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Comparative analysis of two major water bodies (Jalgun and Narawala ponds) in Barwani, Madhya Pradesh, India

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Abstract : Microbiomes form an essential link between ecosystem functioning and human health. In many rural regions, surface water bodies such as ponds serve as important resources for agriculture and domestic use, making an understanding of their microbial composition critical for public health assessment. This study examines the microbial diversity, ecological dynamics, and potential health implications of two major freshwater ponds, Narwala and Jalgun, located in the Barwani district of Madhya Pradesh, India. Water samples were collected in triplicate from both ponds and analyzed using 16S rRNA gene amplicon sequencing targeting the V3–V4 hypervariable regions. Following DNA extraction and quality control, sequencing data were processed through established bioinformatics pipelines for taxonomic classification and diversity analysis. Alpha and beta diversity metrics were used to assess microbial richness, evenness, and community differences, while ecological modeling approaches were applied to understand microbial assembly processes. Both ponds exhibited high microbial diversity dominated by bacterial phyla commonly associated with freshwater environments, including Bacteroidota, Actinomycetota, Cyanobacteriota, Verrucomicrobiota, and Acidobacteriota. Comparative analysis revealed differences in community structure, with Narwala Pond showing a more balanced distribution of bacterial groups, particularly Bacillota, suggesting greater ecological stability. In contrast, Jalgun Pond displayed reduced abundance of some beneficial taxa. The presence of Cyanobacteriota in both ponds indicates nutrient enrichment and potential risks related to algal blooms. Several pathogenic and opportunistic bacteria associated with waterborne diseases were also detected, raising concerns regarding water use without treatment. Overall, the study highlights the influence of agricultural practices, monsoon-driven runoff, and soil–water interactions on pond microbiomes. The findings emphasize the need for regular microbial monitoring and appropriate management strategies, as neither pond water is suitable for direct human consumption without adequate treatment.

Index Terms - **Alpha diversity, Beta diversity, Environmental microbiome, Public health, Bacteria**

I. INTRODUCTION

The "One Health paradigm", which integrates environmental, animal, and human health, is based on the idea of the microbiome, which is the collective genetic material of microbial populations living in a certain environment. In order to maintain terrestrial and aquatic ecosystems, environmental microbiomes support organic matter decomposition, nutrient cycling, and ecological stability. The importance of microbiota in mediating relationships between environmental systems and human health through co-evolution, co-metabolism, and regulatory processes has been highlighted in recent studies.

Both pathogenic and helpful microbes can be found in water microbiomes. Antibiotic resistance genes have become a significant public health problem due to their existence and spread within these microbial communities, especially in developing nations with high agricultural activity and poor sanitation infrastructure. Water-borne illnesses continue to be major causes of morbidity and mortality in Madhya Pradesh, highlighting the necessity of thorough environmental microbiome analysis.

Water bodies are an essential component for a locality and the usage mostly for the population is usually confined in that particular region. This usage pattern make it pertinent to have a close look into the microbial flora of the water body, usually a pond catering to the needs of a village. The water of the pond is not only used for irrigating the agriculture field but also are being used by the common population for drinking purpose. Microbial interchange between soil and water systems happens constantly due to both anthropogenic activities like waste disposal and agriculture as well as natural processes like rainfall, flooding, and surface runoff. Determining environmental pathways of disease transmission and creating evidence-based public health interventions require an understanding of the pond microbial ecosystem. Thus, the goal of this study is to evaluate the microbial dynamics and public health significance of water microbiome of Barwani area which is having two major ponds namely Jalgun and Narawala pond.

2. RESEARCH METHODOLOGY

2.1 Study Area and Sample Classification

Randomly three water samples from the Jalgun and Nawarala ponds were collected from Madhya Pradesh, India's Barwani district. The samples were named NPWS (Narawala Pond Water Sample) and JPWS(Jalgun Pond Water Sample). The three replicates mentioned were NPWS1, NPWS2, NPWS3 and JPWS1, JPWS2, JPWS3.

2.2 DNA Extraction and Sequencing

DNA Extraction from the water samples were done with DNeasy Power Water Kit (14900-100-NF) and the quality of the DNA and QC was done in with Agilent Bioanalyzer before the NGS process. Post PCR, Illumina platform was used to perform 16S rRNA gene amplicon sequencing that targeted the V3–V4 hypervariable areas. To create high-quality, useful tags, raw reads were combined, quality-filtered, and checked for chimeras..

2.3 Bioinformatics and Statistical Analysis

The raw data sequenced were cleared by filtering and splicing. The DADA2 process was used to estimate amplicon sequence variants, which were then taxonomically categorized using a Naïve Bayes classifier against the SILVA 138 database. To evaluate within-sample diversity, alpha diversity metrics such as Chao1, observed features, Shannon index, and Pielou's evenness were computed. Weighted and unweighted UniFrac distances were used to examine beta diversity, and principal coordinates analysis (PCoA) and non-metric multidimensional scaling (NMDS) were used to show the results. ANOSIM, MRPP, and PERMANOVA (Adonis) were used to examine the statistical significance of community differences. PICRUSt2 was used to predict functional potential, whereas beta NTI, NST, and iCAMP analyses were used to assess ecological assembly processes. T-test is performed to determine species with significant variation between groups (p-value < 0.05) at various taxon ranks including phylum, class, order, family, genus, and species.

3. RESULTS AND DISCUSSION

3.1 Alpha Diversity

Significant differences in microbial richness and evenness between samples were found using alpha diversity analysis. Extremely high Chao1 richness values and extremely low coverage were found in several water samples, indicating both a vast pool of uncommon and unidentified microbial species and inadequate sequencing depth.

3.2 Beta Diversity

Microbial communities were clearly clustered according to the environmental medium (water) and chemical treatment status, according to ordination analyses. These results validate ecological difference across sample groups and reveal microbial assemblages peculiar to a given ecosystem. Beta Diversity is a comparative analysis of microbial community composition of different samples. First, according to the taxonomy annotation results of all samples and the abundance information of feature sequences, the feature sequence information of the same classification is combined to obtain the species abundance

3.3 Identification of microbial flora

The microbial communities identified from Jalgun pond and Narawala pond with their abundance values are tabulated in Table 1 and Table 2.

Table 1. List of Bacterial communities identified with their abundance in Jalgun Pond

Bacterial community	**Abundance Value**			
	0-0.25	0.25-0.5	0.5-0.75	0.75-1
Pseudomoadota	✓	✓	✓	✗
Bacillota	✓	✓	✓	✗
Bacteroidota	✓	✓	✓	✓
Chloroflexota	✓	✓	✓	✗
Actinomycetota	✓	✓	✓	✓
Cyanobacteriota	✓	✓	✓	✓
Deinococcota	✓	✓	✓	✓
Verrucomicrobiota	✓	✓	✓	✓
Acidobacteriota	✓	✓	✓	✓

Table 2. List of Bacterial communities identified with their abundance in Narawala Pond

Bacterial Community	**Abundance Value**			
	0-0.25	0.25-0.5	0.5-0.75	0.75-1
Pseudomoadota	✓	✓	✓	✗
Bacillota	✓	✓	✓	✓
Bacteroidota	✓	✓	✓	✓
Chloroflexota	✓	✓	✓	✗
Actinomycetota	✓	✓	✓	✓
Cyanobacteriota	✓	✓	✓	✓
Deinococcota	✓	✓	✓	✓
Verrucomicrobiota	✓	✓	✓	✓
Acidobacteriota	✓	✓	✓	✓

3.4. Comparative Analysis of Narwala and Jalgun Pond Water

Narwala Pond and Jalgun Pond both harbor a diverse range of bacterial phyla that are characteristic of freshwater ecosystems. Although the overall bacterial diversity observed in the two ponds is broadly comparable, notable differences in the relative abundance and distribution of specific bacterial groups provide important insights into water quality and its potential suitability for drinking.

Several bacterial phyla, including Bacteroidota, Actinomycetota, Cyanobacteriota, Deinococcota, Verrucomicrobiota, and Acidobacteriota, were detected at relatively high abundance levels in both ponds, with values reaching up to 0.75–1. The consistent presence of Cyanobacteriota in both water bodies suggests nutrient enrichment, which may increase the likelihood of algal blooms and the production of cyanotoxins, if the water is not adequately treated. Pseudomonadota (*Proteobacteria*), a phylum that includes several opportunistic and potentially pathogenic taxa, were also identified in both ponds. However, their absence from the highest abundance category indicates a moderate microbial load rather than severe contamination.

Despite these similarities, differences in community structure were evident between the two ponds. A key distinction was observed in the distribution of Bacillota. In Narwala Pond, Bacillota were present across all abundance ranges, including the highest category, whereas in Jalgun Pond they were absent from the upper range. This pattern suggests that Narwala Pond supports a more stable and functionally balanced Bacillota population, many members of which are involved in organic matter degradation and are generally associated with lower pathogenic risk. Overall, the microbial community in Narwala Pond appeared more evenly distributed, while Jalgun Pond showed a comparatively reduced abundance of certain beneficial bacterial groups.

High microbial richness coupled with limited sequencing coverage indicates the possible presence of a rare biosphere containing low-abundance but potentially harmful microorganisms. Such hidden microbial diversity may pose a public health concern, particularly in regions where human exposure to untreated surface water and contaminated soil is frequent.

In the Barwani region, agricultural activities and monsoon-driven runoff play a significant role in shaping the ecological connection between soil and aquatic microbiomes. During periods of heavy rainfall, pesticides, fertilizers, and microbial contaminants present in agricultural soils are transported into nearby water bodies, leading to the formation of a dynamic and interconnected environmental microbiome. This process facilitates the spread of waterborne pathogens such as *Vibrio cholerae*, *Salmonella Typhi*, *Leptospira*, and antibiotic-resistant *Escherichia coli*, thereby increasing potential health risks.

Ecological modeling suggests that microbial community assembly in these environments is influenced by both deterministic selection and stochastic dispersal processes. Monsoon flooding acts as a major stochastic force, increasing environmental unpredictability by randomly introducing soil-derived microorganisms into aquatic systems. These interactions complicate public health management and highlight the need for integrated environmental monitoring and coordinated intervention strategies.

3.5. Clinical and Health Significance of the Observed Bacterial Groups

The bacterial groups identified in this study are known to play important roles in physiological processes, metabolic activity, and disease susceptibility (Table 3). Consequently, variations in their relative abundance may have meaningful implications for human health.

Table 3: List of identified pathogen in Jalgun and Narawala Ponds from Barwani, Madhya Pradesh

Pathogen	**Associated Disease**	**Environmental Reservoir**	**Link to Local Conditions**
Vibrio cholerae	Cholera	Contaminated water, untreated sewage	Untreated wastewater discharge into water bodies (Awereet.al. 2025)
Salmonella Typhi	Typhoid fever	Contaminated water, soil, food	Poor sanitation, open defecation (Kim 2023)
Leptospira	Leptospirosis	Contaminated water, soil	Animal urine contamination, floods, and agricultural exposure (Wang & Dunn 2025).
Enterococcus	Fecal contamination indicator	Soil, water, human/animal waste	High levels in agricultural/urban soils, storm water runoff (Ling et.al.2023)
Staphylococcus aureus	Staphinfections, gastroenteritis	Soil, water, human/animal Presence	Proliferation in soils with human activity; transport to water bodies (Ling et.al.2023)

E. coli	Diarrhea, dysentery	Fecal waste, contaminated water	Widespread in India; high prevalence in clinical samples (Hall & Robert 2018)
Shigella	Shigellosis (dysentery)	Contaminated water and food	High prevalence in clinical samples in India. (Das et.al.2015).

Members of the phylum Firmicutes include several short-chain fatty acid (SCFA)–producing bacteria, such as Faecalibacterium and Clostridia. These organisms contribute to gut epithelial integrity and help regulate inflammatory responses. Alterations in Firmicutes abundance have been linked to metabolic disorders, including obesity and insulin resistance, as well as inflammatory bowel diseases.

Bacteroidetes are primarily involved in the degradation of complex carbohydrates and play a central role in energy extraction and nutrient metabolism. Changes in the balance between Firmicutes and Bacteroidetes have frequently been associated with dietary patterns, metabolic health, and chronic inflammatory conditions.

Actinobacteria, particularly species of the genus *Bifidobacterium*, are widely regarded as beneficial components of the normal microbiota. They are associated with immune modulation, suppression of pathogenic organisms, and strengthening of the gut barrier. Reduced abundance of *Actinobacteria* has been reported in aging populations and in individuals with gastrointestinal and metabolic disorders.

An increased presence of *Proteobacteria*, which include opportunistic pathogens such as *Escherichia* and *Klebsiella*, is often considered a marker of microbial imbalance. Elevated levels of this group have been associated with inflammation, gastrointestinal disturbances, and an increased risk of infection.

Lactic acid bacteria, including *Lactobacillus* species, are commonly linked to probiotic functions. These bacteria contribute to mucosal health, limit pathogen colonization through competitive exclusion, and influence immune responses. Their presence is generally considered supportive of both gut and urogenital health.

The detection or enrichment of pathogenic or opportunistic bacterial groups may therefore reflect underlying dysbiosis and increased susceptibility to infection, particularly among immuno-compromised individuals or populations with limited access to treated water.

Taken together, the bacterial profiles observed in this study provide insight into the functional characteristics of the microbial communities present in Narwala and Jalgun ponds and their potential implications for human health. However, any clinical or public health interpretation should be made cautiously and in conjunction with environmental conditions, host factors, and additional microbiological and epidemiological data, as microbial composition alone is not sufficient for definitive risk assessment.

4. Conclusion

This study offers a comparative overview of the microbial communities present in Narwala and Jalgun pond waters and highlights their relevance to water quality and public health. Both ponds support a broad range of bacterial phyla commonly found in freshwater environments, indicating active and complex microbial ecosystems. However, differences in the relative abundance and distribution of certain bacterial groups point to variations in ecological balance between the two water bodies. Narwala Pond showed a more evenly distributed microbial profile, including the consistent presence of Bacillota across all abundance ranges, suggesting relatively stable microbial functioning. In contrast, Jalgun Pond exhibited a reduced representation of some beneficial bacterial groups, which may indicate greater ecological stress or disturbance. The occurrence of *Cyanobacteriota* in both ponds suggests nutrient enrichment, likely linked to surrounding land use and runoff, and raises concerns about the potential for algal blooms and toxin production. The detection of *Proteobacteria*, which include several opportunistic pathogens, further emphasizes the need for caution when considering these waters for domestic use. Seasonal factors, particularly monsoon-driven runoff combined with agricultural activities, appear to play a significant role in shaping microbial diversity and facilitating the movement of soil-associated microorganisms into aquatic systems. Taken together, the findings suggest that although Narwala Pond appears microbiologically more stable than Jalgun Pond, neither source is suitable for direct consumption without treatment. Regular monitoring and appropriate water management practices are essential to minimize potential health risks associated with these surface water resources.

II. ACKNOWLEDGMENT

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