshwori were collected in August, Matatirtha, and Indradaha in September, Machhegaun and Alkohiti in November, 2024 and Gokarna in January 2025. Samples were collected aseptically in sterile and clean bottles and were transported in icebox within an hour to Department of Microbiology, St. Xavier's College, Maitighar for further analysis.

Physicochemical Analysis

The physicochemical parameters of the water samples, including pH, temperature, total dissolved solids (TDS), electrical conductivity (EC), dissolved oxygen (DO), nitrate, chloride, total hardness, iron, ammonia and biological oxygen demand (BOD), were analyzed following the standard methods described by the American Public Health Association.

Temperature and pH were measured on-site using a thermometer and pH meter. Rest of the tests were done in the laboratory. TDS and electrical conductivity were measured using respective calibrated meters. Dissolved oxygen was measured by the Winkler azide modification method. Chloride concentration was determined by argentometric titration, while nitrate and ammonia concentration were estimated using rapid test kit via colorimetric method. Total hardness was measured using the EDTA titrimetric method with Eriochrome Black T indicator. Iron concentration was determined by spectrophotometric method. Biological oxygen demand (BOD) was analyzed by incubating samples at 20 °C for five days followed by determination of dissolved oxygen depletion.

Microbiological analysis

Enumeration of bacteria was performed using the serial dilution and spread plate technique. Water samples were serially diluted to 10^{-4} dilution in sterile water, and dilutions 10^{-3} and 10^{-4} were plated onto Eosin Methylene Blue agar plates. The plates were incubated at 37 °C for 24 hours, and colonies showing characteristic morphology (metallic green sheen) were counted and expressed as colony-forming units per milliliter (CFU/ml). The subcultures were identified by biochemical analysis.

All physicochemical and microbiological analyses were performed according to standard procedures outlined in *Standard Methods for the Examination of Water and Wastewater* (2017) published by the American Public Health Association.

Data Analysis

The observed data were entered in EXCEL 15 and data were analysed using SPSS 28.

Quality Control

All the chemicals used were checked for expiry dates before use. Only those chemicals within the expiry dates were used. Instruments were calibrated using respective chemicals. Incubator and refrigerator were tested for their respective temperature using external thermometer. Purity of the media plates were tested incubating 10% of media at 37 ºC for 24 hours. Sterile plates were used for the study.

Results

Physicochemical and microbiological parameters of holy water samples

The physicochemical parameters like pH, TDS, conductivity, chloride, hardness, iron and ammonia meets the requirements of National Drinking Water Quality Standards, 2005. Nitrate was beyond the limit in BM, AH and MT. Coliform count far exceeds the recommend limits for recreational water use.

Table 1: Physicochemical and microbiological parameters of holy water samples

Parameters	Range	Mean ± S.D.
pH	6.5 -7	6.56 ± 0.32
Temperature (ºC)	17 - 22	18.75 ± 1.67
TDS (mg/l)	84 - 360	187.25 ± 89.7
Conductivity (µS/cm)	116 - 402	259.88 ± 110.6
DO (mg/l)	2.5 - 8	6.06 ± 1.93
BOD (mg/l)	1 - 6	2.5 ± 1.6
Chloride (mg/l)	12.8 – 49.6	23.4 ± 15.79
Hardness (mg/l)	14 -60	33.25 ± 17.56
Nitrate (mg/l)	2 - 100	31.75 ± 39.81
Iron (mg/l)	0.01 – 0.05	0.03 ± 0.02
Ammonia (mg/l)	0.05 – 0.34	0.16 ± 0.11
Coliforms ($\log_{10}$ CFU/ml)	4.903 – 5.279	5.114 ± 4.591

Correlational analysis of different parameters of holy water samples

Pearson correlation analysis revealed several significant relationships among physicochemical and microbiological parameters. Temperature was strongly positively correlated with water hardness (0.81). Electrical conductivity showed a strong positive correlation with chloride (0.75) and nitrate (0.81). BOD was correlated well with ammonia (0.8). A strong positive correlation between nitrate and chloride (0.89) was also observed. Dissolved oxygen exhibited a strong negative correlation with biological oxygen demand (-0.72).

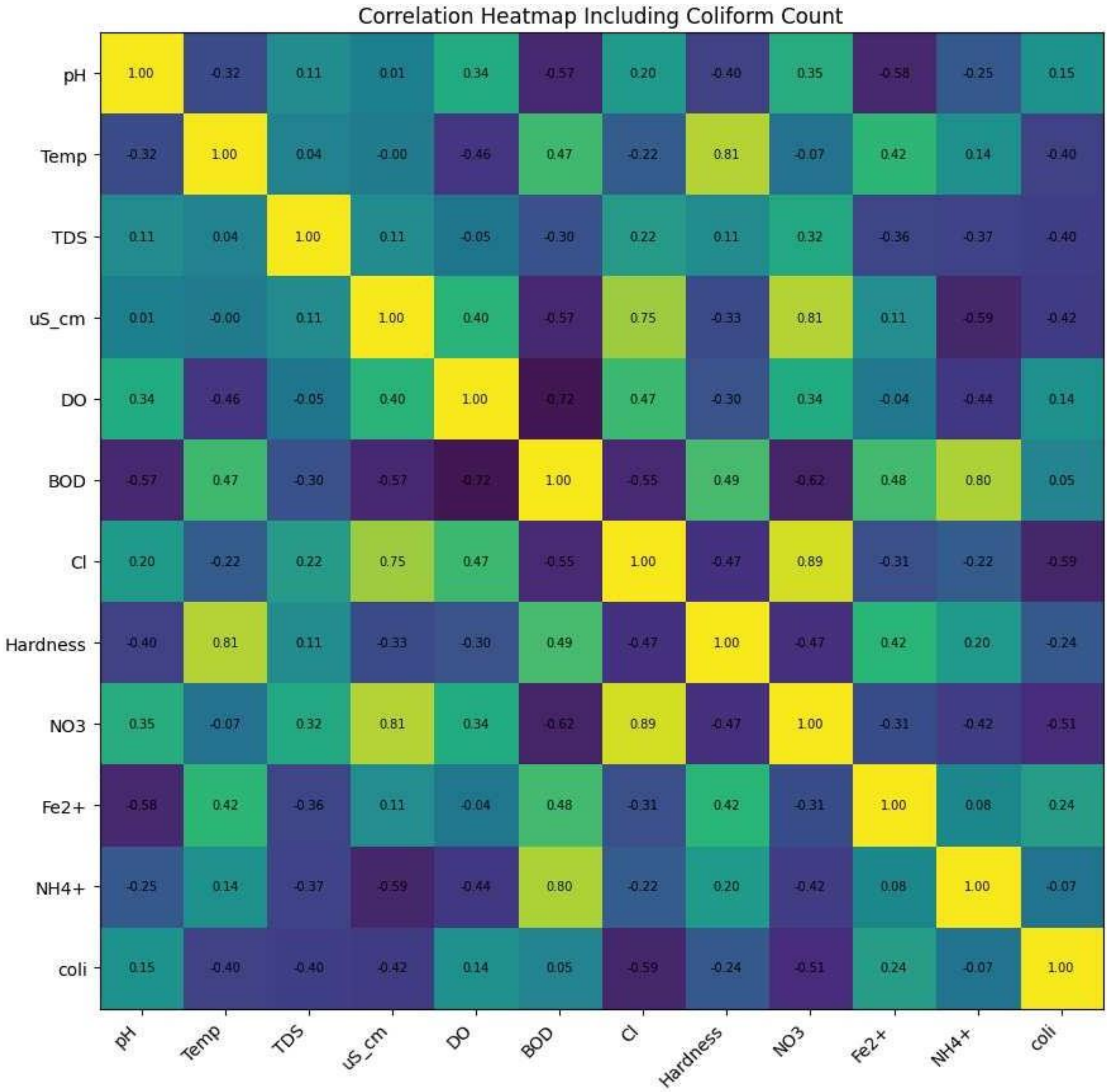

Figure 1: Correlational heatmap of physicochemical parameters and coliforms

Bacterial isolates from holy water samples

All samples showed green metallic sheen on EMB media, suggesting the presence of coliforms and through further biochemical analysis they were confirmed as *E. coli*. *S*ample from MT also showed growth of *Pseudomonas* spp.

Antibiotic susceptibility test of the *E. coli* isolates

The antibiotics susceptibility test of the isolated *E.coli* showed that all the isolates were resistant to at least two of the tested antibiotics. 100% of the isolates were resistant to Cefotaxime. Five isolates showed multidrug resistance pattern which were from PP, KK, GK, MT and AH.

Table 2: Antibiotic susceptibility test of the *E. coli* isolates

Antibiotics	Sensitive	Resistant
Amoxicillin	5 (50%)	5 (50%)
Cefoxitin	6 (60%)	4 (40%)
Cefotaxime	0	10 (100%)
Azithromycin	4 (40%)	6 (60%)
Meropenem	9 (90%)	1 (10%)
Amikacin	6 (60%)	4 (40%)
Cotrimoxazole	7 (70%)	3 (30%)

Discussion

The physicochemical parameters of the holy water samples were within the limits of National Drinking Water Quality Standards, 2005 except for nitrate. Three of the sampled waters exceeded the limits for nitrate (90 mg/l of AH, 50 mg/l of MT and 100 mg/l of BM). The increased level may be attributed to anthropogenic activity. The festive occasion of Janai Purnima when Lord Shiva is placed in the pond when devotees swim and worship which might have some relation with this increase in BM water. But in others samples as other parameters were within limits, sewage contamination may not only be a cause rather, fertilizer use, natural decomposition of organic waste near the source may also have added to greater values.

The mean temperature at the time of sample collection was 18.75 ±1.66 ⁰C which is within the limits of the water temperature of the Bagmati River in the Kathmandu Valley which ranges from 15–25 °C (Department of Environment; Shrestha & Kazama, 2007; Bagmati River Basin Improvement Project, 2018). Since all the sampled waters were surface water, the values were comparable.

The mean DO content is comparable to the surface water with the range 1-8 ((Department of Environment, Nepal). The BOD of the samples was 2.5±1.6 mg/l which indicates the clean water suitable for recreation generally correspond to low BOD (<3–5 mg/L) (US EPA, 2012).

Electrical conductivity showed a strong positive correlation with chloride and nitrate, indicating the influence of their ionic contamination. Temperature was strongly positively correlated with water hardness, suggesting temperature-dependent mineral solubility effects of calcium and magnesium salts. A strong positive correlation between nitrate and chloride further indicates shared anthropogenic sources, likely from domestic or agricultural inputs (APHA, 2017). A strong positive correlation between biological oxygen demand and ammonia might be due to consumption of oxygen during oxidation of ammonia.

Chloride exhibits a strong positive relationship with total dissolved solids (TDS) because it constitutes a major fraction of dissolved ionic content in water and commonly originates from the same anthropogenic sources such as sewage and industrial discharge (APHA, 2017). But in this research no such relation was found indicating presence of other ionic contributors or indirect/less contact with human wastes.

Dissolved oxygen exhibited a strong negative correlation with biological oxygen demand, reflecting increased organic pollution. Microbial oxygen consumption during decomposition of organic matter decreases dissolved oxygen (Metcalf & Eddy, 2014; APHA, 2017).

Coliform counts showed weak to moderate negative associations with chloride, nitrate and electrical conductivity, indicating localized fecal contamination. Though it was visible in case of Bagmati river water of sewage contamination upstream of sampled site, its physicochemical parameters were within limit and therefore, it was included in the correlational analysis. However, its coliform counts were the highest. The coliforms were identified as *E. coli* from all of the samples. The isolated *Pseudomonas* can be attributed to the surroundings vegetation in case of MT.

Coliform counts were too high in all samples. Exceeding WHO guidelines values of ≤ 500 *E. coli* per 100 mL increases higher probability of gastrointestinal illness (WHO, 2003; WHO, 2009). These sources of water are around the highly populated areas and are open, where anthropogenic activities directly or indirectly affect its quality, physicochemically or microbiologically, making it unsuitable even for recreational purpose. The act of sprinkling after washing hands, bathing and swimming in these waters pose a threat of allergies and several enteric diseases. These waters need treatments and the holy sites needed conservation so as to limit infections associated with the holy water and sites.

Antibiotic susceptibility test showed different patterns of resistance among *E. coli* isolated from different sources. However, no isolates were found susceptible to all of the tested antibiotics. All the isolates were resistant to either of the two antibiotics tested. Multidrug resistant isolates were recovered from 50% of the sites. Cefotaxime was ineffective with 100% resistance. This rate of multidrug resistance was less in *E. coli* compared to Purohit et al. (2020), Chaturvedi et al. (2020), Ghimire et al. (2023) but high compared to Mohamed et al. (2014). However, the presence of resistance bacteria can be attributed to direct human and animal activity at a place while indirect access can be attributed to shallow ground water prone to fecal contamination from sewage and urban runoff.

So far, no investigation has been documented addressing religious waters in Kathmandu Valley. This study also limits only 10 sites and studies only *E. coli* for their phenotypic resistivity studies. Larger number of samples and molecular studies involving specific pathogenic bacteria can be included in further studies.

Conclusion

Most of the physicochemical parameters of religious water bodies in Kathmandu Valley were within limits of recreational water use. However, microbiological analysis reveals contamination beyond recommended limits in all samples indicating poor quality of water. The detection of coliforms in these waters suggests harmful nature of these waters. The presence of multidrug resistant *E. coli* in itself is a great threat to use holy waters for any religious or domestic purpose. More so, it warns of possible presence of pathogenic bacteria of faecal origin as well. These findings highlight the need for better sanitation, regular cleaning and monitoring programs, and stronger efforts toward the protection and conservation of sacred water sources. At the current time holy waters should be used with precaution to minimize the risk of water borne diseases.

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Conflict of interest

The authors express no conflict of interest towards research and publication.

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