Mansoura Veterinary Medical Journal

Manuscript 1237

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Mohammed A. Hussein

Alaa Eldin Eissa

Reham M. Eltarabily

Mohamed F. Abdelghany

See next page for additional authors

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Authors

Mohammed A. Hussein, Alaa Eldin Eissa, Reham M. Eltarabily, Mohamed F. Abdelghany, Mahmoud A. Elnakeeb, Eman M. Ismail, Reham H. Ragab, and Amina A. Dessouki

ORIGINAL ARTICLE

Poor Water Quality as a Trigger of Harmful Algal Blooms in Lake Manzala

Mohammed A. Hussein [a], Alaa E. Eissa [b,*], Reham M. Eltarabily [c], Mohamed F. Abdelghany [d], Mahmoud A. Elnakeeb [d], Eman M. Ismail [e], Reham H. Ragab [b], Amina A. Dessouki [f]

[a] Department of Aquaculture Diseases Control, Fish Farming and Technology Institute, Suez Canal University, Ismailia, Egypt
[b] Department of Aquatic Animal Medicine and Management, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[c] Department of Bacteriology, Immunology and Mycology, Suez Canal University, Ismailia, Egypt
[d] Department of Fish Production, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt
[e] Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[f] Department of Pathology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

Abstract

OBJECTIVE: The current study aimed to assess the direct effects of poor water quality on the eruption of heavy harmful algal blooms and to assess the physicochemical properties of water in Lake Manzala, as well as the molecular identification of the retrieved green algae.
PROCEDURES: Subsurface water samples were collected from three different sites in September 2023 for water quality assessment. Moreover, morphological and molecular identification of suspected harmful algae is the most reliable diagnostic tool for this eruption.
RESULTS: This study was conducted in the northern and northeastern regions of Lake Manzala. These regions receive large amounts of municipal, agricultural, and industrial waste effluents that contain organic and inorganic pollutants. High surges in phosphate, ammonia, nitrate, and other water quality measures were remarkable at Boughaz El-Boghdady. Morphological and microbiological analyses of the water samples revealed that *Microcystis aeruginosa* was the most widespread algal bloom species in Lake Manzala. Identity of the retrieved *Microcystis aeruginosa* algae was confirmed using 16S rRNA gene sequencing.
CONCLUSIONS: Such algal blooms have both hypoxic and toxic effects on all living marine species throughout the lake. These results necessitate intense environmental rehabilitation and restoration efforts through increasing numbers of boughazes linked to the Mediterranean Sea, increasing the number of wastewater treatment stations, and regular monitoring of lake water quality.

Keywords: Algal blooms, Cyanobacteria, Lake manzala, *Microcystis aeruginosa*, Water quality

1. Introduction

The Egyptian Mediterranean shore has six lakes extending from the Nile Delta to the east of the Suez Canal: the Burullus, Manzala, Port-Fouad, Marriott, Edku, and Bardawil lakes [1]. Lake Manzala is considered the second most significant source of natural fisheries after El-Burullus Lake. Furthermore, it is estimated to produce ~41.69% of Egypt's delta lakes in 2020. Additionally, the amount of fish produced by Manzala Lake has increased from 42,000 tons in 2016 to 82,541 tons in 2020 [2,3].

Lake Manzala is 50 km long and is situated along the Mediterranean coast in the northeastern section of the Nile Delta between the Suez Canal (east) and Damietta Branch (west) [4]. Lake Manzala's northern boundary is connected to the Mediterranean Sea by the major entrance at the El-Gameel region and

Received 5 April 2024; revised 2 May 2024; accepted 2 May 2024.
Available online 21 June 2024

* Corresponding author at: Department of Aquatic Animal Medicine and Management, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt.
E-mail addresses: aeissa2005@gmail.com, aeissa2012@cu.edu.eg (A.E. Eissa).

https://doi.org/10.35943/2682-2512.1237

New El-Gamil and linked to the Suez Canal via the El-Qabouti canal near Port Said. These discharges provide seawater for the lake [5]. Additionally, freshwater enters the lake through three canals originating from the Damietta branch, which are called Al-Enania, Al-Ratma, and Al-Sufara canals. The primary purpose of the construction of these canals was to provide freshwater to the lake's northwest region during the flood period [6]. The lake's southern and western borders include many inlets that greatly introduce wastewater discharge into the lake. Among these inlets, Bahr El-Baqar, Al-Sirw, Abu Garida, Bahr Hadous, Ramsis, and Faraskur are the most important drains [7].

Cyanobacteria (blue-green algae) are mainly identified in shallow, warm, nutrient-rich, and polluted low-oxygen water. They can grow tremendously to form thick scums that can alter the color of water, thereby creating green blooms [8,9]. The world is focusing on cyanobacterial species, especially *Microcystis* species, because of their ability to create powerful cyanotoxins known as 'Microcystins (MCs)' [10,11]. *Microcystis aeruginosa* (*M. aeruginosa*) is one of the most prevalent *Microcystis* species and can produce cyanotoxins and bloom-forming green blooms in fresh and brackish water [12].

The classification of *Microcystis* species is based on morphological criteria such as cell size, cell arrangement through the algal colonies, presence of gas vesicles, and mucilage properties of the colonies [13]. According to traditional botany definitions, *M. aeruginosa* is a coccoid unicellular *Microcystis* that creates spherical colonies with irregular or net-like organized cells [14]. Additionally, the molecular identification of *Microcystis* was performed using 16S rRNA gene sequencing to distinguish between algal species [15,16].

MCs, the most commonly detected cyanotoxins, pose major risks to aquatic ecosystems and human health. The toxicity mechanism of MCs involves the inhibition of protein phosphatases 1 and 2A [17]. MCs primarily accumulate in the liver and induce hepatotoxicity; however, exposure to MCs can also affect immunological organs including the spleen and head kidney. Numerous investigations have revealed that MCs can have harmful effects on phagocytes and lymphocytes, which can lead to immunodeficiency in fish [17−19].

Water quality is the most significant factor in determining the health status of water bodies [20]. The physiochemical parameters of water are the most crucial factors in determining water quality in aquatic environments, and their impacts on algal blooms in water are considered vital in assessing the health status of water bodies [21]. In recent decades, the occurrence of harmful algal blooms has increased significantly because of poor water quality [22]. The detrimental effects of these blooms result in decreased water clarity, which adversely affects phytoplankton, zooplankton, and aquatic plants [23]. Moreover, the decomposition of algal blooms can result in oxygen deficiency, which consequently leads to the mass death of fish and other aquatic species [24].

At present, Lake Manzala is severely affected by human activities, suffers from exposure to severe climatic change [25], and has high inputs of industrial, domestic, and agricultural discharges, resulting in lower water quality and increased harmful algal blooms [1,26]. Many studies have been conducted on the effects of water quality on phytoplankton species composition and abundance in Lake Manzala [4,6,27].

The northern region of Lake Manzala is heavily influenced by Mediterranean seawater entries and massive discharge of wastewater containing significant concentrations of organic matter, heavy metals, and organic pollutants that enter the lake through many inlets [5]. Hence, Lake Manzala is greatly impacted by pollution from a variety of sources, including domestic, industrial, and agricultural waste. As a result, the water characteristics and quality of the lake drastically decline, which has unfavorable consequences such as an increase in harmful algal blooms [28].

The current research work is a descriptive study aimed at assessing the impact of poor water quality as a trigger for heavy algal blooms in the northern region of Lake Manzala, Egypt, during September 2023 and to identify the causative algae.

2. Patients and methods

2.1. Study area

Lake Manzala is one of the most important fisheries resources in northern Egypt. The lake is also the main water supply for a large number of earthen-pond-based marine fish farms in Damietta. Lake Manzala is located at the most northern region of the Nile Delta between latitudes 31°07′N and 31°30′N and longitudes 31°48′E and 32°17′E with about a length of 64.5 km and a width of 49 km. On the northern side of the lake, there are narrow outlets at El-Boghdady and El-Gmail connected to the Mediterranean Sea, in addition to Ashtoum El-Gmail on the east-north side [29]. A Description of the selected sampling sites is presented in Table 1.

Table 1. Geographical description of the studied sites in the northern of lake Manzala.

Sampling sites	Site code	Latitude	Longitude
Boughaz El-Gmail	St. 1	31°14′49.66′ N	32°11′58.78′ E
Boughaz Ashtoum El-Gmail (New El-Gamil)	St. 2	31°17′17.97′ N	32° 9′53.49′ E
Boughaz El-Boghdady	St. 3	31°20′47.80′ N	31°59′50.94′ E

2.2. Physicochemical analysis of water

Five subsurface water samples were collected from three sites in the northern region of Lake Manzala, Egypt. In September 2023, water samples were collected in a 1L sterile bottle at a depth of 50 cm and preserved in an isothermal ice box until they were sent to the laboratory for water quality assessment. Water temperature, pH, dissolved oxygen, biological oxygen demand (BOD), electrical conductivity (EC), ammonia, nitrate, nitrates, phosphates, total dissolved solids (TDS), total nitrogen (N), and total phosphorus (P) were determined according to American Public Health Association [30]. Temperature, pH, electrical conductivity (EC), and dissolved oxygen (DO) were measured on-site using a portable multi-parameter water quality meter (HI98194, Hanna Instruments, USA). The transparency of the water samples was measured using a Secchi disc (30 cm diameter). For the measurement of nitrate, nitrite, and phosphate at selected water sites, the concentrations of these variables were estimated in the laboratory using a UV−Visible Spectrophotometer according to the standard methods American Public Health Association [30].

2.3. Phytoplankton analysis

For qualitative analysis of phytoplankton, 500 ml of the water sample was preserved with 4% neutral formalin and Lugol's iodine solution, which was then transferred into a glass cylinder with extra Lugol's iodine solution added to faint tea color, covered with aluminum foil, and allowed to settle for 3 days [31]. Ninety % of the supernatant fluid was siphoned off, and the sample volume was adjusted to a fixed volume (50 ml) and transferred to a small plastic vial for microscopic examination. The drop method was used to identify the phytoplankton species. Five microliters of sample were taken and examined under an inverted microscope ZEISS IM 4738, with magnification powers of 40 and 100×. The main references used in phytoplankton identification and classification were [32−34]. The currently accepted nomenclature for all taxa was given according to Guiry and Guiry [35].

2.4. 16S rRNA gene sequencing

Chromosomal DNA was extracted using an enzymatic lysis step followed by a phenol/chloroform extraction technique according to the procedure described by Ausubel *et al.* [36]. DNA purity was measured at 260 and 280 nm using a Nanodrop 1000 spectrophotometer (USA). The 16S rRNA gene was amplified using universal primers (27F: AGAGTTTGATCCTGGCTCAG and 1494R: TACGGCTACCTTGTTACGAC) as described by Neilan *et al.* [18]. For amplifying 16S rRNA, reaction mixtures (30 μl) consisting of 1 μl DNA template, 0.5 μM of both forward and reverse primers, 0.2 mM of (dNTPs), 3 mM MgCl2, 1 U Taq polymerase, 0.1 mg ml^{-1} BSA, and 1 × PCR buffer. The PCR reaction conditions were as follows: initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 70 °C for 1 min, and final extension at 72 °C for 10 min.

Purified PCR amplicons were sequenced in both directions using a 3500/3500 × L Genetic Analyzer (Applied Biosystems, at Faculty of Agriculture Research Park, Faculty Agriculture, Cairo University). The assembled 16S rRNA gene was compared with other 16S rRNA gene sequences using the BLAST search in the GenBank database.

2.5. Phylogenetic analysis

The raw sequence of *M. aeruginosa* was verified and assembled using BioEdit version 7.0 [37]. The assembled sequence was submitted to the GenBank database and compared with related sequences using BLASTN search. A phylogenetic tree was constructed using MEGA X 11 using the neighbor-joining method, with confidence levels estimated using 1000 bootstrap replicates [38].

3. Results

3.1. Study area

In September 2023, some fishermen noticed the massive spread of heavy green algal blooms through the Mediterranean junction with Lake

Manzala. These green algal blooms extended 14 km from the El-Sid Club in Port Said to Boughaz Ashtoum El-Gmail (Fig. 1). Fishermen reported that these green algal blooms have caused severe allergic reactions to the skin and mass of fish.

3.2. Physicochemical properties of water

The results of the water quality assessments at different sampling locations in Lake Manzala are presented in (Table 2). The temperature values at (St. 1, St. 2, and St. 3) were 26.5, 28.5, and 20 °C, respectively. The pH values in (St. 1, St. 2, and St. 3) were 7.9, 8.5, and 8.7, respectively. Transparency values at (St. 1, St. 2, and St. 3) were 28.5, 33 and 41.5 cm, respectively. The highest DO and BOD values of 4 mg/l and 13.7 mg/l were recorded at St. 1 and St. 3, respectively.

The highest values of EC, TDS, phosphate, nitrate, ammonia, TP and N/P ratio (29.5 mmohS/cm, 20.4 g/l, 6.2, 0.65, 1.7, 3.5 mg/l, and 5.5, respectively) were obtained at St. 3. Whereas, the maximum value of

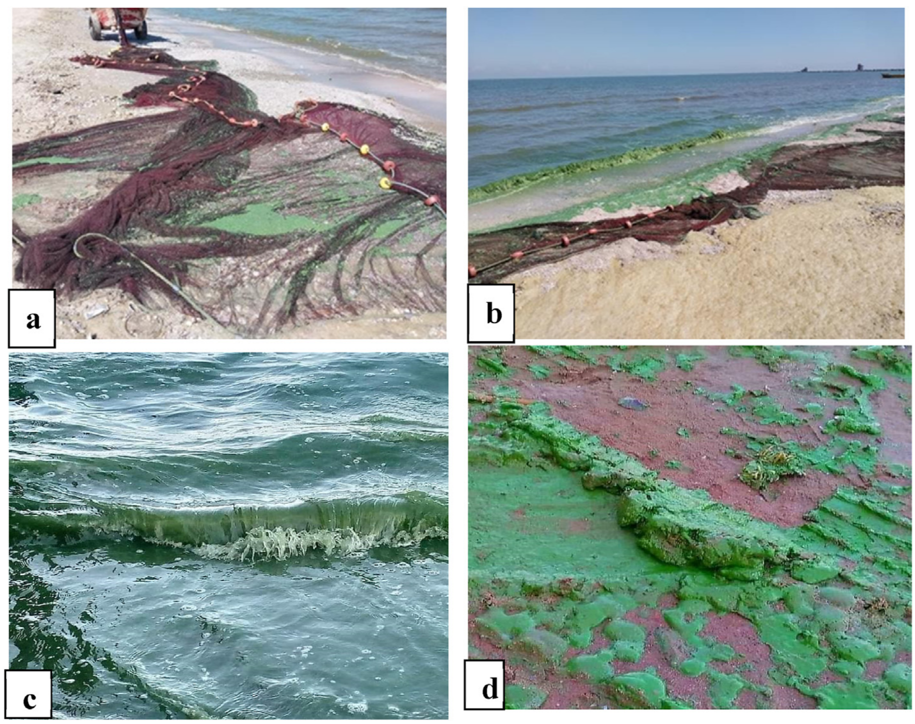

Fig. 1. Heavy green algal bloom of Microcystis aeruginosa in Lake Manzala.

Table 2. Physicochemical parameters of water at three sampling locations in Lake Manzala in September 2023.

Parameters	Sampling sites			Permissible limit[a]
	Boughaz El-Gmail (St. 1)	Boughaz Ashtoum El-Gmail (New El-Gamil) (St. 2)	Boughaz El-Boghdady (St. 3)	
Temperature (°C)	26.5	28.5	29	<35
Transparency (cm)	28.5	33	41.5	20−40
pH	7.9	8.5	8.7	6−9
DO (mg/l)	4	3.5	2.4	6.5−8
BOD (mg/l)	10.4	12.5	13.7	4
TDS (g/l)	11.5	12.7	20.4	<0.5
Ammonia (mg/l)	1.10	0.9	1.7	<0.05
Nitrite (mg/l)	0.18	0.13	0.15	0.125
Nitrate (mg/l)	0.50	0.37	0.65	0.1
Phosphate (mg/l)	4.6	2.3	6.2	0.413
TN (mg/l)	1.5	3.5	1.80	0.3
TP (mg/l)	1.01	2.10	3.5	0.1

Notes: biological oxygen demand (BOD); dissolved oxygen (DO); Total dissolved solid (TDS); total nitrogen (TN); total phosphorus (TP).
[a] Permissible limits of temperature, pH, DO, BOD, TN, and TP according to **EPA, (50)**; ammonia, nitrite, nitrate, and phosphate are according to **GAFRD, (59)**; transparency and TDS according to **WHO, (47)**.

nitrite and TN (0.18 mg/l and 3.5 mg/l) were recorded at St. 1 and St. 2, respectively.

3.3. Morphological characteristics of *Microcystis aeruginosa*

Colony morphology, size, and mucilage characteristics of the *Microcystis* isolates were determined. Microscopically, the cells were unicellular, spherical, irregularly arranged, 4–5 μm in diameter, and contained gas vesicles. Mucilage was colorless, structure-less, and did not form a very wide margin around the cells. According to Komárek *et al.* [34], these morphospecies could be assigned to *M. aeruginosa* isolate (Fig. 2).

3.4. Molecular identification of *Microcystis aeruginosa*

The assembled 16S rRNA gene sequence has been deposited in GenBank under the accession number PP581786. Based on the sequence analysis, the current sequence was confirmed to be *M. aeruginosa*. GenBank accession number (PP581786) was 1423 bp and showed 100% similarity to the accession numbers of *M. aeruginosa* (MG979399 and D89031). The other species were closely clustered into one group with less identity 99% with the *M. aeruginosa* strain (Fig. 3).

4. Discussion

Harmful algal blooms are considered one of the critical problems affecting the economics of water production, and some of these blooms threaten aquatic organisms as well as human health. Cyanobacteria are among the most eligible groups for regular blooms, whether alone or in conjunction with other species. Curiously, the unfavorable outcome is that these blooms mostly create various toxins that pose threats to humans and other aquatic organisms [11,39].

Lake Manzala receives huge amounts of agricultural (pesticides and fertilizers), industrial, and untreated domestic discharge from five main provinces located in northern Egypt: Damietta, Ismailia Port Said, Dakahlia, and Sharkiya. These discharges enrich lake water with large amounts of inorganic nutrients (nitrate, phosphate, and ammonia), combined organic nitrogen, and heavy metals [5]. The continuous discharge of these nutrients into Lake Manzala has a direct impact on its water quality and may lead to many changes in the phytoplankton structure [40].

This study focused on the physicochemical properties of water, as they represent the primary indicator of water quality, which has a great influence on the frequency of *M. aeruginosa* blooms. Such algal blooms have resulted in the washing of high amounts of organic nutrients in the studied

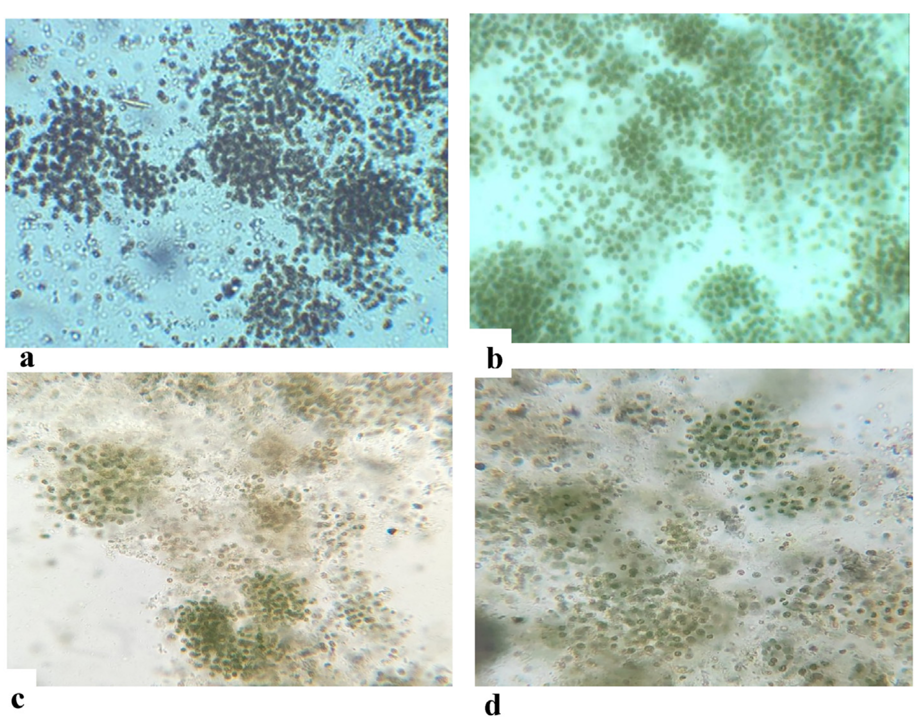

Fig. 2. Microcystis aeruginosa isolated from Lake Manzala waterbodies. They were unicellular spherical, irregularly arranged, and 4–5 μm in diameter.

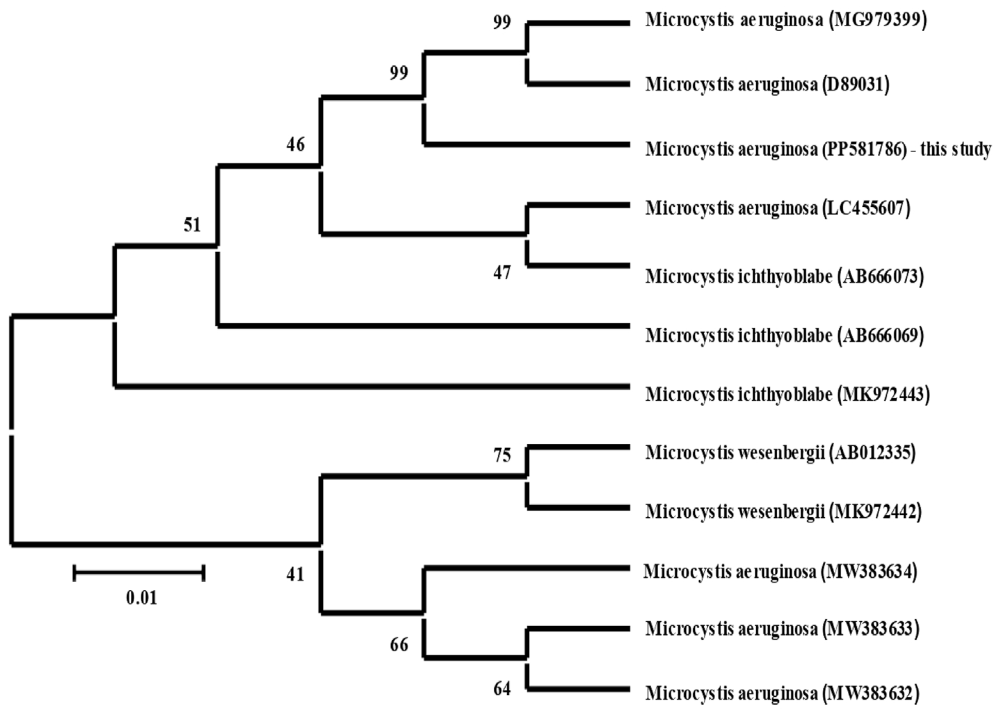

Fig. 3. Phylogenetic tree based on the 16S rDNA sequence for Microcystis aeruginosa with other related members of Microcystis.

areas. Similar results were reported by Yusuf [41], and Mohammed and Mahran [20], who reported that the growth of algae such as *Microcystis* sp. in water bodies is an indication of high concentrations of organic matter and low dissolved oxygen.

The most important physiochemical factors affecting the growth of algal blooms are pH, nitrogenous compounds, phosphorus, BOD, and DO. In the current study, the pH values at the sampling sites were shifted to the alkaline side, which may be attributed to anthropogenic activities [41] and could be related to photosynthesis and the growth of aquatic plants, where photosynthesis consumes CO_2 with consequent elevation in pH values [42]. In accordance with previous assumptions, it was previously reported that alkalinity enhances cyanobacterial growth [43,44].

The high electrical conductivity recorded at St. 3 was due to an increase in total dissolved solids, which may be attributed to the high inorganic salts that dissolve in water originating from urban, industrial, and agricultural runoff [45], which is consistent with previous results recorded by Koçer and Şen [46]. Water transparency varies depending on the amount of suspended matter and the phytoplankton concentration [40,47]. The high turbidity at St. 3 was due to the high organic matter content in the water.

On the other hand, the lower value of dissolved oxygen recorded at St. 3 could be a result of the high water temperature. Koralay *et al.* [48] also reported a negative relationship between the DO and water temperature. It has been proposed that a decline in water's DO level, particularly when it is accompanied by high BOD level, may be an indicator of organic pollutants [49,50].

Ammonia (NH_3) is extremely toxic and is naturally produced in lake water as a result of biological degradation of nitrogenous organic matter [51]. According to our research, the high levels of ammonia found at St. 3 are mostly related to domestic and agricultural pollution, which is then decomposed by the bacterial effect and produces a significant amount of NH_3 [52]. Nitrite is an intermediate product of ammonia and nitrate [53]. Nitrite concentrations in water are affected by nitrifying bacterial activity. The high levels of nitrite recorded at the sampling sites are due to the increase in water temperature in September as nitrifying bacteria become active [54].

Nitrate is considered the most stable and predominant form of inorganic nitrogen in seawater, and it is one of the main nitrogen sources for phytoplankton [55]. The high values of nitrate recorded in our study may be attributed to the decomposition of organic matter and dead algae at high temperatures, the entry of nitrogen fertilizers

[56], and the oxidation of ammonia by nitrifying bacteria [20]. Phosphorus is an important element for phytoplankton growth in aquatic ecosystems [57]. In the present study, the high values of phosphorus at St. 3 may be related to pollution from industrial and human waste [58,59]. Similarly, Rachna and Disha [60], showed that the decomposition of organic nitrogenous compounds and phosphates, in combination with nitrates, induced algal blooms.

It is well-known that the algal blooms increase by the uptake of available phosphorus and nitrogen from water [61]. The most conservative ratio suggests that when the N/P ratio is between 5 and 10, either element could be limiting, and if it is less than 5, nitrogen is the limiting factor for algal growth [62]. Thus, to prevent the unwanted growth of algae and plants, the N/P ratio must be determined.

In the present study, the N/P ratio recorded at St.2 of Lake Manzala was below 5, indicating that nitrogen is the limiting nutrient that may favor the growth of *M. aeruginosa*. Our results agree with Monem *et al.* [21] who reported that *Microcystis* is a non-N-fixing cyanobacterium; therefore, it requires nitrogen to bloom. However, at the other two sites (St. 1 and St. 3) the N/P ratio was above 5, indicating that either element could be a limiting nutrient [63].

In the present study, microscopic examination of *M. aeruginosa* revealed that the cells were unicellular spherical, irregularly arranged, 4–5 μm in diameter, and contained gas vesicles. Our results agree with Deyab *et al.* [27] who identified *M. aeruginosa* in brackish water blooms from Lake Manzala, Damietta, Egypt.

The phylogenetic tree based on 16S rRNA analysis confirmed the identification of the *M. aeruginosa* isolate, as it showed high identity (99–100% with *M. aeruginosa* strains and other *Microcystis* species. Several previous studies using 16S rRNA sequence analysis have demonstrated the ability to group various *Microcystis* species [18,64].

5. Conclusion

Generally, the water quality plays a major role in phytoplankton growth. Seawater intrusion and untreated pollutants, including urban and agricultural runoff, as well as industrial wastewater discharge into Lake Manzala, have a negative impact on water quality. Furthermore, excessive increases in total nitrogen and phosphorous stimulate massive growth of *M. aeruginosa* and consequently increase algal bloom quantity in the aquatic environment. Therefore, massive algal blooms can be used as bioindicators of the poor quality of open water systems.

Declarations

Ethics approval

The study doesn't require the use of animal or human subjects.

Availability of data and materials

All data are included in the manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Funding

No funding was received for this study.

Authors' contributions

All authors contributed equally to this manuscript.

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