EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
MICROBIOLOGY
G

ISSN
2090-0872

WWW.EAJBS.EG.NET

Vol. 14 No. 1 (2022)

Citation: *Egypt. Acad. J. Biolog. Sci. (G.Microbiolog) Vol.14 (1) pp.139- 153 (2022)*
DOI: 10.21608/EAJBSG.2022.230232

Egypt. Acad. J. Biolog. Sci., 14(1):139-153(2022)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
https://eajbsg.journals.ekb.eg/

Ecological Dynamics of the Toxic Bloom-Forming Cyanobacterium *Microcystis aeruginosa* with Its Cyanophage in Surface Freshwater of the River Nile

Seham E. Abu Ahmed[1], Mohamed A. Deyab[1], Jelan Mofeed[2,3*] and Emad H. El-Bilawy [3]
1-Botany and Microbiology Department, Faculty of Science, Damietta University, Damietta, Egypt.
2-Faculty of Fish Resources, Suez University, Suez, Egypt.
3-Faculty of Science, King Salman International University, South-Sinai, Egypt.
*E.Mail: jelan.mofeed@ksiu.edu.eg

ARTICLE INFO
Article History
Received: 6/2/2022
Accepted:22/3//2022
Available:25/3/2022

***Keywords*:**
Harmful blooms, *Microcystis*, Microcystin, Cyanophage, *g91*, 16S rRNA, *mcyA*.

ABSTRACT

The present investigation aims to study the dynamics of the harmful bloom-forming cyanobacterium *Microcystis aeruginosa* with its Cyanophage Ma-LMM01 in the River Nile ecosystem. Two freshwater samples in the Damietta branch of the River Nile represented the inlet and the outlet of a drinking water treatment plant were assessed according to a number of physicochemical and biological parameters. The total dissolved hepatotoxin microcystins in the raw and potable water were tested using the ELISA technique. Quantitative analysis of the 16S rRNA, *mcyA*, and *g91*genes was done in order to estimate microcystin-producing organisms and the Ma-LMM01 cyanophage. The results showed a high growth reached bloom-forming of *Microcystis aeruginosa* in the raw water while the treated one showed a vanishing in the number of *Microcystis aeruginosa.* A high concentration of microcystins was detected during the study period with the highest concentration of 4.4 µg. L^{-1} was recorded in the raw water sample during July. The sequencing result of the amplification products of the *g91* gene proves the presence of cyanophages but generally in a lysogenic state. The results obtained from this study revealed the presence of cyanophage Ma-LMM01 concomitantly with the presence of *Microcystis aeruginosa* bloom without having a high lytic effect.

INTRODUCTION

Freshwater which is the main source of drinkable water exposed to harmful microbial blooms from species with a high competency to produce different types of toxins which seriously affect human health. Microbial Blooms are not static communities, they depend on circumstances of time and space. Sometimes the blooming species belong to unrelated different groups, but more often the identified species belong to one dominant group (Graham *et al.*, 2020; Mohamed *et al.*, 2015). Among those species, Cyanobacteria is the most common group of organisms with the efficiency to produce diverse types of toxins.

The cyanobacterial species, *Microcystis* is at the focus of the world's attention for its super ability to produce the potent hepatotoxins "Microcystins" (Morón-López *et al.,* 2019; Krausfeldt *et al.,* 2019; Oliveira *et al.,* 2019).

Microcystis involves toxic and non-toxic strains whose growth rates are affected by environmental conditions (Dörr *et al.,* 2010; Huang and Chen 2013).Cyanobacterial microcystins toxicity may influence humans and animals and even plants directly by drinking and recreational water or by an indirect route through consuming contaminated foods, where, these toxins accumulated (5-15 times) in edible tissues of vegetables and fish exposed to contaminated waters (Mohamed and Carmichael 2000; Akyol *et al.,* 2021). It is worth mentioning that, most cyanobacterial and algal toxins are acutely efficient even at very low doses (Li *et al.,* 2011). Moreover, during the last decade, multiple species of cyanobacteria have been reported to be involved in microcystin production including *Microcystis*, *Oscillatoria*, *Anabaena*, *Nostoc*, *Gloeotrichia*, and *Planktothrix* (Hisbergues *et al.,* 2003). As a result of the exacerbation of microcystins problems, several strategies have been applied to control its consequences including chemical, physical and biological treatments, however, biological treatment methods especially using cyanophages, represented a safe and specific approach (Morimoto *et al.,* 2018).

Cyanophages were recorded in both fresh and marine water (Xia *et al.,* 2013). Although little is known about freshwater cyanophages, they are thought to have the same effect on cyanobacterial populations as marine cyanophages (Scherer *et al.,* 2017). The Microcystis cyanophages were classified into three families (Microcystaceae, Chroococcaceae and Myoviridae); the Ma-LMM01 strain which belongs to the family Myoviridae was the most studied one (Li *et al.,* 2013).

Cyanophages can alter both metabolism and replications of their cyanobacterial hosts, hence influencing the cyanobacterial community's structure and function (Xia *et al.,* 2013; Naknaen *et al.,* 2021). Yoshida *et al.* (2008) identified a lytic phage and noted that the numbers of *Microcystis aeruginosa* infectious cyanophages negatively correlated to the numbers of the host cells, however, natural environmental factors can influence the attachment of cyanophage to its host (Safferman *et al.,* 1969). The dominance of lysogenic bacteriophages is accompanied by the low abundance of the host due to minimized nutritional resources or high viral lysis rates (Weinbauer, 2004). Phage can persist in this lysogenic state for several generations until an environmental or biochemical 'trigger' event occurs, which causes the phage to switch to the lytic pathway (Brüssow *et al.,* 2004). Thus, applying cyanophages as a biological control requires understanding the environmental impacts on *M. aeruginosa*, its infectious cyanophages, and their interactions. For instance, the lytic T4 bacteriophage causes the lysis of the bacterial cell and consequently the death of the bacterial host, following the steps of viral replication and assembly of the virion progeny (Dimmock *et al.,* 2015).

In the present investigation, we aimed to study the interaction between *Microcystis aeruginosa*, and its infectious cyanophage (Ma-LMM01) in their natural environment. Thus, we monitored the changes of toxic *Microcystis aeruginosa*, and its cyanophage Ma-LMM01 using the molecular identification tools of 16S rRNA, *mcyA*, and *g91* representing the total cyanobacterial species, Toxic *Microcystis aeruginosa*, and cyanophage Ma-LMM01, respectively in relation to environmental physicochemical characteristics in the two freshwater samples of the River Nile during the flourishing period (from April 2020 to September 2020).

MATERIALS AND METHODS

Sampling:

Raw and treated water samples were collected during the flourishing period from April to September 2020 from El-Einania Drinking Water Plant (31°23′25″N 31°48′50″E) which lay on the Damietta branch of the River Nile. Sample (I)

represents the raw water, while Sample (II) is the treated water. Immediately after the collection, samples were subjected to microscopic examination and molecular analysis.

Microscopic Examination:

One liter of raw and drinking water samples was preserved (Prescott, 1978) with 5% Lugol's solution for qualitative and quantitative analysis of cyanobacteria. Fixed planktonic cyanobacteria were identified using OLYMPUS CX41 light microscope according to features described by Komark (2005), Baker and Fabbro (1999) in addition to Bergey's Manual of Systematic Bacteriology (Garrity *et al.*, 2001).

Estimation of Chlorophyll-A Content:

A known volume of raw and drinking water samples was filtered through GF/C filters, grounded using a PTFE/glass tissue grinder in presence of 10 ml of cool 90% aqueous acetone, transferred to centrifuge tubes and kept in dark for 12 hours at 4^{o}C for extraction. Chlorophyll a. was determined according to the formulas of Jeffrey and Humphery (1975).

Physicochemical Analyses of Water:

Water temperature, pH, and Conductivity were measured directly in the field using a Jenway dual temperature and pH meter model 370 and YSI Model 33 conductivity meter, respectively. Total nitrogen, dissolved oxygen, and total phosphorus were determined according to methods described in APHA (Baird, 2017).

Extraction of Microcystin:

The collected water samples were filtered by passing through GF/C filters and kept frozen to be ready for measuring dissolved microcystin. Microcystins were extracted via the sonication of the frozen GF/C with the use of distilled water, then the extract was purified using centrifugation at 20,000 rpm for 15 min. Depending on the density of planktonic particles and to avoid interferences present in the raw water, the final extracts were diluted 20 to 40 times. After purification, the total dissolved microcystins were determined by ELISA technique using Abraxis microcystins ADDA ELISA kits (Abraxis LLC 54 Streamwhistle Drive, Warminster, PA 18974, USA) (Lévesque *et al.*, 2014).

Genomic DNA Extraction:

Through a Millipore filter paper (0.45 μm), 100 ml of each collected water sample was filtered. DNA was extracted through an enzymatic lysis step followed by a phenol/chloroform extraction step as described by Giovannoni *et al.* (1990). The purity of DNA was measured as a ratio at 260 and 280 nm by Nanodrop 1000 spectrophotometer (USA).

Qualitative PCR Screening of 16S rRNA, mcyA and g91 genes:

The DNA primers designed by Neilan *et al.* (1997); Takashima et al. (2007) and Gągała et al. (2014) were used to amplify16S rRNA, *mcyA* and *g91* genes, respectively (Table. 1). For amplifying 16S rRNA and *mcyA* genes, reactions mixtures (30 μL) were consisted of 1 μL DNA template, 0.5μM of both forward and reverse primers, 0.2 mM of (dNTPs), 3mM of $MgCl_2$, 1 U of Taq polymerase,0.1 mg. mL^{-1} of BSA and 1×PCR buffer solution. The optimal condition was adjusted as described by Ha *et al.* (2009): 95 °C for 10 min, 26 cycles at 94 °C for 10 sec,51 °C for 30 sec, and70 °C for 1 min, and a final extension at 72 °C for 10 min. Along with that, to amplify *g91*gene, the reaction mixtures (30 μL) contained 1 μL of DNA template, 0.25 μM of both forward and reverse primers, 0.2 mM of dNTPs, 3 mM of $MgCl_2$, 1× PCR buffer, 0.1 mg mL^{-1} of BSA, and 1 U of Taq polymerase and the optimal condition was adjusted according to Takashima et al. (2007): 95 °C for 5min, 30 cycles of 94 °C for 30 sec, 56 °C for 30 sec, and 72 °C for 30 sec, and a final extension at 72 °C for 10 min. Finally, the PCR products, along with the negative control and DNA ladder (1 kb), were separated by 1.5 % agarose gel. Electrophoresis was performed for 2hrs

with a 70 v/35 mA current. The DNA bands were visualized with a UV transilluminator.

Table 1 PCR primers for 16S rRNA, *mcy*A and *g91* used in the study.

Targeting Gene	Primers	Sequence	Expected product size	Reference
16S rRNA	209F	5'ATGTGCCGCGAGGTGAAACCTAAT 3'	250 bp	Neilan *et al.,* 1997
	409R	5' TTACAATCCAAAGACCTTCCTCCC 3'		
*mcy*A	*mcyA*-f1	5' AACCTATCCCGGTTGCTCAGATG 3'	395 bp	Gągała *et al.,* 2014
	mcyA-r1	5' CACATCTCCAAGGAAAATACACCCC 3'		
g91	SheathRTF	5' ACATCAGCGTTCGTTTCGG 3'	132 bp	Takashima *et al.,*2007
	SheathRTR	5' CAATCTGGTTAGGTAGGTCG 3'		

Quantitative Screening of 16S rRNA, mcyA and g91genes:

Standard curves were created using the DNA extract of *Microcystis* sp. (PCC7820) for 16S rRNA, and *mcyA* genes. The standard curves for *g91* gene analysis were done using the amplified DNA isolated from the water samples. The copy number per microliter was determined. The reaction volumes were 25µL consisting of 1µL DNA templates, 0.3µM of forward and reverse primers, 1×SYBR green qPCR master mix. For 16S rRNA and *mcyA* genes, the initial denaturation step was at 95 °C for 5 min followed by 45 cycles of DNA denaturation at 95 °C for 15 sec; 51 °C for 30 sec, and 72 °C for 1 min. While for *g91*gene, the initial denaturation step was at 95°C for 5 min, 45 cycles of DNA denaturation at 95 °C for 15 sec; 56 °C for 30 sec, and 72 °C for 1 min.

RESULTS

Microscopic Examination:

A glance in Table. 2 revealed the microscopic examination results of the collected samples, where 7 cyanobacterial taxa were recorded; among them, *Microcystis aeruginosa was* the predominant one over other bloom-forming species, where it fluctuated from 483 x 10^6 cell.L^{-1} during April 2020 to 4548 x 10^6 cell.L^{-1} during July within sample I (Fig. 1). Following the *Microcystis aeruginosa,* the highest count of cyanobacterial species within sample I was recorded by *Phormidium* sp (327- 895 x 10^4 cell.L^{-1}), and *Nostoc punctiforme* (198 - 862 x 10^4 cell.L^{-1}) during August, where

Statistical Analysis:

Statistical analysis and correlation analyses were carried out using the SPSS program version 22 in order to test the relationship between different physicochemical and biological parameters. The mean separation test was done according to Duncan's multiple range tests.

Aphanocapsa delicatissima. displayed a relatively high growth within the raw water (Sample I) during September (578 x 10^4 cell. L^{-1}). The cyanobacterial species *Chrococcus turgidus, Merismopedia elegans* and *Leptolyngbya boryana*. also showed a considerable growth (4 – 432 x 10^4 cell. L^{-1}) in raw water samples. In contrast, the treated water samples (Sample II) showed a very low number of *Microcystis aeruginosa* (220 –600x 10^5 cell. L^{-1}) during the entire period of the investigation, at the same time the other cyanobacterial species were almost permanently recorded with nonsignificant numbers if present.

Table 2 Variations in cell number (cell.L^{-1}) of Cyanobacterial species within raw water (sample I) and treated water (sample II) from April to September (2020).

Cyanobacteria Taxa	Sample	Months					
		April	May	June	July	August	September
Aphanocapsa delicatissima	I	98×10^4	198×10^4	354×10^4	397×10^4	423×10^4	578×10^4
	II	ND	ND	ND	1.2×10^4	4.5×10^4	18×10^4
Chroococcus turgidus	I	48×10^4	18×10^4	98×10^4	281×10^4	179×10^4	265×10^4
	II	ND	ND	ND	2.2×10^4	0.9×10^4	3×10^4
Leptolyngbya boryana	I	4×10^4	84×10^4	85×10^4	48×10^4	243×10^4	432×10^4
	II	ND	ND	ND	ND	ND	ND
Merismopedia elegans	I	89×10^4	112×10^4	82×10^4	49×10^4	96×10^4	89×10^4
	II	0.7×10^4	ND	ND	ND	0.8×10^4	ND
Microcystis aeruginosa	I	483×10^6	682×10^6	836×10^6	4548×10^6	2809×10^6	3124×10^6
	II	220×10^5	300×10^5	400×10^5	600×10^5	500×10^5	330×10^5
Nostoc punctiforme	I	198×10^4	245×10^4	598×10^4	758×10^4	862×10^4	589×10^4
	II	ND	ND	ND	ND	ND	ND
Phormidium sp.	I	425×10^4	412×10^4	327×10^4	856×10^4	895×10^4	751×10^4
	II	ND	ND	ND	ND	ND	ND

ND: Not detected

Fig. 1. Bloom of *Microcystis aeruginosa* in the raw water (Sample 1).

Physicochemical Characteristics of Water:

The recorded physicochemical parameters in both untreated and treated water were shown in a Table. 3. Water temperature varied between 16 ±1.6 °C (during April 2020) and 31 ±1.8 °C (during July 2020). Concerning the relation with the other parameters it gives significantly negative correlations with dissolved oxygen (r = -0.52) and pH (r = -0.752), while strongly positive correlated with total-phosphorus (r = 0.816) and total-nitrogen (r = 0.76) at p<0.01 (Table.4). On the other hand, the freshwater pH values in both samples were slightly alkaline (Table.3) and fluctuated in a narrow range (from 7.32± 0.75 to 8.78 ± 0.9). As shown in Table. 4, beside temperature is negatively correlated with Total-Nitrogen (r = -0.53 at p<0.01) and total-phosphorus (r = -0.48 at p<0.05), while positively correlated with DO (r = 0.43) and conductivity (r = 0.41) at p<0.05.

The electrical conductivity increased gradually to give its maximum value (628 ±11.3 µS.cm^{-1}) in August 2020 within the raw water (Sample I); whereas a noticeable lower value was recorded within the treated water sample (Sample II), ranged from 248 ±12.86 to 530 ± 19.64 µS.cm^{-1}. Significant positive correlations appeared between electrical conductivity and total nitrogen (r = 0.704 at p<0.01), total phosphorous (r = 0.423 at p<0.05) and pH (r = 0.410 at p<0.05), while negatively

correlated with DO (r = -0.401 at p<0.05). In the study site, the dissolved oxygen values decreased in the hot months in both samples giving their maximum values during April 2020 in both samples (10.20 ± 0.97 and 9.67 ± 0.21 mg.L^{-1} for samples II and I, respectively), while the minimum value (6.84 ± 0.21 mg.L^{-1}) was recorded during July 2020 for sample I (Table.3). Dissolved oxygen was connecting inversely with temperature (r = -0.52 at p<0.01) and total nitrogen (r= -0.464, p< 0.05). The data pertaining to total nitrogen concentrations in the sample (I) revealed a relatively slight range of variation that fluctuated from 10 ±1.79 to 11.70 ±1.78 mg.L^{-1}, meanwhile, it widely changed from 4.8 ±0.02 to 10.4 ±1.09 mg.L^{-1} on the sample (II). The total nitrogen is significantly correlated with Temperature, electric conductivity and total phosphorus, while negatively correlated with dissolved oxygen (Table.4). The concentrations of total phosphorous followed the same trend as total nitrogen in the studied samples. It ranged from 0.96 ± 0.13 mg. L^{-1} during April 2020 in sample II to 3.2 ± 0.07 mg. L^{-1} during July 2020 in sample I.

Table 3 Monthly variations of physicochemical parameters (mean± SD) within raw water (sample I) and treated water (sample II) from April to September (2020).

Parameter	Sample	Month					
		April	May	June	July	August	September
Water Temp. (°C)	I	16 ± 1.6	20 ± 1.76	24 ± 2.09	30 ± 3.7	27 ± 2.85	23 ± 2.6
	II	17 ± 2.4	22 ± 2.07	26 ± 2.98	31 ± 1.8	26 ± 2.8	22 ± 1.95
pH	I	8.78 ± 0.9	7.86 ± 0.69	7.9 ± 1.26	7.5 ± 0.53	7.8 ± 1.07	8.43 ± 0.81
	II	8.43 ± 0.89	7.79 ± 0.95	7.7 ± 0.68	7.32 ± 0.75	8.0 ± 0.85	8.26 ± 0.83
Conductivity (µScm^{-1})	I	552 ± 15.4	425 ± 11.6	579 ± 15.96	624 ± 17.14	628 ± 11.3	512± 17.53
	II	494 ± 12	248 ± 12.86	347 ± 19.73	376 ± 16.43	530 ± 19.64	270 ± 17.57
DO (mg. L^{-1})	I	9.67 ± 0.21	9.23 ± 0.45	8.45 ± 0.9	6.84 ± 0.21	8.32 ± 0.47	9.20 ± 0.83
	II	10.20 ± 0.97	10.1 ± 0.67	9.1 ± 0.43	7.30 ± 0.34	8.95 ± 0.8	9.75 ± 0.62
TN (mg. L^{-1})	I	11.20 ± 1.04	10 ± 1.79	11.32 ± 1.13	11.70 ± 1.78	11.25 ± 1.89	10.60 ± 1.04
	II	10.4 ± 1.09	6 ± 0.69	4.8 ± 0.02	6.7 ± 0.05	6.9 ± 0.04	9 ± 0.06
TP (mg. L^{-1})	I	1.90 ± 0.03	1.42 ± 0.09	1.50 ± 0.02	3.2 ± 0.07	2.45 ± 0.73	2.2 ± 0.03
	II	0.96 ± 0.13	1.16 ± 0.03	0.78 ± 0.01	1.19 ± 0.01	1.12 ± 0.03	1.05 ± 0.06

Table 4 Pearson's correlation between physicochemical parameters, Chl. a and copy numbers of genes (*g91* representing cyanophages, 16S rRNA representing *Microcystis aeruginosa* and *mcyA* representing toxic *Microcystis aeruginosa*).

	pH	T	EC	DO	TN	TP	Chl-a	*g91*	16s RNA	*mcyA*
pH	1.000									
Temperature	-0.752**	1.000								
Conductivity	0.410*	-0.201	1.000							
DO	0.430**	-0.520	-0.401	1.000						
Total-N	-0.53	0.760	0.704**	-0.464*	1.000					
Total-P	-0.48	0.816**	0.423	-0.536*	0.788	1.000				
Chlorophyll-a	0.335**	0.576*	0.728**	0.106	0.796**	0.566*	1.000			
g91	-0.336*	0.500**	0.286	-0.501**	0.653**	0.072	0.575**	1.000		
16s RNA	0.360*	-0.442**	0.633**	0.241	0.568**	-0.201	0.702**	0.322	1.000	
mcy-A	-0.139	0.301	0.248	-0.231	0.525**	-0.089	0.663**	0.751**	0.297	1.000

Where, **: correlation is significant at p< 0.01 and *: correlation is significant at p< 0.05

Chlorophyll A Content:

As illustrated in Fig.2, a wide range of variations in chlorophyll "a" concentration between the two samples. The maximum concentration of chlorophyll "a" (6.7 ±0.37 mg.L^{-1}) was recorded in July for sample I, while the minimum value (0.4 ±0.1 mg.L^{-1}) was recorded during April in sample II. It was noteworthy to mention that Chl. a gave significant correlations with total nitrogen (r = 0.796), total phosphorus (r = 0.566) and temperature (r = 0.576) at p< 0.01 (Table.4).

Microcystin Content in Untreated and Treated Water:

Levels of total microcystins in both raw and treated water exhibited distinct variations (Fig. 3). Concentrations of total

microcystins in raw water (Sample I) ranged between 1.3 and a maximum value of 4.4 µg.L^{-1} was recorded during July. While total microcystin levels of treated water samples (II) were significantly ($p \leq 0.05$) lower than those of raw water samples and fluctuated between 0.3 and 1.8 µg.L^{-1} with the highest concentration being observed during August.

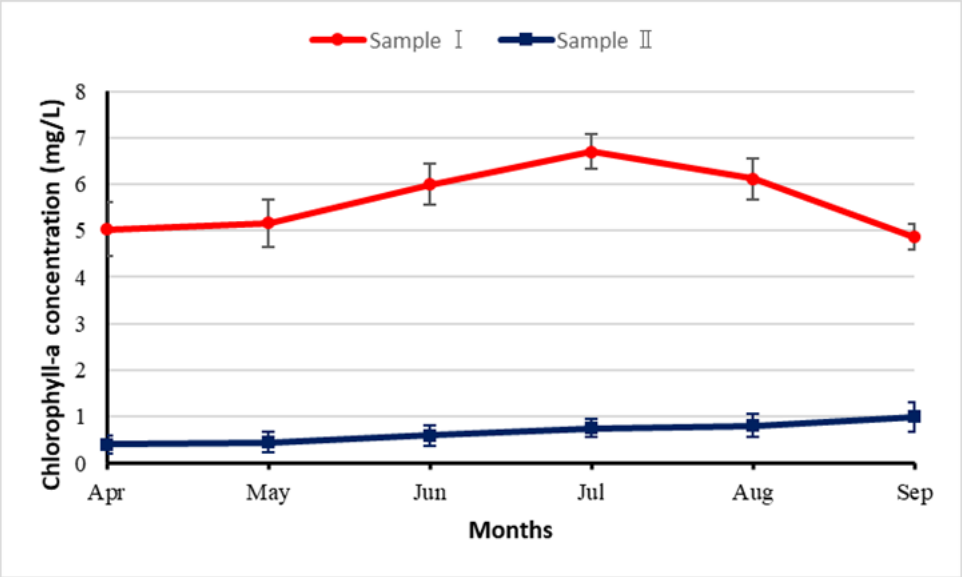

Fig. 2. Monthly variations in chlorophyll-a concentrations (mg.L^{-1}) ± SD within raw water (sample I) and treated water (sample II) from April to September (2020).

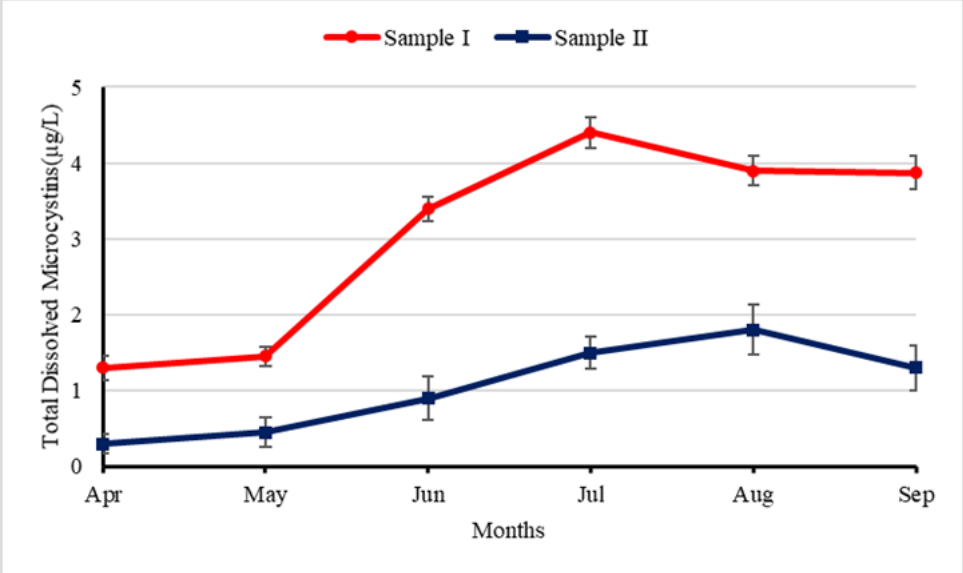

Fig. 3. Concentration of total dissolved microcystins (µg.L^{-1}) ± SD within raw water (sample I) and treated water (sample II) from April to September (2020).

Molecular Analysis:

For all the tested samples, PCR amplified fragments showed bands with the expected sizes (250 bp, 395 bp, and 132 bp) for 16S rRNA, *mcyA*, and *g91* genes, respectively (Fig. 4). The *mycA* copy numbers mirrored the change in the abundance of the toxic *Microcystis aeruginosa* during the study period. The ratio of the *mcyA* to the 16S rRNA copy numbers ranged from 0.44 to 2.63 % in sample I, while it fluctuated from 5.41 to

33.68 % in sample II. The results obtained in Figs.5 & 6 revealed that the percentages of cyanophages infecting *Microcystis aeruginosa* varied greatly among the entire period of investigation for the two samples, where it fluctuated between 68 and 180 gene copy.μL^{-1} to give its maximum peak during the summer months. Table 4 indicates that cyanophages were significantly correlated with temperature ($r = 0.500$, $p < 0.01$). The minimum numbers of *g91* gene copies (representing cyanophages) were detected generally at temperatures below 27 °C. The Cyanophage concentrations changed from an assumed antagonistic coexistence during winter to a stable pattern from mid to late summer. These changes were accompanied by non-prominent changes in environmental conditions. The gene copies of 16s rRNA (total cyanobacterial species) increased greatly with the maximum number of 93×10^3 copy number for sample I during July, while for sample II the highest copy numbers of 16S rRNA gene (2.85×10^2) were recorded during August. In the same context *mycA* copy numbers showed a highly significant relation with cyanophages ($r = 0.751$) at $p<0.001$(Table 4).

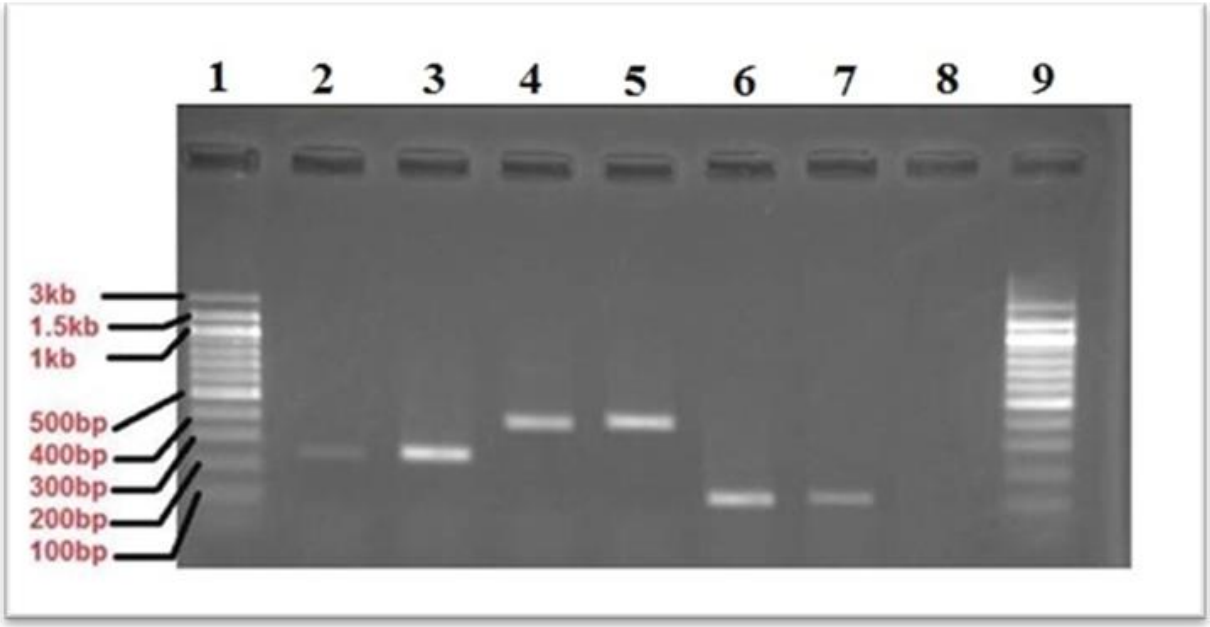

Fig. 4. Gel electrophoresis of PCR products for both samples (I & II). Gene direx 100bp ladder, Inc (H3RTU) (Lane 1), 16S rRNA gene sample (I) (Lane 2), 16S rRNA gene sample (II) (Lane 3), *mcyA* gene sample (I) (Lane 4), *mcyA* gene sample (II) (Lane 5), *g91* gene sample (I) (Lane 6), *g91* gene sample (II) (Lane 7), a negative control (Lane 8).

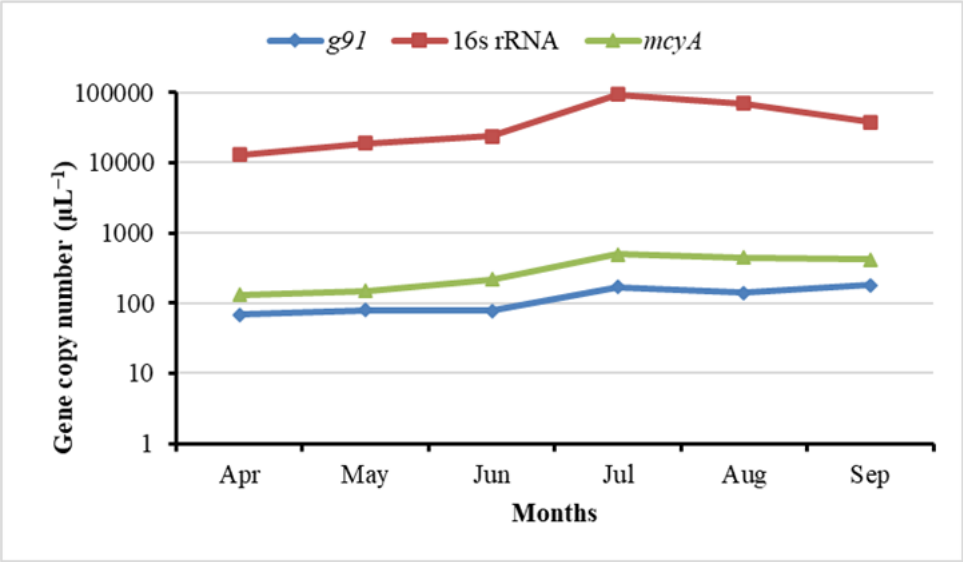

Fig. 5. The numbers of 16s rRNA, *mcyA* and *g91* genes copies per µL (Copies.µL^{-1}) using RT-PCR, representing abundance, toxic genotypes of *Microcystis aeruginosa*, and their cyanophage, respectively in the sample I from April to September (2020).

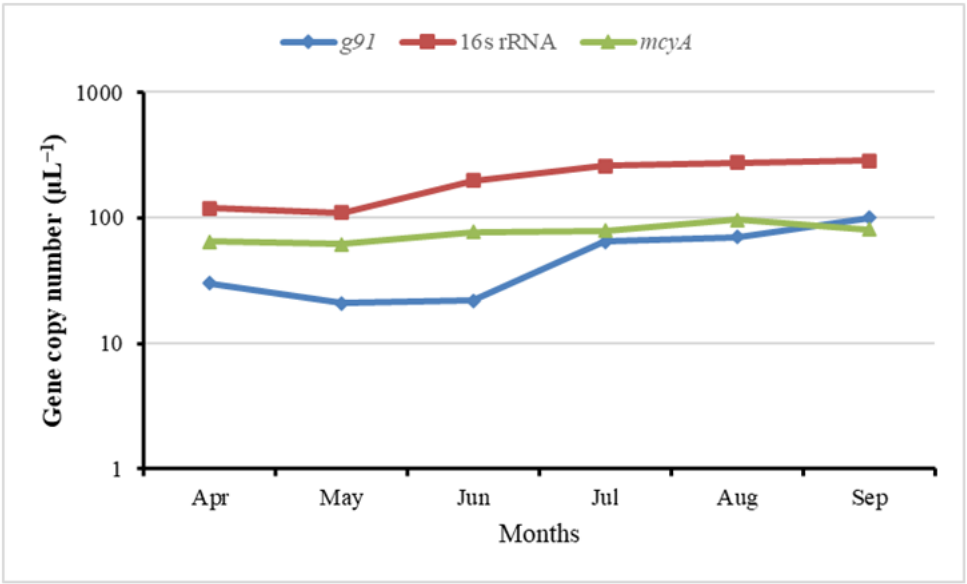

Fig. 6. The numbers of 16s rRNA, *mcyA* and *g91* genes copies per µL (Copies.µL^{-1}) using RT-PCR, representing abundance, toxic genotypes of *Microcystis aeruginosa*, and their cyanophage, respectively in the sample II from April to September (2020).

DISCUSSION

The problem of harmful algal blooms is considered one of the biggest problems that affect the economics of water production, along with that some of these blooms threaten aquatic organisms as well as human health. Freshwater cyanobacteria are among the most eligible group to bloom frequently, whether alone or in the company of other groups. But the undesirable effect is that these species mostly produce different types of toxins with hazardous effects on other living organisms, including humans (Griffith and Gobler 2020; Deyab *et al.*, 2020).

The present study represents a comprehensive investigation focused on the water quality characterization of raw and potable water with emphasis on the studying of the toxic and non-toxic

Microcystis aeruginosa blooms and the ability to use cyanophage as a biocontrol tool to get rid of these blooms. The obtained results revealed that *Microcystis aeruginosa* was the predominant species over 7 cyanobacterial taxa forming a noticeable bloom on the raw water. In contrast, the drinking treated water has a very low number of *Microcystis aeruginosa* and the other cyanobacterial strains were rarely appeared, these results agreed greatly with that of Lam and Prepas (1997) during their study of *Microcystis* algal bloom in the battle river drainage basin in Alberta.

This study paid considerable attention to the physicochemical characteristics of water since they represent the primary image of the water quality which affect greatly in the occurrence of *Microcystis aeruginosa* blooms as well as cyanophages. In general, the results in this investigation were consistent with those of Paerl and Otten (2013) who revealed that the nutrients and their availability are also affecting phytoplankton composition. The freshwater pH values in both samples were slightly alkaline, which may be attributed to the anthropogenic activities (Nassar *et al.*, 2014). Interestingly it was reported previously that the slit alkalinity enhances cyanobacterial growth (Elmorsi *et al.*, 2017). The diversity and succession of the cyanobacterial species were attributed greatly to the variations in temperature, similar results were recorded by Chellappa *et al.* (2009). On the other hand, the electrical conductivity gradually increased to reach its maximum value during July in sample I, the high value of conductivity is due to an increase in dissolved solids, which may be a result of relatively high rates of biodegradation, biogeochemical cycle and the growing practice of human activities, these results are also consistent with previous results recorded by Koçer and Şen (2012). Unlike the electrical conductivity, the dissolved oxygen gradually decreases reaching lower values during the summer months; this may be explained by the rapid increase in photosynthesis due to the phytoplankton fast-growing (Huang and Chen, 2013).

The recorded high concentration of total nitrogen especially during the bloom period yields high cellular N: C ratio hence induction the growth of toxic *Microcystis aeruginosa*. According to the carbon-nutrient balance hypothesis, at low cellular N: C ratios the contents of the nitrogen-rich variant microcystin are lower, reciprocally, a high level of Nitrogen and Carbon concentrations resulted in light-limited conditions (due to dense growth) with high levels of microcystin (Van de Waal *et al.*, 2009).

The obtained total phosphorus values in the present study followed the same trend of total nitrogen, fluctuating in a narrow range (1.12 – 3.2 $mg.L^{-1}$) giving its maximum during July and August. This result agreed with the previous results of Margalef (1978) who clarified that 0.2 and 2.8 $mg.L^{-1}$ concentrations of dissolved phosphorus are favorable for cyanobacterial growth, similarly, Deyab *et al.* (2019) noted that phosphorus is important for the growth and primary production of phytoplankton in aquatic ecosystems.

Chlorophyll-a concentration represented an indicator of primary productivity and water quality (Abdel-Aal and Mofeed, 2020). The obtained results, clarified the high content of Chlorophyll-a within-sample (I) during the entire period of investigation, in contrast to the recorded results in the sample (II). Regarding Carlson's trophic state index on chlorophyll-a measurement, the studied area can be classified as a hypereutrophic environment (Carlson and Simpson 1996), this result agreed with results obtained by Abdel-Hamid *et al.* (2012) who reported high Chlorophyll a. concentration in two sites in the Damietta branch of the River Nile.

In the present work, the ELISA technique detected a wide range of dissolved microcystins concentrations ranging between 0.3 and 4.4 $\mu g.L^{-1}$ The

highest total microcystins levels of raw water were exclusively recorded in summer coincided with the summer maxima of species richness and biomass standing crop of cyanobacteria. This finding seems very logical and explains the significant ($p \leq 0.05$) and strong positive correlations between cyanotoxins and the abundance of cyanobacterial community, these results are harmonious with other previously reported results by Ibrahim *et al.* (2016) and Brena *et al.* (2021).

The molecular quantification during the study period detected potentially a high ratio of *mcyA* to 16S rRNA which revealed the presence of the *Microcystis aeruginosa*, in both samples, from April to September 2020 with levels increased with the increase of total nitrogen, phosphorous and warm temperatures, these results are in accordance with Van de Waal *et al.* (2009). Interestingly, molecular quantification of *g91* gene showed that the cyanophage abundance was high enhanced due to both warm temperature and the number of daylight hours, where light can considerably affect the step of adsorption in freshwater cyanophages' life cycle (Quail *et al.*, 1995; de Oliveira Santos *et al.*, 2021).

The results of the gene copy numbers showed stability in the copy numbers of *mcyA* and g91 with being the copy numbers of *mcyA* always superior to the copy numbers of *g91*. Along with that, there's no significant decline in the count of *Microcystis aeruginosa*, especially during summer months, which is evidence of the coexistence of cyanophage in the lysogenic state rather than the lytic state, these results come consistent with the results of Yoshida *et al.* (2009), who prove the presence of cyanophages with *Microcystis aeruginosa* rather than destroying them.

Conclusion

The excessive increases in total nitrogen and phosphorous encourage the flourishing of *Microcystis aeruginosa* and consequently increase the cyanophage quantity. The obtained results clarify that in the eutrophic aquatic environments; cyanophages coexisted with *Microcystis aeruginosa* rather than destroyed them which supports the hypothesis that the bacteriophage is present in the lysogenic state instead of the lytic state. We are working on further studies to induce cyanophages' conversion from the lysogenicity to the lytic state in order to be used as an effective bio-control tool against harmful *Microcystis aeruginosa* blooms.

Author Statement:

The authors confirm their contributions to the paper as follows: Mohamed Deyab and Jelan Mofeed Design and put study conception. Seham Abu Ahmed, Jelan Mofeed, and Emad El-Bilawy prepared the draft manuscript. Emad El-Bilawy supervised the practical work. Seham Abu Ahmed analyzed the Physico-chemical parameters of water samples. Emad El-Bilway and Seham Abu Ahmed performed morphological and molecular identification of cyanobacteria and cyanophages. Jelan Mofeed performed Statistical Analysis. All authors reviewed the results and approved the final version of the manuscript.

Declaration of Competing Interest:

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

REFERENCES

Abdel-Aal, E. and Mofeed, J. (2020).Mass production of Arthrospira platensis on the livestock manure for use as a protein source in animal feed. *Egyptian Journal of Aquatic Biology and Fisheries*, 24(7-Special issue), 725-739.

Abdel-Hamid, M. I.; Abdel-Halim, E. H. and Azab, Y. A. (2012). Microcystins in raw water, drinking water, and some isolates of Cyanoprokaryotes. *Egyptian Journal of Phycology*, 13(1), 1-25.

Akyol, Ç.; Ozbayram, E. G.; Accoroni, S.; Radini, S.; Eusebi, A. L.; Gorbi, S. and Gigli, F. (2021). Monitoring of

cyanobacterial blooms and assessing polymer-enhanced microfiltration and ultrafiltration for microcystin removal in an Italian drinking water treatment plant. *Environmental Pollution*, 117535.

Baird, R. B. (2017). Standard methods for the examination of water and wastewater, 23rd: Water Environment Federation, American Public Health Association, American.

Baker, P. D. and Fabbro, L. D. (1999). A guide to the identification of common blue-green algae (Cyanoprokaryotes) in Australian freshwaters: Cooperative Research Centre for Freshwater Ecology New South Wales.

Brena, B. M.; Font, E.; Pírez Schirmer, M.; Badagian, N.; Cardozo, E.; Pérez Parada, A. and Bonilla, S. (2021). Microcystin ELISA in water and animal serum for an integrated environmental monitoring strategy. *International Journal of Environmental Analytical Chemistry*, 1-13.

Brüssow, H.; Canchaya, C. and Hardt, W. D. (2004). Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. *Microbiology and molecular biology reviews*, 68(3), 560-602.

Carlson, R. E., and Simpson, J. (1996). A coordinator's guide to volunteer lake monitoring methods. *North American Lake Management Society*, 96, 305.

Chellappa, N.; Câmara, F. and Rocha, O. (2009). Phytoplankton community: indicator of water quality in the Armando Ribeiro Gonçalves reservoir and Pataxó channel, Rio Grande do Norte, Brazil. *Brazilian Journal of Biology*, 69, 241-251.

de Oliveira Santos, L.; Guedes, I. A. and Pacheco, A. B. F. (2021). Occurrence and diversity of viruses associated with cyanobacterial communities in a Brazilian freshwater reservoir. *Brazilian Journal of Microbiology*, 52(2), 773-785.

Deyab, M.; Ahmed, S. A. and Ward, F. (2019). Comparative studies of phytoplankton compositions as a response of water quality at North El-Manzala Lake, Egypt. *International Journal of Environmental Science and Technology*, 16(12), 8557-8572.

Deyab, M.; Mofeed, J.; El-Bilawy, E. and Ward, F. (2020). Antiviral activity of five filamentous cyanobacteria against coxsackievirus B3 and rotavirus. *Archives of Microbiology*, 202(2), 213-223.

Dimmock, N. J.; Easton, A. J. and Leppard, K. N. (2015). Introduction to modern virology: John Wiley & Sons.

Dörr, F. A.; Pinto, E. and Soares, R. M. (2010). Microcystins in South American aquatic ecosystems: Occurrence, toxicity and toxicological assays. *Toxicon*, 56(7), 1247-1256.

Elmorsi, R. R.; Hamed, M. and Abou-El-Sherbini, K. (2017). Physicochemical properties of Manzala lake, Egypt. *Egyptian Journal of Chemistry*, 60(4), 519-535.

Gągała, I.; Izydorczyk, K.; Jurczak, T.; Pawełczyk, J.; Dziadek, J.; Wojtal-Frankiewicz, A. and Mankiewicz-Boczek, J. (2014). Role of environmental factors and toxic genotypes in the regulation of microcystins producing cyanobacterial blooms. *Microbial ecology*, 67(2), 465-479.

Garrity, G. M.; Holt, J. G.; Whitman, W. B.; Keswani, J.; Boone, D. R.; Koga, Y. and Chong, S. C. (2001). Phylum All. Euryarchaeota phy. nov Bergey's Manual® of

Systematic Bacteriology (pp. 211-355): Springer.

Giovannoni, S.; DeLong, E.; Schmidt, T. and Pace, N. (1990). Tangential flow filtration and preliminary phylogenetic analysis of marine picoplankton. *Applied and environmental microbiology*, 56(8), 2572-2575.

Graham, J. L.; Dubrovsky, N. M.; Foster, G. M.; King, L. R.; Loftin, K. A.; Rosen, B. H. and Stelzer, E. A. (2020) .Cyanotoxin occurrence in large rivers of the United States. *Inland Waters*, 10(1), 109-117.

Griffith, A. W. and Gobler, C. J. (2020). Harmful algal blooms: a climate change co-stressor in marine and freshwater ecosystems. *Harmful Algae*, 91, 101590.

Ha, J. H.; Hidaka, T., and Tsuno, H. (2009). Quantification of toxic Microcystis and evaluation of its dominance ratio in blooms using real-time PCR. *Environmental science & technology*, 43(3), 812-818.

Hisbergues, M.; Christiansen, G.; Rouhiainen, L.; Sivonen, K. and Börner, T. (2003). PCR-based identification of microcystin-producing genotypes of different cyanobacterial genera. *Archives of Microbiology*, 180(6), 402-410.

Huang, Y. and Chen, M. (2013).Variation of dissolved oxygen in the experiments of occurrence & disappearance for Microcystis bloom. *Procedia Environmental Sciences*, 18, 559-566.

Ibrahim, W. M., Salim, E. H., Azab, Y. A., & Ismail, A. H. M. (2016). Monitoring and removal of cyanobacterial toxins from drinking water by algal-activated carbon. *Toxicology and industrial health*, 32(10), 1752-1762.

Jeffrey, S. T. and Humphrey, G. (1975).New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochemie und physiologie der pflanzen*, 167(2), 191-194.

Kocer, M. A. T. and ŞEN, B. (2012). The seasonal succession of diatoms in phytoplankton of a soda lake (Lake Hazar, Turkey). *Turkish Journal of Botany*, 36(6), 738-746.

Komárek, J. (2005). The modern classification of cyanoprokaryotes [Cyanobacteria]. Oceanological and Hydrobiological studies, 34(Suppl. 3).

Krausfeldt, L. E.; Farmer, A. T.; Castro Gonzalez, H. F.; Zepernick, B. N.; Campagna, S. R. and Wilhelm, S. W. (2019). Urea is both a carbon and nitrogen source for Microcystis aeruginosa: tracking 13C incorporation at bloom pH conditions. *Frontiers in microbiology*, 10, 1064.

Lam, A. K. and Prepas, E. E. (1997). In situ evaluation of options for chemical treatment of hepatotoxic cyanobacterial blooms. *Canadian Journal of Fisheries And Aquatic Sciences*, 54(8), 1736-1742.

Lévesque, B.; Gervais, M. C.; Chevalier, P.; Gauvin, D.; Anassour-Laouan-Sidi, E.; Gingras, S. and Bird, D. (2014). Prospective study of acute health effects in relation to exposure to cyanobacteria. *Science of The Total Environment*, 466, 397-403.

Li, S.; Ou, T. and Zhang, Q. (2013). Two virus-like particles that cause lytic infections in freshwater cyanobacteria. *Virologica Sinica*, 28(5), 303-305.

Li, Y.; Chen, J. A.; Zhao, Q.; Pu, C.; Qiu, Z.; Zhang, R. and Shu, W. (2011). A cross-sectional investigation of chronic exposure to microcystin in relationship to childhood liver damage in the Three Gorges Reservoir Region, China.

Environmental health perspectives, 119(10), 1483-1488.

Margalef, R. (1978). Life-forms of phytoplankton as survival alternatives in an unstable environment. *Oceanologica acta*, 1(4), 493-509.

Mohamed, Z. A.; Deyab, M. A.; Abou-Dobara, M. I.; El-Sayed, A. K. and El-Raghi, W. M. (2015). Occurrence of cyanobacteria and microcystin toxins in raw and treated waters of the Nile River, Egypt: implication for water treatment and human health. *Environmental Science and Pollution Research*, 22(15), 11716-11727.

Mohamed, Z., and Carmichael, W. (2000). Seasonal variation in microcystin levels of river Nile water at Sohag City, Egypt. *Annls Limnology*, 36 (4) 227-234

Morimoto, D.; Kimura, S.; Sako, Y. and Yoshida, T. (2018). Transcriptome analysis of a bloom-forming cyanobacterium Microcystis aeruginosa during Ma-LMM01 phage infection. *Frontiers in microbiology*, 9, 2.

Morón-López, J.; Nieto-Reyes, L.; Senán-Salinas, J.; Molina, S. and El-Shehawy, R. (2019). Recycled desalination membranes as a support material for biofilm development: A new approach for microcystin removal during water treatment. *Science of The Total Environment*, 647, 785-793.

Naknaen, A.; Oramas Suttinun, K. S.; Khan, E. and Pomwised, R. (2021). A Novel Jumbo Phage PhiMa05 Inhibits Harmful Microcystis sp. *Frontiers in microbiology*, 12.????

Nassar, M. Z.; Mohamed, H. R.; Khiray, H. M. and Rashedy, S. H. (2014). Seasonal fluctuations of phytoplankton community and physico-chemical parameters of the north western part of the Red Sea, Egypt. *The Egyptian Journal of Aquatic Research*, 40(4), 395-403.

Neilan, B. A.; Jacobs, D.; Blackall, L. L.; Hawkins, P. R.; Cox, P. T. and Goodman, A. E. (1997). rRNA sequences and evolutionary relationships among toxic and nontoxic cyanobacteria of the genus Microcystis. *International Journal of Systematic and Evolutionary Microbiology*, 47(3), 693-697.

Oliveira, C. Y.; Oliveira, C. D.; Almeida, A. J.; Gálvez, A. O. and Dantas, D. M. (2019). Phytoplankton responses to an extreme drought season: A case study at two reservoirs from a semiarid region, *Northeastern Brazil. Journal of Limnology*, 78(2).

Paerl, H. W. and Otten, T. G. (2013). Harmful cyanobacterial blooms: causes, consequences, and controls. *Microbial ecology*, 65(4), 995-1010.

Quail, P. H.; Boylan, M. T.; Parks, B. M.; Short, T. W.; Xu, Y. and Wagner, D. (1995). Phytochromes: photosensory perception and signal transduction. *Science*, 268(5211), 675-680.

Safferman, R.; Schneider, I.; Steere, R.; Morris, M. and Diener, T. (1969). Phycovirus SM-1: a virus infecting unicellular blue-green algae. *Virology*, 37(3), 386-395.

Scherer, P. I.; Millard, A. D.; Miller, A.; Schoen, R.; Raeder, U.; Geist, J. and Zwirglmaier, K. (2017). Temporal dynamics of the microbial community composition with a focus on toxic cyanobacteria and toxin presence during harmful algal blooms in two South German lakes. *Frontiers in microbiology*, 8, 2387.

Takashima, Y.; Yoshida, T.; Yoshida, M.; Shirai, Y.; Tomaru, Y.; Takao, Y. and Nagasaki, K. (2007).

Development and application of quantitative detection of cyanophages phylogenetically related to cyanophage Ma-LMM01 infecting Microcystis aeruginosa in freshwater. *Microbes and Environments*, 22(3), 207-213.

Van de Waal, D. B.; Verspagen, J. M.; Lürling, M.; Van Donk, E.; Visser, P. M. and Huisman, J. (2009). The ecological stoichiometry of toxins produced by harmful cyanobacteria: an experimental test of the carbon-nutrient balance hypothesis. *Ecological Letter*, 12(12), 1326-1335.

Weinbauer, M. G. (2004). Ecology of prokaryotic viruses. *FEMS microbiology reviews*, 28(2), 127-181.

Xia, H.; Li, T.; Deng, F. and Hu, Z. (2013). Freshwater cyanophages. *Virologica Sinica*, 28(5), 253-259.

Yoshida, M.; Yoshida, T.; Yoshida-Takashima, Y.; Kashima, A. and Hiroishi, S. (2009). Real-time PCR detection of host-mediated cyanophage gene transcripts during infection of a natural Microcystis aeruginosa population. *Microbes and Environments*, 1007200209-1007200209.

Yoshida, T.; Nagasaki, K.; Takashima, Y.; Shirai, Y.; Tomaru, Y.; Takao, Y. and Ogata, H. (2008). Ma-LMM01 infecting toxic Microcystis aeruginosa illuminates diverse cyanophage genome strategies. *Journal of Bacteriology*, 190(5), 1762-1772.