



# Investigating Physicochemical Parameters, Microbiome, and Heavy Metals in the Nile River, Greater Cairo, Egypt

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## Abstract

**Introduction:** Seasonal variation of physicochemical parameters and metal concentrations in water sources can affect the type and density of microorganisms. These microorganisms might include pathogenic bacteria, parasites, and viruses. This study aims to investigate contamination of Nile River water at Greater Cairo sites by examining the type of microbiome, physicochemical characteristics, and heavy metal concentrations.

**Methods:** Nile water samples were seasonally collected and centrifuged, and their total DNA was extracted and amplified by polymerase chain reaction (PCR) for qualitative detection of eubacteria, eukaryotes, and adenoviruses. Heavy metals were analyzed in water samples using an atomic absorption spectrometer.

**Results:** No adenoviruses were detected; 97.92% of the samples were positive for eukaryotes, 93.75% for Gram-positive bacteria, and 6.25% for Gram-negative bacteria. Although iron, cadmium, calcium, and copper concentrations were within permissible limits, lead and zinc exceeded the limits at certain sites, with maxima of 93.4 µg/L and 100 mg/L, respectively. Principal component analysis indicated that eukaryotes and Gram-positive bacteria showed positive correlations with temperature and pH, and negative correlations with Zn, Cd, Ca, and Fe. On the other hand, Gram-negative bacteria were positively correlated with dissolved oxygen, and negatively correlated with temperature, pH, total dissolved solids, and Pb.

**Conclusion:** Nile River water in Greater Cairo may be contaminated with pathogenic bacteria, parasites, and fungi. In addition, the physicochemical characteristics and metal pollution of Nile River water correlated with the microbiome type and density in the investigated sites.

**Keywords:** Eukaryotes, Gram-positive bacteria, Gram-negative bacteria, Lead, Temperature

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## Introduction

Freshwater ecosystems harbor complex, highly dynamic microbial communities that are strongly influenced by local environmental conditions and biota (1). Freshwater microorganisms play a substantial functional role in the global carbon cycle (2-4). They are responsible for most of the uptake and emission of greenhouse gases (GHGs), such as carbon dioxide and methane, in freshwater systems (5), and thus affect climate change by controlling the carbon balance (6). The incremental increase in city populations and urbanization alters freshwater ecosystems by affecting land cover and use, aquatic systems, biodiversity, and climate conditions (7). In addition, anthropogenic activities, such as the introduction of sewage water containing fecal bacteria, as well as industrial wastewater containing heavy metals,

into natural water systems, cause eutrophication, along with biological and chemical pollution that may alter the composition of the natural microbial community in water courses (1). These new microbial communities may also favor the proliferation of pathogens, whereas natural communities in less contaminated, nutrient-deficient waters may inhibit their growth (8).

The freshwater microbiome has been widely studied, with bacterial communities correlated with the water's physicochemical parameters (9). Most studies have been conducted on temperate lakes or streams, while studies on other watercourses in different geographical regions are scarce (10). The available studies mainly focus on bacteria rather than the entire freshwater microbiome, which includes archaea and eukaryotes (11). Although freshwater microbes across habitats can be structurally



similar, pronounced differences may occur (12).

There are a few studies on the effects of human activities on the microbiome of freshwater systems (13-15,1). This study represents a recent addition to the previously published papers in this field, especially those on the Nile River. Hence, the present work aims to determine the extent of Nile River water contamination by investigating the type of microbiome, physicochemical characteristics, and heavy metal concentrations at Greater Cairo sites, in addition to the correlation between these parameters.

## Materials and Methods

### Investigated area

This study was carried out on Nile River water samples collected seasonally from 12 sites representing Greater Cairo from 2019 to 2020. These sites were Kobry El-Maraziq, Helwan Water Station, El-Hwamdeya, El-Maady, El-Manial, El-Andalos, Rod El-Farag, Gezeret El-Warraq, Ring Road-Warraq, West Cairo Electric Station, Gezeret El-Qurateyeen, and El-Kanater.

### Physicochemical parameters

Temperature, pH, total dissolved solids (TDS), dissolved oxygen (DO), and electrical conductivity (EC) were measured in situ using portable HANNA™ instruments. The heavy metals in water samples, including lead (Pb), iron (Fe), zinc (Zn), cadmium (Cd), copper (Cu), and calcium (Ca), were analyzed using an atomic absorption spectrometer (GBC AVANTA™).

### Quantitative and qualitative PCR analysis of the water microbiome

Different water samples (50 ml each) were centrifuged at 2560 rpm for 15 min. The total DNA was extracted using a commercial kit (Favogen Soil DNA Isolation Mini Kit™) following the manufacturer's instructions. The purified DNA was dissolved in 50 µL water, measured on a Nanodrop ND-2000c (Thermo Fisher Scientific, Waltham, MA, USA™), and stored at -20 °C for further analysis. Genomic DNA was quantified, and then PCR-amplified for qualitative detection of eubacteria, eukaryotes, and adenoviruses using specific primers (16-19). The primer sequences are listed in Table S1. The detailed program for eukaryotes was as follows: an initial denaturation step for 3 min at 93 °C; then, 35 cycles, 30 s each, at 93 °C, annealing for 30 s at 55 °C, extension for 1 min at 72 °C, and a final elongation step for 10 min at 72 °C. For eubacteria and adenoviruses, the annealing temperatures were 60 °C and 62 °C, respectively. The reactions were performed at a 25 µL volume. PCR products were resolved on an agarose gel, electrophoresed in a Bio-Rad electrophoresis chamber using 5 µL of 100-1000 bp DNA ladder RTU as a marker, and visualized by ethidium bromide staining. The gel image was analyzed using the Cleaver Micro DOC Gel Documentation System.

### Statistical analysis

Minimum, median, maximum, 25%, and 75% percentile

values of heavy metals, as well as principal component analysis, Eigenvalues, and percentages of variance, were calculated and illustrated using GraphPad Prism (version 9).

## Results

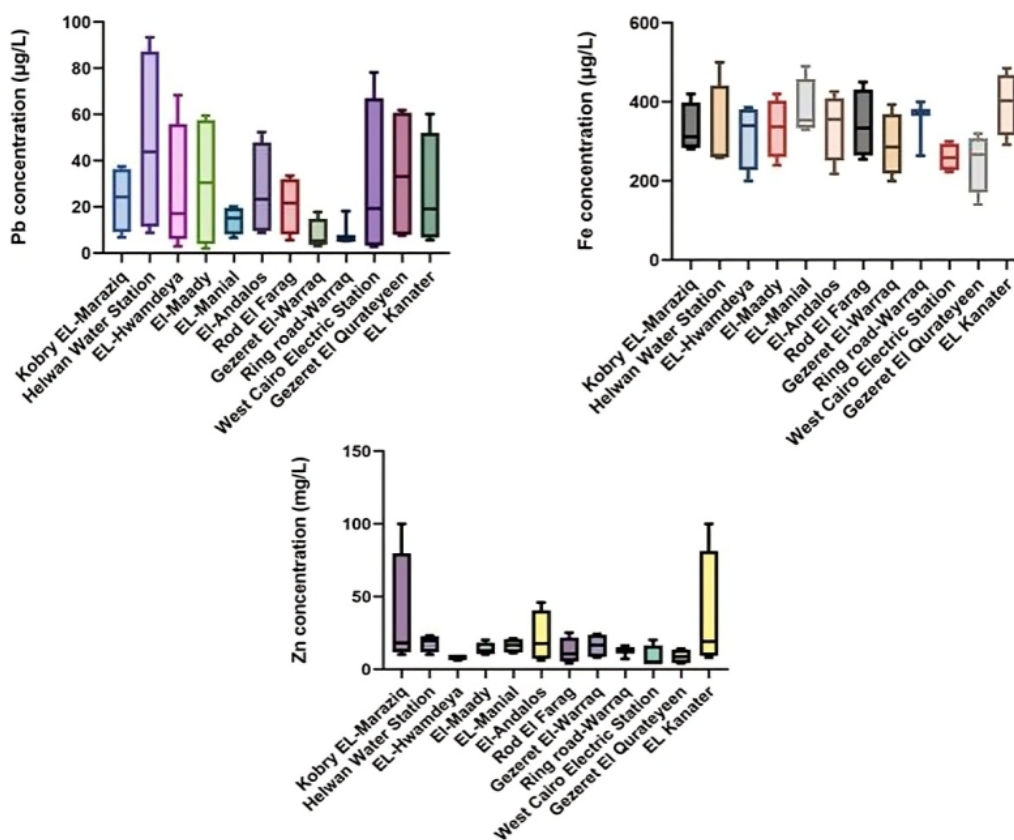
### Physicochemical parameters

Figure (S1) shows that generally, the highest temperature was recorded in summer in all the investigated sites, except Kobry El-Maraziq, Helwan Water Station, and El-Kanater, where the highest temperature was observed in spring. The maximum temperature values were 31.5 °C and 31 °C in Gezeret El-Warraq and Rod El-Farag, respectively. Regarding pH, the highest value (8.2) was noticed in Gezeret El-Warraq in winter, followed by Ring Road-Warraq (7.9) in autumn and El-Hwamdeya (7.8) in spring (Figure S1). Total dissolved solids (TDS) were the highest in Helwan Water Station (397 mg/L), El-Andalos (367 mg/L), and El-Manial (338 mg/L) in autumn, winter, and summer, respectively. As for electrical conductivity (EC), the highest value was recorded in El-Hwamdeya (795 µmohs/cm), followed by El-Manial (480 µmohs/cm) and El-Andalos (475 µmohs/cm) in winter (Figure S1). The dissolved oxygen (DO) results (Figure S1) indicated that the lowest values (4.25 mg/L to 5.92 mg/L) were observed in all sites during summer. The highest DO values were detected in Kobry El-Maraziq (12.2 mg/L) and Helwan Water Station (11.7 mg/L) during spring.

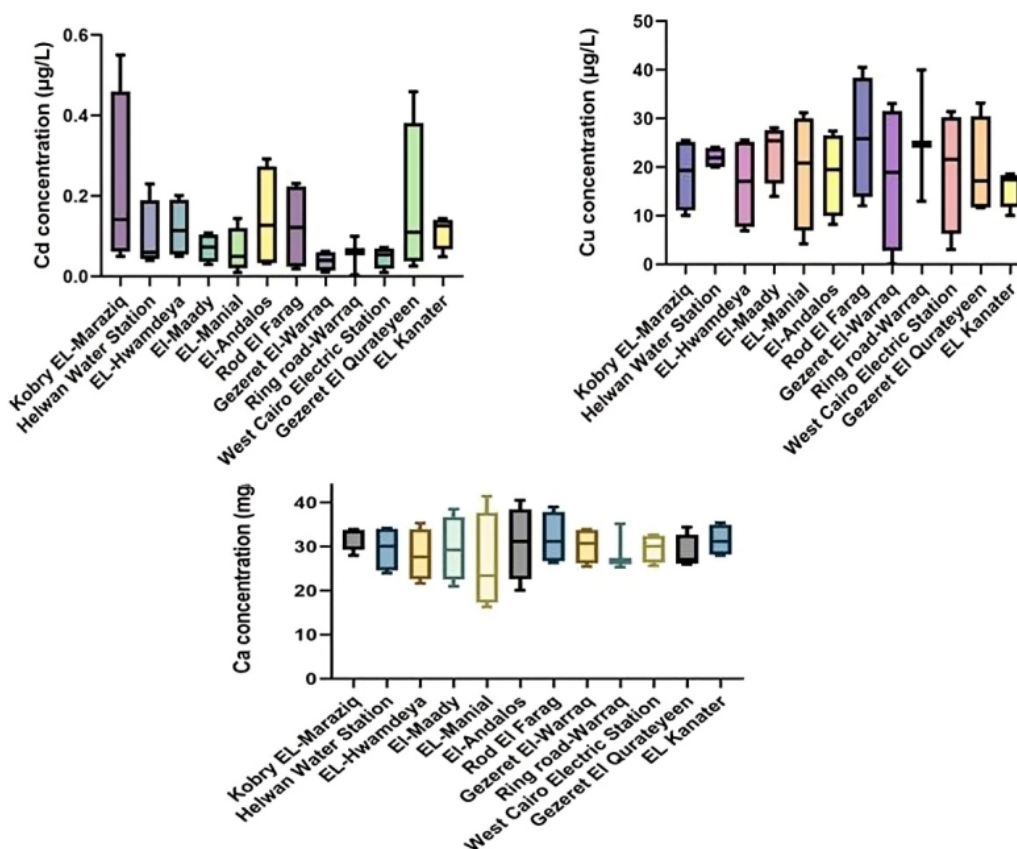
### Heavy metals

The concentrations of 6 metals (Pb, Fe, Zn, Cd, Cu, and Ca) were investigated at 12 sites along the Nile River. Figure 1 shows that the highest median Pb concentration was observed at Helwan Water Station (43.80 µg/L), followed by Gezeret El-Qurateyeen (33.10 µg/L). Also, the highest maximum concentration of Pb was observed at Helwan Water Station (93.40 µg/L), followed by 78.10 µg/L at West Cairo Electric Station. In the last two sites, Pb concentrations exceeded the permissible limit (50 µg/L). Regarding Fe, the highest median value was observed in El-Kanater (402.5 µg/L), followed by Ring Road-Warraq (373 µg/L). The highest maximum concentrations were 500 and 490 µg/L at Helwan Water Station and El-Manial, respectively (Figure 1). The highest median Zn concentration was recorded at Helwan Water Station, followed by El-Kanater, at 19 and 19.5 mg/L, respectively. On the other hand, the highest maximum concentration was 100 mg/L in both Kobry El-Maraziq and El-Kanater (Figure 1).

Figure 2 shows that the highest median and maximum concentrations of Cd were recorded in Kobry El-Maraziq, 0.14 µg/L and 0.55 µg/L, respectively. Also, El-Andalos recorded a high median Cd concentration (0.126 µg/L), while Gezeret El-Qurateyeen recorded a maximum concentration of 0.45 µg/L. For Cu, the highest median and maximum concentrations were observed in Rod El-Farag: 25.80 and 40.5 µg/L, followed by El Maady (25.40 µg/L) and Ring Road-Warraq (40 µg/L). Regarding



**Figure 1.** Minimum, median, and maximum concentrations of Pb, Fe, and Zn in Nile River water samples collected from Greater Cairo sites. The lowest line in the arm represents the minimum concentration, the highest line in the arm represents the maximum concentration, and the line inside the box represents the median concentration



**Figure 2.** Minimum, median, and maximum concentrations of Cd, Cu, and Ca in Nile River water samples collected from Greater Cairo sites. The lowest line in the arm represents the minimum concentration, the highest line in the arm represents the maximum concentration, and the line inside the box represents the median concentration

Ca, Kobry El-Maraziq showed the highest median concentration (33.3 mg/L), followed by El-Andalos, Rod El-Farag, and El-Kanater (31.2 mg/L). In addition, the highest maximum Ca concentration was observed in El-Manial (41.4 mg/L), followed by El-Andalos (40.5 mg/L) (Figure 2).

Figure (3) shows seasonal variation of Pb, Fe, and Zn concentrations, as spring and winter witnessed the highest median and maximum concentrations of Pb, which were 35.5 and 34.4  $\mu\text{g/L}$ , respectively, as median values, and 78.1 and 93.4  $\mu\text{g/L}$ , respectively, as maximum concentrations. For Fe, the highest concentrations were observed in summer and spring, as the median concentrations were 380 and 362.9  $\mu\text{g/L}$ , while the maximum concentrations were 500 and 490  $\mu\text{g/L}$ , respectively. Regarding Zn, the highest median was recorded in summer (20 mg/L), followed by spring (15 mg/L), whereas the highest maximum concentration was 100 mg/L in summer, followed by 46 mg/L in the autumn. Seasonal variations of Cd, Cu, and Ca concentrations are shown in Figure 4. It was found that the highest median Cd concentration was observed in autumn (0.12  $\mu\text{g/L}$ ), followed by spring (0.055  $\mu\text{g/L}$ ), while the highest maximum concentrations were detected in winter (0.55  $\mu\text{g/L}$ ) and autumn (0.45  $\mu\text{g/L}$ ). For Cu, the highest median concentration was recorded in spring (25.1  $\mu\text{g/L}$ ), followed by autumn (24.25  $\mu\text{g/L}$ ). On the other hand, the highest concentrations were observed in autumn (40.5  $\mu\text{g/L}$ ), followed by winter (33.2  $\mu\text{g/L}$ ). Regarding Ca, autumn followed by winter showed the highest median concentrations (35.2 and 31.8 mg/L) and maximum concentrations (41.4 and 34.4 mg/L), respectively.

### Microbiome composition

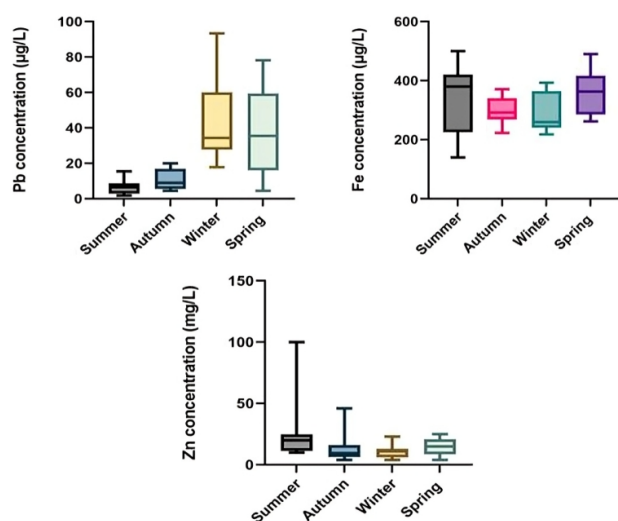
Table 1 shows that amongst 96 examined water samples, no adenoviruses were detected. On the other hand, 97.92% of the investigated samples were positive for eukaryotes,

including microscopic fungi, microalgae, and parasites. In addition, 93.75% of samples contained Gram-positive bacteria, and 6.25% contained Gram-negative bacteria (Table 1). The largest number of positive Gram-positive bacteria samples were recorded in summer and autumn, while the richest season for Gram-negative bacteria was spring. The highest percentage of positive eukaryotic samples was observed in summer (26.67%) (Table 2).

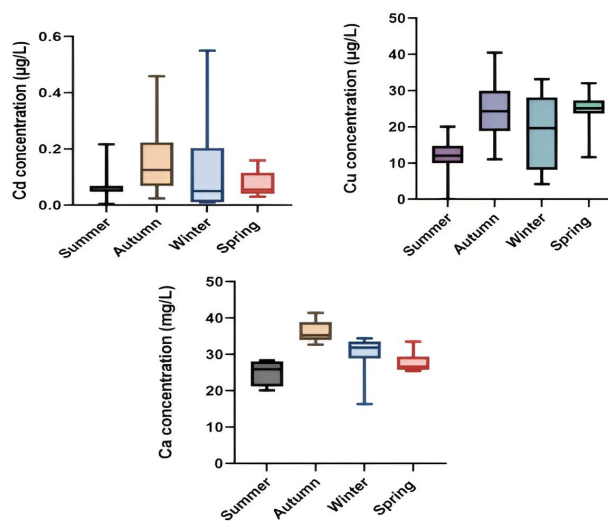
DNA concentrations in different Nile River sites in the four seasons of the study are shown in Figure 5. The highest DNA concentration in summer was observed in Gezeret El-Qurateyeen (6 ng/ $\mu\text{L}$ ), followed by El-Hwamdeya (2.8 ng/ $\mu\text{L}$ ). In autumn, El-Kanater and El-Manial witnessed the highest DNA concentrations: 8.4 and 7.6 ng/ $\mu\text{L}$ , respectively (Figure 5). Gezeret El-Qurateyeen (16.3 ng/ $\mu\text{L}$ ) and West Cairo Electric Station (13.3 ng/ $\mu\text{L}$ ) showed the highest DNA concentrations in winter, whereas Gezeret El-Warraq and Ring Road-Warraq were the highest in DNA concentrations in spring; 19.3 and 19.2 ng/ $\mu\text{L}$ , respectively (Figure 5). On the other hand, the lowest DNA concentrations in summer, autumn, winter, and spring of the present work were recorded in West Cairo Electric Station (0.4 ng/ $\mu\text{L}$ ), El-Hwamdeya (1.5 ng/ $\mu\text{L}$ ), El-Andalos (5.4 ng/ $\mu\text{L}$ ), and Helwan Water Station (7.7 ng/ $\mu\text{L}$ ), respectively (Figure 5).

Figure 6 shows that the highest total DNA concentration was observed in Gezeret El-Qurateyeen (40.7 ng/ $\mu\text{L}$ ), followed by Ring Road-Warraq (37.2 ng/ $\mu\text{L}$ ) and Gezeret El-Warraq (34.7 ng/ $\mu\text{L}$ ). In comparison, Helwan Water Station showed the lowest total DNA concentration (24.4 ng/ $\mu\text{L}$ ) throughout the study year. Seasonal variation in total DNA concentration is shown in Figure 7, with spring recording the highest value (168 ng/ $\mu\text{L}$ ) and summer the lowest (26.4 ng/ $\mu\text{L}$ ).

Figure S2 and Table 3 show that the correlation between the variables of the study was extracted into 11 principal components (PC), but six PCs that had



**Figure 3.** Seasonal variation of minimum, median, and maximum concentrations of Pb, Fe, and Zn in Nile River water samples collected from Greater Cairo sites. The lowest line in the arm represents the minimum concentration, the highest line in the arm represents the maximum concentration, and the line inside the box represents the median concentration



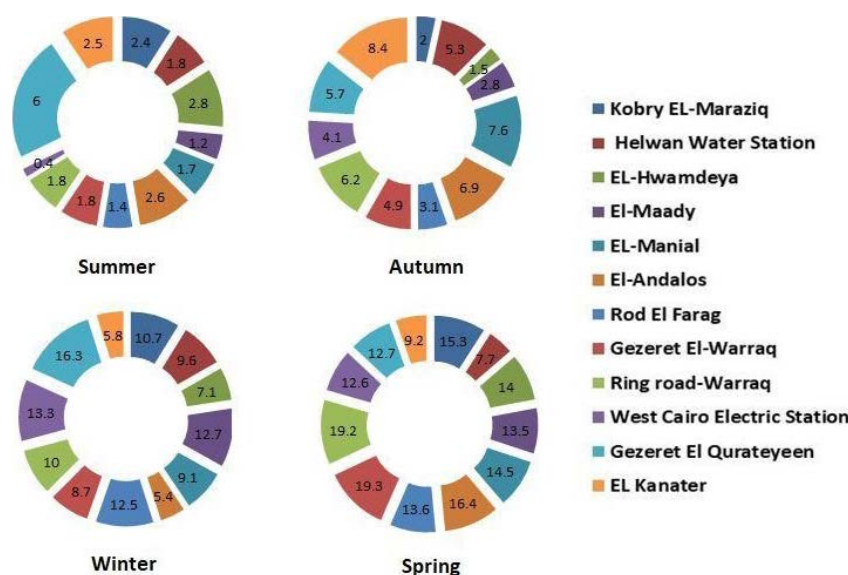
**Figure 4.** Seasonal variation of minimum, median, and maximum concentrations of Cd, Cu, and Ca in Nile River water samples collected from Greater Cairo sites. The lowest line in the arm represents the minimum concentration, the highest line in the arm represents the maximum concentration, and the line inside the box represents the median concentration

**Table 1.** Numbers and percentages of positive and negative water samples for eukaryotes, adenoviruses, and Gram-positive and Gram-negative eubacteria from Nile River sites at Greater Cairo (July 2019 to June 2020)

Samples Site	Samples examined for Eubacteria (200b.p.) (n= 96)				Samples examined for Eukaryotes (400-900b.p.) (n=96)				Samples examined for adenoviruses (301b.p.) (n=96)			
	Gram Positive n=90 (93.75 %)		Gram Negative n=6 (6.25%)		Positive n= 94 (97.92%)		Negative n= 2 (2.08 %)		Positive n= 0 (0 %)		Negative n= 96 (100 %)	
	No	%	No	%	No	%	No	%	No	%	No	%
KobryEL-Maraziq	6	6.67	2	33.33	8	8.5	0	0	0	0	8	8.33
Helwan Water Station	8	8.89	0	0	6	6.38	2	100	0	0	8	8.33
EL-Hwamdeya	6	6.67	2	33.33	8	8.5	0	0	0	0	8	8.33
El-Maady	8	8.89	0	0	8	8.5	0	0	0	0	8	8.33
El-Manial	8	8.89	0	0	8	8.5	0	0	0	0	8	8.33
El-Andalos	8	8.89	0	0	8	8.5	0	0	0	0	8	8.33
Rod El Farag	8	8.89	0	0	8	8.5	0	0	0	0	8	8.33
Gezeret El-Warraq	8	8.89	0	0	8	8.5	0	0	0	0	8	8.33
Ring road-Warraq	6	6.67	2	33.33	8	8.5	0	0	0	0	8	8.33
West Cairo Electric Station	8	8.89	0	0	8	8.5	0	0	0	0	8	8.33
Gezeret El Qurateyeen	8	8.89	0	0	8	8.5	0	0	0	0	8	8.33
El Kanater	8	8.89	0	0	8	8.5	0	0	0	0	8	8.33

**Table 2.** Numbers and percentages of positive and negative water samples for eukaryotes, adenoviruses, and Gram-positive and Gram-negative eubacteria from Nile River sites at Greater Cairo in each season (July 2019 to June 2020)

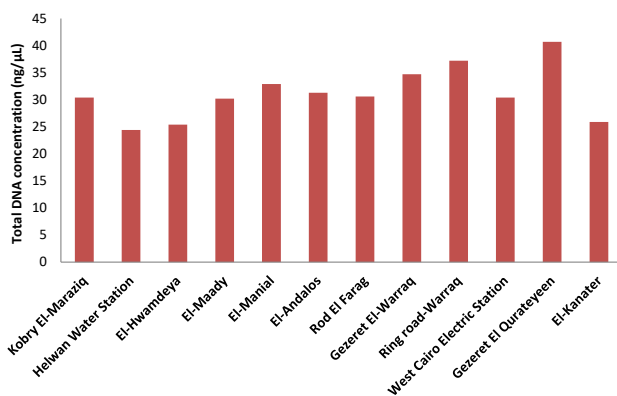
Samples Season	Samples examined for eubacteria (200 b.p.) (n=96)				Samples examined for eukaryotes (400–900 b.p.) (n=96)				Samples examined for adenoviruses (301 b.p.) (n=96)			
	Gram Positive n= 90 (93.75 %)		Gram Negative n= 6 (6.25%)		Positive n= 94 (97.92%)		Negative n=2 (2.08 %)		Positive n= 0 (0 %)		Negative n=96 (100 %)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Summer	24	26.67	0	0	24	25.53	0	0	0	0	24	25
Autumn	24	26.67	0	0	22	23.4	2	100	0	0	24	25
Winter	22	24.44	2	33.33	24	25.53	0	0	0	0	24	25
Spring	20	22.22	4	66.67	24	25.53	0	0	0	0	24	25



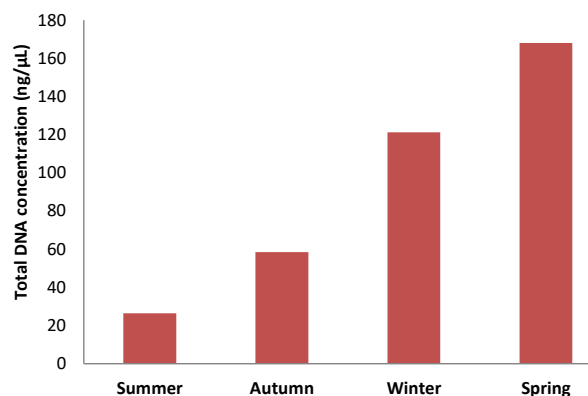
**Figure 5.** DNA concentration (ng/µL) in water samples representing the Nile River at Greater Cairo

eigenvalues greater than 1 were selected. The first PC (PC1) contained temperature (0.9214) and pH (0.8682), which showed strong negative correlation with Zn (−0.7400), Cd (−0.5808), Ca (−0.5359), and Pb (−0.4110) and positive correlation with electrical conductivity (0.6958) and eukaryotes (0.4472). Eukaryotes showed negative correlations with Pb (−0.411), Zn (−0.7400),

Cd (−0.5808), Ca (−0.5359), and Fe (−0.2087) (Figure S2 and Table 3). Additionally, PC2 contained Gram-positive bacteria (−0.7926), which were negatively correlated with Gram-negative bacteria (0.7926), eukaryotes (0.6020), Fe (0.2962), Zn (0.2358), Cd (0.081), and Ca (0.2283). Although Gram-positive bacteria showed a strong positive correlation with total dissolved solids (TDS) (−0.5084)



**Figure 6.** Total DNA concentration (ng/µL) in water samples representing the Nile River at Greater Cairo



**Figure 7.** Seasonal variation of total DNA concentration (ng/µL) from water samples representing the Nile River at Greater Cairo

**Table 3.** Principal components of variables

Variable	PC1	PC2	PC3	PC4	PC5	PC6
Gram-P	0.0807	-0.7926	0.5744	-0.0424	-0.0126	-0.1022
Gram-N	-0.0807	0.7926	-0.5744	0.0424	0.0126	0.1022
EUK	0.4472	0.6020	0.3209	-0.3391	-0.1117	-0.2283
Temp	0.9214	0.0834	0.0264	-0.2823	-0.0006	0.0609
pH	0.8682	0.2217	0.1622	0.1395	-0.0059	-0.2275
DO	-0.5290	-0.1018	-0.5089	0.3836	-0.0297	-0.3397
EC	0.6958	-0.0886	-0.2983	-0.4132	0.32513	0.1192
TDS	0.4278	-0.5084	-0.3227	0.1339	0.4890	0.0726
Pb	-0.4110	-0.5517	-0.2890	-0.5583	-0.0393	0.0291
Fe	-0.2087	0.2962	0.3389	0.0739	0.7173	0.4471
Zn	-0.7400	0.2358	0.4124	0.0761	0.4237	-0.0959
Cd	-0.5808	0.0810	-0.2919	-0.6579	0.11044	0.0114
Cu	0.0657	-0.0968	-0.0326	0.1910	-0.4392	0.8411
Ca	-0.5359	0.2283	0.4392	-0.3327	-0.2739	0.0897
Eigenvalue	4.1523	2.4679	1.8899	1.4568	1.3345	1.1891
Proportion of variance (%)	29.66	17.63	13.50	10.41	9.53	8.49
Cumulative proportion of variance (%)	29.66	47.29	60.79	71.19	80.73	89.22

The six selected principal components are those with eigenvalues > 1; Gram-P: Gram positive bacteria, Gram-N: Gram negative bacteria, EUK: eukaryotes, Temp: temperature, DO: dissolved oxygen, EC: electrical conductivity, TDS: total dissolved solids, Pb: lead, Fe: iron, Zn: zinc, Cd: cadmium, Cu: copper and Ca: calcium

and Pb (-0.5517), Gram-negative bacteria and eukaryotes were negatively correlated with these two parameters (Figure S2 and Table 3).

In PC3, there was a positive correlation between Gram-positive bacteria (0.5744) and both temperature (0.0264) and pH (0.1622), whereas Gram-negative bacteria (-0.5744) were negatively correlated with both. The correlation between dissolved oxygen (-0.5089) and Gram-negative bacteria (-0.5744) was positive, while it was negative with Gram-positive bacteria (0.5744), as observed in PC3 (Figure S2 and Table 3).

**Discussion**

Generally, the highest temperatures were recorded in summer at most of the investigated sites, with maximum values of 31.5 °C and 31 °C. Similarly, Saad et al. (20) and Okasha et al. (21) recorded the highest water temperatures in water bodies of Giza Governorate in summer, with

maximum values of 27.3 °C and 27.5 °C, respectively. In addition, Abdel-Satar et al. (22) reported that the highest mean temperature (27.4 °C) in Nile River water occurred in summer. The temperature rise observed in the current study, compared with previous work on Egypt’s watercourses, indicates the impact of climate change. Regarding pH, the highest value (8.2) was recorded in autumn and spring. In the same vein, Abdel-Satar et al. (22) observed high pH values, averaging 8.3, in Nile River water in autumn. However, our results contradict those reported in previous studies on water canals in Giza Governorate, which reported near-neutral pH values at all sites (21,23,24).

Total dissolved solids (TDS) in the current work were the highest in autumn and winter. As for electrical conductivity (EC), the highest value was recorded in winter. Our results are in line with those of Abdel-Satar et al. (22), Okasha et al. (21), and Abdel-Wareth and

Sayed (24), in which the highest EC and TDS values were detected in Nile River water and Giza canals in winter and autumn. Mostly, EC and TDS are related, and EC is a good indicator of the total amount of dissolved mineral salts in water (25). It was reported that high EC values might indicate that these watercourses receive substantial discharge from land runoff or industrial effluents (26,27). Dissolved oxygen (DO) results indicated that the lowest values were observed in all sites during summer, whereas the highest DO values were detected during spring. It was reported that low temperatures promote greater oxygen dissolution, while high temperatures (as observed in summer) favor a lower dissociation coefficient of oxygen in aquatic ecosystems (28).

In addition, higher temperatures favor decomposers, which also consume a significant portion of dissolved oxygen. This may be the cause of lower DO levels during the summer. On the contrary, in previous studies conducted in watercourses in Egypt, the highest DO values were recorded in autumn (20) and winter (22,24). Generally, the changes observed in the values of different physicochemical parameters and the seasons that showed the highest values, as compared to previous studies in Egypt, are expected as the years pass, for two main reasons. These include increased urbanization, which in turn leads to more anthropogenic activities contaminating water sources, and global warming, which threatens the environment worldwide and affects different ecological systems, including aquatic ecosystems.

Heavy metals pose a health threat because they are non-biodegradable and accumulate in the tissues of different organisms. Regular monitoring of surface waters is mandatory to protect aquatic fauna and human beings (29). In the current study, the highest median Pb concentration was observed at Helwan Water Station, followed by Gezeret El-Qurateyeen. Also, the highest maximum Pb concentration was observed at Helwan Water Station, followed by West Cairo Electric Station. In 2016, Abdel-Satar et al. (22) reported a maximum Pb concentration of 51.4 µg/L from Nile River samples. They reported that lead sources include municipal sewage and industrial waste, as well as ship effluents discharged into the Nile River (30,31). The consequences of exposure to high lead concentrations include inhibition of hemoglobin synthesis, dysfunction of the reproductive and renal systems, and teratogenic effects (32,33).

Regarding Fe, the highest median value was detected in El-Kanater, followed by Ring Road-Warraq. In contrast, the highest maximum concentrations were observed at Helwan Water Station and El-Manial. The detected Fe levels were within the permissible limit (1000 µg/L). By the same token, Abdel-Khalek et al. (34) recorded Fe concentration of 230 µg/L in the Nile River at El-Kanater, whereas Abdel-Satar (35) and Abdel-Satar et al. (22) reported high Fe concentrations in Nile samples, 1180 and 2211 µg/L, respectively. It was found that different metallurgical industries, pigments, paints, and textile industries, in addition to fertilizers, herbicides, and

corrosion of iron pipes, are the main sources of Fe in water (30). Iron contamination can cause stomach problems, nausea, diarrhea, vomiting, gastrointestinal tract damage, liver disorders, and brain damage (36).

The highest median Zn concentration in the present work was recorded at Helwan Water Station, followed by El-Kanater. On the other hand, the highest maximum concentration was observed in both Kobry El-Maraziq and El-Kanater. The detected Zn concentrations exceeded the permissible limit (5 mg/L). In the same vein, Abdel-Satar et al. (22) recorded high Zn concentrations (115 µg/L) in many sites along the Nile River. It was demonstrated that the major sources of Zn in water are electroplating, smelting and refining, biocides and fertilizers, batteries, pigments, and paint industries (30). High Zn concentrations result in vomiting, diarrhea, jaundice, and liver and kidney damage (32,33).

The highest median and maximum Cd concentrations were recorded in Kobry El-Maraziq. Also, El-Andalos had the highest median Cd concentration, while Gezeret El-Qurateyeen had the highest concentration. For Cu, the highest median and maximum concentrations were observed in Rod El-Farag, followed by El Maady (second-highest median) and Ring Road-Warraq (second-highest maximum). Regarding Ca, Kobry El-Maraziq showed the highest median concentration, followed by El-Andalos, Rod El-Farag, and El-Kanater. In addition, the highest maximum Ca concentration was observed in El-Manial, followed by El-Andalos. Fortunately, these concentrations were within the accepted limits for Cd (5 µg/L), Cu (2000 µg/L), and Ca (200 mg/L). Our results match those of Abdel-Satar et al. (22), who reported Cu and Ca concentrations within permissible levels, with a maximum Cu concentration of 50.5 µg/L. At the same time, the Ca concentration in Nile River water was 41.16 mg/L; however, they detected Cd concentrations above the permissible limit (8.1 µg/L). The main sources of Cd in water are anthropogenic activities, metal smelting and refining, fossil fuel burning, phosphate fertilizers, and sewage sludge (30). High concentrations of Cd lead to hepatic, pulmonary, reproductive, and renal disorders (32,33). Copper, on the other hand, comes from biocides, fertilizers, fuel, the electroplating industry, smelting, refining, and pigment, paint, and alloy industries (30). Increased Cu concentration can lead to headache, nausea, vomiting, diarrhea, and kidney dysfunction (32,33).

The present work demonstrated seasonal variation in Pb, Fe, and Zn concentrations, with spring and winter showing the highest median and maximum concentrations of Pb. In contrast, the highest concentrations of Fe were observed in summer and spring. Regarding Zn, the highest median was recorded in summer, followed by spring, whereas the highest maximum concentration was observed in summer, followed by autumn.

The highest median Cd concentration was observed in autumn, followed by spring, while the highest maximum concentrations were observed in winter and autumn. For Cu, the highest median concentration was recorded

in spring, followed by autumn. On the other hand, the maximum concentrations were observed in autumn, followed by winter. Regarding Ca, autumn followed by winter showed the highest median and maximum concentrations. In the same vein, Abdel-Satar et al. (22) observed the highest Ca concentrations in autumn (41.16 mg/L) and winter (28.62 mg/L), and a pronounced increase in Cd concentrations in winter. Generally, temporal and spatial variations in metal concentrations in water bodies depend on factors such as weathering, climate conditions, soil type, pH, redox potential, and dilution capacity (37). Moreover, water flow may determine increases or decreases in metal concentrations in a given season (22).

Amongst 96 examined water samples, no adenoviruses were detected. Adenoviruses are among the best-studied DNA viruses; they are usually more abundant than RNA viruses in surface waters, making them a suitable indicator of viral contamination in aquatic systems (38). Fortunately, they were not detected in Nile River samples from the sites we investigated. However, they had been previously detected in rivers and other surface waters in New Zealand (39), South Korea (40), the USA (41), Benin (42), and Japan (43). This indicated that there were no emerging viral pollutants in the investigated sites of the current work. On the other hand, 97.92% of the investigated samples in the present work were positive for eukaryotes, including microscopic fungi, microalgae, and parasites. These results coincide with the findings of El-Khayat et al. (44), who reported that 100% of the samples collected from Nile River water at Greater Cairo sites were positive for *Naegleria* sp., 52.08% for *Acanthamoeba* sp., and 33.33% for *Cryptosporidium* oocysts. Also, in our study, 93.75% of samples contained Gram-positive bacteria, while 6.25% were positive for Gram-negative bacteria. These results are in line with those of El-Khayat et al. (44), who reported the occurrence of coliforms at all investigated sites in the Nile River, with five sites exceeding the permissible limit for total coliforms in surface water (500 MPN/100 mL) (45). It was reported that Gram-negative bacteria are among the top human bacterial pathogens and exhibit the highest resistance. The most critical group on this list includes members of Enterobacteriaceae (i.e., *Klebsiella*, *Escherichia coli*, *Serratia*, and *Proteus*), *Acinetobacter*, and *Pseudomonas*. They could cause health problems such as bloodstream infections and pneumonia, which can lead to mortality (46).

The current study indicated that the highest number of positive Gram-positive samples was recorded in summer and autumn, while the richest season for Gram-negative bacteria was spring. This matches the findings of El-Khayat et al. (44), who reported the highest mean total coliform count, including Gram-negative bacteria belonging to the *Enterobacter*, *Klebsiella*, and *Citrobacter* genera, as well as *E. coli*, in Nile River samples collected in spring. Furthermore, Reittera et al. (47) postulated that summer had a higher number of detected coliform bacteria than winter, and they attributed this to the role of

high temperatures, which enhance bacterial proliferation. The highest percentage of positive eukaryotic samples in the current study was observed in summer. This coincides with the results of Miranda and Krishnakumar (48), who demonstrated that microalgal density increased in the dry months, characterized by lower water levels due to evaporation, thereby increasing nutrient concentration and promoting an increase in the diversity and density of microalgae. Moreover, Kumar and Datta (49) reported that algal growth was highest in summer. Only two samples in the autumn of the current study were negative for eukaryotes. These two samples were collected from Helwan Water Station. This station was shown to be negative for *Acanthamoeba* sp. and *Cryptosporidium* oocysts in the study of El-Khayat et al. (44) on parasites of Nile River at Greater Cairo.

Seasonal variations of DNA concentrations in different Nile River sites were investigated. The highest DNA concentration in summer was observed in Gezeret El-Qurateyeen, followed by El-Hwamdeya. Similarly, El-Khayat et al. (44) found that Gezeret El-Qurateyeen witnessed the highest number of total coliforms (1100 MPN/100 mL) in summer. In the autumn of the present study, El-Kanater, followed by El-Manial, witnessed the highest DNA concentrations. Also, El-Khayat et al. (44) reported a high number of total coliforms in El-Manial in autumn. In the current study, Gezeret El-Qurateyeen and West Cairo Electric Station showed the highest DNA concentrations in winter. In contrast, Gezeret El-Warraq and Ring Road-Warraq showed the highest in spring. These results are in line with those of Saad et al. (50) and El-Khayat et al. (44), who recorded high total coliform in Nile River samples from Warraq in spring. On the other hand, the lowest DNA concentrations in summer, autumn, winter, and spring of the present work were recorded at West Cairo Electric Station, El-Hwamdeya, El-Andalos, and Helwan Water Station. By the same token, El-Khayat et al. (44) showed low total coliform numbers in El-Andalos and Helwan Water Station in winter and spring, respectively. Also, Reittera et al. (47) found lower coliform bacterial counts in winter and reported that low temperatures are not suitable for the growth of most bacteria.

The highest total DNA concentration in the present study was observed in Gezeret El-Qurateyeen, followed by Ring Road-Warraq and Gezeret El-Warraq. At the same time, Helwan Water Station showed the lowest total DNA concentration during the study year. These results are consistent with those of El-Khayat et al. (44), who demonstrated that Gezeret El-Qurateyeen was among the most contaminated sites for total coliforms. In contrast, Helwan Water Station was the least contaminated site for coliforms and parasites. The Gezeret El-Qurateyeen and El-Warraq areas are crowded with casinos and boats that discharge their wastewater directly into the Nile River. Furthermore, fishermen at Gezeret El-Qurateyeen use fermented tree waste as bait to catch fish, thereby contaminating the Nile's water with more bacteria and

parasites (44).

Our results on seasonal variation in total DNA concentration indicated that spring had the highest concentration, while summer had the lowest. El-Khayat et al. (44) reported a similar result, with spring as the richest season for total coliforms (633.42 MPN/100 mL), *Acanthamoeba* sp. (18.75% positive samples), *Cryptosporidium* oocysts (12.5% positive samples), and *Naegleria* sp.

The correlation matrix for the study variables was divided into 11 principal components (PCs). The positive correlation between temperature and eukaryotes in the current work matches the findings of Saad et al. (20), who reported a strong positive correlation between temperature and one of the most ubiquitous fungal genera in watercourses of Gharbeya Governorate, Egypt: *Aspergillus* sp. Moreover, Abdel-Wareth and Sayed (24) demonstrated that most fungal genera that were isolated from water bodies in the same governorate were positively correlated with temperature. This can be explained by the ability of filamentous fungi to tolerate a wide range of temperatures (16.05 °C–30.75 °C) (20). In addition, as temperature increases, evaporation rates increase, concentrating nutrients in the water, which eventually leads to enhanced microalgal productivity (51).

The observed positive correlation between pH and eukaryotes in the present study is consistent with the findings of Saad et al. (20), who reported a positive correlation between pH and a group of fungi known as phycomyces. Similarly, Abdel-Wareth and Sayed (24) reported a positive relationship between *Mucor* sp. and *Rhizopus* sp. (phycomyces) and pH in water canals in Giza Governorate, Egypt. Also, Asan et al. (52) observed a positive correlation between waterborne fungal abundance and pH. Eukaryotes in the current work showed negative correlations with Pb, Zn, Cd, Ca, and Fe. This might be attributed to the toxicity of these metals to fungi and microalgae, or to the ability of these microorganisms to sequester them via specific mechanisms (21,53).

Additionally, PC2 contained Gram-positive bacteria, which were negatively correlated with Gram-negative bacteria, eukaryotes, Fe, Zn, Cd, and Ca. Gram-positive bacteria are more sensitive to xenobiotics than Gram-negative bacteria (54); hence, the latter can coexist with different heavy metals without being affected. Although Gram-positive bacteria in the current study showed a strong positive correlation with total dissolved solids (TDS) and Pb, Gram-negative bacteria and eukaryotes were negatively correlated with these two parameters. A similar positive correlation was observed between fecal streptococci (Gram-positive bacteria) and TDS, whereas *E. coli* (Gram-negative bacterium) was negatively correlated with TDS in Nile River water samples (55). The negative correlation between eukaryotes and TDS can be explained by the fact that, as TDS increases, it acts as a barrier to light penetration into the water, thereby preventing photosynthetic microalgae from growing well and decreasing their density (48).

In PC3, there was a positive correlation between Gram-positive bacteria and both temperature and pH, whereas Gram-negative bacteria were negatively correlated with those. In the same context, Abdel Rahim et al. (55) recorded a positive correlation between temperature and fecal streptococci, but a negative relation with *E. coli*. It was found that higher temperatures increase the productivity of a water body by boosting bacterial metabolism and nutrient cycling rates (56). The correlation between dissolved oxygen and Gram-negative bacteria in our study was positive, while dissolved oxygen and Gram-positive bacteria were negatively correlated. By the same token, Abdel Rahim et al. (55) observed a negative correlation between DO and Gram-positive fecal streptococci, whereas a positive correlation was observed between DO and *E. coli* in Nile River samples from Sohag Governorate, Egypt.

The main limitations of this study are the unavailability of cheap, rapid tools that can detect microbiome composition both qualitatively and quantitatively, and the lack of sensitive, cost-effective heavy-metal detectors that can determine the concentrations of multiple heavy metals in situ simultaneously.

## Conclusion

Microbiome type and density varied during the year of the study. Eukaryotes were not detected in one of the investigated sites, and Gram-positive bacteria were more dominant than Gram-negative bacteria. The detection of these bacteria indicated biological pollution of the Nile's water at the investigated sites. Moreover, the dominance of eukaryotes might indicate the presence of pathogenic fungi and parasites. Also, changes in the physicochemical characteristics of Nile River water, along with contamination with high concentrations of Pb and Zn, demonstrated that Nile water was exposed to excessive effluents. The correlation between physicochemical parameters and the microbiome showed that the observed seasonal variations might favor the proliferation of existing pathogenic microorganisms or the emergence of new pathogens. Regular monitoring of surface water is necessary to collect recent data. Also, urbanization should be regulated by law so that socioeconomic activities are reduced, and their impact on water quality and, consequently, on human health is controlled. Future studies should focus on enhancing monitoring and data collection methods, studying interactions among various parameters, developing more advanced detection and analytical techniques for the microbiome, and developing predictive models that incorporate physicochemical and biological factors to address the future status of Nile River water quality.

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### Competing Interests

The authors declare that there are no competing interests.

### Ethical Approval

The authors hereby certify that all data collected during the study are as stated in the manuscript, and no data from the study have been or will be published separately elsewhere.

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### Supplementary Files

Supplementary File 1 contains Table S1, and Figures S1 and S2.

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