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Metagenomic analysis of bacterial communities of Wadi Namar Lake, Riyadh, Saudi Arabia

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ABSTRACT

Wadi Namar lake is a new touristic attraction area in the south of Riyadh. Human activities around the lake may lead to changes in water quality with subsequent changes in microenvironment components including microbial diversity. The current study was designed to assess possible changes in bacterial communities of the water at Wadi Namar Lake. Therefore, water samples were collected from three different locations along the lake: L1 (no human activities, no plants), L2 (no human activity, some plants) and L3 (human activities, municipal wastes and some plants). The total DNA of the samples was extracted and subjected to 16S rDNA sequencing and metagenomic analysis; water pH, electrical conductivity (EC), total dissolved solids (TDS) as well as the concentration of Na⁺, K⁺, Cl⁻ and total N were analysed. Metagenomic analysis showed variations in relative abundance of 17 phyla, 31 families, 43 genera and 19 species of bacteria between the locations. Proteobacteria was the most abundant phylum in all locations; however, its highest abundance was in L1. Planctomycete phylum was highly abundant in L1 and L3, while its abundance in L2 was low. The phyla Acidobacteria, Candidatus Saccharibacteria, Nitrospirae and Chloroflexi were associated with high TDS, EC, K⁺ and Cl⁻ concentrations in L3; various human activities around this location had possibly affected microbial diversity. Current study results help in recognising the structure of bacterial communities at Wadi Namar Lake in relation to their surroundings for planning to environment protection and future restoration of affected ecosystems.

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1. Introduction

Water bodies are natural boundaries between humans and various types of organisms; they are prone to contamination from numerous sources with consequent alterations in water status and quality. Water subjected to organic and inorganic contaminants might become a source of pollution threatening the surrounding ecosystem: wastes, such as agricultural, industrial, and municipal wastes that find a way to water sources lead to alterations in water quality; therefore, decreased water quality and alteration in nutrient cycles as consequences of pollution are

important concerns in the aquatic environment (Isaza et al., 2020). Furthermore, water contaminants, including microbes, could be of high risk for human health directly, or indirectly through other environmental elements, as water quality is a main contributor to human health. Lamb et al. (2017) noted the connection between human activities and the diversity of aquatic organisms including microflora, corals and seagrass as well as the significance of the seagrass to human health. One of the essential water resources for humans are the lake's networks in which microorganisms are important components. Microbes with varying metabolic features might help in better degradation of water pollutants, such as organic matters. In addition, microbial communities in lake networks could be appropriate bioresources for industry, agriculture, and relevant divisions (Yadav et al., 2018). Bacteria is considered the most important component of the microbial community in aquatic ecosystems that is responsible for vital global ecological processes (Han et al., 2019). Bacterial type and concentration can provide information about the water quality and contamination source. Furthermore, bacterial community construction in polluted water could assist in defining the

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influence of human activities on water ecologies. Generally, low water quality is usually indicated by the detection of *Escherichia coli* or coliforms; however, the development of a direct water monitoring system is important. For a better understanding of the microbial community structure and function, metagenomic studies could be undertaken since they provide better insight of the present microbes. Metagenomics is a means for monitoring the natural composition of microbiota by using direct DNA separation from a natural sample containing various microbiota to identify the exact microbial structure (Ngara and Zhang., 2018; Ahmad et al., 2019). It could also be a significant dataset that represents how variations in microbial communities arise due to variations in water characteristics and pollution levels. Many recent studies identified microbial communities in water systems in relation to water quality (Ahmad et al., 2021; Betiku et al., 2021; Fournier et al., 2021; Zhang et al., 2021). For making metagenomic investigation more available through targeted metagenomics, the Next Generation Sequencing (NGS) has been considered (Amrane and Lagier, 2018). Lake microbiome links to many water characteristics, such as temperature, pH and nutrient concentration as well as anthropogenic actions (Maguvu et al., 2020). In the present study, 16S metagenomic analysis was used to investigate the bacterial community of Wadi Namar Lake - a new tourist attraction area located in the Riyadh region, Saudi Arabia, as common picnic spot, around which pollution by municipal wastes was noticed. Three different locations along the lake were sampled to investigate water chemical properties and possible variations in bacterial communities in relation to the surroundings. Two locations lack human activities, and one was near human activities. Such connection would help in better planning to environment protection and management as well as future restoration or improvement of affected ecosystems.

2. Materials and methods

2.1. Study site and sample collection

The water samples were collected from three locations in Wadi Namar Lake, Riyadh, SA in May 2020: L1 (24°33'57.0"N 46°40'20.5"E) was far from human activities and no plants were observed, L2 (24°34'06.4"N 46°40'22.2"E) was covered by different small grasses, but also far from human activities and L3 (24°34'16.7"N 46°40'34.3"E) is near human activities where municipal wastes and small grasses were noticed. Study locations are presented in Fig. 1. From each location, five liters were collected. The samples were sent to the microbiology and molecular biology laboratory of the Unit of Animal Health and Safety of Animal Products, UofK, Sudan for bacteriological investigation and to the Chemistry Laboratory of the Department of Biology, KSU, SA for chemical analysis.

2.2. DNA extraction and 16S metagenomic analysis

Genomic DNA from the water sample of each location was extracted in two separate experiments as follows: One litre of water was filtered through 0.2 µm filter (Merck Millipore, Darmstadt, Germany). The filter paper was then cut into pieces and put into 2 ml Eppendorf tube. DNA was extracted from the filter paper using the MagMax Microbiome Ultra Nucleic Acid Isolation Kit (Applied Biosystems, USA) according to manufacturer's instructions. Briefly, 800 µL of the lysis buffer were added together with the beating beads to the filter paper and mixed using Intelli-Mixer's™ (ELMI, Riga, Latvia) at room temperature for 30 min followed by centrifugation at 14000× g for 2 min. The DNA in the supernatant was purified using the binding beads solution

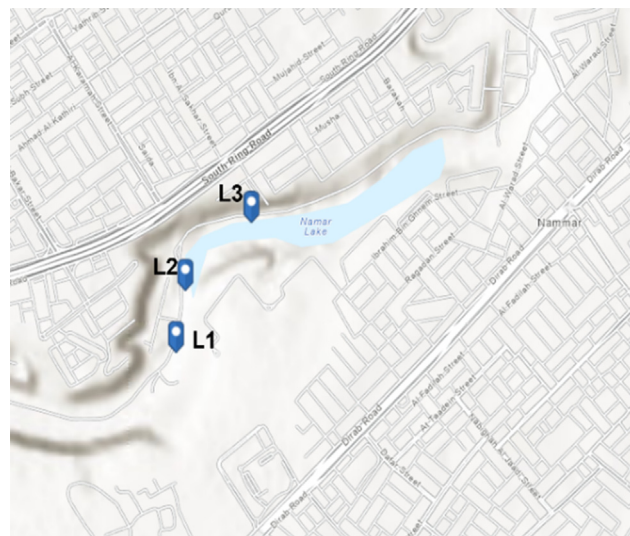


Fig. 1. Map of Wadi Namar Lake displaying the three locations (L1, L2 and L3) of water samples collection. The map was prepared using ArcGIS programme (Esri, Redlands, California, U.S.).

prepared using the kit chemicals, followed by washing steps with the washing buffer and 80% alcohol. DNA elution was made by heating at 75 °C for 5 min with the elution buffer followed by pelleting the binding beads against magnet rack; the clear solution contained the DNA. The DNA concentration was measured using Qubit 4.0. fluorometer and Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, Waltham, MA, USA). A total of 25 ng DNA was sent to a commercial company (Microsynth, Balgach, Switzerland) for 16S rDNA sequencing and metagenomic analysis.

2.3. PCR amplification

A two-step protocol PCR amplification of the V34 of the 16S rRNA gene was used. In a first step, the locus-specific sequence (~460 bp) was amplified using the primers 341F_ill (5' CCTACGGGNGGCWGCAG 3') and 802R_ill (5' GACTACHVGGGTATCTAATCC 3') as described by Klindworth et al. (2013). In the second step, the Illumina sequencing adaptors and indices were added.

2.4. NGS sequencing

Sequencing was performed on the Illumina MiSeq system, which allows high throughput profiling at low cost, supporting read lengths up to 2x300 bp.

2.5. Chemical analysis

The pH was measured by pH-meter, EC was measured using EC meter, Na and K were measured using flamephotometer. Cl and total N were measured using a colorimetric method flowing digestion (Fournier et al., 2021). The total dissolved solids were measured by a multi- 350 probe analyser (Merck, Darmstadt, Germany) according to a method described elsewhere (Maguvu et al., 2020).

2.6. Statistical and bioinformatics analysis

Means and standard deviations were calculated using MICROSOFT EXCEL 2019, and GraphPad Prism Software version 9.1 (San Deigo, CA, USA) was used for statistical analysis and creation of

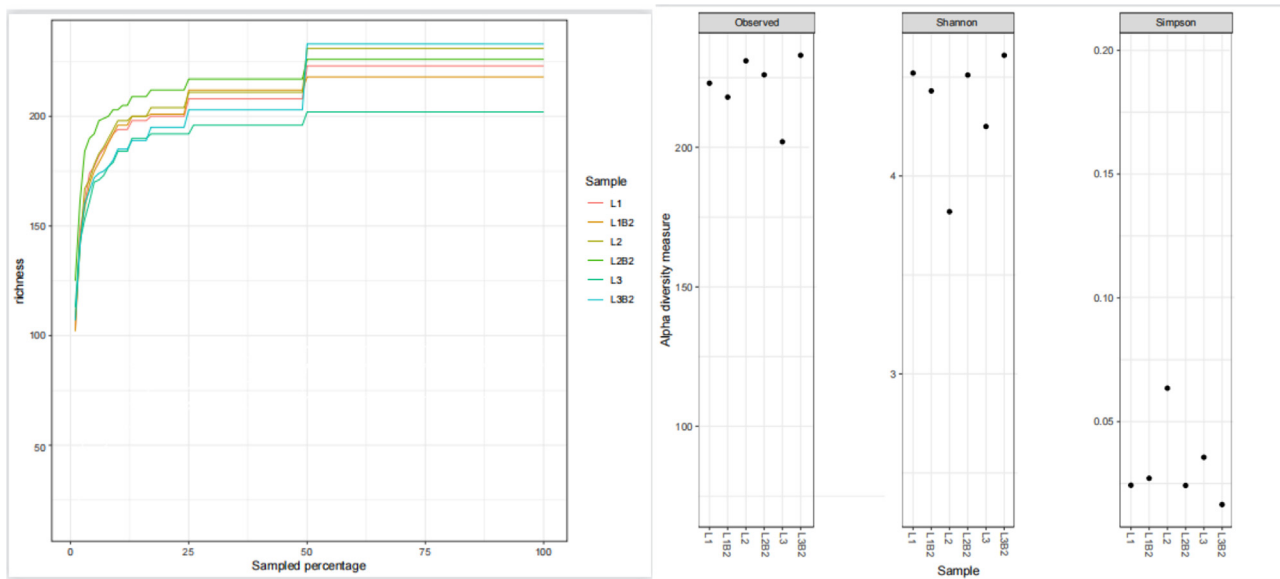


Fig. 2. Richness and alpha diversity results of the microbial sequence data of the water samples collected from the three different locations (L1, L2 and L3) of Wadi Namar Lake, Riyadh, SA.

the heatmaps. For bioinformatics analysis, the quality of the sequence data was assessed by the FastQC tool designed by Simon Andrews at the Babraham Institute in Cambridge (UK) and aggregated quality reports were made by the MultiQC tool (Ewels et al., 2016). Alpha diversity measures and richness were calculated with the R programme package v3.6.0 (The R Foundation for Statistical Computing, Vienna, Austria). Assignment of generated sequences to operational taxonomic units (OTU) and taxonomic classification were made to online available databases.

3. Results

3.1. Taxonomic classification of Wadi Namar water bacterial communities

Metagenomic analysis is a powerful tool in environmental studies to identify the microbial community at specific locations and might be helpful in understanding the interaction between microbial communities and the environment.

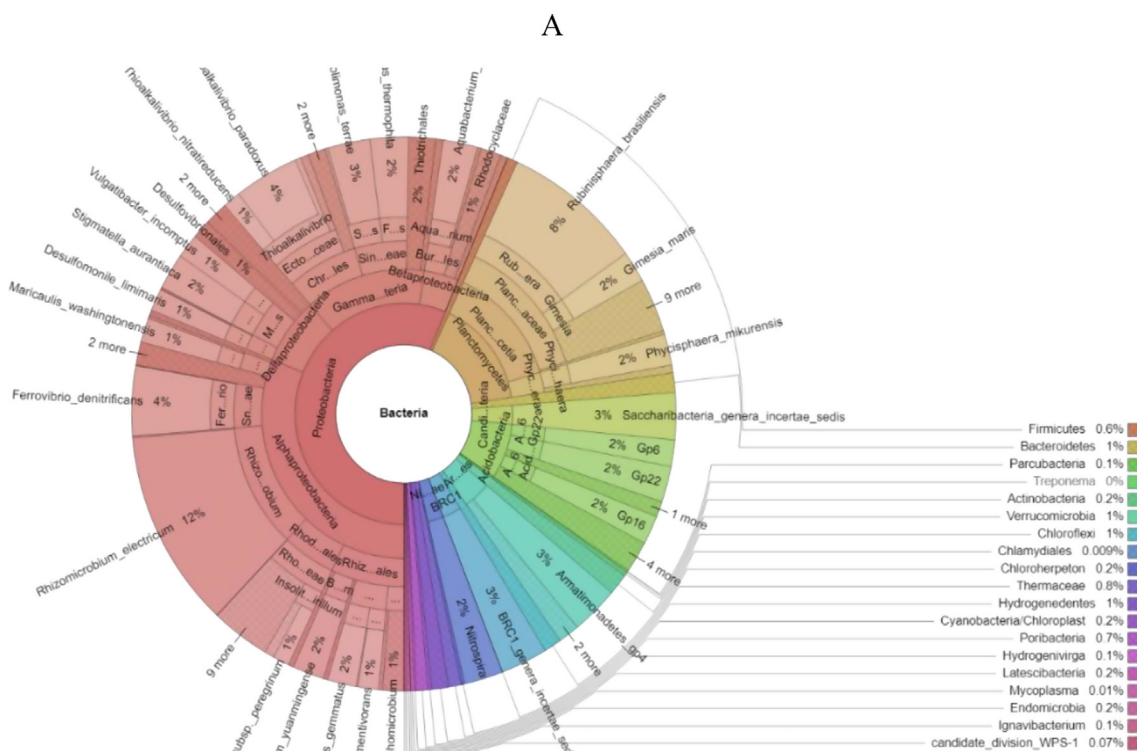
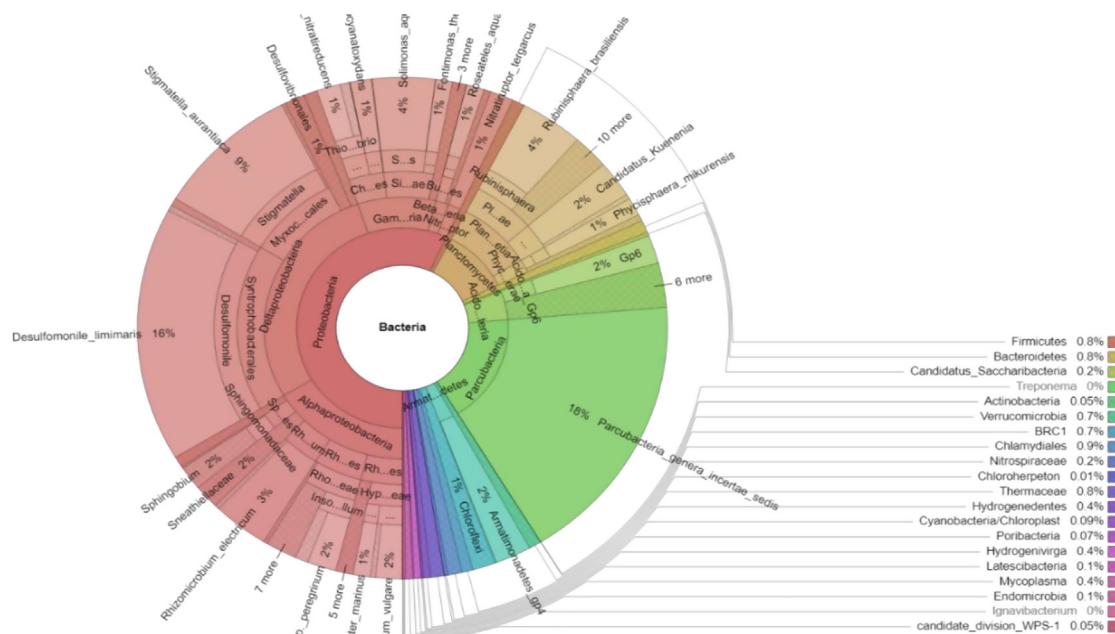


Fig. 3. Krona charts representation of metagenomic and taxonomic classification of bacterial communities in water samples collected from three different locations: L1 (A), L2 (B) and L3 (C), at Wadi Namar Lake, Riyadh, SA. Indicated numbers are the bacterial abundance (%).

B



C

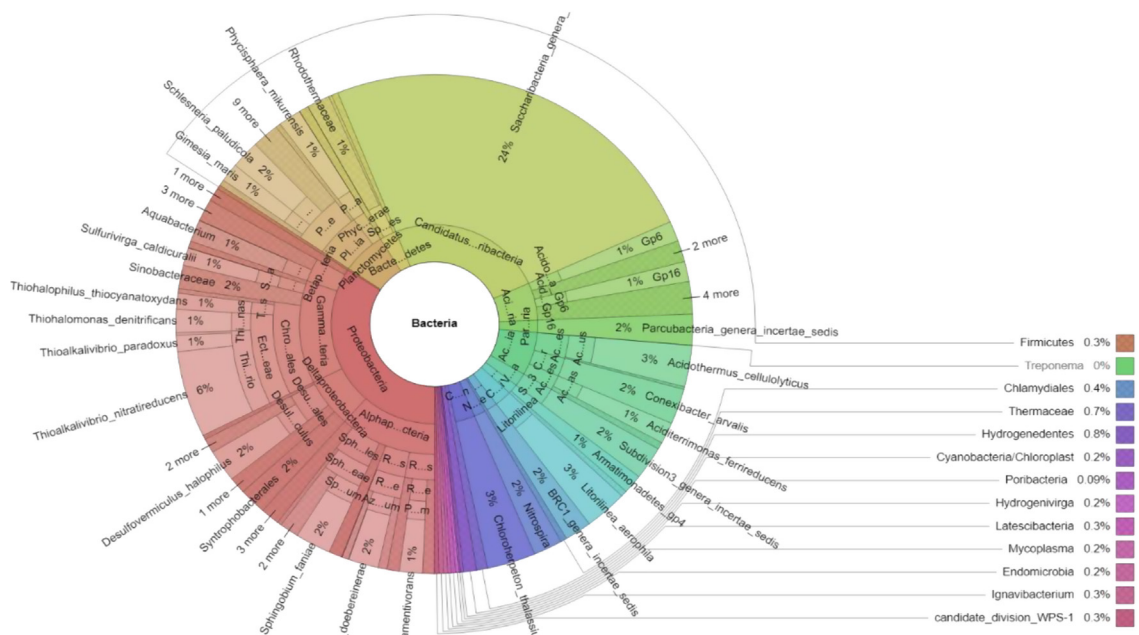


Fig. 3 (continued)

The total number of reads in the samples was between 22.0 K and 42.0 K and the assigned OTU was between 202 and 233. Bioinformatics tools used for analysis showed high quality of sequences obtained by Illumina NGS platform (Fig. 2). Microbial diversity at different taxa levels of the water samples collected from the study site at the three different locations are presented in Figs. 3–8. Krona charts (Fig. 3), the eye-catching approach, show the relative abundance of taxa in relation to numerous points of the hierarchy

in the three samples. The number of reads were 22026, 41,966 and 31,977 in L1, L2 and L3 respectively.

Variation between the locations in bacterial abundance is obvious. The significantly abundant OTU were Alphaproteobacteria class, Proteobacteria phylum, Planctomycetaceae family, Ferrovibrio genus and the Ferrovibrio denitrificans species. Abundance patterns of the above-mentioned taxa were consistent; however, all were highly abundant in L1 compared to L2 and L3. At the phylum level,

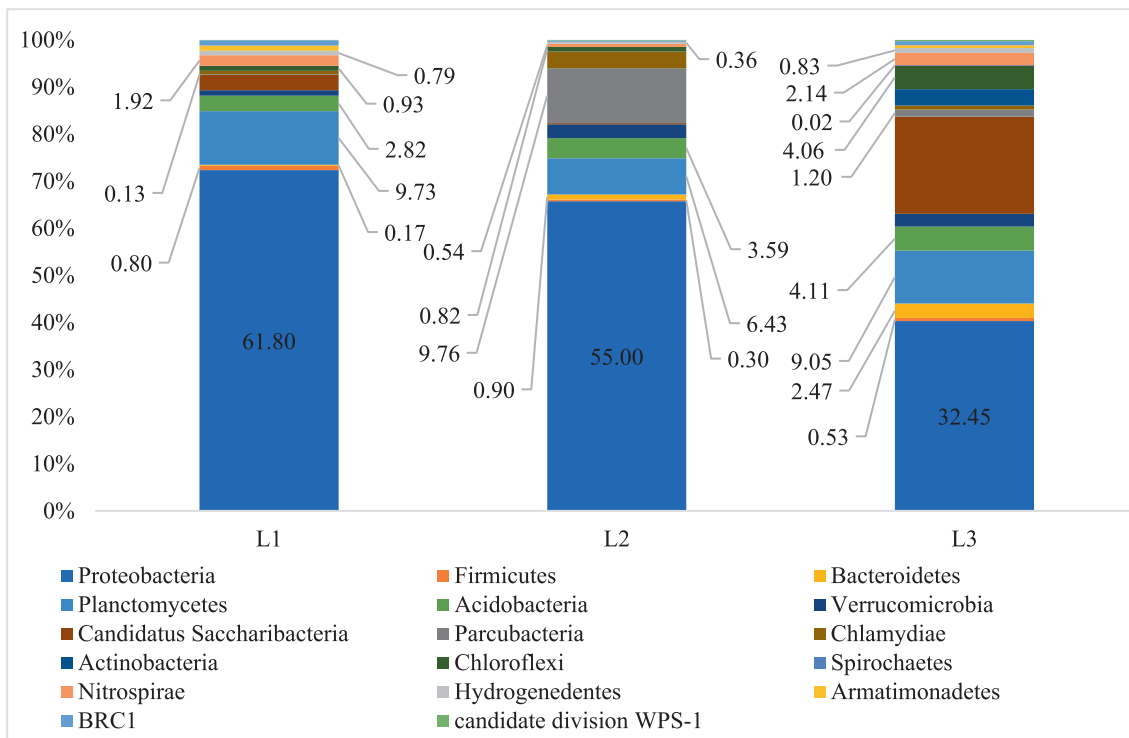


Fig. 4. Relative abundance (%) of the bacterial phyla identified by 16S metagenomics in water samples taken from three different locations (L1, L2 and L3) of Wadi Namar Lake, Riyadh, SA. Numbers indication the relative abundance of the top.

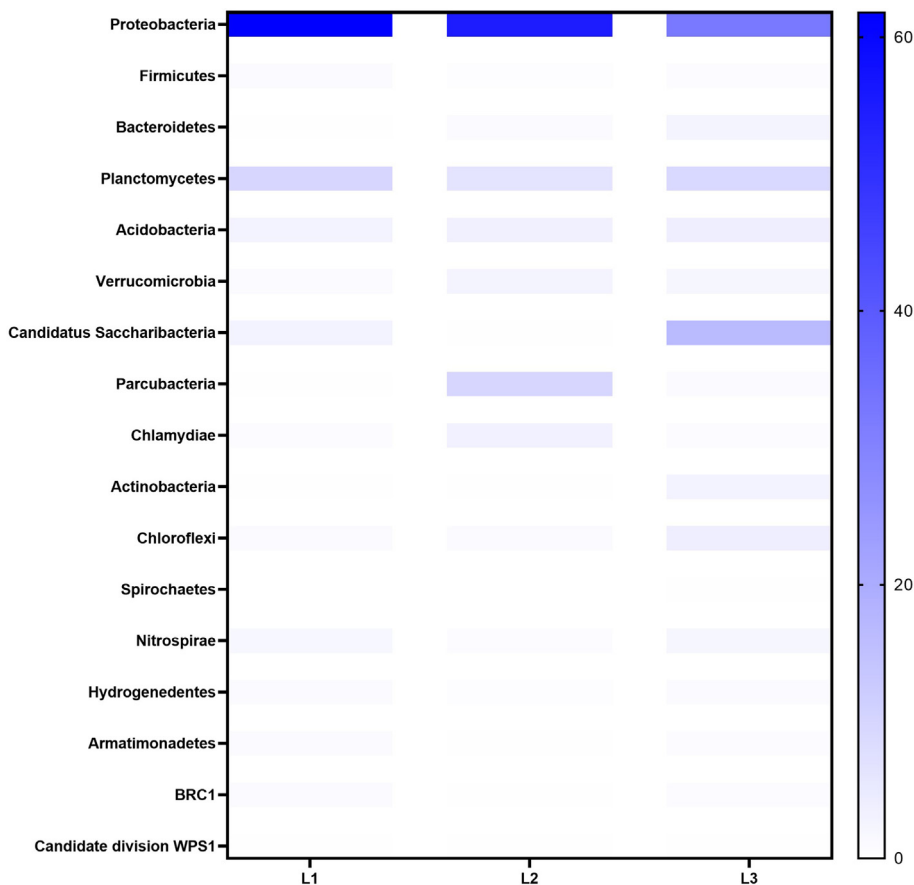


Fig. 5. Heat map representing relative abundance of the bacterial phyla identified by 16S metagenomic sequencing in water samples taken from three different locations (L1, L2 and L3) of Wadi Namar Lake, Riyadh, SA.

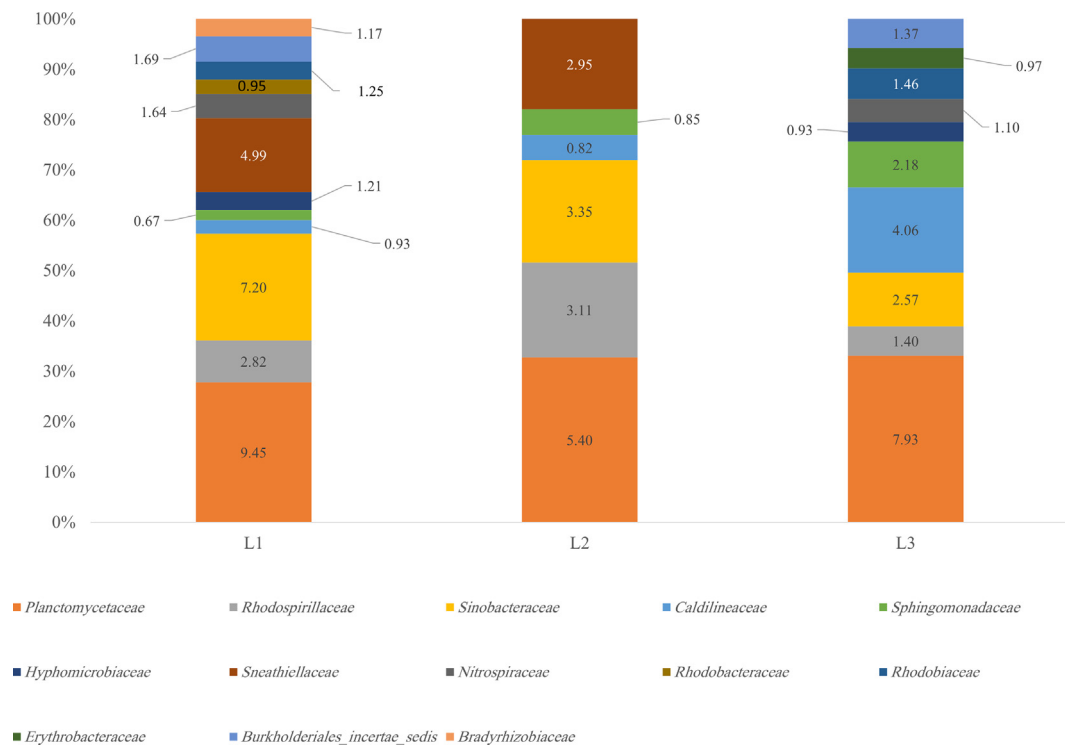


Fig. 6. Relative abundance (%) of the bacterial families identified by 16S metgenomic sequencing in water samples taken from three different locations (L1, L2 and L3) of Wadi Namar Lake, Riyadh, SA.

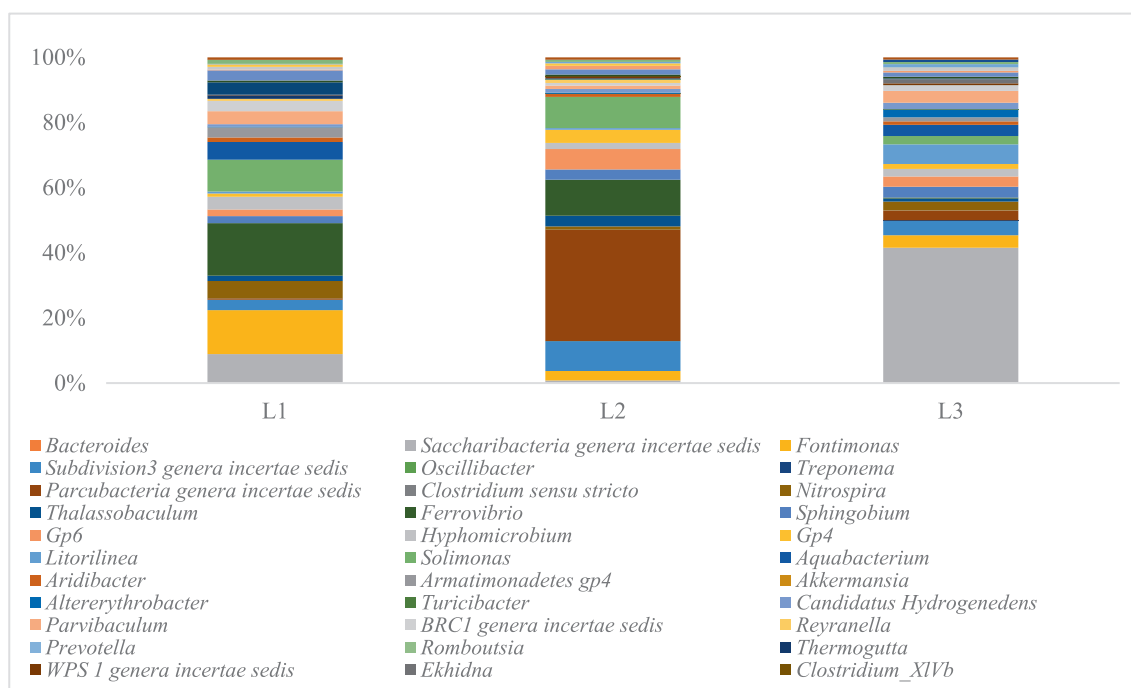


Fig. 7. Relative abundance (%) of the bacterial genera identified by 16S metgenomic sequencing in water samples taken from three different locations (L1, L2 and L3) of Wadi Namar Lake, Riyadh, SA.

the unassigned sequences were increasing from L1 to L3 (14.6 to 19.5%). The community structure observed showed that bacteria assigned to Proteobacteria were the most abundant in all water samples, accounting for 61.8% in L1, decreasing to 55% in L2 and sharply decreasing in L3 to 32.45% (Fig. 4). Candidatus Saccharibac-

teria phyla ranked second in abundance in L3 with 16.63%, but it sharply decreased to 2.8% and 0.2 % in L1 and L2, respectively. Planctomycetes ranked second in abundance in L1 and third in L3 (9.73% and 9.05%, respectively), but its abundance was about 30% lower in L2. Acidobacteria increased in abundance gradually

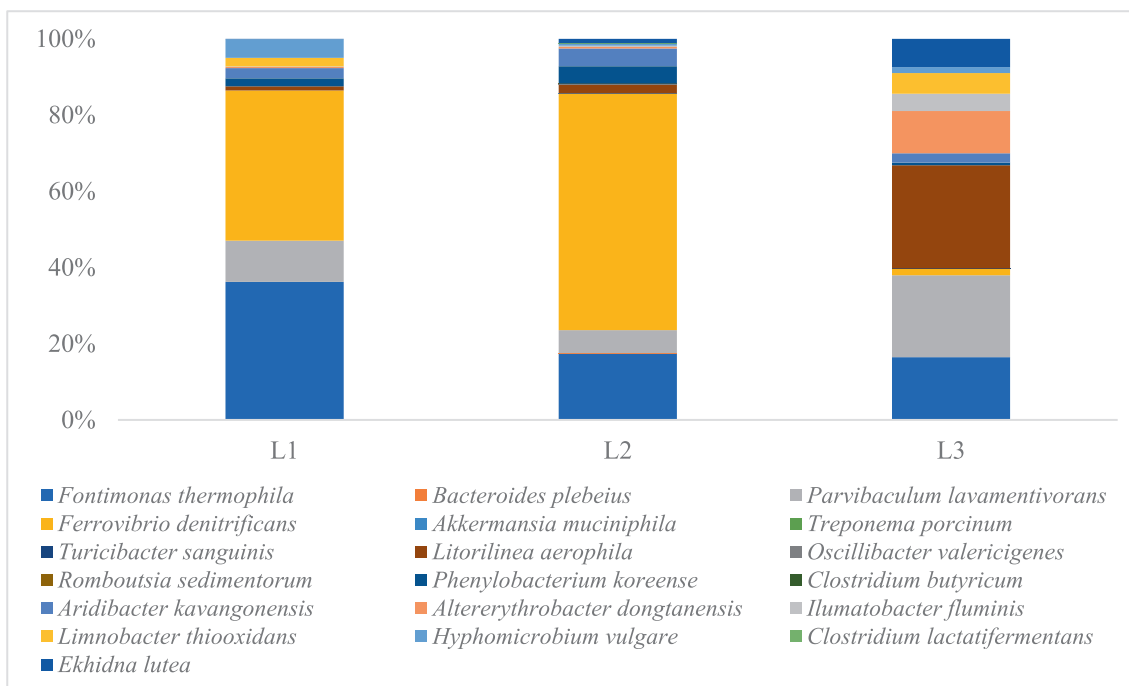


Fig. 8. Relative abundance (%) of the bacterial species identified by 16S metagenomic sequencing in water samples taken from three different locations (L1, L2 and L3) of Wadi Namar Lake, Riyadh, SA.

from L1 to L3, Chloroflexi and Nitrospirae were relatively more abundant in L3 than in L1 and L2. However, the phylum Armatimonadetes was only noted in L1 and L3. The top 10 phyla are presented in Fig. 5 and Table 1.

Regarding the family level, unassigned bacteria were higher in L2 (80.2%) and relatively lower in L1 (64.45%). The number of families that were identified was 31 and the families with abundance about 1% or more are shown in Fig. 6. The top 10 are presented in Table 2, among which 7 families had abundance between 0.9 and 1.7%. *Planctomycetaceae* was the most abundant family in all locations: 9.45% in L1, about 40% lower in L2 (5.4%) than in L1. *Planctomycetaceae*, *Sinobacteraceae* and *Sneathiellaceae* were the most abundant in L1. *Sinobacteraceae* and *Sneathiellaceae* decreased gradually from L1 to L3. *Rhodospirillaceae* is the only family that is abundant in L2 more than in L1 and L3. All families with low abundance (*Bradyrhizobiaceae*, *Burkholderiales incertae sedis*, *Erythrobacteraceae*, *Rhodobacteraceae*, *Rhodobiaceae*, *Nitrospiraceae* and *Hyphomicrobiaceae*) were very low in L2 (Table 2). Abundance of *Caldilineaceae* and *Sphingomonadaceae* was comparatively higher in L3 than in both L1 and L2.

Table 1
Relative abundance of the top 10 bacterial phyla in water samples taken from three different locations (L1, L2 and L3) of Wadi Namar Lake, Riyadh, SA. Data are presented as mean % ±SD.

Phylum	L1	L2	L3
Proteobacteria	61.8 ± 9.9 ^a	55.0 ± 2.0 ^b	32.5 ± 3.2 ^c
Planctomycetes	9.7 ± 4.8 ^a	6.4 ± 0.0 ^b	9.0 ± 5.2 ^a
Acidobacteria	2.8 ± 1.5 ^b	3.6 ± 0.8 ^{ab}	4.1 ± 1.0 ^a
Candidatus Saccharibacteria	2.8 ± 0.0 ^b	0.2 ± 0.0 ^b	16.6 ± 9.4 ^a
Nitrospirae	1.9 ± 0.6 ^a	0.5 ± 0.5 ^b	2.1 ± 0.5 ^a
Verrucomicrobia	1.0 ± 0.3 ^c	2.5 ± 2.4 ^a	2.2 ± 0.1 ^b
BRC1	1.0 ± 0.5 ^a	0.2 ± 0.1 ^c	0.7 ± 0.5 ^b
Armatimonadetes	1.0 ± 0.4 ^a	0.0 ± 0.0	0.5 ± 0.2 ^b
Chloroflexi	0.9 ± 0.2 ^b	0.8 ± 0.4 ^b	4.1 ± 1.9 ^a
Hydrogenedentes	0.8 ± 0.1 ^a	0.4 ± 0.2 ^b	0.8 ± 0.2 ^a

Table 2
Relative abundance of the top 10 bacterial families identified by 16S metagenomic sequencing in water samples taken from three locations (L1, L2 and L3) of Wadi Namar Lake, Riyadh, SA. Data presented as mean % ±SD.

Family	L1	L2	L3
<i>Planctomycetaceae</i>	9.5 ± 4.7 ^a	5.4 ± 0.1 ^b	7.9 ± 4.1 ^{ab}
<i>Sinobacteraceae</i>	7.2 ± 3.2 ^a	3.3 ± 2.1 ^b	2.6 ± 1.5 ^b
<i>Sneathiellaceae</i>	5.0 ± 1.3 ^a	3.0 ± 3.0 ^b	0.1 ± 0.1 ^c
<i>Rhodospirillaceae</i>	2.8 ± 1.5 ^a	3.1 ± 2.5 ^a	1.4 ± 0.9 ^b
<i>Burkholderiales incertae sedis</i>	1.7 ± 0.5 ^a	0	1.4 ± 0.1 ^a
<i>Nitrospiraceae</i>	1.6 ± 0.5 ^a	0.3 ± 0.3 ^c	1.1 ± 0.0 ^b
<i>Rhodobiaceae</i>	1.2 ± 0.0 ^a	0.3 ± 0.0 ^b	1.5 ± 0.0 ^a
<i>Hyphomicrobiaceae</i>	1.2 ± 0.7 ^a	0.5 ± 0.3 ^b	0.9 ± 0.4 ^a
<i>Bradyrhizobiaceae</i>	1.2 ± 1.0	0	0.1 ± 0.0
<i>Caldilineaceae</i>	0.9 ± 0.2 ^b	0.8 ± 0.4 ^b	4.1 ± 1.9 ^a

Table 3
Relative abundance of the top 10 bacterial genera identified by 16S metagenomic sequencing in water samples taken from the three different locations (L1, L2 and L3) of Wadi Namar Lake, Riyadh, SA. Data presented as mean % ±SD.

Genus	L1	L2	L3
<i>Ferrovibrio</i>	5.0 ± 1.3 ^a	3.0 ± 3 ^b	0.1 ± 0.1 ^c
<i>Fontimonas</i>	4.2 ± 2.7 ^a	0.8 ± 0.3 ^c	1.5 ± 1.1 ^b
<i>Solimonas</i>	3.0 ± 0.5 ^a	2.6 ± 1.8 ^b	1.0 ± 0.4 ^c
<i>Saccharibacteria genera incertae sedis</i>	2.8 ± 0 ^b	0.2 ± 0 ^c	16.6 ± 9.4 ^a
<i>Aquabacterium</i>	1.7 ± 0.5 ^a	0	1.4 ± 0.1 ^b
<i>Nitrospira</i>	1.6 ± 0.5 ^a	0.3 ± 0.3 ^c	1.1 ± 0 ^b
<i>Parvibaculum</i>	1.2 ± 0 ^b	0.3 ± 0 ^c	1.5 ± 0 ^a
<i>Hyphomicrobium</i>	1.2 ± 0.7 ^a	0.5 ± 0.3 ^c	0.9 ± 0.4 ^b
<i>Bradyrhizobium</i>	1.2 ± 1 ^a	0	0.1 ± 0 ^b
<i>Subdivision3 genera incertae sedis</i>	1.0 ± 0.3 ^c	2.5 ± 2.4 ^a	1.8 ± 0.5 ^b

At the genus level, variations in abundance were also noted between the three locations. The number of identified genera was 43 (Fig. 7), where *Ferrovibrio*, *Fontimonas* and *Solimonas* were highly abundant at L1. However, *Saccharibacteria genera incertae sedis* showed significantly high abundance at L3 compared to L1 and L2 (Table 3). *Aquabacterium* and *Bradyrhizobium* were not

Table 4

Relative abundance of the top 10 bacterial species identified by 16S metagenomic sequencing in water samples taken from three different locations L1, L2 and L3) of Wadi Namar Lake, Riyadh, SA.

Species	L1	L2	L3
<i>Ferrovibrio denitrificans</i>	4.5 ± 1.5 ^a	2.8 ± 2.8 ^b	0.1 ± 0.1 ^c
<i>Fonitimonas thermophila</i>	4.2 ± 2.7 ^a	0.8 ± 0.3 ^c	1.1 ± 1.0 ^b
<i>Parvibaculum lavamentivorans</i>	1.2 ± 0.0 ^b	0.3 ± 0.0 ^c	1.5 ± 0.0 ^a
<i>Hyphomicrobium vulgare</i>	0.6 ± 0.6 ^a	0	0.1 ± 0.1 ^b
<i>Aridibacter kavangonensis</i>	0.3 ± 0.1 ^a	0.2 ± 0.1 ^b	0.2 ± 0.1 ^b
<i>Limnobacter thiooxidans</i>	0.3 ± 0.4 ^b	0	0.4 ± 0.5 ^a
<i>Phenylobacterium koreense</i>	0.2 ± 0.1 ^a	0.2 ± 0.1 ^a	0
<i>Litorilinea aerophila</i>	0.1 ± 0.1 ^b	0.1 ± 0.0 ^b	1.8 ± 0.5 ^a
<i>Altererythro bacter dongtanensis</i>	0	0	0.8 ± 1.0 ^a
<i>Ekhidna lutea</i>	0	0	0.5 ± 0.1 ^a
<i>Ilumatobacter fluminis</i>	0	0	0.3 ± 0.2 ^a

Figures with different letters are significantly different ($P > 0.05$).

Table 5

Chemical composition of water samples taken from three locations of Wadi Namar Lake, Riyadh, SA.

Measure	Location		
	L1	L2	L3
pH	8.31 ± 0.2 ^a	7.75 ± 0.1 ^c	7.90 ± 0.1 ^b
EC (dS.m ⁻¹)	5.19 ± 0.1 ^b	5.20 ± 0.2 ^b	5.30 ± 0.3 ^a
Total dissolved solids (TDS) mg/L	3321.6 ± 6.3 ^c	3328.0 ± 7 ^b	3392.0 ± 8.3 ^a
Na ⁺ (meq L ⁻¹)	32.30 ± 0.7 ^a	28.39 ± 0.8 ^b	25.68 ± 0.5 ^c
K ⁺ (meq L ⁻¹)	0.43 ± 0.1 ^c	0.47 ± 0.1 ^b	0.52 ± 0.1 ^a
Cl ⁻ (meq L ⁻¹)	26.33 ± 0.3 ^b	26.08 ± 0.5 ^b	27.00 ± 0.5 ^a
Na ⁺ and Cl ⁻ ratio	1.23	1.1	0.95
Total N (mg/100 ml)	0.03 ± 0.01 ^b	0.07 ± 0.01 ^a	0.03 ± 0.01 ^b

Figures with different letters are significantly different ($P > 0.05$).

noticed in L2. Subdivision 3 genera *incertae sedis* showed significantly high abundance at L2 compared to L1 and L3 (Table 3).

On the other hand, abundance in relation to species showed that unassigned bacteria were higher in L2 (95.5%) and decreased gradually in L3 (93.2%) and L1 (88.5%). The number of species identified was 19 (Fig. 8). *Ferrovibriodenitrificans* was the most abundant species in samples from L1 and L2 accounting for 4.5% and 2.8%, respectively, while it was only 1% in L2 (Fig. 8, Table 4). *Fonitimonas thermophila* ranked second in abundance in L1. *Litorilinea aerophila*, *Parvibaculum lavamentivorans*, *Hyphomicrobium vulgare*, *Limnobacter thiooxidans*, *Altererythro bacter dongtanensis*, *Ekhidna lutea* and *Ilumatobacter fluminis* were significantly higher in abundance in L3, with the last three identified in L3 only. In addition, *Hyphomicrobium vulgare* and *Limnobacter thiooxidans* were not detected in L2, while *Phenylobacterium koreense* was not noticed in L3.

3.2. Chemical analysis of the water samples

The chemical composition of water varied between the different locations of the study site. Higher pH and Na⁺ were found in L1 than in L2 and L3, while EC, TDS, K⁺ and Cl⁻ values were higher in L3 than those in L1 and L2. Finally, the total N was twice higher in L2 than in the other two locations (Table 5).

4. Discussion

Microbial structures or communities, basically bacteria, of the water system contribute significantly to the aquatic ecosystem due to their role in biogeochemical processes (Han et al., 2019). Such communities have a high diversity level that enhances their function and stability (Lu et al., 2020). However, the abundance

of microbial communities is highly affected by their microenvironment as well as other ecological factors (Gibbons et al., 2014). In the current study, the possible effect of different microenvironments on the bacterial structure at Wadi Namar Lake was investigated. The locations were chosen based on variations in surroundings that might affect their microenvironment. Metagenomic analysis results indicated variations in the composition at taxonomic levels of bacterial communities across the three different locations of the lake that could be related to the surrounding conditions in each location. Generally, variations at the phylum level might provide a better indication of community structure shifts in response to changes in the microenvironments along Wadi Namar Lake. Bacterial communities of low abundance were in total the main drivers of general responses at phylum level (Dawson et al., 2017). Therefore, phylum-level will be considered here for further interpretation. Proteobacteria, Planctomycetes, and Acidobacteria were the most dominant phyla in the current investigation. These phyla represent bacterial phyla in water system (Zhang et al., 2018). Proteobacteria was the most populating phyla in the three studied locations with different levels of abundance. Proteobacteria is a fast-growing microbial community with high ability in some biogeochemical processes, therefore, adapted well to high nutrients environments (Krishna et al., 2020). A recent metagenomic study by Ahmad et al. (2021) on Himalayan urban freshwater lake, found Proteobacteria as the main abundant phylum in all study locations and its highest abundance was noted in the location where human interference was observed. Alpha/Beta/Gamma-proteobacteria comprise families of N-fixing bacteria (Marín and Arahal, 2014) and favour high pH (Núñez Salazar et al., 2020). Furthermore, such phylum has been reported to have high abundance in low saline environments, which correlated with low EC (Smith et al., 2002). Therefore, low EC beside high pH could be good explanations for the higher abundance of Proteobacteria in L1 than in the other two locations. Furthermore, Siles and Margesin (2016) reported a negative correlation between microbial diversity and EC when bacterial, archaeal, and fungal communities were identified in alpine forest soils. On the other hand, it was noted that this phylum was the main taxon in the water that suffered anthropogenic contamination (Yadav and Sharma, 2019). Similarly, Proteobacteria was the main phylum in L3 where the continuous discharge of human waste was noted; however, its relative lower abundance in L1 and L2 could be related to the low anthropogenic contamination.

The second most abundant phylum was Planctomycetes, which showed higher abundance in L1 and L3 than in L2. Planctomycetes and Proteobacteria are non-cyanobacterial diazotrophs predominant in the aquatic system, with Planctomycetes phylum was more abundant in seagrass sediments in relation to different marine ecosystems (Delmont et al., 2018). Such ability to live in association with seagrass could be due to nutrients released during their metabolism that seagrasses make use of. Habitat with pH ranging from 2 to 11.6 with a wide range of salinity is preferred by Planctomycetes species (Kaboré et al., 2020). The nitrogen bio-accessibility in aquatic system is highly linked to Planctomycetes (Delmont et al., 2018). However, a negative correlation between total N and Planctomycetes abundance has been noted in the current study in L2. Pollet et al (2014) also noted a reduction in the abundance of lake Planctomycetes in high N sample. The negative effect of N on some microorganisms could be a reason for their lower abundance in high N concentration in L2 in relation to L1 and L3. Furthermore, some seagrasses were found in L2 and L3, which might support the presence of Planctomycetes, but the low abundance of Planctomycetes in L2 was noted which might be related to lower EC and TDS in relation to L3. Therefore, considering a specific factor in relation to microbial abundance could be difficult due to varied microenvironments in all locations.

Furthermore, Acidobacteria, Candidatus Saccharibacteria, Nitrospirae, and Chloroflexi were also abundant in L3 and their abundance decreased in L1 and L2. High abundance level of Acidobacteria was also noted in the water stream in Japan (Nunoura et al., 2005) and soil samples in Korea (Butterfield et al., 2016). Acidobacteria and Chloroflexi phyla showed high abundance in soil samples (Kim et al., 2016). Acidic condition (pH between 3 and 6.5) is preferred by most Acidobacteria for better growth and development (Ward et al., 2009). Therefore, lower pH in L2 and L3 might have supported the growth of Acidobacteria better than the high pH condition in L1. Similar findings on pH and Acidobacteria abundance were noted before (Chu et al., 2010; Shen et al., 2013). Acidobacteria and Chloroflexi abundances were high in L3 where EC and TDS were the highest. The study of Kim et al. (2016) on the microbial abundance in greenhouse soils reported that Acidobacteria and Chloroflexi were correlated with EC. L3 was located near the road and where contamination by human activities were noted, which may increase the nutrients and soluble compounds resulting in increased EC and TDS, thus supporting the high abundance of these phyla. It is well known that shortage in elements concentration may limit microbial growth and development (Finney and O'Halloran, 2003); therefore, high EC and TDS would have enhanced such bacterial growth. On the other hand, an acidic environment is preferred by Nitrospirae and some of its taxa are known nitrifiers in acidic environments at low pH (Takahashi et al., 2020). The reason for the abundance of this phylum in L3 besides the pH could be due to the fact that high TDS at this location increased the water turbidity that might affect the concentrations of dissolved oxygen, which created a suitable environment for Nitrospirae growth (Almstrand et al., 2013). The significantly high abundance of Candidatus saccharibacteria in L3 might be related to the expected high organic compounds in this location due to contamination by human activities since such phylum favours biodegradation and utilization of contaminants (Ma et al., 2017). Verrucomicrobia was highly abundant in L2, in which high total N concentration was detected; Verrucomicrobia was found as a dominant phylum in a high N polluted river sediment (Lin et al., 2019). Generally, contamination noted in L3 increased the EC and TDS, K^+Cl^- that supported the abundance of some phyla, such as Acidobacteria, Candidatus Saccharibacteria, Nitrospirae and Chloroflexi.

5. Bacterial community and the water physico-chemical properties

The chemical characteristics of water varied among the different locations. Higher pH and Na^+ were noted in L1 than in L2 and L3. EC, TDS, K^+ and Cl^- were higher in L3 than those in L1 and L2. Furthermore, the total N in L2 was twice more than in the other two locations. Water was taken from the same surface level, therefore, conditions regarding oxygen and average temperature could be the same in all locations, but variations in other properties in the study locations were noted. The pH value of 7.75–8.31 at the three locations would affect microbial growth and phytoplankton as well as their biological activities. Most often, open surface water has a pH range from 8.0 to 8.5, which could be a consequence of the uptake of CO_2 (Wu et al., 2018). The relatively lower pH noted in L2 and L3 could be related to their surrounding environment, where some seagrasses and other contaminants were noted that may affect the ability of water for CO_2 uptake and, therefore, reduced the pH. Sodium and chloride are known road salts that appear in urbanized lakes (Kaushal et al., 2018). Sodium contributes to alkalinity enhancement (Shanley, 1994), which might explain the correlation between the pH and Na con-

centration at L1. The Na and Cl ratio was >1 in L1 and L2, <1 at L3, although it was expected to be >1 ; this also can be due to the fact that L3 is the nearest location to the road; however, other types of contaminants might have contributed in reducing Na concentration in L3. High pH was noted in L1, which could be related to the absence of pollution by human activities; however, its low value in L2 and L3 might be due to contamination from human wastes and the watergrass that had heavily grown there. It is well known that microbial abundance is highly correlated with organic carbon compounds content (Prest et al., 2016): however, the most important factor explaining the variation in microbial abundance could be chloride and total nitrogen concentrations (Fournier et al., 2021). Some of the top ten phyla in the three locations were correlated with some water characteristics such as Proteobacteria and Planctomycetes, the abundances of which were correlated with pH and Na^+ content: Acidobacteria, Candidatus Saccharibacteria, Nitrospirae and Chloroflexi abundances were correlated with EC, TDS, K^+ and Cl^- . On the other hand, TDS, the possibly suspended material in water, may contribute to a significant increment in its turbidity, which affects the microbial abundance. Correlation between electrical conductivity and total dissolved solids was noted, which might be vital factors guiding the microbial community structure, since from the top ten phyla, 50% were at higher abundance in L3, where the conductivity and TDS were the highest. L3 is highly vulnerable to contamination by organic and inorganic compounds due to human activities. Linkage of EC and TDS in the aquatic system was also noted when the metagenomic study was conducted at Lake Talquin (Betiku et al., 2021). Generally, variations in bacterial abundance could also be related to some factors that were not investigated in the present study.

6. Conclusions

In the present investigation, 16S rDNA metagenomics were used to identify the bacterial structure of three different locations along Wadi Namar Lake, Riyadh, SA in order to establish a connection between microbiota and physicochemical characteristics of the water system that might have resulted from the human activities near the lake. Our results indicated that differences in the microbial community structure at the studied locations are more likely consequences of the water physicochemical characteristics noted. Although other non-investigated factors could be of great effect, identified water characteristics and surrounding microenvironment would help in predicting microbial abundance in relation to contamination level and type. Therefore, our findings will help in understanding factors influencing the water microbiome at Wadi Namar Lake for the development of an environmental management system and planning for the future restoration of affected ecosystems.

Author Contributions

MA, AEM and KHE: conceptualization, investigation. AEM and KHE: data curation and statistical analysis, writing and editing; MA; funding; AEM: finalized, prepared the final version and submission.

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Data Availability Statement

Data of this manuscript are displayed in Figs. 1–8. The facts and raw data analyzed are available from the corresponding author upon request.

CRedit authorship contribution statement

Modhi O. Alotaibi: Conceptualization, Investigation. **Afrah E. Mohammed:** Conceptualization, Investigation, Data curation, draft preparation. **Kamal Eltom:** Conceptualization, Investigation, Methodology, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- Ahmad, T., Gupta, G., Sharma, A., Kaur, B., El-Sheikh, M.A., Alyemeni, M.N., Farooq, S., 2021. Metagenomic analysis exploring taxonomic and functional diversity of bacterial communities of a Himalayan urban fresh water lake. *PLoS ONE* 16 (3), e0248116.
- Ahmad, T., Singh, R.S., Gupta, G., Sharma, A., Kaur, B., 2019. Metagenomics in the search for industrial enzymes. In: *Adva. Enzy. Tech.*. Elsevier, pp. 419–451.
- Almstrand, R., Daims, H., Persson, F., Sörensson, F., Hermansson, M., 2013. New methods for analysis of spatial distribution and coaggregation of microbial populations in complex biofilms. *Appl. Environ. Microbiol.* 79 (19), 5978–5987.
- Amrane, S., Lagier, J.C., 2018. Metagenomic and clinical microbiology. *Human Micro.* 9, 1–6.
- Betiku, O.C., Sarjeant, K.C., Ngatia, L.W., Aghimien, M.O., Odewumi, C.O., Latinwo, L. M., 2021. Evaluation of microbial diversity of three recreational water bodies using 16S rRNA metagenomic approach. *Sci. Total Environ.* 771, 144773. <https://doi.org/10.1016/j.scitotenv.2020.144773>.
- Butterfield, C.N., Li, Z., Andeer, P.F., Spaulding, S., Thomas, B.C., Singh, A., Hettich, R. L., Suttle, K.B., Probst, A.J., Tringe, S.G., Northen, T., Pan, C., Banfield, J.F., 2016. Proteogenomic analyses indicate bacterial methylotrophy and archaeal heterotrophy are prevalent below the grass root zone. *PeerJ* 4, e2687.
- Chu, H., Fierer, N., Lauber, C.L., Caporaso, J.G., Knight, R., Grogan, P., 2010. Soil bacterial diversity in the Arctic is not fundamentally different from that found in other biomes. *Environ. Microbiol.* 12, 2998–3006.
- Dawson, W., Hor, J., Egert, M., van Kleunen, M., Pester, M., 2017. A small number of low-abundance bacteria dominate plant species-specific responses during rhizosphere colonization. *Fron. Micro.* 8, 975.
- Delmont, T.O., Quince, C., Shaiber, A., Esen, Ö.C., Lee, S.T.M., Rappé, M.S., McLellan, S. L., Lucker, S., Eren, A.M., 2018. Nitrogen-fixing populations of Planctomycetes and Proteobacteria are abundant in surface ocean metagenomes. *Nat. Microbiol.* 3 (7), 804–813.
- Ewels, P., Magnusson, M., Lundin, S., Käller, M., 2016. MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics* 32 (19), 3047–3048.
- Finney, L.A., O'Halloran, T.V., 2003. Transition Metal Speciation in the Cell: Insights from the Chemistry of Metal Ion Receptors. *Science* 300 (5621), 931–936.
- Fournier, I.B., Lovejoy, C., Vincent, W.F., 2021. Changes in the Community Structure of Under-Ice and Open-Water Microbiomes in Urban Lakes Exposed to Road Salts. *Fron. Micro.* 12, 12.
- Gibbons, S.M., Jones, E., Bearquiver, A., Blackwolf, F., Roundstone, W., Scott, N., Hooker, J., Madsen, R., Coleman, M.L., Gilbert, J.A., Ibekwe, A.M., 2014. Human and environmental impacts on river sediment microbial communities. *PLoS One* 9 (5).
- Han, B., Addo, F.G., Mu, X., Zhang, L., Zhang, S., Lv, X., Li, X., Wang, P., Wang, C., 2019. Epiphytic bacterial community shift drives the nutrient cycle during Potamogeton malaianus decomposition. *Chemosphere* 236, 124253. <https://doi.org/10.1016/j.chemosphere.2019.06.223>.
- Isaza, D.F.G., Cramp, R.L., Franklin, C.E., 2020. Living in polluted waters: a meta-analysis of the effects of nitrate and interactions with other environmental stressors on freshwater taxa. *Environ. Pollut.* 261, 114091.
- Kaboré, O.D., Godreuil, S., Drancourt, M., 2020. Planctomycetes as host-associated bacteria: A perspective that holds promise for their future isolations, by mimicking their native environmental niches in clinical microbiology laboratories. *Front. Cell. Infect. Microbiol.* 10, 729.
- Kaushal, S.S., Likens, G.E., Pace, M.L., Utz, R.M., Haq, S., Gorman, J., Grese, M., 2018. Freshwater salinization syndrome on a continental scale. *Proc. Natl. Acad. Sci.* 115 (4), E574–E583.
- Kim, J.M., Roh, A.-S., Choi, S.-C., Kim, E.-J., Choi, M.-T., Ahn, B.-K., Kim, S.-K., Lee, Y.-H., Joa, J.-H., Kang, S.-S., Lee, S.A., Ahn, J.-H., Song, J., Weon, H.-Y., 2016. Soil pH and electrical conductivity are key edaphic factors shaping bacterial communities of greenhouse soils in Korea. *J. Microbiol.* 54 (12), 838–845.
- Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* 41 (1).
- Krishna, M., Gupta, S., Delgado-Baquerizo, M., Morriën, E., Garkoti, S.C., Chaturvedi, R., Ahmad, S., 2020. Successional trajectory of bacterial communities in soil are shaped by plant-driven changes during secondary succession. *Sci. Rep.* 10 (1), 1–10.
- Lamb, J.B., van de Water, J.A.J.M., Bourne, D.G., Altier, C., Hein, M.Y., Fiorenza, E.A., Abu, N., Jompa, J., Harvell, C.D., 2017. Seagrass ecosystems reduce exposure to bacterial pathogens of humans, fishes, and invertebrates. *Science* 355 (6326), 731–733.
- Lin, X., Gao, D., Lu, K., Li, X., 2019. Bacterial community shifts driven by nitrogen pollution in river sediments of a highly urbanized city. *Int. J. env. Res. Pub. Health.* 16 (20), 3794.
- Lu, G., Xie, B., Cagle, G.A., Wang, X., Han, G., Wang, X., Hou, A., Guan, B.o., 2020. Effects of simulated nitrogen deposition on soil microbial community diversity in coastal wetland of the Yellow River Delta. *Sci. Total Environ.* 757, 143825. <https://doi.org/10.1016/j.scitotenv.2020.143825>.
- Ma, X., Wang, X., Liu, Y., Gao, J., Wang, Y., 2017. Variations in toxicity of semi-coking wastewater treatment processes and their toxicity prediction. *Ecotox. Environ. Safety* 138, 163–169.
- Maguvu, T.E., Bezuidenhout, C.C., Kritzing, R., Tsholo, K., Plaatjie, M., Molale-Tom, L.G., Mienie, C.M., Coertze, R.D., Aziz, R.K., 2020. Combining physicochemical properties and microbiome data to evaluate the water quality of South African drinking water production plants. *PLoS One* 15 (8), e0237335.
- Marín, I., Arahal, D., 2014. The family Beijerinckiaceae. Springer, Berlin, pp. 115–133.
- Ngara, T.R., Zhang, H., 2018. Recent advances in function-based metagenomic screening. *Gen. Prot. Bioinform.* 16 (6), 405–415.
- Nunoura, T., Hirayama, H., Takami, H., Oida, H., Nishi, S., Shimamura, S., Suzuki, Y., Inagaki, F., Takai, K., Neelson, K.H., Horikoshi, K., 2005. Genetic and functional properties of uncultivated thermophilic crenarchaeotes from a subsurface gold mine as revealed by analysis of genome fragments. *Environ. Micro.* 7 (12), 1967–1984.
- Pollet, T., Humbert, J.-F., Tadonlélé, R.D., 2014. Planctomycetes in lakes: poor or strong competitors for phosphorus? *Appl. Environ. Micro.* 80 (3), 819–828.
- Prest, E.I., Hammes, F., van Loosdrecht, M.C.M., Vrouwenvelder, J.S., 2016. Biological stability of drinking water: controlling factors, methods, and challenges. *Front. Microbiol.* 2016 (7), 45.
- Salazar, N.R., Aguirre, C., Soto, J., Salinas, P., Salinas, C., Prieto, H., Paneque, M., 2020. Physicochemical parameters affecting the distribution and diversity of the water column microbial community in the high-altitude Andean lake system of La Brava and La Punta. *Microorganisms* 8 (8), 1181.
- Shanley, J.B., 1994. Effects of ion-exchange on stream solute fluxes in a basin receiving highway deicing salts. *J. Environ. Qual.* 23, 977–986.
- Shen, C., Xiong, J., Zhang, H., Feng, Y., Lin, X., Li, X., Liang, W., Chu, H., 2013. Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. *Soil Biol. Biochem.* 57, 204–211.
- Siles, J.A., Margesin, R., 2016. Abundance and diversity of bacterial, archaeal, and fungal communities along an altitudinal gradient in alpine forest soils: what are the driving factors? *Micro. Ecol.* 72 (1), 207–220.
- Smith, J.L., Halvorson, J.J., Bolton, H., 2002. Soil properties and microbial activity across a 500 m elevation gradient in a semi-arid environment. *Soil Biol. Biochem.* 34 (11), 1749–1757.
- Takahashi, Y., Fujitani, H., Hirono, Y., Tago, K., Wang, Y., Hayatsu, M., Tsuneda, S., 2020. Enrichment of comammox and nitrite-oxidizing Nitrospira from acidic soils. *Fron. Micro.* 11, 1737.
- Ward, N.L., Challacombe, J.F., Janssen, P.H., Henrissat, B., Coutinho, P.M., Wu, M., 2009. Three genomes from the phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. *Appl. Environ. Microbiol.* 75, 2046–2056. <https://doi.org/10.1128/aem.02294-08>.
- Wu, X., Wu, L., Liu, Y., Zhang, P., Li, Q., Zhou, J., Hess, N.J., Hazen, T.C., Yang, W., Chakraborty, R., 2018. Microbial Interactions with Dissolved Organic Matter Drive Carbon Dynamics and Community Succession. *Front. Microbiol.* 9, 1234.
- Yadav, N., Kour, D., Yadav, A.N., 2018. Microbiomes of freshwater lake ecosystems. *J. Microbiol. Exp.* 6, 245–248.
- Yadav, N., Sharma, S., 2019. Pollution shapes the bacterial community of a river: a case study. *Int. J. Environ. Sci. Tech.* 33, 1–14.
- Zhang, J., Ding, X., Guan, R., Zhu, C., Xu, C., Zhu, B., Zhang, H., Xiong, Z., Xue, Y., Tu, J., 2018. Evaluation of different 16S rRNA gene V regions for exploring bacterial diversity in a eutrophic freshwater lake. *Sci. Total Environ.* 618, 1254–1267.
- Zhang, R., Liu, W., Liu, Y., Zhang, H., Zhao, Z., Zou, L., 2021. Impact of water quality variations on the microbial metagenome across coastal waters in Shenzhen, south China. *Ocean Coast. Man.* 208, 105612. <https://doi.org/10.1016/j.ocecoaman.2021.105612>.