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Spatiotemporal Analysis of the Water and Sediment Nile Microbial Community Along an Urban Metropolis

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Abstract

Assessing microbial identity, diversity, and community structure could be a valuable tool for monitoring the impact of xenobiotics and anthropogenic inputs in rivers, especially in urban and industrial settings. Here, we characterize the Nile River microbial community in water and sediments in summer and winter at five locations that span its natural flow through the Cairo metropolis. 16S rRNA gene datasets were analyzed to identify the role played by sample type (sediment versus water), season, and location in shaping the community, as well as to predict functional potential of the Nile River microbiome. Microbial communities were mostly influenced by sampling type (sediments versus water), while seasonal effects were only observed in water samples. Spatial differences did not represent a significant factor in shaping the community in either summer or winter seasons. Proteobacteria was the most abundant phylum in both water and sediment samples, with the order Betaproteobacteriales being the abundant one. Chloroflexi and Bacteroidetes were also prevalent in sediment samples, while Cyanobacteria and Actinobacteria were abundant in water samples. The linear discriminative analysis effect size (LEfSe) identified the cyanobacterial genus *Cyanobium PCC-6307* as the main variable between summer and winter water. Sequences representing human and animal potential pathogens, as well as toxin-producing Cyanobacteria, were identified in low abundance within the Nile microbiome. Functionally predicted metabolic pathways predicted the presence of antibiotic biosynthesis, as well as aerobic xenobiotic degradation pathways in the river microbiome.

Keywords Nile River · 16S rRNA gene sequencing · Microbiome · Freshwater · Sediment

Introduction

Rivers shape lives, start civilizations, and are the indispensable sources of water for residential, agricultural, and industrial activities around the world [1]. Rivers are, however, under continuous threat from obstruction of flow, drought, and most importantly anthropogenic activities such as urbanization, industrialization, and agriculture [2]. Microbial communities are highly dynamic and sensitive to environmental fluctuations and hence could be used as potential indicators for exogenous environmental input [3–7]. The Nile River is the main source of freshwater in Egypt [8] and exercises a dominating influence on all social and industrial aspects of the Egyptian society as the country depends on it for almost 90% of its water supply, particularly in irrigation [8–10]. The Nile originates in Lake Victoria as the Victorian or White Nile, which is joined in Khartoum, the capital of North Sudan with the Blue Nile, springing from Lake Tana in the Ethiopian peninsula. During the high-flow season, the Blue Nile drains into the Nile River, contributing to almost 90% of its water [11]. The Nile River continues to flow up north until it drains in the Mediterranean coast of Egypt through its two tributaries (Damietta and Rosetta) both of which branch out north of the Egyptian capital, Cairo, at El-Qanatir. A considerable seasonal variation in the water levels occurs in the Blue Nile related to rainy summers followed by dry winters, in the Ethiopian peninsula where it originates. Despite the presence

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of the Aswan High Dam at the southernmost city of Aswan in Egypt to store excess water, the seasonal variation can still be detected even in Cairo [12].

Water quality in the Nile River is constantly under threat due to (1) increased variability in raining events at the Nile source due to global climate change; (2) construction of the Grand Ethiopian Renaissance Dam (GERD) along Blue Nile, at Benishangul-Gumuz, 15 km off the border region between Ethiopia and Sudan; (3) anthropogenic activities, e.g., drainage of untreated agricultural flows, sewage and industrial wastewater, and input of untreated sewage as well as industrial waste; and (4) a pattern of explosive population growth in all countries through which the river runs.

Cairo is the capital of Egypt and the largest metropolis in the Middle East and Africa. It is one of the most populous cities in the world. With over 23 million inhabitants in what is known as the "Greater Cairo" area, such a megacity suffers from extreme pollution that deeply affects the environment [13]. Within the limits of the Greater Cairo area, several industrial plants have been established along the banks of the Nile flanking the city from its southern tip as the Nile enters the city and its northern end as the Nile leaves the city before branching into its two tributaries. The industrial settings range from iron and steel, cement industries, and sugar refineries on the south side to textile industries in the north. Such facilities are expected to contribute to altering the type and level of pollutants in the river by pounding waters with varying arrays of pollutants. In addition to industrial waste, inadequate disposal of Cairo's sewage flowing to the Nile River post treatment can cause waterborne outbreaks and constitute a serious health threat [14–16]. Pathogen contamination of water can result in unsafe drinking water leading to health implications for consumers, restrictions on recreations opportunities, and closure of shellfish beds [17].

Our earlier investigation provided a preliminary view of microbial taxa in the Nile River waters as it enters Cairo metropolis [18]. Here, we expand our research by providing a detailed comparative study that examines the impact of three distinct factors (sample type, sampling location, and sampling time) on the diversity and community structure within the Nile River around Cairo metropolis. In addition, functional profile of the Nile microbiome was predicted based on the 16S rRNA gene sequences through mapping to Kyoto Encyclopedia of Genes and Genome (KEGG) Orthology (KO) Database for prediction of the different metabolic functions.

Materials and Methods

Sampling Location

Five sampling locations were chosen. From each location, water (from the midstream of the river) and sediment samples (from the riverbanks) were collected in two seasons (June, representing summer, and November, representing winter). Following the river's flow from south to north, these locations are (1) Saff (29° 34′ 24.1572″ N, 31° 16′ 12.0612″ E), the southern entry point, representing the "pristine" conditions relative to other locations where there was no clear impact of any industrial waste, before the river enters the city; (2) Helwan (29° 52′ 27.5988″ N, 31° 17′ 8.3076″ E), a location bound by iron/steel works and cement factories and sugar refineries; (3) Tahrir (30° 2′ 39.912″ N, 31° 14′ 8.5632″ E), the downtown area, representing the main business and commercial area in Cairo; (4) Warraq (30° 7′ 2.2656″ N, 31° 14′ 18.4956″ E), an uptown (north) location that is home to multiple fabric and textile factories; and (5) Qanatir (30° 10′ 21.4392″ N, 31° 8′ 24.5616″ E) at the northern tip of Cairo metropolis before the Nile branches into its two main tributaries (Fig. 1). Air, water temperatures, and any precipitation levels were monitored in all locations. In the Cairo area, the average precipitation rates in the winter months is normally between 3.8 and5.9 mm per month and in the summer months, it falls to zero mm. All samples (both water and sediment) were collected at days with no or prior precipitation. For the water samples, Electric conductivity, pH, total dissolved salts, and dissolved oxygen (DO) were measured by appropriate metered electrodes. Chemical oxygen demand, biochemical oxygen demand (BOD), and chemical analysis for different elements, including ammonia, nitrate, total nitrogen, carbonate, bicarbonate, and phosphate, were measured.

Sample Collection, Processing, and DNA Extraction

Water samples were collected from a depth of 0.5 m according to the standard methods for the examination of water and wastewater [19]. From each sampling location, 6 l of water were filled in sterile nonreactive borosilicate glass bottles (1 l/bottle). The total volume of water was pooled together and mixed in a sterile conical flask, passed through sterile cheesecloth, and then filtered through 0.22-μm pore size membrane filters (47 mm diameter, Sartorius, Germany) under vacuum. DNA was extracted from the filters using Epicentre's Metagenomic DNA Isolation Kit for Water (Madison, WI, USA), as per the manufacturer's protocol.

Sediment samples from Nile riverbanks were collected in sterile 50-ml Falcon tubes and directly transported in an ice-box to the laboratory. The sediments were sieved through 1/4-inch then 1/8-inch mesh to remove coarse non-sediment materials. Sediment DNA extraction was performed using the PowerPlant® Pro DNA Isolation Kit according to the manufacturer's instructions. The extracted DNA was kept at − 20 °C until further use. Purification of the DNA extracted from both water and sediment samples was done using the PowerClean® Pro DNA MO BIO Clean-Up Kit (Qiagen, Carlsbad, CA, USA) to obtain high-purity DNA for more successful polymerase chain reaction (PCR) amplification.

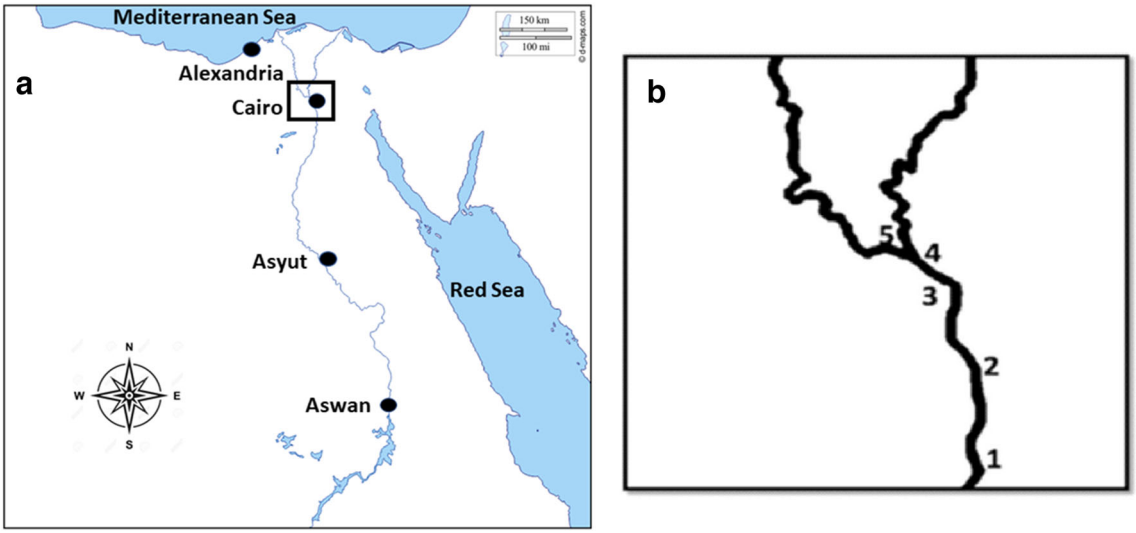

Fig. 1 (A) An overall map of Egypt showing the Nile River; box indicates the covered area. (B) Zoom in on the study area (Cairo Metropolis) and the five sampling sites. City limits are between points 2 (south) and 4 (north); point 1 represents the entry point and just before water flows to the city and point 5 represents the exit point of water just before the Nile River branches to its two tributaries

Finally, the purified DNA was quantified using Qubit® fluorometer (Life technologies, Carlsbad, CA, USA).

16S rRNA Illumina Sequencing and Data Analysis

The V4 region of the 16S rRNA gene was amplified by PCR using the following primers with Illumina adapters (underlined): Hyb515F_rRNA 5\ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTA-3\ and Hyb806R_rRNA 3\ TAATCTWTGGGVHCATCAGGGACAGAGAATAGTGTAGAGGCTCGGGTGCTCTG-5\. Both PCR amplification and Illumina sequencing were conducted at the Genomic Sequencing and Analysis Facility at the University of Texas at Austin. The paired-end sequencing was performed on an Illumina MiSeq sequencing platform [20]. Sequence reads were first trimmed of primer sequences and multiplexing barcodes. Sequences that had unknowns (sequences with N's), had at least one ambiguous base, and had > 8 nt homopolymers or those which had short sequences < 80 bp were removed. Sequences were aligned and subsequently classified against the SILVA database (V. 132) [21]. Most of the analysis of sequence data was essentially performed with the MOTHUR (v. 1.35.1) software package [22]. The MiSeq SOP available online was used as a guide for the analysis of the produced sequences (www.mothur.org/wiki/MiSeq_SOP). Sequences were then clustered into operational taxonomic units (OTUs) at 3% sequence divergence cutoff (the putative species level, 0.03) as well as 6% sequence divergence cutoff (the putative genus level, 0.06). Alpha diversity and richness indices as Chao1 richness estimators, ACE (Abundance-based Coverage Estimator), and the Shannon's index were measured using summary.single command [23–26]. Beta diversity analysis was computed based on Bray–Curtis measure of dissimilarity and visualized in Microbiome analyst software [27] through PCoA plots comparing the different analyzed groups. Identification of discriminative taxa in the water samples and potential biomarkers in the sediment samples were done using the linear discriminative analysis effect size (LEfSe) applying the appropriate cutoffs [28]. All water sequences were deposited in the Sequence Read Archive database under accession number SRP150523. Sediment sample sequences are under accession number: PRJNA672140.

Functional Prediction of Microbial Communities in Nile River

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was used to predict the functional profile of the Nile metagenome based on the 16S rRNA gene sequences [29]. Filtered, denoised reads were first aligned to the Greengenes database taxonomy (V 13.8) [30] at a 97% identity using UCLUST [31], and then exported in the required format to Galaxy software (http://huttenhower.sph.harvard.edu/galaxy/) to predict metabolic functions through mapping to Kyoto Encyclopedia of Genes and Genome (KEGG) Orthology (KO) Database [32, 33]. LEfSe classification tool was used for plotting of the metabolic pathways' biomarkers identified.

Results

Environmental and Physiochemical Parameters

The average air temperature in the summer ranged between 37 °C and 38 °C, while water temperature was 29–32 °C. The

average air temperature during winter ranged between 24 and 26 °C and the water temperature was 20 °C. It is worth mentioning that precipitation days in the Cairo area are normally scarce during the whole year and no precipitation was recorded in any of the collection days or before. Physiochemical parameters for water samples were analyzed (Supplementary Table 1).

Overview of Sequencing Output

817,422 sequences were generated from water samples. Only 739,244 sequences were included in the analysis after removing low-quality sequences. On average, 73,924 sequences were obtained per sample (Table 1). Coverage estimates [34] ranged between 98 and 99% at a putative genus sequence divergence cutoff ($OTU_{0.06}$). For the sediment samples, a total of 245,399 sequences were generated in this study. After the removal of low-quality sequences, 220,582 sequences were included in the analysis. On average, 24,509 sequences were obtained per sample (Table 1). Coverage estimates ranged between 80 and 91% at $OTU_{0.06}$. As the summer season sample sequence reads of the fifth location (Qanatir/Summer) were significantly lower than those of the rest of the samples, they were excluded from further analysis.

Diversity Patterns

Alpha diversity indices are presented in Table 1. Based on Shannon diversity index, a parameter that considers both richness and evenness level of microbial communities' diversity levels was significantly higher in sediment versus water samples (Mann-Whitney, $p < 0.0001$) (Fig. 2A). On the other hand, no significant seasonal variation in diversity levels was observed (Mann-Whitney, $p > 0.999$) (Fig. 2B). As well, no significant difference in diversity was observed between any of the different studied locations (Kruskal-Wallis, $p = 0.646$) (Fig. 2C).

Taxonomic Classification

All sequences clustered into 62 phyla, 117 classes, 232 orders, 383 families, and 606 genera. Twelve different phyla (Proteobacteria, Cyanobacteria, Chloroflexi, Acidobacteria, Spirochaetes, Firmicutes, Latescibacteria, Actinobacteria, Bacteroidetes, Planctomycetes, Verrucomicrobia, and Armatimonadetes) accounted for about 74.3–98.4% of the total sequenced bacterial community (Fig. 3, Supplementary Table 2). Proteobacteria was the most abundant phylum in all the samples ranging from 24.1 to 46.8% with the majority of Proteobacteria sequences affiliated with the Gammaproteobacteria and Alphaproteobacteria. Cyanobacteria was the second major phylum in water samples, constituting up to 17.8% of the total phyla, followed by the Actinobacteria (8.7–30.8%) and Bacteroidetes (5.9–14.1%) (Fig. 3, Supplementary Table 2). However, Cyanobacteria were only detected as a minor phylum in the sediment community ranging from 0.4 to 2.5%. On the other hand, sequences affiliated with the phyla Chloroflexi, Acidobacteria, Spirochaetes, and Firmicutes were detected as major constituents of sediment samples but represented a minor fraction of water community. Other phyla identified in both water and sediment samples above 1% in at least one sample were Planctomycetes (2.5–11.9%), Verrucomicrobia (2.5–12.5%), Acidobacteria (0.1–13.5%), and Armatimonadetes (0.1–5.8%). The percentages of the unassigned sequences at the phylum level ranged from 1.24% to 6.3% with an average of 3.27% for water samples and 5.1% to 12.95 with an average of 7.44% for the sediments (Fig. 3 and Supplementary Table 2).

At the order level, in water samples, the Cyanobacteria order Synechococcales was especially abundant (3.0–17.0%), followed by Frankiales (Actinobacteria, 4.4–23.8%), Bacteroidetes orders (Chitinophagales 1.8–6% and Flavobacteriales 2.1–5.2%), and Steroidobacterales order (Proteobacteria, 0.6–2.8%) (Supplementary Table 3). Sediment samples were dominated by order Betaproteobacteriales (belonging to Proteobacteria (5.2–25.7%), followed by the order Anaerolineales (Chloroflexi, 1.5–8.4%) and the Bacteroidetes orders (Chitinophagales (1.0–3.4%) and Bacteroidales (0.8–4.3%) (Supplementary Table 5).

On the genus level, water samples were characterized by the abundance of the cyanobacterial genus *Cyanobium PCC-6307* 2–16.8% (phylum Cyanobacteria, order Synechococcales), followed by members of the genus *hgcI clade* 23.9–4.5% (phylum Actinobacteria), *CL500-29 marine group* 1.7–4.5% (phylum Actinobacteria), *Pirellula* 1.1–5.4% (phylum Planctomycetes), *Limnohabitans* 1.5–9.7% (phylum Proteobacteria), *Fluviicola* 0.6–2% and *Dinghuibacter* 0.4–8% (phylum Bacteroidetes), and *Chthoniobacter* 0.2–2.4% (phylum Verrucomicrobia) (Supplementary Table 4). In sediment samples, most of genera members were detected in small proportions (< 1%) with no major genera except for few members representing a percentage of about 2% (Supplementary Table 6).

Notably, within the rare (< 1% relative abundance) community in all datasets, we identified sequences belonging to specific genera, several members of which were previously implicated as human and/or animal pathogens. These include members of the genus *Acinetobacter* (0.03–0.14%), *Aeromonas* (0.16–0.4%), and *Legionella* (0.02–0.03%). Further, sequences affiliated with the enteric pathogen *Listeria monocytogenes* (0.01–0.02%) were also detected in the sediment samples. Finally, some blooming cyanobacteria capable of toxin production, e.g., *Microcystis* (0.1–0.5%) and *Cylindrospermopsis* (0.04–0.16%), were also identified in the

Table 1 Sequence analysis for the generated reads, the reads after filtration and reprocessing, and alpha diversity estimates in Nile samples at different locations and seasons

	Water samples										Sediment samples									
	S_Su	H_Su	D_Su	W_Su	Q_Su	S_Win	H_Win	D_Win	W_Win	Q_Win	S_Su	H_Su	D_Su	W_Su	S_Win	H_Win	D_Win	W_Win	Q_Win
Number of sequences	73,377	114,078	10,7395	11,1568	16,1983	36,840	40,949	33,830	30,627	28,597	41,501	35,193	5,158	33,987	14,922	37,752	27,969	15,001	9099
Number of OTU	1288	1490	1486	1442	1527	1095	1023	1056	909	930	1823	1890	1038	1560	1627	1981	1849	1420	1236
Coverage ($OTU_{0.06}$)	99%	99%	99%	99%	99%	98%	99%	98%	99%	99%	91%	90%	80%	93%	82%	90%	87%	80%	84%
Chao	1437	1531	1522	1488	1545	1282	1207	1298	1008	1123	1978	1986	1549	1711	1989	2077	2031	1652	1650
ACE	1417	1511	1522	1480	1538	1251	1198	1252	1001	1129	1927	1960	1538	1693	1905	2044	1979	1666	1727
Shannon	4.27	4.62	4.27	4.23	4.28	3.9	4.4	4	4	3.86	6.47	6.55	5.9	6.22	6.52	6.69	6.59	6.63	6.06

Su summer, *Win* winter, *S* Saf, *H* Helwan, *D* Downtown, *W* Warraq, *Q* Qanatir, N_{seqs} number of sequences in the sample. Alpha diversity indices and number of OTUs were calculated at 0.06 cutoff

water samples. Additional supplementary file has all taxa assignments according the OTUs identified.

Community Structure

Variability in the Nile microbiome was examined using principal coordinate analysis (PCoA) based on Bray–Curtis measure of dissimilarity. Water samples showed season-dependent rather than location-dependent clustering (Fig. 4A). The community structure of summer samples was significantly different from the winter samples (PERMANOVA, *F*-value: 6.0553; R-squared: 0.43082; p value < 0.01). Unlike water samples, the community structure of sediment samples was not significantly different between summer and winter seasons (PERMANOVA, F-value: 1.045; R-squared: 0.12989; p value < 0.221) (Fig. 4B).

LEfSe analysis was performed for the definition of taxa that drive taxonomic shifts in summer and winter at different development stages (Fig. 5). In water samples, the phyla Cyanobacteria and Verrucomicrobia served as strong discriminants for water community in summer and winter, respectively, while at the genus level, different discriminative taxa including *Cyanobium PCC_6307* in summer and *Terrimicrobium* in winter were identified. On the other hand, LEfSe analysis of sediment samples identified the genera *Enterococcus* (Enterococcaceae), *Staphylococcus*, *Desulfuromonas*, *Idiomarina* (Idiomarinaceae), *Wenyingzhuangia* (Flavobacteriaceae), *Acinetobacter*, *Desulfurivibrio*, *Arenimonas*, *Marinimicrobium*, and *Wenzhouxiangella*; the species *Lactobacillus fermentum*, *Listeria monocytogenes*, and *Escherichia coli TOP293_4*; and the families Hydrogenophilaceae and Archangiaceae (order Myxococcales) were the most prominent biomarkers. In winter samples, the genera *Nitrospirae 4_29_1*, *Geobacter*, *Methanosarcina* (family Methanosarcinaceae), *Syntrophobacter*, *MSBL7* (family Desulfobulbaceae), *Desulfomicrobium*, *Leptospiraceae RBG_16_49_21*, and *Acidobacteria Subgroup6 RBG_16_49_21* (Family Leptospiraceae) were the most prominent biomarkers (Supplementary Fig. 1).

Function Prediction of Nile River Ecosystem Metagenome

PICRUSt was used to infer bacterial functions using genomic composition of closely related organisms to the 16S rRNA composition of the studied communities. Pathways differentially inferred to be enriched in water samples include degradation of various xenobiotics, e.g., benzoate, aminobenzoate, taurine/hypotaurine, caprolactam, geraniol, dioxins, atrazine, xylene, bisphenol A, and ethylbenzene. On the other hand, the major metabolic pathways enriched in sediment were the biosynthesis of different antibiotics (vancomycin, streptomycin,

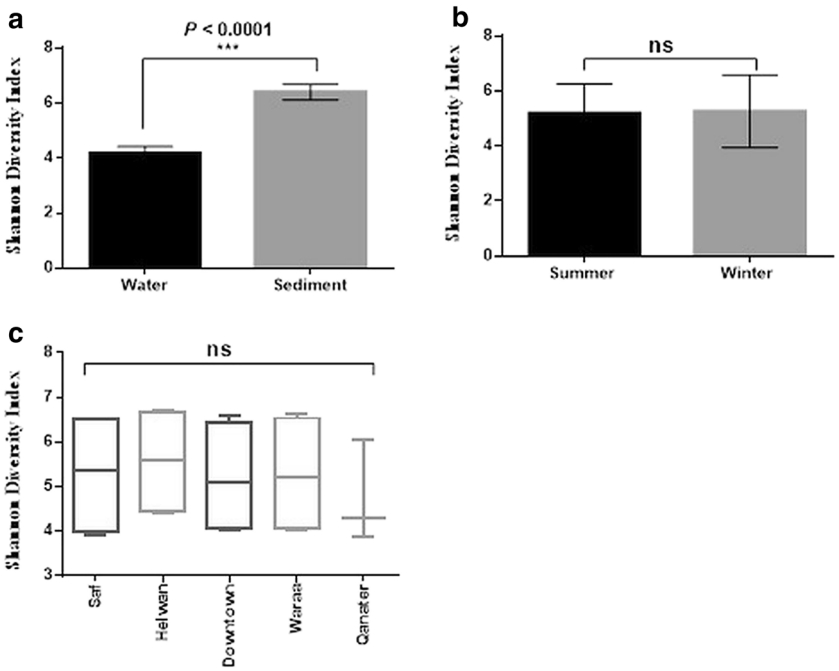

Fig. 2 Shannon diversity index of Nile River samples. Shannon diversity comparisons between sample types (water/sediment) (2A), seasons (summer and winter) (2B), and locations (2C). Error bars represent standard error of average values (*n* ranges from 4 to 10)

and novobiocin antibiotics), amino acids (leucine, isoleucine, and valine), folate, and glycan; in addition, carbon fixation and carbohydrates metabolism were also significantly abundant in sediment samples.

Discussion

The Nile River is the major source of freshwater in Egypt and about 95% of Egyptians live along the Nile making its valley one of the world's most densely populated areas, especially in Greater Cairo. Accordingly, there is a great need to explore and understand the microbiota of the Nile River and the extent of contribution of different pollutant sources to the composition of the "main artery" of the Egyptian population. As the journey of the Nile River across Cairo metropolis brings it through various industrial and domestic areas with subsequent variations in the water quality and characteristics, sampling sites for water quality evaluation were selected at five different locations in summer and winter of 2016 and 2017. The

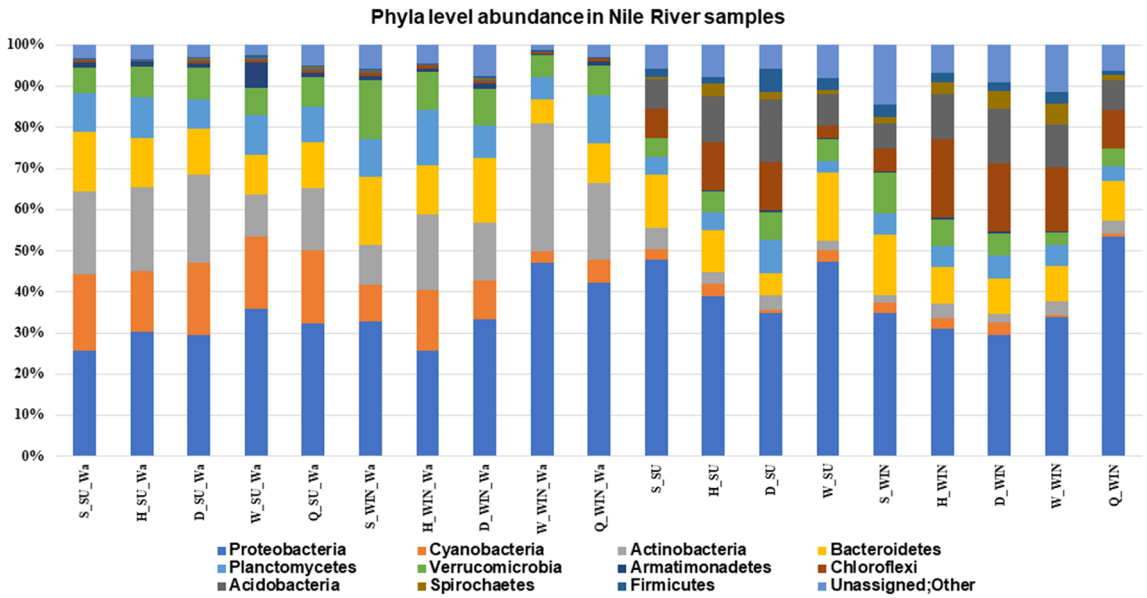

Fig. 3 Phylum taxa level abundance in Nile samples in summer and winter. Bar charts represent relative proportions of the most predominant phyla detected in both water and sediment samples at each of the five locations in summer and winter seasons. Wa, water; Su, summer; Win, winter; S, Saff; H, Helwan; D, Downtown; W, Warraq; Q, Qanatir

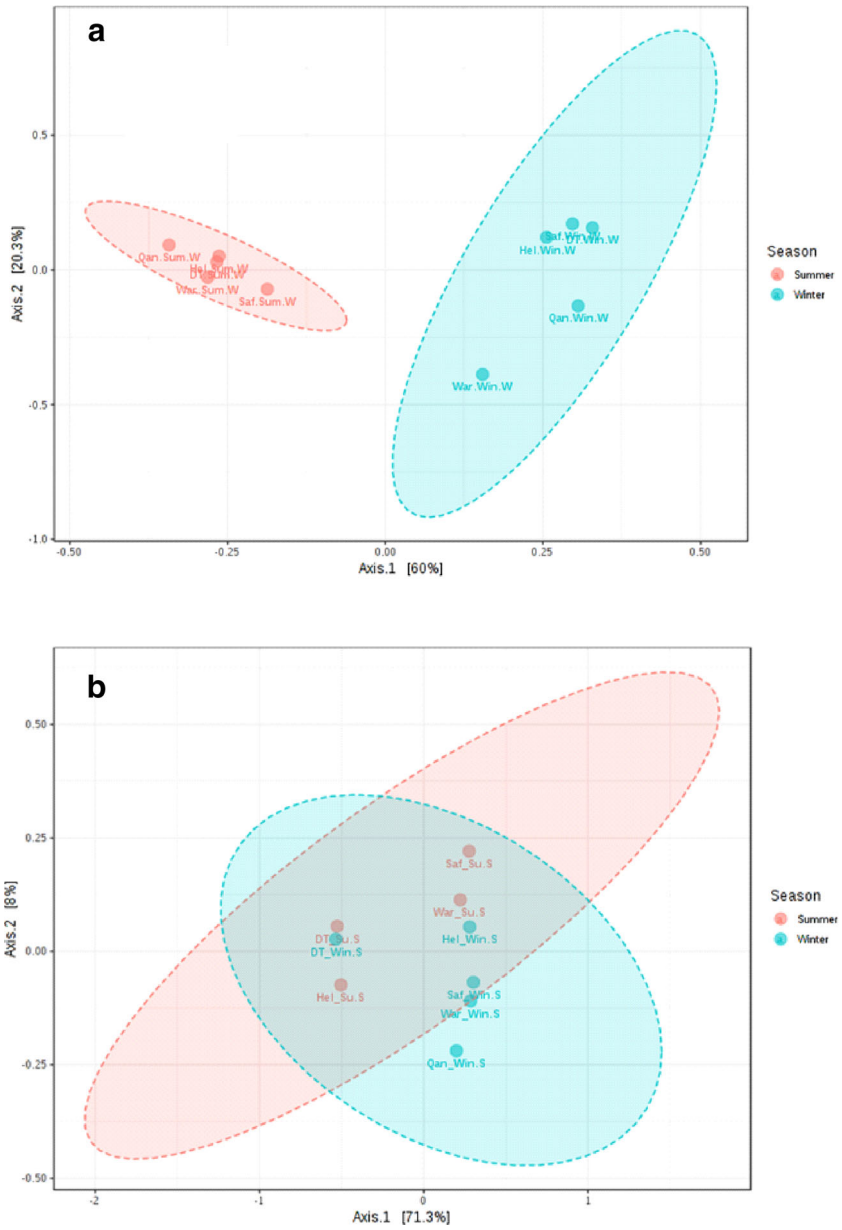

Fig. 4 Principal coordinates analysis (PCoA) for Bray–Curtis measure of dissimilarity of Nile water samples (4A) and Nile sediment samples (4B) in summer and winter. The *x*- and *y*-axes are indicated by the first and second coordinates, respectively. The percentages of the community variation explained are depicted by the percentages in parentheses on each axis 60% and 20.3%, respectively, for water samples with ellipses denoting significant clustering (p value < 0.001, PERMANOVA) according to season. For sediment samples, the percentages of the community variation explained are depicted by the percentages in parentheses on each axis 71.3% and 8%, respectively, with ellipses denoting no significant clustering (p value < 0.221, PERMANOVA) according to season

selected sampling sites, as indicated in Fig. 1, were chosen to extend over a considerable distance along the natural river path across this vast metropolis.

Water samples exhibited an overall lower level of diversity when compared to sediment samples. This could be attributed to the transient nature of a water sample as opposed to the cumulative nature of a sediment sample. Sediment samples are the product of sedimentation processes of particulate matters over years, even decades. Microorganisms attach to particulates in water column that subsequently reach the sediments at the bottom of the river [5, 35, 36]. Such particulates (composed of organic matter and cell detritus) have higher organic content and hence represent hotspots of diversity within a water body. The expected higher levels of diversity might explain the higher percentages of unassigned sequences in sediments compared to water. The higher cell loads usually encountered in sediment samples also promotes the development of a more diverse communities mediating degradation of various organic components of dead microbial cells, hence allowing for a more diverse community compared to water samples [37].

PCoA analysis demonstrated significant seasonal difference between summer and winter microbiota in water samples, while no such differences were observed between locations (Fig. 4A). LEfSe tool identified predominance of Cyanobacteria (mainly genus *Cyanobium PCC-6307*) and the Verrucomicrobia. The Cyanobacteria, primary producing phototrophs, are known to thrive in water bodies during summer months due to the relatively higher temperatures and longer hours of light exposure [38]. In winter months, this

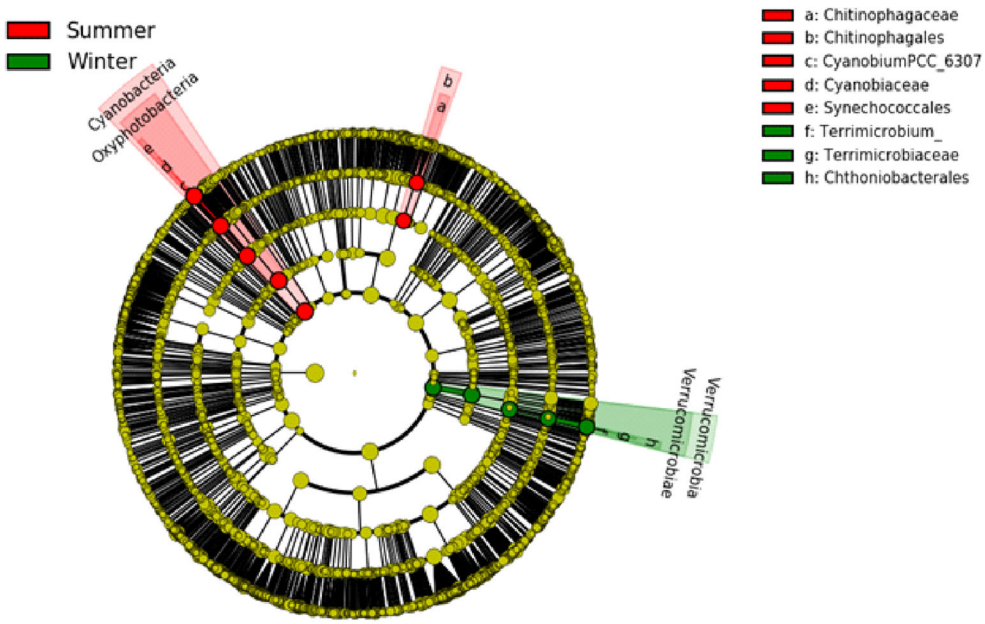

Fig. 5 Taxonomic cladogram showing significantly abundant taxa that differentiate between summer and winter communities of water samples at different development stages at p value cutoff of 0.05 and LDA score cutoff of 4. Microbial taxa are colored according to the season

biomass of cyanobacterial detritus acts as a substrate for heterotrophic organisms in a seasonal carbon cycle. Many members of the Verrucomicrobia, enriched during winter months, are known for their ability to degrade cell detritus, especially cell wall polysaccharides due to their possession of an array of carbohydrate active enzymes (CAZymes) [39, 40]. Unlike

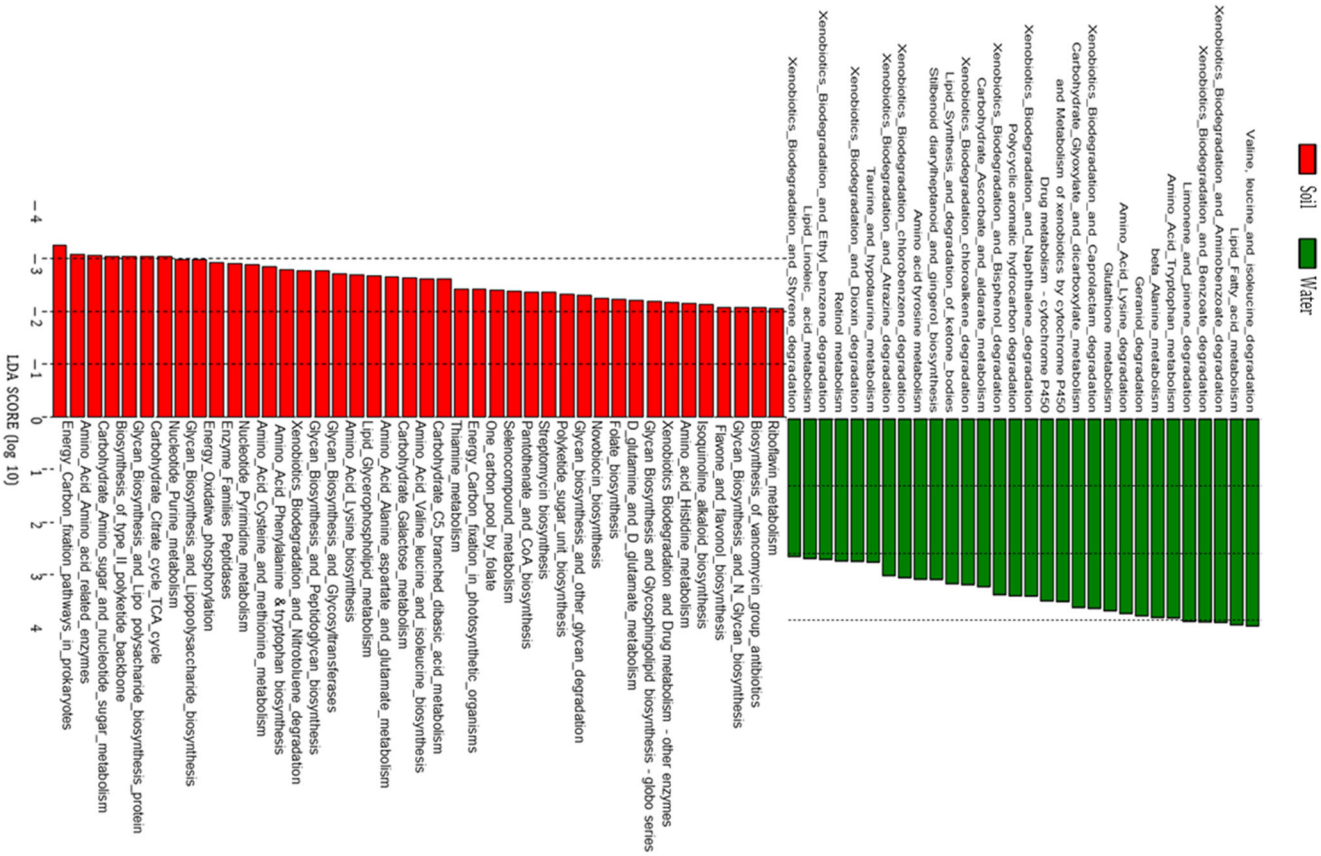

Fig. 6 LEfSe analysis of differentially abundant metabolic pathways between sediment and water communities. Histogram of linear discriminant analysis (LDA) scores computed for metabolic pathways, differently enriched in sediment (red) and water (green) communities at p value cutoff of 0.05 and LDA score cutoff of 2

water samples, no significant differences in the microbial community of sediment samples were observed between seasons. This re-emphasizes the cumulative nature and relative stability of sediment samples, when compared to water samples in various types of freshwater and marine water bodies.

Multiple predominantly rare taxa (less than 1% abundance) were identified in all samples. Interestingly, some of these samples belong to taxa known for their pathogenic potential. Despite the fact that we cannot generalize on all species belonging to a particular genus that they are actual pathogens [41], several members of *Acinetobacter*, *Aeromonas*, *Legionella*, and *Listeria* were found to be associated with certain diseases. Similarly, others are known for their capability to generate microtoxins (*Microcystis* and *Cylindrospermopsis*). Potential pathogenic *Acinetobacter* include *Acinetobacter baumannii* and organism that gained medical attention because of the Gulf war syndrome and the association of *A. baumannii* with multiresistant hospital infections [42, 43]. It is known by its resistance to harsh environment factors allowing it to spread rapidly and develop resistance to all conventional antimicrobial agents [44]. *Legionella* spp. are known for their parasitism of amoebas [45, 46]. Certain *Legionella* spp. may cause Legionnaires' disease in humans which is a fatal pneumonia-like illness that affects the human lungs [47]. According to the Centers for Disease Control and Prevention (CDC), 9,933 cases of Legionnaires' disease were recorded in 2018, which was the highest since the disease was first identified in 1976 and it is also estimated by The National Academies of Sciences, Engineering, and Medicine that around 70,000 people suffer from these diseases every year. The majority of the species belonging to the genus *Aeromonas* are human pathogens associated with wound infections, septicemia, and meningitis in immunocompromised people, and diarrhea and dysenteric infections in the elderly and children [48]. Other species of *Aeromonas* are also well-known as fish pathogens. *A. salmonicida* is one of the most intensively studied fish pathogenic bacteria that caused marked economic losses in commercial farming of different fish species [49]. Finally, *Listeria monocytogenes* is an enteric pathogen identified as a potential biomarker in our sediment/summer samples. *Listeria monocytogenes* is a serious pathogen that causes listeriosis disease mainly affecting pregnant women and immunocompromised patient leading to life-threatening complications, including septicemia or meningitis [50]. Although the reported cases of listeriosis is small, 0.1 to 10 cases per 1 million people per year depending on the countries, the infection is of high public health concern because of its high death rate (20–30%) [51]. It is also estimated in a study of de Noordhout et al. [52] that listeriosis causes 273 stillbirths globally annually. In a previous study of Gad, Al-Herrawy [53], *Acanthamoeba* was detected in Egyptian aquatic environments such as the Nile River and ground and tap water. This free-living amoeba causes several types of illness to humans and acts as a natural host of many bacteria including *Listeria monocytogenes*. This study also reported that the highest occurrence of *Acanthamoeba* spp. in the Nile water was recorded in summer agreeing with our detection of *Listeria monocytogenes* as a potential biomarker in summer samples.

The toxin producers, *Cylindrospermopsis* and *Microcystis*, were also detected at a relatively low abundance in our samples. The genus *Microcystis* is one of the most common bloom-forming Cyanobacteria worldwide. Different members of *Microcystis* have the gene cluster responsible for the biosynthesis of cyanotoxins including anatoxin-a, cyanopeptolin, micocystin, and others. These cyanotoxins have been reported to be highly detrimental for the survival of several aquatic organisms and human life [54].

Functional analysis of the Nile microbiome by using PICRUST and mapping to Kyoto Encyclopedia of Genes and Genome (KEGG) Orthology (KO) database revealed the enrichment of metabolic pathways mediating xenobiotic degradation of benzoate, aminobenzoate, taurine/hypotaurine, caprolactam, geraniol, and others in water communities. Degradation of highly toxic xenobiotics including dioxins, atrazine, xylene, bisphenol A, and ethylbenzene was also detected as a biomarker metabolic pathway in our study. The high levels of xenobiotics degradation could be a pointer to the increased input of industrial waste into the Nile ecosystem. In addition, a notable enrichment in genes mediating antibiotic biosynthesis was observed, putatively explained by the predominance of phylum Actinobacteria in the tested samples. Whether such pathways coding genes are transcriptionally active in the Nile ecosystem remains to be seen. Different biomarker pathways including biosynthesis of different amino acids (leucine, isoleucine, and valine), folate, glycan, and others were also detected (Fig. 6). Carbon fixation in prokaryotes (a biomarker metabolic pathway in soil) may be under the effect of class Anaerolineales (1.5–8.4%) of the phylum Chloroflexi. These microorganisms can degrade organic compounds and cellular compounds derived from dead biomass and metabolites of anaerobic ammonium oxidation [55]. Pathways of carbohydrate metabolism are linked to the different classes of Firmicutes, particularly Lactobacillales (0.1–2.6%) and Clostridiales (0.3–1.4%). Their physiology as "lactic acid bacteria" allows them to ferment the carbohydrates present in the soil [56].

In conclusion, this study provides a baseline analysis of the Nile microbiome by testing various locations spanning the Cairo metropolis in the Greater Cairo area. Our results highlight the apparent level of stability and resilience of the Nile microbiome to changes of the tested locations and differences in water level encountered in summer and winter. Data from community analysis, potential biomarkers, and functional predicted metabolic pathways show a number of potential pathogens and fecal indicators in the Nile River. It also revealed that the Nile ecosystem may be loaded with different

pollutants and xenobiotics despite being a reservoir of different secondary metabolites-producing taxa. There is a need to investigate deeper layers of water through water columns and deeper sediment layers in order to capture potential variations in the bacterial communities.

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Compliance with Ethical Standards

Conflict of Interest All authors declare that they have no competing interests of any sort.

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