

Microbiological Analysis and Biochemical Characterization of Bacterial Isolates from Water Samples

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ABSTRACT

This study provides a comparative analysis of water quality across three freshwater ecosystems in Kollam District, Kerala, Ashtamudi Lake, Ayathil Lake, and Vattakkayal. The primary objective was to evaluate the microbial quality of water and identify key biochemical traits of bacterial isolates from these selected sites. Comprehensive physicochemical assessments were conducted, measuring turbidity, pH, total alkalinity, electrical conductivity, total dissolved solids, total hardness, and concentrations of calcium, magnesium, chloride, sulphate, fluoride, iron, and nitrate. Notable spatial variations were observed, with Ashtamudi Lake exhibiting elevated turbidity, likely due to sedimentation, anthropogenic discharges, and organic matter accumulation. Vattakkayal, by contrast, displayed relatively low turbidity and clearer water.

All three lakes showed alkaline pH values, influenced by buffering capacity and natural decomposition processes. The conductivity and hardness values indicated varying ionic composition and mineral content, reflective of both natural inputs and human-induced pressures. Microbiological analysis revealed the presence of general heterotrophic bacteria on nutrient agar, with no growth observed on selective media for coliforms or pathogenic strains, suggesting an absence of faecal contamination. Biochemical profiling identified the isolates as Gram-negative rods exhibiting catalase and oxidase activity, indicative of their ecological role in organic matter degradation and nutrient cycling. These findings underscore the importance of continuous monitoring and sustainable management of freshwater resources to preserve ecological integrity and public health.

Key Words: Water quality assessment, Physicochemical parameters, Anthropogenic impact, Ecological monitoring, Biochemical characterization, Heterotrophic bacteria.

INTRODUCTION

Water is an essential resource for sustaining life, yet less than 1% of the Earth's freshwater is directly accessible for human consumption and use (WWAP, 2024). Surface water bodies, such as lakes, rivers, and canals are indispensable for domestic, agricultural, and industrial activities. However, they are increasingly exposed to pollution stemming from unregulated urbanization, industrial effluents, sewage discharge, and intensive agricultural practices (NITI Aayog, 2023; FAO, 2021).

In Kerala, renowned for its dense hydrological network comprising 44 rivers and expansive backwaters, the average annual rainfall ranges between 2,800 and 3,200 mm. Despite this abundance, water quality has shown a declining trend due to contamination from poorly managed septic systems, leach pits, agricultural runoff, and unregulated industrial discharge (CGWB, 2023). The state's high density of open wells, many of which lack protective linings, renders them highly vulnerable to microbial contamination, particularly during the monsoon season when surface runoff and rising water tables facilitate the transport of pathogens into aquifers (Kumar et al., 2022).

In districts like Kollam, surface water sources such as Ashtamudi Lake, Ayathil Lake, and Vattakkayal are increasingly under ecological stress. Microbial contamination is of particular concern due to the discharge of untreated domestic

sewage and the influx of organic matter. Pathogenic microorganisms, including coliform bacteria, *Vibrio* spp., and *Pseudomonas* spp.-pose significant threats to both ecological integrity and public health (Sherlyn et al., 2014).

Microbiological water quality is typically assessed through the estimation of heterotrophic bacterial counts and the detection of coliform and faecal indicator organisms using selective media. The presence and density of microbial colonies provide insight into the hygienic status of water bodies and help identify potential pollution sources. Additionally, biochemical characterization, such as Gram staining, oxidase, and catalase tests, can aid in the identification of environmental bacterial isolates and their ecological roles. Hence, microbial water analysis remains a crucial tool in evaluating ecosystem health, identifying pollution pathways, and developing evidence-based water resource management strategies. Addressing water pollution requires integrated monitoring, policy support, and public awareness to protect ecosystem services and ensure clean water for future generations.

Freshwater ecosystems are vital for public health, biodiversity, and livelihoods, yet they are increasingly threatened by urbanization, agriculture, and industrial activities. This study investigates the physicochemical and microbiological characteristics of selected freshwater bodies in Kollam District, Kerala, to assess water quality and ecological status. Lakes are sensitive to land use changes and pollution, which can disrupt their chemical and biological balance. Globally, water pollution causes over 14,000 deaths daily, including around 580 in India, mainly due to waterborne diseases and poor sanitation (Mishra et al., 2021; WHO, 2022; UNICEF, 2023). This study provides a baseline assessment of lake water quality, facilitating evidence-based approaches for lake restoration, sustainable water resource management, and the protection of aquatic biodiversity in rapidly urbanizing landscapes. Microbiological indicators such as total and faecal coliforms, particularly *Escherichia coli*, reflect faecal contamination and potential health risks. Biochemical profiling of isolates further reveals their ecological roles and resistance traits. This integrated assessment supports sustainable water management, public health protection, and progress toward Sustainable Development Goals on clean water, health, and ecosystem conservation.

MATERIALS AND METHODS

Assessing water quality is essential for understanding the overall health of aquatic ecosystems, identifying pollution sources, and guiding sustainable management practices. Core indicators like dissolved oxygen, nutrients such as nitrates and phosphates, and pollutants including heavy metals and organic compounds offer valuable insights into how ecosystems function and how human activities may be impacting them. By studying these parameters over time and across locations, we can detect early warning signs of environmental stress and trace both direct and indirect pollution.

Alongside chemical and physical assessments, microbiological analysis provides a crucial layer of understanding, especially when it comes to human health and environmental safety. Testing for bacteria like *Escherichia coli* and other coliforms helps reveal whether water has been contaminated by sewage or other faecal matter. These microbes act as warning signals, alerting us to the possible presence of harmful pathogens. Traditional culture methods, as well as advanced techniques like molecular diagnostics, allow for a clearer picture of microbial diversity and contamination levels. Together, these assessments inform practical actions from pollution control to conservation policies, while helping to protect biodiversity and ensure safe, clean water for communities and ecosystems alike.

Study Area

Kollam district in Kerala, India, is characterized by a dense network of water bodies, including rivers, lakes, canals, and backwaters. However, rapid urbanization, industrialization, and unsustainable land use practices have significantly compromised the quality of these aquatic systems. Notable water bodies such as Ashtamudi Lake, Ayathil Lake, and

Vattakkayal are experiencing increasing pollution from untreated domestic sewage, industrial effluents, agricultural runoff, and solid waste accumulation. These pollutants have led to eutrophication, oxygen depletion, and the loss of aquatic biodiversity, posing risks to public health and ecosystem services. The degradation of these water bodies underscores the urgent need for integrated watershed management, community-based conservation initiatives, and strict enforcement of environmental regulations. Addressing these challenges is vital for restoring ecological balance, preserving biodiversity, and ensuring the sustainable use of water resources in the region.



Figure 1: Selected study sites

Sampling Sites

Kollam, a coastal city in Kerala, India, faces serious water pollution challenges across its key aquatic ecosystems. Major sources include industrial effluents, urban runoff, agricultural discharge, and domestic waste. Sampling sites such as Ashtamudi Lake, Ayathil Lake, and Vattakkayal highlight the combined impact of human activities and natural factors on water quality. Understanding these dynamics is essential for designing effective remediation strategies to protect both ecological integrity and community health. This study offers a detailed assessment of water quality and environmental stressors affecting these polluted sites.

Ashtamudi Lake: Ashtamudi Lake, spanning about 61 km², is one of India's largest and ecologically vital wetlands. This study aims to evaluate the lake's ecological status, identify pollution sources, and provide scientific insights for conservation, pollution control, and sustainable ecosystem management.

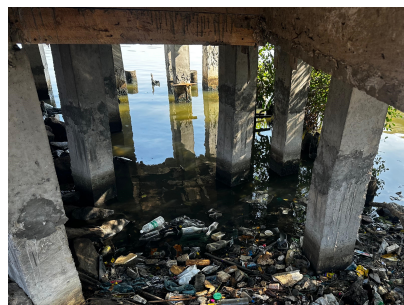


Figure 2: Ashtamudi Lake

Ayathil Lake: Located in Kollam district, Kerala, Ayathil Lake is a freshwater body of ecological and cultural importance. Surrounded by lush greenery, it supports diverse aquatic life and offers essential ecosystem services to nearby communities. This study aims to evaluate the lake's ecological status, identify pollution sources, and recommend sustainable management practices.



Figure 3: Ayathil Lake

Vattakkayal: Vattakkayal, a freshwater lake in Kollam district, Kerala, is valued for its ecological significance and natural beauty. Surrounded by wetlands and marshes, it supports rich biodiversity and provides vital ecosystem services like water purification and flood control. This study aims to assess the environmental condition of Vattakkayal, identify key pollution sources, and suggest strategies for conservation and restoration to safeguard its ecological integrity for future generations.



Figure 4: Vattakkayal

Collection and Preservation of Samples

Water samples for physicochemical and biological analysis were collected from Ashtamudi Lake, Ayathil Lake and Vattakkayal in acid washed, dried polyethylene bottles. Temperature, pH and dissolved oxygen were estimated on site. The samples for bacteriological analysis were collected in pre sterilized bottles and preserved at 4°C. The samples were preserved in buffered formaldehyde (4%) for further analysis.

Water Quality Parameters

Physico-chemical analysis of Water sample

The samples were collected aseptically in sterilized containers and tested by a predefined laid down guidelines by WHO and ICMR in 'WHO guidelines for Drinking Water Quality ' and 'Manual of Standards of Quality for Drinking Water Supplies' (WHO, 2010). The water samples were filtered through whatman filter paper and analysed for hydro-chemical parameters. Analysis of hydro-chemical parameters were carried out following standard methods prescribed by APHA AWWA-WPCF (2006).

Microbiological Analysis of Water Samples

A total of 200 mL water samples was collected from each source in sterile, glass-stoppered bottles for microbiological examination. Sampling, transport, and storage were conducted as per standard guidelines.

Bacteriological contamination in the water samples was evaluated using the multiple-tube fermentation technique, which estimates the Most Probable Number (MPN) of coliforms per 100 mL of water. This method remains a widely accepted approach for detecting faecal contamination in environmental samples (APHA, 2017). To isolate and identify potential waterborne pathogens, a combination of selective agar media was used, including MacConkey agar, Xylose-Lysine-Deoxycholate (XLD) agar, and Membrane Faecal Coliform (M-FC) agar. These media support the differentiation and identification of key microbial groups such as *Escherichia coli*, *Enterobacter*, *Staphylococcus aureus*, *Pseudomonas*, and *Vibrio* spp., which are among the most common and concerning pathogens associated with water pollution (Momba et al., 2020).

For assessing the total heterotrophic bacterial load, nutrient agar composed of peptone (5 g/L), NaCl (5 g/L), beef extract (3 g/L), and agar (15 g/L) was used. A small inoculum (0.2 µL) from each water sample, along with a 10:1 dilution, was spread onto nutrient agar and other selective media. All inoculated plates were incubated at 37°C for 20 to 36 hours, except for the M-FC agar plates, which were incubated at 45°C as recommended for thermotolerant coliforms (EPA, 2021). Following incubation, colony morphology was carefully recorded based on characteristics such as size, form, pigmentation, margin, elevation, and opacity (Tambekar et al., 2022).

Biochemical Characteristics of the Isolates

To identify and differentiate the bacterial isolates, a series of standard biochemical tests were performed following aseptic techniques. Each test was carried out on 24-hour-old pure cultures under controlled laboratory conditions, and results were interpreted based on established microbial diagnostic criteria.

- a. **Catalase Test** (Sivaraman et al., 2022).
- b. **Oxidase Test** (Davis et al., 2021).
- c. **Triple Sugar Iron (TSI) Agar Test** (Mahato et al., 2023).
- d. **Motility Indole Urea (MIU) Test** (Patel & Prajapati, 2020).
- e. **Casein Hydrolysis Test** (Thakur & Singh, 2022).

RESULTS AND DISCUSSION

The analysis of water quality parameters in Ashtamudi Lake, Ayathil Lake, and Vattakkayal offers critical insights into the ecological status and environmental pressures affecting these freshwater ecosystems. By comparing indicators such as turbidity, pH, nitrate levels, and other key factors, the study highlights variations in water quality and their potential causes (Table 1). Understanding these differences is vital for developing effective management and conservation strategies to sustain the health and ecological balance of these important aquatic systems.

Table 1: Physicochemical parameters of Water

Parameters	Site 1	Site 2	Site 3
Turbidity (NTU)	24	21	9
Total Alkalinity (mg/l)	422	163	422
Total Dissolved Solids (mg/l)	>10000	365	1 705
Total Hardness (mg/l)	3296	132	618
Calcium (mg/l)	578	43	248
Magnesium (mg/l)	451	6	<1
Chloride (mg/l)	3600	128	1000
Electrical Conductivity mhos/cm	28850	636.7	2957

Acidity (mg/l)	200	27	300
Sulphate (mg/l)	273.41	7.01	62.45
Fluoride (mg/l)	0.66	<0.01	0.35
Iron (mg/l)	1.4	2.69	0.82
Nitrate (mg/l)	11.01	10.79	8.36
pH	7.23	7.14	6.9
Site 1: Ashtamudi Lake; Site 2: Ayathil Lake; Site 3: Vattakkayal			

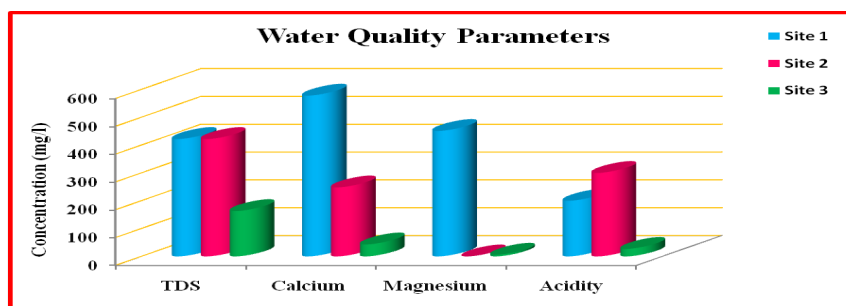


Figure 5: Water Quality Parameters

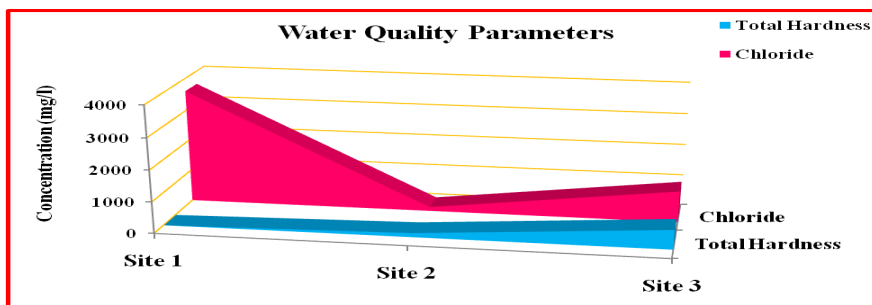


Figure 6: Water Quality Parameters

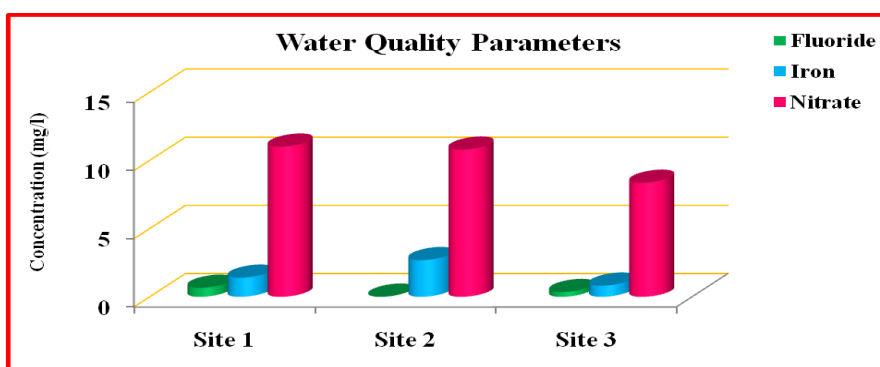


Figure 7: Water Quality Parameters

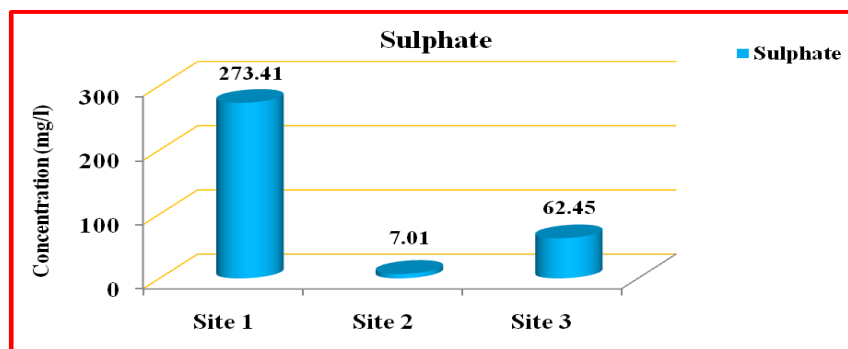


Figure 8: Water Quality Parameters

Turbidity: Turbidity, an important indicator of water quality, reflects the presence of suspended particles such as silt, organic matter, and pollutants. Among the sites studied, Ashtamudi Lake showed the highest turbidity (24 NTU), followed by Ayathil Lake (21 NTU) and Vattakkayal (9 NTU). Elevated levels, especially during summer, are likely due to surface runoff, sewage discharge, and sediment resuspension. High turbidity reduces light penetration, limiting photosynthesis and impacting aquatic biodiversity. Regular monitoring is essential to protect ecosystem health (APHA, 2017; WHO, 2017; USEPA, 2021; Kumar et al., 2022).

pH: The pH values of Ashtamudi (7.23), Ayathil (7.14), and Vattakkayal (6.90) lakes indicate near-neutral to slightly alkaline conditions, typical of Indian freshwater systems. The alkalinity in Ashtamudi and Ayathil is likely due to bicarbonate buffering, biological activity, and organic matter decomposition. The lower pH of Vattakkayal may reflect less buffering or more organic input. pH plays a vital role in regulating nutrient availability and biological functioning (BIS, 2012).

Total Alkalinity: Total alkalinity, representing the water's acid-neutralizing capacity, was highest in Ashtamudi and Vattakkayal (422 mg/L), and lower in Ayathil (163 mg/L). High values suggest active decomposition, CO₂ release, and bicarbonate formation, intensified by summer evaporation and nutrient-rich runoff. Such elevated levels may indicate pollution or eutrophic conditions, potentially stressing aquatic life (Wetzel, 2001).

Electrical Conductivity (EC): EC, a measure of dissolved ions, was highest in Ashtamudi (28,850 μ S/cm), reflecting heavy salt and pollutant loads, likely from sewage, industrial discharges, and saltwater intrusion. Vattakkayal recorded 2957 μ S/cm, influenced by domestic waste and runoff, while lower EC (636.7 μ S/cm) of Ayathil showed moderate mineralization. Seasonal fluctuations show summer concentration and monsoon dilution. Elevated EC highlights anthropogenic pressures and calls for continuous monitoring (Ramesh et al., 2021).

Total Dissolved Solids (TDS): TDS indicates the concentration of dissolved minerals, salts, and organic matter in water, influencing its taste, usability, and ecological health. Ashtamudi Lake shows an alarmingly high TDS level of over 10,000 mg/L, far exceeding the WHO (2017) and BIS (2012) safe limit of 500 mg/L for drinking water. This suggests intense pollution from industrial discharge, saline water intrusion, and sewage runoff. In contrast, Ayathil Lake records a safer TDS value of 365 mg/L, while Vattakkayal stands at 1705 mg/L, still above permissible levels, hinting at anthropogenic influence. Persistent high TDS levels can disrupt aquatic life, impair crop irrigation, and reduce drinking water quality (Khan et al., 2022).

Total Hardness (TH): Water hardness is influenced by the presence of calcium and magnesium salts. Ashtamudi Lake registers extremely high hardness at 3296 mg/L, likely due to saltwater intrusion, mineral weathering, and evaporation.

Vattakkayal also shows significant hardness (618 mg/L), while Ayathil Lake, at 132 mg/L, falls into the moderately hard category. Excessive hardness affects aquatic life and can damage infrastructure through scaling (Sawyer et al., 2023).

Calcium (Ca^{2+}): Calcium plays a vital role in aquatic ecosystems, supporting shell formation, buffering capacity, and plant cell structure. Ashtamudi Lake records high calcium levels (578 mg/L), peaking in summer, possibly due to organic matter breakdown, mineral leaching, and evaporation. Vattakkayal shows 248 mg/L, while Ayathil Lake reports 43 mg/L. These variations highlight geogenic sources and seasonal nutrient dynamics (Bhatt et al., 2020; Choudhury & Das, 2021).

Magnesium (Mg^{2+}): Magnesium is essential for aquatic productivity and photosynthesis, particularly as a central component of Chlorophyll. Ashtamudi Lake has high magnesium levels (451 mg/L), while Ayathil Lake (6 mg/L) and Vattakkayal (<1 mg/L) show much lower concentrations. These differences may reflect dilution effects during monsoon and evaporative concentration during drier seasons (Zhang et al., 2023).

Chloride (Cl^-): Chloride is a reliable marker of sewage, industrial, and agricultural pollution. Ashtamudi Lake shows extremely high chloride content (>5000 mg/L), especially during summer, pointing to intense evaporation and anthropogenic loading. Vattakkayal (1000 mg/L) and Ayathil Lake (128 mg/L) also reflect human influence, with seasonal peaks. Elevated chloride disrupts freshwater balance and signals ecosystem stress (Bhatnagar et al., 2020).

Sulphate (SO_4^{2-}): Sulphate ions enter freshwater systems through natural weathering and anthropogenic sources such as fertilizers, detergents, and industrial effluents (USEPA, 2021; Singh et al., 2022). While necessary in low concentrations, elevated sulphate levels can harm aquatic organisms and affect human health. In this study, Ashtamudi Lake recorded a high sulphate concentration of 273.41 mg/L, suggesting significant anthropogenic input and potential risks of eutrophication and hydrogen sulphide formation. Vattakkayal showed moderate levels (62.45 mg/L), while Ayathil Lake had low concentrations (7.01 mg/L), reflecting varying pollution intensities across sites.

Fluoride: Fluoride (F^-), naturally occurring in freshwater, aids in dental health when maintained within the WHO-recommended range of 0.5–1.5 mg/L (WHO, 2022). Ashtamudi Lake recorded 0.66 mg/L, within safe limits, while Vattakkayal (0.35 mg/L) and Ayathil Lake (<0.01 mg/L) showed suboptimal levels. Though all sites remained below toxicity thresholds, sustained monitoring is essential, and fluoride supplementation may be needed in deficient zones to support oral health.

Iron: Iron, though naturally present in freshwater, can indicate contamination when levels exceed recommended limits. The BIS and WHO set the acceptable limit at 0.3 mg/L for drinking water due to aesthetic and microbial concerns (BIS, 2012; WHO, 2022). In this study, Ayathil Lake showed the highest concentration (2.69 mg/L), followed by Ashtamudi (1.40 mg/L) and Vattakkayal (0.82 mg/L). These elevated levels suggest inputs from sediment leaching, industrial effluents, or sewage, and may impair water quality while fostering microbial proliferation.

Nitrate: Nitrate (NO_3^-), commonly introduced into freshwater through agricultural runoff, sewage, and organic waste, is regulated at 50 mg/L in drinking water to prevent methemoglobinemia (WHO, 2022). Concentrations in Ashtamudi (11.01 mg/L), Ayathil (10.79 mg/L), and Vattakkayal (8.36 mg/L) remained below the limit but suggest anthropogenic nutrient input. Continued accumulation may promote eutrophication, oxygen depletion, and disruption of aquatic ecosystems.

Microbial Assessment and Isolate Characterization

Water samples from Ashtamudi, Ayathil, and Vattakkayal were tested for microbial presence. Growth was observed only on Nutrient Agar, indicating the presence of general heterotrophic bacteria, while no colonies grew on selective media

like MacConkey, XLD, or Faecal Coliform Agar, suggesting no detectable pathogenic or coliform bacteria in the samples.

Biochemical tests identified the bacteria as Gram-negative rods, with positive reactions for oxidase and catalase enzymes (Table 2). These are commonly found in natural aquatic environments and play important roles in breaking down organic matter and recycling nutrients. While they are not harmful, their increased numbers can signal ecological imbalance or pollution from human activities.

Table 2: pH value of sample mineral waters and microbial load in different media.

Sample No.	Sample Name	pH	Growth on Nutrient Agar (CFU/ml)	Growth on Mac-Conkey Agar (CFU/ml)	Growth on Membrane Faecal Coliform Agar (CFU/ml)	Growth on Xylose Lysine Deoxycholate Agar (CFU/ml)
1	ALW	7.5	5.00×10^2	NIL	NIL	NIL
2	AyLW	6.4	6.20×10^2	NIL	NIL	NIL
3	VLW	7.2	1.6×10	NIL	NIL	NIL
AL- Ashtamudi Lake Water, AyL- Ayathil Lake Water, VL- Vattakkayal Lake Water						

Water samples from three aquatic sites, Ashtamudi, Ayathil, and Vattakkayal were analyzed to evaluate microbial contamination and general water quality. The pH ranged from mildly acidic to neutral: Ayathil measured 6.4, Vattakkayal 7.2, and Ashtamudi 7.5, all within the acceptable pH range for surface waters (WHO, 2023). However, slight pH shifts can significantly affect microbial metabolism and the solubility of nutrients and metals in aquatic systems (USEPA, 2023; APHA, 2022). Ayathil Lake exhibited the highest bacterial load (6.2×10^2 CFU/mL), followed by Ashtamudi (5.0×10^2 CFU/mL) and Vattakkayal (1.6×10^1 CFU/mL), likely due to variable organic input, surface runoff, and anthropogenic disturbances. Elevated microbial counts may indicate faecal contamination or eutrophic conditions, warranting site-specific intervention and continuous surveillance (Mohan et al., 2023).

Bacterial growth was recorded only on nutrient agar, which supports a broad range of heterotrophic microorganisms. Among the samples, Ayathil exhibited the highest colony count (6.20×10^2 CFU/ml), followed by Ashtamudi (5.00×10^2 CFU/ml), while Vattakkayal had a notably lower load (1.6×10^1 CFU/ml). No growth was observed on selective media such as MacConkey agar, membrane faecal coliform agar, or XLD agar, indicating the absence of detectable coliforms or enteric pathogens at the time of sampling. The elevated heterotrophic count in Ayathil may suggest organic pollution or nutrient enrichment, even in the absence of faecal contamination (Akter et al., 2021; Fang et al., 2022). Regular monitoring is essential, as high heterotrophic bacterial loads can indicate early warning signs of environmental stress and declining water quality (Sharma et al., 2024).

Biochemical Characteristics of the Isolates

The colony characteristics of the isolated colony were differing from each other. All the biochemical tests result of two isolated organisms were observed and the organisms were identified as *E coli* and *Staphylococcus* sp. (Table 3).

Table 3: Biochemical tests of the isolates collected from nutrient agar.

Isolate no	Sample Name	Isolate Name	Gram Stain		TSI				MIU			Catalase	Oxidase	CHI	Organism
			+	-	BUTT	SLANT	H ₂ S	Gas	Motility	Indole	Urease				
1.	ALW	Ast	-	cocci	K	K	-	-	+	+	+	+	-	+	<i>E coli</i>

Isolate no	Sample Name	Isolate Name	Gram Stain		TSI				MIU			Catalase	Oxidase	CHI	Organism
			+/-	Shape	BUTT	SLANT	H ₂ S	Gas	Motility	Indole	Urease				
2.	AyLW	Ayl	+	cocci	A	A	+	+	+	-	+	+	-	-	<i>S.aureus</i>
3.	VLW	Vkl	+	cocci	A	A	-	-	+	+	+	+	-	+	<i>E coli</i>

K = Alkaline reaction, A = Acidic reaction, + = Positive reaction; - = Negative reaction

Microbiological analysis of water samples from three freshwater sites in Kollam District, Kerala, was conducted to identify potential bacterial contaminants. Isolate 1 (Ast) from Ashtamudi Lake was identified as Gram-negative cocci with a K/K reaction in the Triple Sugar Iron (TSI) test, indicating no carbohydrate fermentation. It showed positive results for motility, indole, catalase, oxidase, and citrate utilization, while H₂S, gas, and urease production was absent. These biochemical features are consistent with *Escherichia coli*, a widely recognized faecal indicator organism (FIO) in environmental microbiology, known for its role in assessing sanitary quality of water (WHO, 2017; Jang et al., 2021). Similarly, isolate 3 (Vkl) from Vattakkayal Lake shared comparable biochemical characteristics with positive acid reaction (A/A) in TSI, and was likewise identified as *E. coli*. The repeated detection of *E. coli* in different sites raises concerns over possible faecal contamination, likely arising from domestic sewage discharge, agricultural runoff, or other anthropogenic activities impacting these ecosystems (Li et al., 2023).

In contrast, isolate 2 (Ayl) from Ayathil Lake was Gram-positive cocci and exhibited an A/A reaction in TSI, indicating fermentation of glucose and other sugars. This isolate was catalase-positive, oxidase-negative, and positive for motility, indole, and citrate utilization, while urease activity was negative, traits that support its identification as *Staphylococcus aureus*. This bacterium, commonly associated with human skin and mucosal flora, may enter aquatic environments through direct human activity, wastewater discharge, or surface runoff (Otto, 2022).

CONCLUSION

The investigation of the water quality parameters of polluted water bodies in Kollam district, Kerala, has unearthed concerning trends that demand immediate attention. From elevated levels of pollutants like heavy metals, organic matter, and microbial contaminants to compromised pH levels and diminished oxygen content, the data paints a vivid picture of environmental degradation. These findings not only highlight the imminent threats to aquatic ecosystems but also underscore the looming public health risks for communities reliant on these water sources. The presence of pollutants poses a grave danger to human health, ranging from waterborne diseases to long-term health complications.

To combat these challenges effectively, a multifaceted approach is imperative. This entails robust regulatory frameworks to enforce pollution control measures, investment in wastewater treatment infrastructure, and community engagement initiatives to raise awareness and foster stewardship of local water resources.

Furthermore, sustainable land management practices and reforestation efforts can mitigate runoff and erosion, curbing the influx of pollutants into water bodies. Additionally, promoting the adoption of eco-friendly agricultural practices and incentivizing industries to implement cleaner production methods are pivotal steps toward restoring water quality and ecological balance.

By rallying collective action and fostering partnerships among stakeholders, we can pave the way for tangible improvements in water quality across Kollam district. Together, let us embark on a journey toward rejuvenating these vital lifelines, safeguarding both the environment and the well-being of present and future generations.

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