

RESEARCH ARTICLE

Seasonal dynamics of fish-borne pathogens and water quality in Lake Nasser, Egypt: Environmental correlates and One Health implications



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ABSTRACT

Background and Aim: Seasonal variation profoundly influences aquatic ecosystems, altering water quality, microbial ecology, and food safety. In Egypt's Lake Nasser, one of the world's largest artificial freshwater reservoirs, climatic fluctuations may shape pathogen dynamics, affecting fish health and public safety. This study investigated the seasonal variations in physicochemical parameters, heavy metals, and the phenotypic and genotypic profiles of major fish-borne pathogens in Lake Nasser, Egypt, within a One Health framework.

Materials and Methods: A total of 300 water and 300 Nile tilapia (*Oreochromis niloticus*) samples were collected seasonally from five lake sectors. Physicochemical indicators (temperature, pH, dissolved oxygen [DO], electrical conductivity, and six heavy metals) were analyzed following the American Public Health Association and the Association of Official Analytical Collaboration standards. Bacteriological examinations were performed according to ISO protocols to enumerate total bacterial counts (TBCs) and to isolate *Staphylococcus aureus*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, and *Aeromonas hydrophila*. Molecular confirmation of species and virulence determinants was achieved using polymerase chain reaction (PCR) assays targeting *16S ribosomal RNA* and virulence genes. Correlations between environmental parameters and pathogen prevalence were evaluated using Pearson's analysis ($p < 0.05$).

Results: Water temperature peaked in summer (29.7°C) while DO and pH were highest in winter (8.05 mg/L and 8.7, respectively). While zinc, copper, cadmium, and lead exceeded the Canadian Council of Ministers of the Environment thresholds for aquatic life, all heavy metals were below World Health Organization limits for drinking water. TBCs increased significantly during summer (3.59×10^5 Colony Forming Unit/g). *S. aureus* and *V. cholerae* predominated in summer, *P. aeruginosa* in spring, and *A. hydrophila* in autumn. Temperature positively correlated with bacterial counts ($r = 0.82$, $p < 0.001$), whereas DO showed a negative association ($r = -0.71$, $p = 0.001$). PCR confirmed multiple virulence genes in all isolates.

Conclusion: Seasonal climatic fluctuations strongly influence microbial contamination in Lake Nasser. Although water quality remains within acceptable limits, elevated temperatures and reduced oxygen during summer promote pathogen proliferation, posing food safety risks. Continuous One Health-based surveillance integrating environmental, microbiological, and climatic indicators is recommended to safeguard aquatic ecosystems and public health under changing climate conditions.

Keywords: Egypt, fish-borne pathogens, Lake Nasser, One Health, seasonal variation, water quality.

INTRODUCTION

Fish play a vital role in human nutrition, providing high-quality protein, omega-3 fatty acids, essential amino acids, vitamins, and minerals such as calcium and phosphorus, thereby serving as a critical component of balanced diets worldwide [1]. Egypt is a leading aquaculture producer, accounting for approximately 73.8% of Africa's total output and ranking ninth globally, contributing about 1.54% of global fish production [2].

Foodborne pathogens, microorganisms capable of causing illness when ingested through contaminated food or water, remain a major global public health concern. According to the World Health Organization (WHO), one

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in every ten individuals becomes ill each year as a result of consuming contaminated food, leading to millions of infections and thousands of deaths worldwide [3].

Fish species occupy a unique position at the interface of environmental change and public health. As sentinel organisms, they respond rapidly to fluctuations in water temperature, oxygen concentration, and contaminant load, making them sensitive indicators of climate variability and aquatic pollution [4]. Monitoring the microbial load and pathogen composition of aquatic ecosystems thus provides valuable insights into environmental health and potential risks to consumers of contaminated fish or water [5].

The Intergovernmental Panel on Climate Change defines climate change as a long-term alteration in average weather patterns and variability, typically spanning decades or longer [4]. The global climate system is influenced by both natural factors, such as solar radiation, oceanic and atmospheric circulation, and human activities that intensify greenhouse gas emissions [6]. These drivers have far-reaching impacts on global ecosystems, with aquatic environments being particularly vulnerable. Seasonal and anthropogenic changes, including altered water management practices, drainage, and pollution runoff, can disrupt aquatic homeostasis and promote microbial proliferation [7].

Environmental parameters such as temperature, precipitation, humidity, and soil characteristics play a crucial role in determining the distribution and persistence of zoonotic pathogens. Climatic shifts, characterized by elevated ambient temperatures, irregular rainfall, and water scarcity, may enhance the survival and transmission of pathogenic microorganisms [8]. Consequently, seasonal fluctuations linked to climate change can compromise food systems, increasing the incidence of foodborne diseases and negatively affecting both food quality and safety [9].

Adopting a One Health perspective underscores the interdependence of environmental, animal, and human health. In aquatic ecosystems, declining water quality not only threatens fish well-being but also increases the risk of transmission of fish-borne pathogens to humans. Although previous studies by Abdel-Satar *et al.* [10] and Imam *et al.* [11] have assessed water quality and pollution indicators in Egyptian freshwater systems, most have concentrated on physicochemical characteristics without adequately exploring their relationship to the occurrence, virulence, and public health significance of fish-borne pathogens.

Despite extensive research on the physicochemical and ecological characteristics of Egyptian freshwater bodies, a critical knowledge gap remains in understanding how seasonal climatic variations influence the microbiological safety of fish and water within a One Health framework. Previous studies on Lake Nasser and other Nile-based ecosystems have primarily focused on chemical pollution, nutrient load, or heavy metal accumulation, while overlooking the seasonal dynamics of fish-borne pathogens and their genotypic virulence determinants. Moreover, earlier works have relied exclusively on phenotypic identification of bacteria, without integrating molecular confirmation through *16S ribosomal RNA (rRNA)* and virulence gene profiling, which are essential for accurately assessing zoonotic potential. In addition, there has been limited correlation analysis linking water quality indices, such as temperature, pH, dissolved oxygen (DO), and conductivity, with pathogen prevalence across different seasons. The lack of such integrative studies constrains the ability to predict how climate-induced environmental fluctuations affect microbial ecology, fish health, and public health risks. Addressing this gap is crucial for developing early-warning systems and evidence-based management strategies that safeguard both aquaculture sustainability and food safety in Egypt and comparable arid-region reservoirs.

This study aimed to investigate the seasonal variations in fish-borne pathogens and water quality parameters in Lake Nasser, Egypt, under the One Health framework. Specifically, it sought to:

1. Evaluate seasonal changes in physicochemical parameters (temperature, pH, DO, electrical conductivity [EC], and heavy metal concentrations) of lake water across different sectors
2. Determine the total bacterial load and isolate key fish-borne pathogens, including *Staphylococcus aureus*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, and *Aeromonas hydrophila*, from Nile tilapia (*Oreochromis niloticus*) samples collected seasonally
3. Characterize the phenotypic and genotypic profiles of these pathogens using conventional microbiological assays and polymerase chain reaction (PCR) targeting species-specific *16S rRNA* and virulence genes
4. Assess correlations between environmental factors and pathogen prevalence to identify key drivers of microbial proliferation under varying seasonal conditions
5. Interpret the findings within a One Health context, emphasizing implications for food safety, environmental health, and climate-adaptive aquaculture management.

Through this integrative approach, the study provides novel insights into the environment–pathogen–host interplay in Lake Nasser and establishes a foundational framework for seasonal microbial risk assessment and One Health-based monitoring systems to mitigate foodborne hazards in aquatic ecosystems.

MATERIALS AND METHODS

Ethical approval

All procedures involving fish handling and sampling were conducted following the guidelines of the Institutional Animal Care and Use Committee of Aswan University, Egypt, under protocol No. 09-11-2022 FVM. The study design, animal handling, and reporting adhered to the Animal Research: Reporting of *In Vivo* Experiments guidelines, ensuring the humane treatment of animals and the ethical use of aquatic organisms in research.

Study period and location

This study was conducted from January to December 2023 in Lake Nasser, located in Aswan Governorate, Egypt. Lake Nasser, a man-made reservoir in southern Egypt, was formed following the construction of the Aswan High Dam and is located between latitudes 22°00' and 23°58' N and longitudes 31°19' and 33°19' E (Figure 1). It is one of the world's largest artificial reservoirs and serves as a major freshwater reserve for Egypt. The lake includes several side extensions and occupies an area of approximately 5248 km² [2], situated in a desert region between the Mediterranean and subtropical climatic zones [10].



Figure 1: Satellite map of Nasser Lake, Egypt.

In Egypt, the lake is called Lake Nasser, whereas in Sudan, it is known as Lake Nubia. It is of great importance to Egypt, as it provides over 95% of the nation's freshwater needs [10]. The reservoir is generally divided into three main areas:

1. The southern area, near the river in the southern part of Lake Nubia
2. The northern area, extending from Tushka/Amada to the Aswan Dam in Egypt
3. The middle area, which exhibits river-like characteristics during the flood season and lake-like characteristics for the remainder of the year [11].

Water sampling

Water samples were collected seasonally (four times per year: spring, summer, autumn, and winter) from five sites along the main channel of Lake Nasser: Aswan (24°5'20.1768" N, 32°53'59.3880" E), Wadi Abyade (24°34'00" N, 34°55'00" E), El-Madiq (22°52'00" N, 32°35'00" E), Tushka (23°06'00" N, 30°53'60" E), and Abu-Simbel (22°20'12.56" N, 31°37'31.91" E).

At each site, three subsampling locations were selected, representing the eastern, western, and central sections of the main channel, to capture spatial variability. Five independent water samples were collected from each subsampling location using a 2-L polyvinyl chloride Van Dorn plastic flask, maintained at 4°C ± 1°C in insulated iceboxes, and transported to the laboratory within 6 h of collection for analysis.

This design yielded 75 water samples per season (5 sites × 3 locations × 5 samples = 75) and 300 over the entire study period. All samples were treated as biological replicates, representing independent environmental

collections within a site and season. Physicochemical analyses were performed in triplicate for each biological sample, and the mean values were used for statistical analysis. Water samples were handled and stored according to the American Public Health Association (APHA) [12] standard methods to ensure sample integrity until laboratory examination.

Fish sampling

A total of 300 Nile tilapia (*O. niloticus*) specimens were randomly captured directly from Lake Nasser using gill nets operated by local fishermen under the supervision of the research team. Market-sourced fish were not used to avoid possible contamination during handling or storage.

Sampling was conducted at five sites along Lake Nasser (Aswan, Wadi Abyade, El-Madiq, Tushka, and Abu-Simbel) for 1 year, and was performed seasonally, winter (January–March), spring (April–June), summer (July–September), and autumn (October–December), with 75 fish obtained per season (approximately 15 fish/site), ensuring uniform spatial and temporal representation.

Only apparently healthy fish of comparable size (190 ± 10 g) and length (27 ± 2 cm) were included to minimize physiological variability. Fish with visible lesions, deformities, or signs of disease were excluded. Immediately after capture, specimens were placed in sterile polyethylene bags, transported on ice (4°C), and processed within 6 h at the Food Analysis Laboratory, Faculty of Veterinary Medicine, Aswan University.

During fieldwork, temperature loggers were used to ensure continuous monitoring of transport conditions and to maintain sample integrity and representativeness. Each fish was considered a biological replicate representing an independent sampling unit.

All microbiological analyses were conducted in triplicate for each biological sample, and mean values were used for statistical comparisons among seasons. To reduce analytical bias, all fish and water samples were coded alphanumerically on collection, with only sample type and sequence included. The sampling site and season were concealed from laboratory personnel conducting microbiological and molecular analyses. Data decoding and association with sampling metadata (location, season, and environmental parameters) were performed only after all laboratory analyses were completed. This blinding protocol ensured objective data processing and interpretation throughout the investigation.

Meteorological data

Meteorological data were obtained from the Aswan Meteorological Station, operated by the Egyptian Meteorological Authority, to assess the influence of seasonal climatic variations on water quality. Parameters recorded included average air temperature ($^{\circ}\text{C}$), total monthly rainfall (mm), and mean relative humidity (%) for each sampling season during the study (January–December 2023). These data were used to contextualize observed seasonal fluctuations in physicochemical parameters and bacterial loads (Table S5).

Physicochemical analysis of water

The following water quality parameters were assessed on-site: temperature and pH were recorded using a calibrated digital pH meter (WTW Bench 7110, Germany) with buffer solutions at pH 4.0, 7.0, and 10.0. DO content and EC were analyzed using a waterproof portable meter (HI98192, USA), calibrated with the manufacturer-supplied reference standards, following APHA methods [12].

Heavy metals – manganese (Mn), iron (Fe), cadmium (Cd), zinc (Zn), lead (Pb), and copper (Cu) – were extracted by the nitric acid digestion technique and analyzed using atomic absorption spectrophotometry (iCE-3000 series, Thermo Scientific, USA) according to AOAC Official Methods 999.10 and 2011.19 [13].

To avoid contamination, all glassware and sampling bottles were pre-washed with 10% nitric acid and rinsed thoroughly with deionized water. Analytical limits of detection (LOD) and limits of quantification (LOQ) were established based on a signal-to-noise ratio of 3:1 and 10:1, respectively. The LODs ranged from 0.001–0.005 mg/L, and LOQs ranged from 0.003–0.015 mg/L, depending on the metal analyzed. Quality assurance was maintained by analyzing certified reference materials (NIST SRM 1643f–Trace Elements in Water, USA), method blanks, and duplicate samples with each analytical batch. Spike recovery tests were conducted by fortifying blank samples with known amounts of each metal, yielding recoveries of 94%–104%. Instrument calibration was performed daily using five-point standard curves ($R^2 \geq 0.999$) prepared from Merck (Germany) stock standard solutions. These procedures ensured analytical accuracy, precision, and reproducibility throughout the study.

Bacteriological analysis

Before dissection, fish specimens were cleaned with sterile distilled water to remove debris, surface-

sterilized by immersion in 70% ethanol for 2 min, and washed with sterile physiological saline (0.85% sodium chloride). The fish were then placed on sterile aluminum foil in a Class II biosafety cabinet and dissected using sterile instruments. The scales, head, fins, tails, and bones were removed, and the two back fillet muscles were aseptically excised using flame-sterilized scalpels and forceps to minimize cross-contamination. All instruments were re-sterilized between samples. These procedures ensured that the isolated bacteria originated from internal tissues rather than external surfaces [14].

Bacteriological analysis included the following procedures

TBC

Plate counts were determined using plate count agar (M091A, HiMedia, Mumbai, India) and the pour plate technique. Plates were incubated for 48 h at 37°C, and colony numbers (30–300 CFU/g) were counted using a digital colony counter (DC-8 OSK 100086, Kayagaki, Japan). Results were expressed as CFU/g [15].

S. aureus

One milliliter of homogenate was plated on Baird-Parker Agar Base (Oxoid CM 0275, UK) supplemented with egg yolk tellurite emulsion and incubated at 37°C for 48 h. Colonies appearing black and glossy with translucent zones were enumerated (CFU/g). The isolates were purified and biochemically identified following ISO 6888-1:2021 [16]. Positive control strains (*S. aureus* American Type Culture Collection [ATCC] 25923) were used alongside negative controls to confirm selective growth and verify media sterility [17].

V. cholera

A loopful of pre-enrichment broth was streaked on thiosulfate–citrate–bile–salt–sucrose agar (GM189, HiMedia) and incubated at 37°C for 24 h [18]. Yellow, flat colonies were counted (CFU/g), purified, and identified biochemically according to ISO/TS 21872-1 [19]. Positive control: *V. cholerae* ATCC 14035.

P. aeruginosa

Samples were spread on *Pseudomonas* agar base (HiMedia) with glycerol and incubated at 25°C for 48 h [20]. Greenish-yellow colonies were purified and re-streaked on nutrient agar (37°C, 24 h). Colony counts (CFU/g) were determined, and isolates were biochemically confirmed following ISO 16266 [21]. Positive control: *P. aeruginosa* ATCC 27853.

A. hydrophila

Pre-enrichment cultures were streaked on Aeromonas isolation medium base (HiMedia-M884) supplemented with ampicillin and incubated at 35°C for 24 h. Pale green translucent colonies with darker centers were purified and identified following ISO 10272-1:2017 [22, 23]. Positive control: *A. hydrophila* ATCC 7966.

Molecular confirmation and virulence gene detection

At the Dzhelepov Laboratory of Nuclear Problems, Joint Institute for Nuclear Research (Dubna, Russia), PCR was used to confirm bacterial identification and detect species-specific virulence genes.

Presumptive isolates obtained from selective media and biochemically confirmed were validated using species-specific PCR assays targeting *16S rRNA* and virulence genes. DNA was extracted using the Gene JET Genomic DNA Purification Kit (Thermo Scientific, Waltham, MA, USA), and DNA quality was measured with a NanoPhotometer NP80 Touch (Implen, Munich, Germany).

PCR reactions were prepared using a GenPak® PCR Core Kit (ISOGENE Laboratory®) in 25 µL total volume, containing 12.5 µL Master Mix, 1 µL of each primer (20 pmol), 6 µL of DNA template, and 4.5 µL of nuclease-free water. Primers and cycling conditions were obtained from published validated sources (Supplementary Tables S1-S4). Each primer set was synthesized by Willowfort Company, London, UK and validated with positive control strains.

PCR-grade water was used as a negative control, and authenticated genomic DNA from reference strains was used as a positive control. PCR products were separated by 1.5% agarose gel electrophoresis (Bio-Rad Laboratories, Hercules, CA, USA) with GeneRuler DNA Ladder Mix (Thermo Scientific), and visualized using a Gel Doc XR+ Gel Documentation System (Bio-Rad Laboratories).

This molecular approach provided genotypic confirmation of the isolates and identified key virulence genes directly implicated in foodborne pathogenesis, supporting the study's One Health framework linking environmental changes to food safety risks.

Statistical analysis

Data analysis was performed using Statistical Package for the Social Sciences version 25.0 (IBM Corp., Armonk, NY, USA).

- Shapiro-Wilk test was applied to verify data normality, and Levene's test to assess variance homogeneity
- When assumptions were met, parametric analyses were conducted
- One-way analysis of variance was used to evaluate seasonal and geographical differences in water quality and bacterial counts
- Tukey's honestly significant difference *post hoc* test was applied for pairwise comparisons among groups showing significance
- Pearson's correlation analysis was performed to determine associations between physicochemical parameters (temperature, pH, DO, EC, and heavy metals) and bacterial prevalence.

All results are expressed as mean \pm standard error, and statistical significance was determined at $p < 0.05$.

RESULTS

Water physicochemical parameters

Table 1 presents the seasonal variations in water physicochemical parameters, temperature, pH, DO, and EC, recorded in Lake Nasser throughout the study.

A significant increase in water temperature ($p < 0.05$) was observed during the summer compared with winter. The recorded temperature ($^{\circ}\text{C}$) ranged from 24.6 ± 1.26 to 29.62 ± 1.43 in summer, which was higher than that observed in autumn (21.4 ± 0.18 to 23.7 ± 1.04), spring (18.4 ± 0.33 to 22.5 ± 1.53), and winter (16.2 ± 1.03 to 18.6 ± 1.11).

The pH values exhibited moderate seasonal variation, remaining within alkaline ranges across all seasons. The lowest pH values were recorded during summer (8.19 ± 0.04 to 8.58 ± 0.03), whereas higher readings were observed in autumn (8.24 ± 0.04 to 8.64 ± 0.02), spring (8.28 ± 0.02 to 8.69 ± 0.04), and winter (8.27 ± 0.02 to 8.71 ± 0.04). The decrease in pH during summer was statistically significant ($p < 0.05$) compared with other seasons.

Table 1: Measurements of water parameters during different seasons in Nasser Lake.

Sector	Parameters	Season			
		Winter	Spring	Autumn	Summer
Aswan	Temp ($^{\circ}\text{C}$)	$16.24 \pm 1.03^{\text{c}}$	$18.42 \pm 0.33^{\text{b}}$	$21.46 \pm 0.18^{\text{a}}$	$24.61 \pm 1.26^{\text{a}}$
	pH	$8.27 \pm 0.02^{\text{a}}$	$8.28 \pm 0.02^{\text{a}}$	$8.24 \pm 0.04^{\text{a}}$	$8.19 \pm 0.04^{\text{b}}$
	EC ($\mu\text{S}/\text{cm}$)	$283.6 \pm 4.2^{\text{a}}$	$278.3 \pm 3.4^{\text{a}}$	$263.5 \pm 7.3^{\text{b}}$	$254.2 \pm 6.1^{\text{b}}$
	DO (mg/L)	$7.42 \pm 0.32^{\text{a}}$	$6.88 \pm 0.24^{\text{b}}$	$6.22 \pm 0.37^{\text{b}}$	$5.89 \pm 0.33^{\text{b}}$
Wadi Abyade	Temp ($^{\circ}\text{C}$)	$16.63 \pm 1.02^{\text{d}}$	$18.64 \pm 1.61^{\text{c}}$	$21.78 \pm 1.01^{\text{b}}$	$26.74 \pm 1.22^{\text{a}}$
	pH	$8.43 \pm 0.02^{\text{a}}$	$8.39 \pm 0.01^{\text{a}}$	$8.33 \pm 0.04^{\text{a}}$	$8.22 \pm 0.03^{\text{b}}$
	EC ($\mu\text{S}/\text{cm}$)	$279.3 \pm 3.6^{\text{a}}$	$275.2 \pm 4.2^{\text{a}}$	$259.3 \pm 6.2^{\text{b}}$	$250.4 \pm 8.3^{\text{b}}$
	DO (mg/L)	$7.47 \pm 0.21^{\text{a}}$	$6.47 \pm 0.26^{\text{b}}$	$6.19 \pm 0.31^{\text{b}}$	$5.78 \pm 0.42^{\text{b}}$
El-Madiq	Temp ($^{\circ}\text{C}$)	$17.37 \pm 1.04^{\text{d}}$	$20.26 \pm 0.84^{\text{c}}$	$22.83 \pm 0.61^{\text{b}}$	$27.25 \pm 1.41^{\text{a}}$
	pH	$8.65 \pm 0.03^{\text{a}}$	$8.67 \pm 0.02^{\text{a}}$	$8.59 \pm 0.04^{\text{a}}$	$8.48 \pm 0.03^{\text{b}}$
	EC ($\mu\text{S}/\text{cm}$)	$268.8 \pm 8.3^{\text{a}}$	$260.2 \pm 9.3^{\text{a}}$	$252.6 \pm 5.8^{\text{b}}$	$246.7 \pm 3.6^{\text{b}}$
	DO (mg/L)	$7.63 \pm 0.22^{\text{a}}$	$7.02 \pm 0.37^{\text{a}}$	$6.31 \pm 0.25^{\text{b}}$	$5.69 \pm 0.17^{\text{b}}$
Lusaka	Temp ($^{\circ}\text{C}$)	$17.83 \pm 1.03^{\text{c}}$	$21.44 \pm 1.08^{\text{b}}$	$23.47 \pm 1.14^{\text{b}}$	$29.32 \pm 1.28^{\text{a}}$
	pH	$8.68 \pm 0.02^{\text{a}}$	$8.62 \pm 0.04^{\text{a}}$	$8.59 \pm 0.06^{\text{a}}$	$8.54 \pm 0.02^{\text{b}}$
	EC ($\mu\text{S}/\text{cm}$)	$262.3 \pm 5.8^{\text{a}}$	$258.7 \pm 6.4^{\text{a}}$	$256.1 \pm 4.9^{\text{b}}$	$246.5 \pm 7.4^{\text{b}}$
	DO (mg/L)	$7.87 \pm 0.42^{\text{a}}$	$7.13 \pm 0.35^{\text{a}}$	$6.49 \pm 0.27^{\text{b}}$	$5.72 \pm 0.36^{\text{c}}$
Abu-Simbel	Temp ($^{\circ}\text{C}$)	$18.63 \pm 1.11^{\text{c}}$	$22.53 \pm 1.53^{\text{b}}$	$23.71 \pm 1.04^{\text{b}}$	$29.72 \pm 1.43^{\text{a}}$
	pH	$8.71 \pm 0.04^{\text{a}}$	$8.69 \pm 0.04^{\text{a}}$	$8.64 \pm 0.02^{\text{a}}$	$8.57 \pm 0.03^{\text{b}}$
	EC ($\mu\text{S}/\text{cm}$)	$255.6 \pm 4.2^{\text{a}}$	$258.3 \pm 8.2^{\text{a}}$	$248.5 \pm 10.3^{\text{b}}$	$241.3 \pm 6.7^{\text{b}}$
	DO (mg/L)	$8.05 \pm 0.33^{\text{a}}$	$7.76 \pm 0.24^{\text{a}}$	$6.29 \pm 0.62^{\text{b}}$	$5.61 \pm 0.37^{\text{c}}$

Temp = Temperature, DO = Dissolved oxygen content, EC = Electrical conductivity. Data were represented as mean \pm standard error. Values with a different superscript letter in the same raw are significantly different between seasons Analysis of Variance with *post hoc* Tukey test, $p < 0.05$.

The EC values ($\mu\text{S}/\text{cm}$) exhibited a significant decrease ($p < 0.05$) during the summer (254.2 ± 6.1 to 241.3 ± 6.7) compared with spring (278.3 ± 3.4 to 258.3 ± 8.2) and winter (283.6 ± 4.2 to 255.6 ± 4.2), but no significant difference was observed relative to autumn (263.5 ± 7.3 to 248.5 ± 10.3).

Conversely, DO concentrations (mg/L) decreased significantly ($p < 0.05$) in summer compared with winter,

reflecting oxygen depletion associated with elevated water temperature.

The seasonal concentrations of heavy metals ($\mu\text{g/L}$), including Mn, Fe, Cd, Zn, Pb, and Cu, were all within the permissible limits of international water quality regulations (Table 2), confirming that heavy metal contamination did not exceed recommended thresholds during the study.

Table 2: Heavy metal assessment ($\mu\text{g/L}$) in Nasser Lake in different seasons.

Sector	Parameter	Season				WHO (2017)	CCME (2007)
		Winter	Spring	Autumn	Summer		
Aswan	Fe	263.3 \pm 21.6 ^a	243.8 \pm 43.2 ^b	238.4 \pm 37.5 ^c	228.6 \pm 41.3 ^c	300	300
	Zn	28.76 \pm 6.3 ^a	26.83 \pm 3.5 ^a	26.22 \pm 2.7 ^a	25.59 \pm 4.8 ^a	4000	7
	Mn	46.63 \pm 7.4 ^a	45.62 \pm 12.4 ^a	43.83 \pm 9.5 ^a	41.87 \pm 8.2 ^b	400	-
	Cd	1.73 \pm 0.33 ^a	1.66 \pm 0.27 ^a	1.58 \pm 0.31 ^a	1.52 \pm 0.21 ^b	3	0.18
	Cu	8.93 \pm 0.76 ^a	8.56 \pm 0.55 ^a	8.26 \pm 0.47 ^a	7.96 \pm 0.56 ^a	2000	2
	Ab	8.84 \pm 2.04 ^a	8.45 \pm 1.02 ^a	7.34 \pm 1.37 ^b	6.38 \pm 1.22 ^c	10	1
Wadi Abyade	Fe	272.6 \pm 32.5 ^a	258.3 \pm 44.2 ^b	252.7 \pm 35.3 ^b	238.3 \pm 33.5 ^c	300	300
	Zn	27.23 \pm 3.7 ^a	27.82 \pm 4.8 ^a	25.65 \pm 4.9 ^a	24.77 \pm 7.2 ^a	4000	7
	Mn	43.73 \pm 8.3 ^a	44.67 \pm 10.3 ^a	41.69 \pm 14.6 ^a	39.75 \pm 7.9 ^b	400	-
	Cd	1.64 \pm 0.27 ^a	1.67 \pm 0.37 ^a	1.59 \pm 0.62 ^a	1.56 \pm 0.23 ^b	3	0.18
	Cu	8.33 \pm 0.69 ^a	8.46 \pm 0.72 ^a	8.16 \pm 0.77 ^a	7.88 \pm 0.67 ^a	2000	2
	Pb	8.94 \pm 1.13 ^a	7.88 \pm 2.02 ^b	8.05 \pm 1.62 ^a	6.65 \pm 1.36 ^c	10	1
El-Madiq	Fe	268.7 \pm 19.7 ^a	265.6 \pm 22.8 ^a	236.3 \pm 39.2 ^b	242.7 \pm 28.3 ^b	300	300
	Zn	31.43 \pm 8.2 ^a	30.26 \pm 4.3 ^a	27.44 \pm 8.3 ^a	25.58 \pm 6.6 ^a	4000	7
	Mn	39.87 \pm 9.4 ^a	38.93 \pm 7.7 ^b	41.68 \pm 10.8 ^a	36.82 \pm 6.8 ^b	400	-
	Cd	1.53 \pm 0.26 ^a	1.55 \pm 0.47 ^a	1.52 \pm 0.35 ^a	1.44 \pm 0.71 ^b	3	0.18
	Cu	7.98 \pm 0.86 ^a	7.82 \pm 0.33 ^a	8.35 \pm 0.66 ^a	7.57 \pm 0.39 ^a	2000	2
	Pb	9.12 \pm 2.05 ^a	8.76 \pm 1.48 ^a	8.07 \pm 1.65 ^a	7.36 \pm 1.28 ^b	10	1
Lusaka	Fe	276.2 \pm 36.2 ^a	264.3 \pm 41.2 ^a	252.6 \pm 25.8 ^b	254.8 \pm 36.3 ^b	300	300
	Zn	29.62 \pm 4.7 ^a	33.25 \pm 6.4 ^a	27.29 \pm 7.2 ^a	26.75 \pm 5.7 ^a	4000	7
	Mn	44.38 \pm 6.3 ^a	42.97 \pm 11.6 ^a	41.52 \pm 8.9 ^a	39.87 \pm 7.8 ^a	400	-
	Cd	1.93 \pm 0.22 ^a	1.86 \pm 0.26 ^a	1.74 \pm 0.31 ^b	1.66 \pm 0.41 ^c	3	0.18
	Cu	9.03 \pm 0.66 ^a	8.94 \pm 0.74 ^a	8.64 \pm 0.83 ^a	8.27 \pm 0.53 ^a	2000	2
	Pb	9.64 \pm 3.07 ^a	9.22 \pm 2.07 ^a	8.35 \pm 1.76 ^a	7.78 \pm 1.72 ^b	10	1
Abu-Simbel	Fe	287.3 \pm 38.4 ^a	278.4 \pm 29.4 ^a	259.7 \pm 47.2 ^b	246.3 \pm 48.3 ^b	300	300
	Zn	32.48 \pm 5.6 ^a	30.85 \pm 3.9 ^a	26.48 \pm 4.7 ^a	24.89 \pm 6.7 ^a	4000	7
	Mn	48.72 \pm 6.5 ^a	47.47 \pm 10.2 ^a	44.58 \pm 9.5 ^a	42.68 \pm 11.4 ^a	400	-
	Cd	2.08 \pm 0.43 ^a	1.88 \pm 0.29 ^b	1.74 \pm 0.48 ^c	1.87 \pm 0.22 ^c	3	0.18
	Cu	9.67 \pm 0.72 ^a	9.43 \pm 0.58 ^a	8.87 \pm 0.49 ^a	8.66 \pm 0.68 ^a	2000	2
	Pb	9.86 \pm 2.06 ^a	8.68 \pm 2.26 ^b	7.92 \pm 1.87 ^c	7.79 \pm 2.42 ^c	10	1

WHO = World Health Organization, CCME: Canadian Council of Ministers of the Environment. Fe= Iron, Zn = Zinc, Mn = Manganese, Cd = Cadmium, Cu = Copper, Pb = lead. Data were represented as mean \pm standard error. Values with a different superscript letter in the same raw are significantly different between seasons at $p < 0.05$.

Bacteriological examination

TBC

As shown in Table 3, there were significant seasonal variations ($p < 0.05$) in the TBC across fish samples. The highest TBC was recorded during the summer ($3.59 \times 10^5 \pm 0.03 \times 10^3 \text{ CFU/g}$), indicating enhanced microbial proliferation under warmer conditions. Despite these increases, all examined samples remained within acceptable limits for human consumption, as defined by the Egyptian National Food Safety Authority (NFSA).

Pathogen prevalence across seasons

Table 4 shows the mean bacterial counts (CFU/g) of the examined samples. The prevalence of *S. aureus*, *V. cholerae*, and *A. hydrophila* was notably higher during summer than in winter. The highest count of *P. aeruginosa* was recorded in spring, whereas *A. hydrophila* reached its peak in autumn.

There was a significant difference ($p < 0.05$) in the mean bacterial counts between seasons, confirming that microbial profiles were season-dependent.

Overall bacterial distribution

Bacteriological analysis (Figure 2) revealed that *A. hydrophila* and *S. aureus* were the most frequently detected species across all seasons, with overall prevalence rates of 48.3% and 45.3%, respectively, followed by *P. aeruginosa* (36.7%) and *V. cholerae* (32.7%).

Table 3: Total bacterial count (CFU/g) of the examined samples in different seasons (n = 75 each).

Season	Minimum	Maximum	Mean \pm SE	NFSA	Accepted samples	
					No.	%
Winter	3.63×10^2	2.47×10^3	$6.43 \times 10^2 \pm 0.01 \times 10^{2c}$		100	100
Spring	5.48×10^2	4.27×10^3	$8.54 \times 10^2 \pm 0.02 \times 10^{2c}$		100	100
Autumn	5.72×10^2	8.22×10^4	$4.88 \times 10^3 \pm 0.01 \times 10^{2b}$	$<10^6$ CFU/g	100	100
Summer	4.33×10^3	7.36×10^6	$3.59 \times 10^5 \pm 0.03 \times 10^{3a}$		100	100

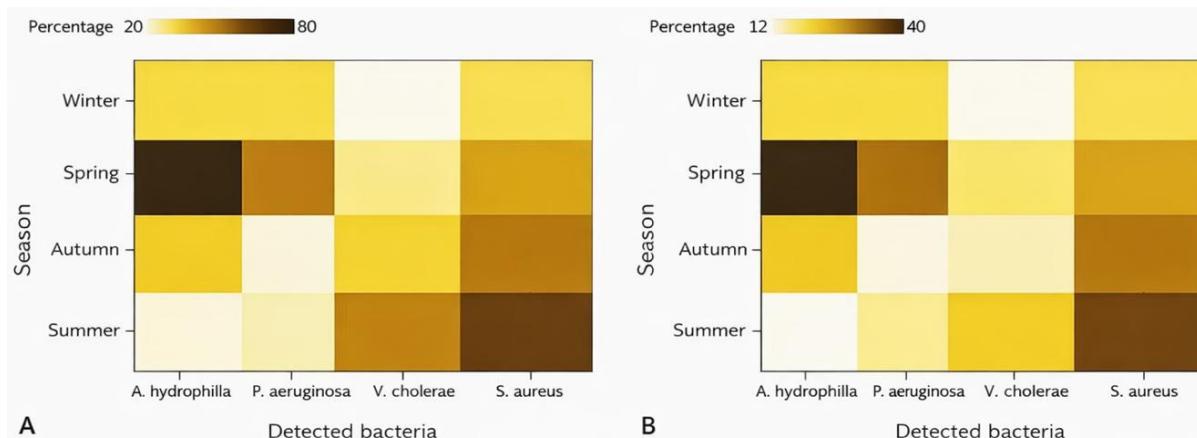
NFSA = Egyptian National Food Safety Authority for fresh fish. $p \leq 0.05$ is considered a significant difference. The mean values with the same letters do not show a significant difference. CFU = Colony forming unit, SE = Standard error.

Table 4: Mean count of the bacteria (CFU/g) in the examined samples in different seasons (n = 75 each).

Season	<i>Staphylococcus aureus</i>	<i>Vibrio cholerae</i>	<i>Pseudomonas aeruginosa</i>	<i>Aeromonas hydrophila</i>
Winter	$5.47 \times 10^2 \pm 0.03 \times 10^c$	$0.85 \times 10^2 \pm 0.001 \times 10^c$	$4.47 \times 10^4 \pm 0.03 \times 10^{2a}$	$7.29 \times 10^2 \pm 0.01 \times 10^d$
Spring	$8.63 \times 10^2 \pm 0.02 \times 10^c$	$1.03 \times 10^2 \pm 0.02 \times 10^c$	$5.33 \times 10^3 \pm 0.02 \times 10^b$	$4.13 \times 10^3 \pm 0.01 \times 10^c$
Autumn	$6.22 \times 10^3 \pm 0.06 \times 10^b$	$7.62 \times 10^2 \pm 0.01 \times 10^b$	$3.82 \times 10^3 \pm 0.01 \times 10^b$	$8.85 \times 10^3 \pm 0.02 \times 10^2a$
Summer	$7.58 \times 10^4 \pm 0.05 \times 10^a$	$5.77 \times 10^3 \pm 0.06 \times 10^a$	$2.17 \times 10^2 \pm 0.02 \times 10^c$	$6.78 \times 10^3 \pm 0.01 \times 10^b$

The investigated samples differ significantly at ($p < 0.05$). Mean values with the same letters in each column are not significantly different. CFU = Colony forming unit.

- During summer, *S. aureus* and *V. cholerae* were predominant, detected in 69.3% and 56% of samples, respectively
- In spring, *A. hydrophila* and *P. aeruginosa* showed the highest occurrence rates at 76% and 52%, respectively.

**Figure 2:** Heatmap showing the prevalence of bacterial species across each season depending on (A) phenotypic and (B) molecular characterization, where darker colors represent a higher prevalence of bacterial species.

A. hydrophila dominance was most evident in autumn, coinciding with moderate water temperature and nutrient-rich conditions.

Overall, seasonal differences in bacterial distribution patterns reflected the direct influence of environmental factors, particularly temperature and DO, on bacterial growth dynamics.

Correlation between water quality and bacterial prevalence

Pearson correlation analysis revealed several significant associations between physicochemical parameters and bacterial prevalence:

- Water temperature was strongly and positively correlated with:
 - TBC ($r = 0.82$, $p < 0.001$)
 - S. aureus* ($r = 0.76$, $p < 0.001$)
 - V. cholerae* ($r = 0.69$, $p = 0.002$), indicating that elevated temperatures in summer-enhanced bacterial proliferation.
- DO demonstrated a negative correlation with TBC ($r = -0.71$, $p = 0.001$), suggesting that lower oxygen availability favored microbial growth
- EC exhibited a moderate positive correlation with *P. aeruginosa* ($r = 0.53$, $p = 0.007$) and *A. hydrophila* ($r = 0.58$, $p = 0.009$), implying that increased ion concentration supported bacterial persistence

- Heavy metal concentrations showed no significant correlation ($p > 0.05$) with bacterial counts, indicating that within permissible limits, these metals did not exert a measurable influence on pathogen occurrence.

These results collectively highlight that temperature and DO were the dominant environmental determinants of microbial activity in Lake Nasser, while heavy metals remained within safe thresholds and had a negligible impact on bacterial abundance.

Molecular characterization of bacterial isolates

PCR analysis confirmed the genotypic identity of the isolates and the presence of key virulence genes across seasons (Figure 2). A total of:

- 102 of 163 *S. aureus*,
- 65 of 98 *V. cholerae*,
- 77 of 110 *P. aeruginosa*, and 96 of 145 *A. hydrophila* isolates tested positive for the species-specific 16S rRNA gene (Figure 3).

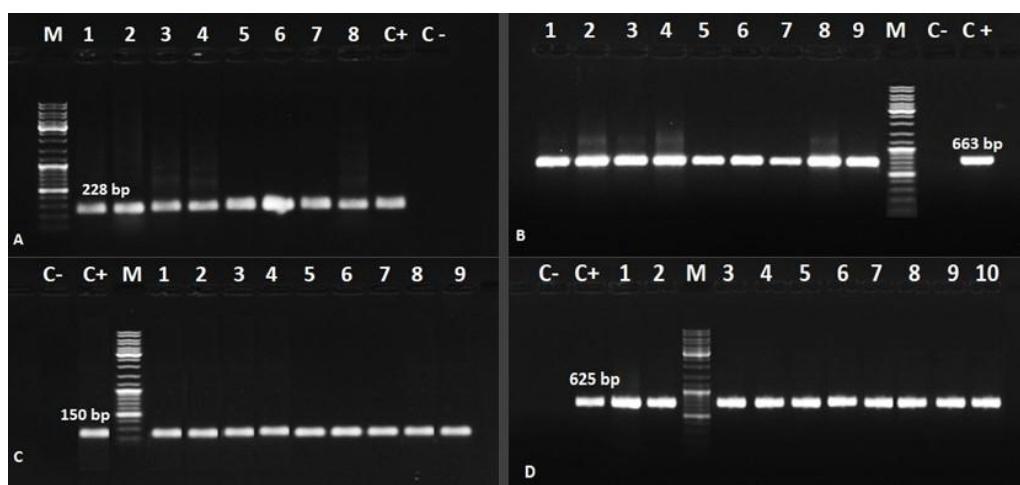


Figure 3: Electrophoretic profile of the amplification products of the 16S ribosomal RNA gene of the samples being studied; (A) *Staphylococcus aureus* (228 bp), (B) *Vibrio cholerae* (663 bp), (C) *Pseudomonas aeruginosa* (150 bp), (D) *Aeromonas hydrophila* (625 bp). M: 100 bp ladder, C+ = Positive control, C = Negative control.

Furthermore, all confirmed isolates harbored one or more virulence genes, as shown in Figures 4-7, signifying their potential pathogenicity.

Seasonal distribution of virulence-positive isolates

- The highest detection rates of *S. aureus* and *V. cholerae* occurred in summer (31/102 and 22/65, respectively), followed by autumn (29/102 and 17/65), spring (24/102 and 14/65), and winter (18/102 and 12/65)
- For *P. aeruginosa*, the highest detection occurred in spring (28/77), followed by winter (19/77), summer (17/77), and autumn (13/77)
- A. hydrophila* demonstrated its highest occurrence in spring (39/96) and autumn (24/96), followed by winter (21/96) and summer (12/96).

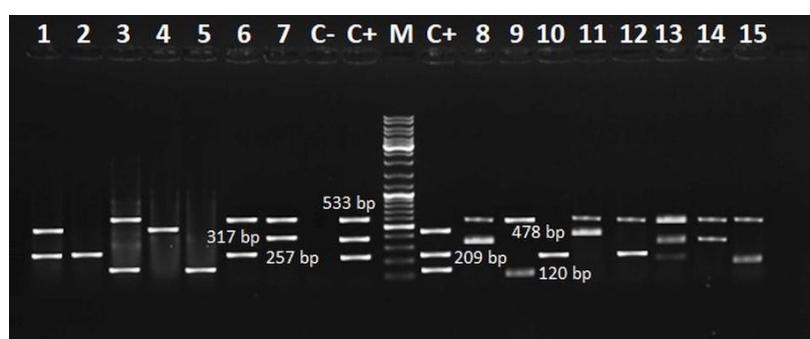


Figure 4: Agarose gel electrophoresis of multiplex polymerase chain reaction of the samples being studied for detection of *Staphylococcus aureus* enterotoxin genes: *mecA* (533 bp), *sea* (120 bp), *see* (478 bp), *sec* (257 bp), *sed* (317 bp), and *see* (209 bp). M: 100 bp ladder. C+: Positive control.

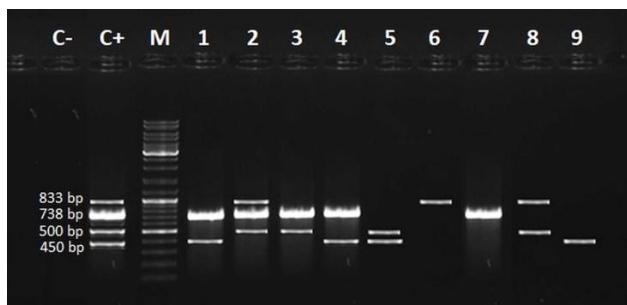


Figure 5: Agarose gel electrophoresis of multiplex polymerase chain reaction of the samples being studied for detection of *Vibrio cholera* virulence genes; *toxR* (833 bp), *hlyA* (738), *Trh* (500 bp), and *Tlh* (450 bp). M: 100 bp ladder. C+ = Positive control, C = Negative control.

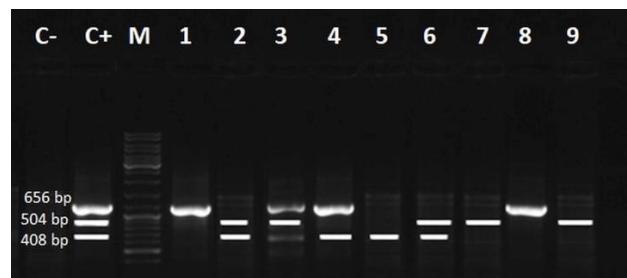


Figure 6: Agarose gel electrophoresis of multiplex polymerase chain reaction of the samples being studied for detection of *Pseudomonas aeruginosa* virulence genes; *PsA* (656 bp), *oprL* (504 bp), and *pilB* (408 bp). M: 100 bp ladder. C+ = Positive control, C = Negative control.

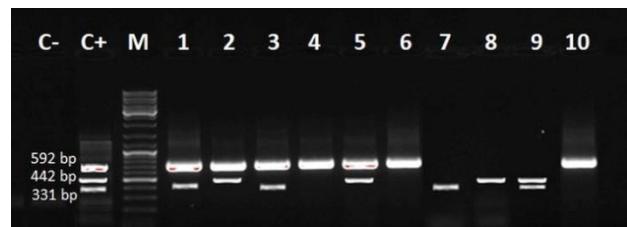


Figure 7: Agarose gel electrophoresis of multiplex polymerase chain reaction of the samples being studied for detection of *Aeromonas hydrophila* virulence genes; *hlyA* (592 bp), *alt* (442 bp), and *ast* (331 bp). M: 100 bp ladder. C+ = Positive control, C = Negative control.

These molecular findings confirm that seasonal variation significantly influences the occurrence and virulence expression of fish-borne pathogens in Lake Nasser, with the summer and spring seasons being the most conducive for the proliferation of *S. aureus*, *V. cholerae*, *P. aeruginosa*, and *A. hydrophila*.

DISCUSSION

Impact of climate change and seasonal variation on aquatic ecosystems

Seasonal changes driven by climate variability exert profound effects on global ecosystems, particularly on aquatic environments that are critical for food production and public health [24]. Natural phenomena associated with climate change, such as flooding, extreme heat, and water scarcity, adversely influence food safety, disease transmission, economic stability, and population displacement. Recognizing these interlinked threats, the WHO has designated climate change as the greatest global health hazard of the 21st century [25].

Seasonal variations have both direct and indirect impacts on the aquaculture sector, influencing cultured fish and the resources required for aquaculture, including water, land, feed, seed, and energy. Changes in rainfall patterns and temperature regimes alter water quality parameters such as pH, DO, and salinity, which in turn affect pond productivity, fish growth, survival, and reproduction. Moreover, climatic shifts elevate physiological and oxidative stress in fish, reducing immunity and rendering them more vulnerable to infection [4].

Temperature dynamics and their ecological implications

Temperature governs the rate of biological and chemical reactions in aquatic ecosystems and is vital for the well-being of aquatic organisms, from microbes to fish [26]. The present study demonstrated that the surface water temperature of Lake Nasser exhibited substantial temporal variation, closely following air temperature

fluctuations. A significant increase in water temperature was recorded during the summer months (Table 1), primarily attributed to elevated evaporation rates and reduced water volume.

Lake Nasser functions as a warm meromictic system, exhibiting a single circulatory cycle during the cooler months. The recorded temperature values remained within the Canadian Council of Ministers of the Environment (CCME) guidelines for aquatic life [27], confirming that the thermal conditions were within the tolerance limits for most freshwater organisms.

pH, EC, and DO trends

Lake Nasser water is naturally alkaline, with pH readings between 6.5 and 9.0, aligning with CCME recommendations for aquatic ecosystems [27]. The elevated alkalinity observed is likely due to carbon dioxide accumulation and the release of bicarbonate ions from sediment layers.

EC, an indicator of salinity and ionic concentration, typically ranges between 0 and 1,500 $\mu\text{S}/\text{cm}$ for freshwater systems. This study documented a seasonal decrease in EC during the flood (summer) season, which may be attributed to dilution effects and southward flow. The observed EC variation was statistically significant ($p < 0.01$), confirming the suitability of Lake Nasser water for livestock and aquaculture.

DO, a key determinant of aquatic life sustainability, exhibited pronounced spatial and seasonal variation ($p < 0.001$). DO ranged from $5.61 \pm 0.37 \text{ mg/L}$ in summer to $8.05 \pm 0.33 \text{ mg/L}$ in winter. The lower DO levels observed during summer frequently approached or fell below the CCME aquatic life threshold ($>5.5 \text{ mg/L}$) [27], likely reflecting reduced photosynthetic activity and increased microbial oxygen demand. Elevated temperatures further diminish oxygen solubility while heightening metabolic oxygen requirements [26]. These findings corroborate earlier studies linking summer oxygen depletion to elevated temperatures in Lake Nasser and other arid freshwater systems [10, 11].

Heavy metal distribution and environmental safety

Heavy metals are persistent environmental contaminants of global concern due to their toxicity, bioaccumulation, and long-term ecological persistence [28]. Both natural sources (atmospheric deposition, weathering, erosion, and leaching) and anthropogenic inputs (industrial, agricultural, and urban runoff) contribute to their presence in aquatic environments [29].

In this study, iron (Fe) was unevenly distributed across sampling sites but remained below the CCME (300 $\mu\text{g/L}$) and WHO acceptable limits for wildlife and drinking water [27, 30], consistent with previous studies by Imam *et al.* [11], Abdel-Satar *et al.* [31], and Goher *et al.* [32]. Concentrations of Mn, Zn, Cd, Cu, and Pb were also within WHO safety thresholds [30], though Zn, Cd, Cu, and Pb marginally exceeded the CCME's guidelines for the protection of aquatic life [27], implying potential ecological stress under chronic exposure.

Spatial variability in metal concentrations was significant ($p < 0.002$) for most elements, except Zn and Cu. The co-occurrence patterns suggest shared geochemical origins and transport pathways. Overall, the physicochemical characteristics of Lake Nasser water complied with the WHO [30] and Egyptian [33] drinking water standards, confirming its high-quality and suitability for multiple uses.

Climate influence on microbial dynamics and fish health

Climate change poses an increasing threat to food and water safety, as rising temperatures and reduced oxygen availability create favorable conditions for bacterial proliferation [34–36]. Egypt, ranked among the top five countries most affected by sea-level rise [37], produces more than 1.14 million tons of aquaculture annually, making it the leading African producer and the 10th globally [36].

Fish meat, particularly muscle tissue, is inherently sterile; hence, microbial contamination serves as a critical indicator of environmental hygiene and post-harvest handling [38]. The TBC, a key measure of sanitary quality, showed a significant seasonal variation, peaking during summer ($p \leq 0.05$). Despite these increases, all TBC values remained below the Egyptian NFSA [39] threshold of 10^6 CFU/g , confirming their acceptability for human consumption.

Meteorological data from the Aswan Meteorological Station supported these observations: the summer period recorded the highest mean air temperature ($\approx 34.6^\circ\text{C}$) and the lowest relative humidity ($\approx 30\%$), both of which promoted elevated water temperature and oxygen depletion. These conditions favored mesophilic pathogens, including *S. aureus* and *V. cholerae*, which reached their highest prevalence during summer. Conversely, the winter season ($\approx 19.8^\circ\text{C}$; high humidity) was characterized by increased oxygen solubility and reduced microbial activity, which explains the lower bacterial counts.

These findings align with global studies linking thermal stress and hypoxia to the enhanced proliferation of opportunistic bacteria in aquatic systems [34, 40–42]. Similar seasonal patterns have been observed by Ali [40] and Magouz *et al.* [42] in tilapia aquaculture in Egypt, where increased *Aeromonas* and *Vibrio* loads corresponded to summer mortality events.

Correlations between environmental parameters and pathogen prevalence

The negative correlation between DO and bacterial counts, particularly for *P. aeruginosa*, indicates that oxygen depletion enhances bacterial persistence, a phenomenon typically associated with warmer, organic-rich waters. Meanwhile, the moderate positive correlation between EC and *A. hydrophila* suggests that ionic strength and nutrient availability contribute to bacterial metabolism and colonization [31].

Collectively, these findings demonstrate that physicochemical and climatic variability exert direct effects on the distribution and abundance of fish-borne pathogens, reinforcing the necessity for integrated environmental–microbial monitoring systems.

Virulence gene detection and zoonotic potential

Molecular characterization confirmed that all target pathogens harbored at least one species-specific virulence gene, underscoring their zoonotic potential within the Lake Nasser ecosystem.

- *V. cholerae* isolates carried *toxR*, *hlyA*, *Trh*, and *Tlh* genes
- *P. aeruginosa* isolates contained *PsIA*, *oprL*, and *piB*
- *A. hydrophila* isolates harbored *hlyA*, *alt*, and *ast*
- *S. aureus* isolates possessed *mecA*, *sea*, *seb*, *sec*, *sed*, and *see*.

These results concur with previous studies for *P. aeruginosa* [2, 14, 43–48].

Variability among regions, fish species, immune responses, and environmental factors may explain differences in gene detection frequencies.

Temperature-induced metabolic acceleration promotes faster microbial growth, mutation, and gene transfer, which may enhance virulence and adaptation [49]. Thus, warmer conditions not only increase bacterial abundance but also potentially amplify pathogenicity through molecular mechanisms of stress-induced gene regulation.

CONCLUSION

This study provides the first integrated assessment of the seasonal dynamics of water quality, bacterial contamination, and virulence gene distribution in *O. niloticus* from Lake Nasser, Egypt, under changing climatic conditions. The findings revealed that water temperature, pH, DO, and EC exhibited significant seasonal variation, directly influencing the abundance and composition of fish-borne pathogens. The summer season recorded the highest temperature (24.6°C–29.6°C) and lowest DO (\approx 5.6 mg/L), conditions that coincided with the maximum TBC (3.59×10^5 CFU/g) and the highest prevalence of *S. aureus*, *V. cholerae*, and *A. hydrophila*. Despite these increases, all samples remained within the Egyptian NFSA limit ($\leq 10^6$ CFU/g), confirming that fish were safe for consumption. Heavy metal concentrations (Mn, Fe, Cd, Zn, Pb, and Cu) were within permissible WHO thresholds, indicating that microbial rather than chemical stressors dominated the ecosystem health risks.

These results highlight the interdependence of environmental and microbial factors within the One Health framework, demonstrating that elevated temperature and oxygen depletion enhance bacterial proliferation and activate virulence gene expression in zoonotic pathogens. The detection of toxigenic genes, *toxR*, *hlyA*, *Trh*, *Tlh*, *mecA*, *sea–see*, *hlyA*, *alt*, *ast*, *oprL*, and *piB*, in multiple species signifies the public health potential of tilapia as a vehicle for pathogen transmission when post-harvest hygiene or cooking is inadequate. These findings support the establishment of risk-based monitoring programs integrating environmental parameters (temperature, DO, EC) with molecular pathogen surveillance to strengthen food safety systems in tropical aquaculture zones.

The strength of this study lies in its multi-layered approach combining physicochemical analysis, culture-based enumeration, and PCR-based virulence profiling, enabling precise identification of microbial hazards. It is the first longitudinal survey documenting climate-linked bacterial dynamics and virulence gene prevalence in Lake Nasser fish, with blinded sampling and triplicate analyses ensuring objectivity and reproducibility. However, the study was limited to 1 year and a single fish species (Nile tilapia), which may not fully capture interannual or multispecies variability. Nutrient parameters such as nitrogen and phosphorus were not analyzed, and although PCR confirmed species and virulence genes, DNA sequencing and antimicrobial resistance (AMR) profiling were

not conducted, limiting molecular resolution.

Future studies should extend over multiple years and species to capture climate-cycle variability, incorporate nutrient, AMR, and sequencing data to map pathogen diversity and resistance trends, and develop predictive models or early-warning systems that integrate meteorological, hydrological, and microbiological data for real-time risk forecasting. Adaptive aquaculture management strategies, such as aeration systems and controlled feeding regimes, may also help mitigate thermal and oxygen stress.

In conclusion, seasonal climatic fluctuations significantly influence water quality and pathogen dynamics in Lake Nasser. The synergistic effects of high temperature and low DO during summer created favorable conditions for pathogen proliferation and virulence expression, representing a potential seasonal food safety hotspot. Nevertheless, the overall physicochemical quality of the lake remained within safe limits, reflecting its resilience and suitability for aquaculture. Integrating environmental monitoring with molecular pathogen detection under a One Health surveillance system will be vital for protecting public health, ensuring sustainable fisheries, and maintaining ecological balance in Egypt's freshwater ecosystems.

DATA AVAILABILITY

All the generated data are included in the manuscript.

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AUTHORS' CONTRIBUTIONS

NE: Conceptualization, sampling, experimental work, methodology, and drafted and edited the manuscript. EK: Conceptualization, molecular analysis, and reviewed and edited the manuscript. KT: Investigation and molecular analysis. MB: Sampling, supervision, and reviewed and edited the manuscript. All authors have read and approved the final version of the manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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