

Comprehensive assessment of bacterial and functional diversity using next-generation high-throughput sequencing of two crucial lakes of Haryana, India

Anjali Yadav¹, Rishikesh Kumar Laxmi², Kirti Amresh Gautam^{1*}

¹ Department of Basic and Applied Sciences, School of Engineering & Sciences, GD Goenka University, Haryana, India

² Department of Zoology, Bhaskaracharya College of Applied Sciences, University of Delhi, Delhi, India.

* Corresponding author E.mail: emails2kirti@gmail.com

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Abstract

Lakes are dynamic and complex ecosystems where bacterial diversity is a significant biological indicator of water quality. In few decades, in India, several water bodies are reported to have pesticide and heavy metal pollutants. The present study conducted on two substantial lakes of Haryana, India, for analysing bacterial diversity using high-throughput Illumina 16S rRNA sequencing-MiSeq platform. Sequencing analysis showed that, in Karna lake, predominant phylum was Proteobacteria accounting (76.38%), Planctomycetes (6.42%), Verrucomicrobia (5.28 %) and Bacteroidetes (5.06%). Although, in Tilyar lake, predominant phylum was Proteobacteria (46.91%), Verrucobacteria (22.87%), Bacteroidetes (16.34 %), Planctomycetes (8.49%) and Acidobacteria (5.75 %). Tilyar lake exhibited higher alkalinity resulting in dominance of alkaliphilic phyla such as Verrucomicrobia (22.87%) and Bacteroidetes (16.34%). On contrary, Karna Lake with a relatively lower pH, showed a dominance of Proteobacteria (76.38%). The high DO in Tilyar lake, signifies better oxygenation attributing to greater abundance of aerobic bacterial communities. Differences in TDS, TSS and EC between the lakes reflect difference in nutrient influx, pollution sources and pollutant levels. A comparison of bacterial genera revealed 529 (47.4%) shared taxa in both lakes and 232 (20.8%) taxa unique to Karna lake and 356 (31.9%) taxa unique to Tilyar lake. Overall, higher microbial diversity and functional potential observed in Tilyar Lake suggest a more stable and ecologically resilient environment capable of withstanding environmental disturbances. While, dominance of a few specific taxa in Karna Lake indicates potential environmental constraints that may limiting microbial diversity.

Keywords: *Lake Tilyar, Lake Karna, Bacterial profile, Bacterial diversity, 16S rRNA Sequencing, Illumina MiSeq, V3-V4.sequence.*

Introduction

Microbial diversity is a substantial biological indicator for any ecosystem, it plays a significant role in different biological processes, decomposition and mineralization of the organic matter (Benson et al., 2019), and nutrient cycling (Lamim et al., 2021). Spatial and temporal scales microbial diversity of aquatic ecosystems varies by several geological, ecological and anthropogenically factors (Prakash et al., 2020). Several published research articles potentially explain bacteria are the primary factor of aquatic ecosystems involved in biogeochemical

processes, transferring energy from dissolved organic matter to higher tropic level via protozoa and zooplankton and therefore, directly correlates with lake functions (Zhang et al., 2025; Kim et al., 2024; Xin et al., 2024). Aquatic bacterial profile and human health are closely linked via positive impacts on health by limiting the growth of disease-causing pathogens, supporting mental and physical well-being and proving key services for recreation, irrigation, drinking water, recharging groundwater, sustaining biodiversity (Heino et al., 2020). Negative impacts are also found when bacterial

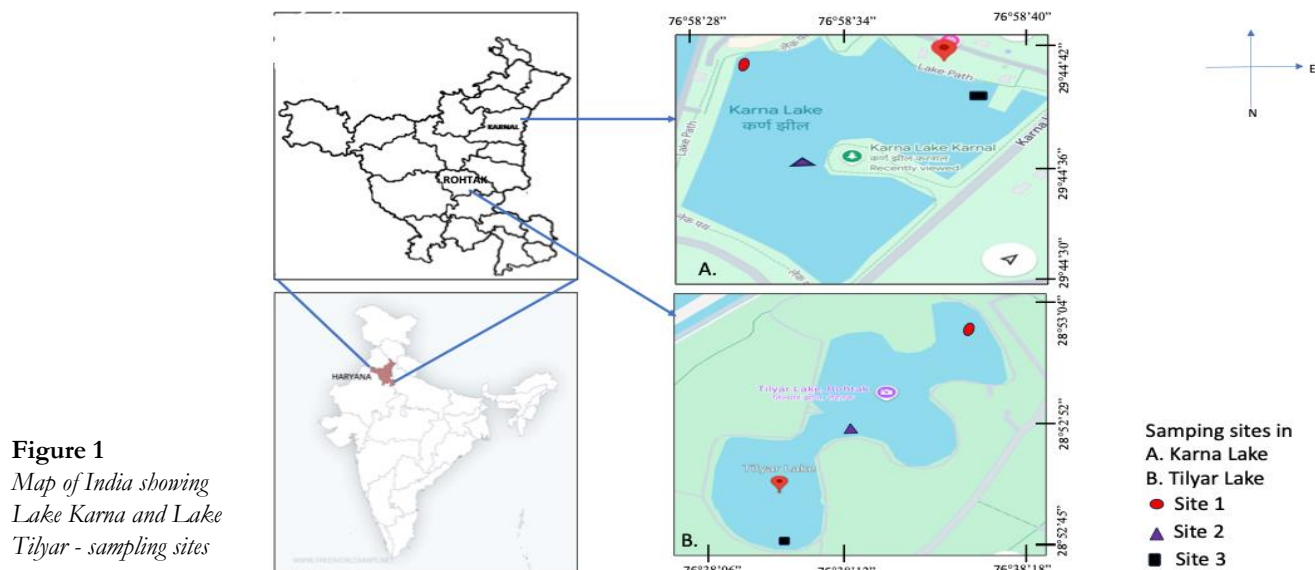
diversity is disrupted (dysbiosis) for example, bacterial shift to toxin producing cyanobacteria, pathogen proliferation-water borne diseases and antibiotic resistance (Chatterjee et al., 2023; Wang et al., 2023). Intrinsically, the preceding explained affecting factors influence physicochemical parameters of any aquatic ecosystem that ultimately modulate and establish the bacterial profile of any water body like river, lakes, ponds (Haftu Z and Estifanos S, 2020; Lu et al., 2020). Understanding of bacterial profile along with other environmental factors is crucial for ecological stability and resilience thereby, to achieve SDG 3-good health and well-being and SDG 6-clean water and sanitation for safeguard of environment, humans and animal health. In recent years, besides its comparatively small part of the Earth's surface, lakes as received worldwide attention because of its crucial ecological services. Lakes are dynamic and complex ecosystems where bacterial community is significantly coupled with geographical locations, ecosystem structure, nutrient status and metabolic balance (Castro Márquez et al., 2025). Karna lake and Tilyar lake are substantial lakes of Haryana and serve as vital recreational hubs for the residents of Rohtak, Karnal and nearby district and offering opportunities for various outdoor activities. Jawahar Lal Nehru (JLN) canal and surface runoff during monsoon are the primary source of water supply to the lake. Lakes are susceptible to pollution by domestic and industrial effluents, agricultural runoff, seasonal variations, and anthropogenic activities therefore, periodical assessment of lakes water quality via monitoring physicochemical and microbiological characteristics is important (Betiku et al., 2021; Heino et al., 2020). Moreover, in India, several water bodies

are reported to have pesticide and heavy metal pollutants (Prasanna et al., 2024; Kashyap et al., 2024). Various metagenomic studies on diverse environments as soil, sediments, surface waters (Alteio et al., 2020), deep sea (Tringe et al., 2005), contaminated ground-water (Smith et al., 2012) have been undertaken however, studies with objective impact of water quality on bacterial profile is scarce. In India, scanty of reports specially from south and north India are available those demonstrated the correlation between lake bacterial profile and aquatic ecosystem function while none of the potential research is undertaken on the lakes in Haryana using NGS (Bag et al., 2025; Kumar et al., 2024; Panwar et al., 2023). The present study will significantly enhances the understanding of microbiota in freshwater ecosystem in Haryana and provide baseline information for using microbiota as ecological health indicators, illuminating their ecological role and potential use for formulating the conservation and management strategies of freshwater ecosystems.

Materials and methods

Study site and sample collection

Primarily, two Litre water samples were collected in sterile bottles from each three distinct sites in May 2024 of the two significant lakes located in Haryana state, India including Tilyar Lake ($28^{\circ}52'53''\text{N}$; $76^{\circ}38'12''\text{E}$) and Karna Lake ($29^{\circ}44'632''\text{N}$; $76^{\circ}58'574''\text{E}$) as illustrated in Figure 1 and transported to laboratory for further analysis as per CPCB guidelines. Primary data of water quality assessment were recorded as per National lake conservation plan (NLCP) guidelines, 2008.



Furthermore, one Litre water samples were collected and carefully transferred into sterile glass container using an integrated sampling method, ensuring that samples have been collected at a depth of 30 cm below the surface water (Sirbu et al., 2022). The depth was chosen to capture a representative profile of the microbial diversity in the surface water. To maintain the integrity of water sample and prevent DNA degradation, water samples were stored at 4°C in the ice box and transported to laboratory on the same day. To create a comprehensive representation of the bacterial diversity in each lake, water samples from each lake were pooled together and hence two representative samples one from each lake were processed.

Metagenomic DNA extraction

Metagenomic DNA isolation was performed using the Qia-gen DNeasy Powerwater Kit (Catalogue no. 14900-100-NF), as per manufacturer's protocol provided with the kit manual. The purified and concentrated DNA was assessed for quality and concentration using Nanodrop-1000 spectrophotometer (Thermo Fisher Scientific, United States). The extracted DNA samples were stored at -20°C until further processing.

DNA amplification and 16S rRNA sequencing

Amplification and purification of 16S rRNA genes was performed followed by library preparation that specifically targeted the hypervariable V3-V4 region of bacterial 16S rRNA gene using Xploregen 16S Amplicon V3-V4 library preparation kit with primer 16S Forward 5' AGAGTTTGA TGMTGGCTCAG 3' and 16S Reverse 5' TTACCGCG GCMGCSGGCAC 3' (Shi et al., 2023). Subsequently PCR amplification, a fragment length of approximately 500-700 bp was obtained in the sequencing library which represents

the combined length of adapters and the inserts corresponding to the 16S V3-V4 region. Sequencing was done in Illumina MiSeq sequencer (Illumina, San Diego, United State) to generate 67000 paired end reads per samples. The data obtained from the sequencing was further analysed for bacterial diversity following bioinformatics pipeline.

Bacterial profile analysis

In sequenced data, read quality was assessed using FastQC (v.0.12.1) and MultiQC (v.0.21), adapters were removed using TrimGalore, subsequently, high-quality reads achieving quality control (QC) were imported into QIIME2 for operational taxonomic units (OUTs) identification. The reads were filtered to remove sequences containing unambiguous bases, denoised and merged using DADA2 algorithm to predict Amplicon Sequence Variants (ASVs). To optimize the data for the analysis, the forward reads were truncated to a length of 290 bp while the reverse reads were truncated to 270 bp from their 3' ends. Further, the primers were also removed from the 5' ends of the reads. Chimeras were identified and removed using DADA2 algorithm. The obtained ASVs were used for taxonomic assignment using QIIME2-Naivebayes, Greengenes2 (v.2022) and (PKSSU 4.0)-EZbiocloud. The filtered reads were utilized to construct taxon table. The rarefaction curves of both Tilyar and Karna lakes initially exhibit an exponential growth pattern before stabilizing. α -diversity indices (Chao index, Shannon index and Simpson index) were calculated for the microbial diversity evaluation.

Results

Physico-chemical properties

The physico-chemical parameters of both the lakes are summarized in the Table1. Preliminary work of this study for Tilyar lake is already published (Yadav and Gautam,

Table 1. Physico-chemical parameters for Tilyar lake and Karna Lake. BLQ: Below the level of quantification

Parameters	Tilyar Lake			Karna Lake		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
pH	9.55 ± 0.01	9.52 ± 0.07	9.54 ± 0.02	8.29 ± 0.18	8.63 ± 0.23	8.83 ± 0.12
Temperature (°C)	24.36 ± 0.1154	24.46 ± 0.1154	25.36 ± 0.2309	24.3 ± 0.1527	24.33 ± 0.2886	24.36 ± 0.3214
TDS (mg/l)	172.67 ± 1.15	174.67 ± 2.08	173.67 ± 2.88	177.00 ± 2.00	171.67 ± 1.53	178.67 ± 1.53
TSS (mg/l)	173.67 ± 2.89	174.66 ± 1.52	173.66 ± 2.89	177.00 ± 1.00	174.00 ± 1.00	175.33 ± 1.53
EC (µs/cm)	217.67 ± 1.52	215.67 ± 3.51	219.33 ± 1.52	289.00 ± 4.00	281.00 ± 7.55	276.67 ± 0.58
Turbidity (NTU)	<1	<1	<1	<1	<1	<1
Nitrate (mg/l)	2.40 ± 0.1	2.57 ± 0.23	2.50 ± 0.20	2.6 ± 0.10	2.47 ± 0.58	2.53 ± 0.2
TH (mg/l)	76.67 ± 0.58	77.33 ± 1.52	75.33 ± 1.52	107.00 ± 2.00	107.33 ± 0.58	106.00 ± 1.00
DO (mg/l)	7.33 ± 0.15	7.37 ± 0.20	7.50 ± 0.17	6.93 ± 0.25	6.83 ± 0.06	6.60 ± 0.20
(BOD) (mg/l)	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
COD (mg/l)	BLQ	BLQ	BLQ	16.26 ± 0.1154	16.23 ± 0.2516	16.13 ± 0.1154
Nickel (mg/l)	BLQ	BLQ	BLQ	0.00133 ± 0.0005	0.0033 ± 0.00152	0.0023 ± 0.0005
Arsenic (mg/l)	0.004	0.004	0.004	0.003	0.003	0.003

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2025). Electric conductivity (EC) was higher in Karna lake as compared to Tilyar lake. The concentration of dissolved oxygen (DO) and water hardness were greater in Tilyar lake than in Karna lake. Biological oxygen Demand (BOD) and Chemical Oxygen Demand (COD) levels in Tilyar lake were below the quantification limit of 0.05 mg/L, however, COD in Karna lake from all the three sites found in range of 16 mg/L. Arsenic was detected in both the lakes, however, nickel was only quantified in the Karna lake.

Pattern of Bacterial distribution

From Karna lake, a total of 51 phyla, 67 classes, 137 orders, 284 families, 584 genera and 1031 species have been reported. In contrast, Tilyar Lake showcase a rich diversity with 35 phyla, 82 classes, 160 orders, 337 families, 659 genera and 1063 species identified. The taxonomic composition of each lake has been depicted in the Krona chart (Figure 3 A & B). There were 529 shared and unique taxa among both the lakes.

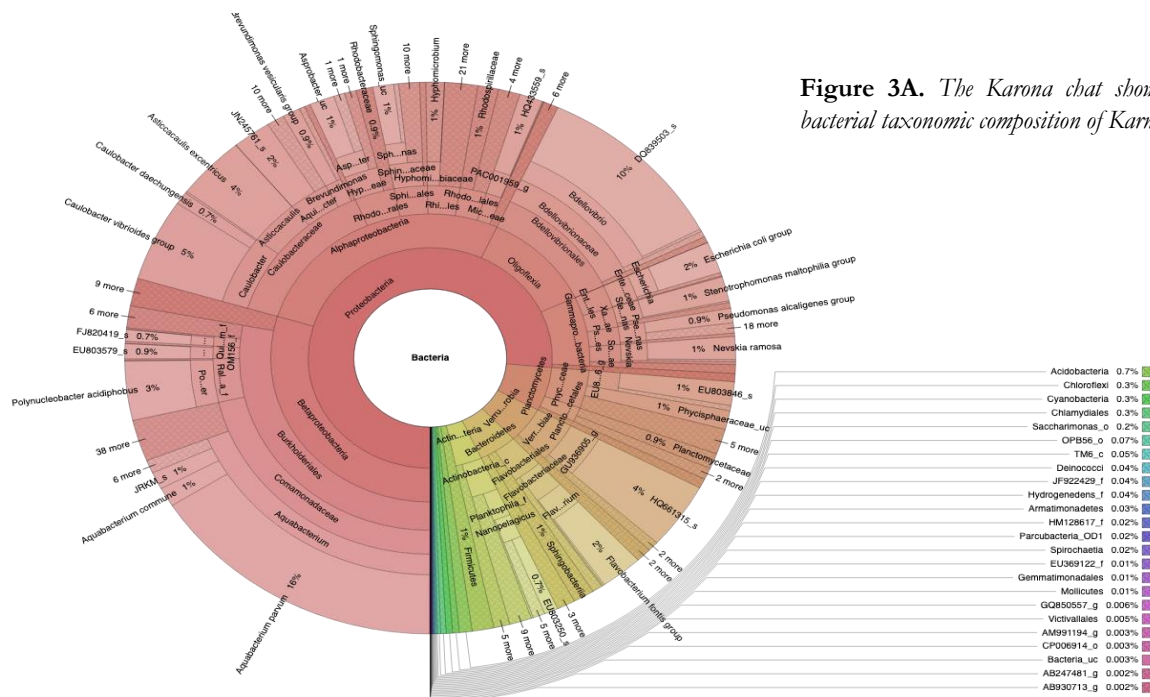


Figure 3A. The Krona chart showing full bacterial taxonomic composition of Karna Lake

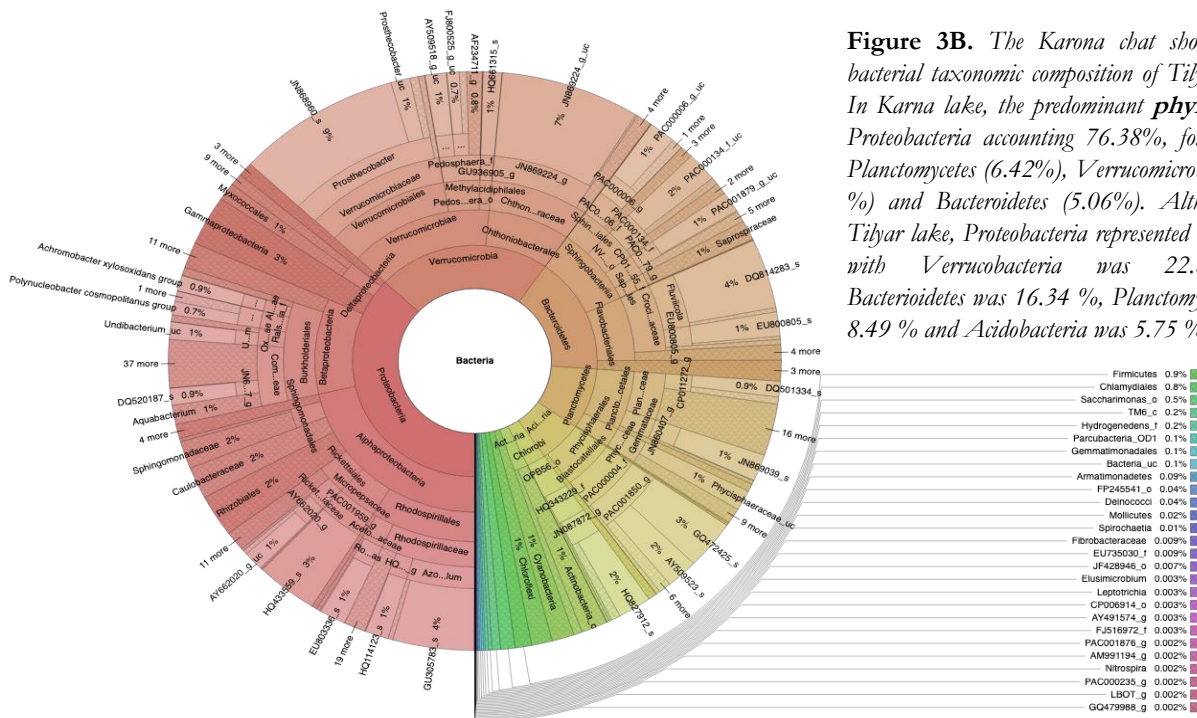


Figure 3B. The Krona chart showing full bacterial taxonomic composition of Tilyar Lake. In Karna lake, the predominant phylum was Proteobacteria accounting 76.38%, followed by Planctomycetes (6.42%), Verrucomicrobia (5.28%) and Bacteroidetes (5.06%). Although, in Tilyar lake, Proteobacteria represented 46.91% with Verrucobacteria was 22.87%, Bacteroidetes was 16.34%, Planctomycetes was 8.49% and Acidobacteria was 5.75%.

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The relative abundance of bacterial genera in Karna Lake included *Aquabacterium* (18.93%), *Bdellovibrio* (10.53%) and *Caulobacter* (5.66%), while Tilyar Lake contained *Prostheco bacter* (10.14%). A comparison of phyla and genera with more than 1% abundance is illustrated in the accompanying stacked histogram (Fig. 5).

The alpha diversity indices for both lakes were calculated as follows; for Karna lake, the Chao1 index was 1893.56, the Shannon index was 4.48 and the Simpson index was 0.047. For Tilyar lake, these values were 2291.80, 5.36 and 0.08 respectively (Fig. 5).

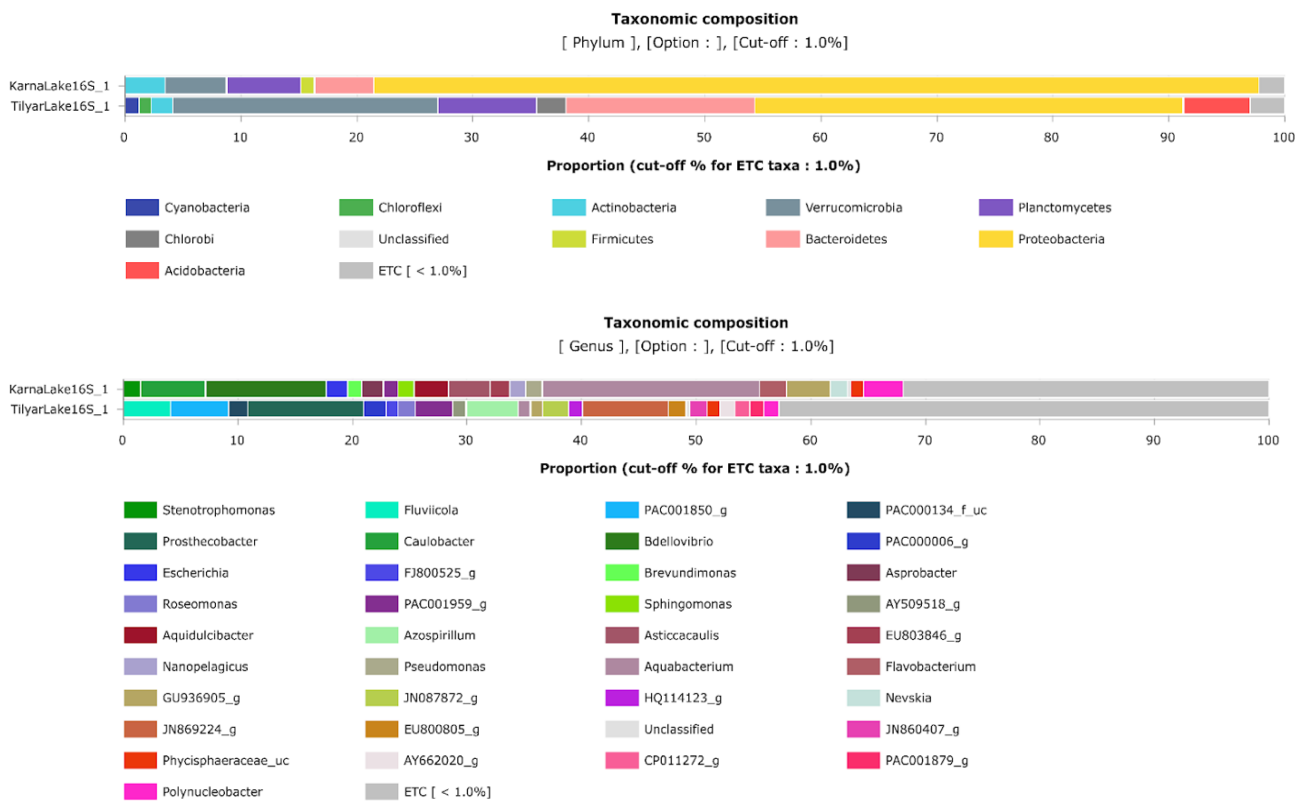


Figure 5. Taxonomic composition showing comparison of phylum and genus of Karna and Tilyar lake with >1% abundance.

Enzyme and pathways abundance

The analysis of relative enzyme abundance (>1%) based on 16S rRNA sequencing data for Karna lake and Tilyar lake showed with enzymes NADH: ubiquinone reductase (H⁺) translocating 1.2261 and 1.2495; histidine kinase 1.0105 and 0.8350; DNA-directed DNA polymerase 1.2055 and 1.2665; DNA helicase 1.1301 and 1.1677, respectively. The relative abundance (>1%) of different pathway were also analysed on the basis of 16S rRNA sequencing data from Karna and Tilyar lake that was L-isoleucine biosynthesis I (from threonine) pathway 0.8446 and 1.0213; aerobic respiration I (cytochrome c) pathway 1.6847 and 1.7588; cis-vaccenate biosynthesis 0.8272 and 1.0048; pyruvate fermentation to isobutanol 0.8574 and 1.0390; gondoate biosynthesis (anaerobic) pathway 0.8538 and 1.0210, respectively.

Discussion

The physico-chemical characteristics of water bodies are fundamental determinants of microbial diversity and community structure (Jordaan and Bezuidenhout, 2016). The current study found that both Tilyar and Karna lakes exhibit a basic pH, with Tilyar Lake displaying a higher level of alkalinity which may create a selective environment favouring alkaliphilic or alkali-tolerant microorganisms. For instance, the dominance of Verrucomicrobia (22.87%) and Bacteroidetes (16.34%) in Tilyar lake aligns with studies linking these phyla to alkaline habitats, where they contribute to organic matter degradation and nutrient cycling (Zhao et al., 2022). While, Karna lake with lower pH and higher conductivity, a proxy for dissolved ion concentration may explain the dominance of Proteobacteria (76.38%), a phylum known for metabolic versatility in diverse conditions, including ion-rich environments.

The observed alkaline nature of these lakes suggests facilitative environment for bacterial communities adapted by affecting enzymatic activities and membrane integrity (Banda et al., 2020; Pu et al., 2023). The relatively stable temperature profiles across both lakes can be attributed to the uniformity of seasonal sampling, thereby eliminating temperature-related variability in microbial composition. Total dissolved solids (TDS) and total suspended solids (TSS) were found to be noncomparable between both lakes, indicating a relatively similar load of organic and inorganic substances. The higher DO concentration in Tilyar Lake signifies better oxygenation, which may support a greater diversity of aerobic bacterial communities compared to Karna Lake. Interestingly, BOD and COD were below the quantification limits, also indicating relatively low levels of organic pollution. This suggests minimal anthropogenic contamination, supporting the fact that natural processes regulate water quality in these lakes (Vasistha and Ganguly, 2020). The higher level of both DO and water hardness in Tilyar lake, suggesting greater aerobic conditions and mineral content (e.g., calcium, magnesium). Elevated DO often supports aerobic metabolizers, reflected in Tilyar's higher abundance of pathways like aerobic respiration I (cytochrome c). Instead, Karna's lower DO might favour facultative anaerobes. The detection of arsenic in both lakes, along with nickel in Karna Lake, raises concerns regarding potential contamination sources, possibly stemming from agricultural runoff or industrial effluents (Gupta et al., 2021). Absence of detectable pesticide residues suggests a lack of recent agricultural contamination or effective degradation mechanisms within these ecosystems. These metals could exert selective pressures, enriching metal-resistant taxa like Proteobacteria harbour resistance genes, while inhibiting sensitive species (Xu et al., 2022). High-throughput sequencing approach used in the current study enabled a comprehensive assessment and the rarefaction curves indicated that sequencing depth was sufficient to capture the majority of microbial diversity, with both lakes demonstrating a saturation pattern. Results indicate that Karnal Lake harbours a slightly lower diversity of bacteria than Tilyar Lake, with fewer phyla, classes, orders, families, and genera. Despite Karna Lake harbouring more phyla (51 vs. 35), Tilyar Lake exhibited greater species-level richness (Chao1: 2291.80 vs. 1893.56) and diversity (Shannon: 5.36 vs. 4.48). This paradox, higher phylum-level diversity but lower species richness in Karna may reflect ecological stress (e.g., heavy metals, conductivi-

ty) limiting niche specialization, allowing only generalist phyla to thrive. Tilyar's stable might support finer niche partitioning, fostering species diversification within fewer phyla (Jiang et al., 2021). Proteobacteria dominated both lakes but were more prevalent in Karna (76.38%), likely due to their adaptability to variable conditions, including heavy metal presence. Subgroups like *Aquabacterium* (18.93% in Karnal) are biofilm-forming betaproteobacteria, potentially mitigating metal toxicity through extracellular polymeric substance (EPS) production (Nyoyoko, 2022). *Verrucomicrobia* and *Bacteroidetes* phyla thrived in Tilyar, often associated with polysaccharide degradation and oligotrophic conditions. The prominence of *Prostheco*bacter (10.14%), a stalked bacterium, suggests adaptation to nutrient-limited environments through surface attachment and resource scavenging. This compositional variation reflects differences in nutrient cycling, organic matter degradation, and habitat conditions - the lakes (Vesamäki et al., 2024; Zhu et al., 2022). The differences in dominant genera between the two lakes underscore the influence of environmental parameters α diversity indices (Chao1, Shannon, Simpson) position Tilyar lake as ecologically healthier, with higher richness and evenness. High diversity often correlates with ecosystem stability, resilience, and functional redundancy, traits critical for mitigating environmental perturbations. Karna's lower diversity, coupled with elevated conductivity and nickel levels, signals potential anthropogenic stress, possibly reducing its capacity to buffer disturbances. The Simpson index (0.08 in Tilyar vs. 0.04 in Karna) further supports this, as lower values indicate dominance by fewer species (Karna), an indicator of disturbed systems. Functional predictions based on 16S rRNA sequencing revealed distinct enzymatic and metabolic pathway abundances in both lakes. Tilyar's higher abundance of NADH: ubiquinone reductase (1.2495 vs. 1.2261) and DNA-directed DNA polymerase (1.2665 vs. 1.2055) suggests robust electron transport chains and replication activity, consistent with its aerobic, nutrient-cycling communities. This could be correlated with the higher DO levels observed in Tilyar Lake, promoting aerobic respiration and energy metabolism (Piatka et al., 2022). Karna's elevated histidine kinase (1.0105 vs. 0.8350) implies greater stress signalling, potentially linked to metal toxicity or ionic imbalances. This enzyme is also crucial for environmental sensing and adaptation, implying that bacterial communities in Karna Lake may possess a greater ability to respond to environmental fluctuations. DNA polymerase and DNA helicase, both essen-

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tial for DNA replication and repair, exhibited marginally higher abundance in Tilyar Lake, potentially contributing to enhanced microbial growth and survival. Metabolic pathway analysis revealed significant differences between the two lakes. L- isoleucine/L- valine biosynthesis pathways were enriched in Tilyar (1.0213 vs. 0.8446), suggesting active amino acid production, possibly supporting a diverse food web. Aerobic respiration-I abundance in Tilyar aligns with its higher DO, favouring oxidative phosphorylation. Cis-vaccenate and gondoate biosynthesis (anaerobic pathways) were scarce in Karnal, likely suppressed by aerobic conditions, whereas Tilyar's moderate levels hint at microniche anaerobiosis. These pathways are integral to microbial adaptation, survival, and biogeochemical cycling, reinforcing the ecological significance of bacterial profile in sustaining lake ecosystems (Porchas and Albores, 2017). Future studies employing shotgun meta-genomics and meta-bolomics could provide deeper insights into microbial functional traits and metabolic interactions. Additionally, long-term monitoring of microbial communities and environmental parameters will be crucial in assessing ecosystem health and developing conservation strategies.

Conclusions

Overall, higher microbial diversity and functional potential observed in Tilyar Lake suggest a more stable and ecologically resilient environment capable of withstanding environmental disturbances. While, dominance of a few specific taxa in Karnal Lake indicates potential environmental constraints that may limiting microbial diversity. The presence of arsenic and nickel in these lakes, albeit at low concentrations, necessitates further investigation into their sources and potential impacts on microbial and aquatic life. These results offer valuable insights into freshwater microbial ecology and highlighting the need of ongoing monitoring and conservation efforts to safeguard aquatic biodiversity and water quality. Overall, the study underscores the value of integrating physicochemical and microbial data for assessing aquatic health, guiding sustainable management strategies in rapidly urbanizing regions.

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