Seasonal Development of Cyanobacteria and Microcystin Production in a Shallow Freshwater Lake (North-Eastern, Algeria)

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ABSTRACT

Lake Oubeira, a shallow Mediterranean lake located in El-Kala National Park, northeastern Algeria, has faced frequent cyanobacterial blooms during the summer and autumn seasons, primarily due to human activities. While prior studies have investigated cyanobacteria abundance and toxin levels in the lake's fauna, they have not identified the specific genera responsible for toxin production or the environmental factors influencing their fluctuations. This study, conducted between 2016 and 2017, involved sampling the lake at one-meter depth across four seasons and identified 23 genera from five orders, with nine genera accounting for 93.68% of total abundance. The most prevalent genera included *Microcystis* (44.51%), *Planktothrix* (16.27%), *Pseudanabaena* (8.38%), *Aphanocapsa* (6.06%), *Synechococcus* (3.73%), *Cylindrospermum* (3.98%), *Cylindrospermopsis* (3.93%), *Dolichospermum* (3.17%) and *Aphanizomenon* (3.65%). Seasonal variations in distribution and abundance were noted, with significant blooms primarily composed of Microcystis and Planktothrix occurring during summer and autumn, coinciding with peak microcystin concentrations. The highest levels recorded were in the summer (9.57μg MC-LR equivalent. L-1) and autumn (10.09μg MC-LR equivalent. L-1). Strong positive correlations were observed between microcystin levels, chlorophyll-a concentration, and the abundance of Microcystis and Planktothrix. Microcystin was detected in all samples, with the lowest concentration (1.31μg MC-LR equivalent. L-1) found in spring. The presence of potentially toxic cyanobacterial genera was consistent throughout the year, regardless of seasonal or weather changes. To mitigate risks for lake users, continuous monitoring is essential to identify and quantify cyanobacteria and their toxins, particularly focusing on seasonal variations in this shallow lake ecosystem.

INTRODUCTION

Shallow lakes are among the most widespread water bodies globally and are particularly sensitive to external influences, making them susceptible to switch easily to a new trophic state. Changes in hydrological conditions and climate can trigger harmful

cyanobacterial blooms, which pose significant risks to ecological balance and human health (**Huang *et al.,* 2021**). Due to their long water residence times and generally low current velocities, lakes provide a time-integrated response to external factors. Nutrient over-enrichment, primarily from agricultural, urban, and industrial activities, leads to eutrophication in freshwater ecosystems (**Olsson *et al.,* 2022**). This process significantly increases cyanobacterial occurrences, as these organisms are highly competitive within phytoplankton communities (**Paerl & Otten, 2013**). Cyanobacterial growth is favored by nutrient availability, high temperatures, solar radiation, stable water columns, and elevated pH levels (**Paerl & Huisman, 2008; Carey *et al.,* 2012; Paerl & Otten, 2013**). Their adaptations include the ability to regulate buoyancy with gas vacuoles, form dormant akinetes during adverse conditions, and fix atmospheric nitrogen through specialized heterocysts (**Komarek & Anagnostidis, 1999, 2005**). Additionally, cyanobacteria produce various secondary metabolites including cyanotoxins that threaten aquatic ecosystems and human health by degrading water quality (**Kulabhusan & Campbell, 2024**). Among these toxins, microcystins (MCs), particularly prevalent in freshwater systems, are cyclic heptapeptides with over 240 identified structural variants (**Spoof & Catherine, 2016; Humbert & Fastner, 2017; Du *et al.,* 2019**). Numerous cyanobacterial genera—including *Microcystis, Dolichospermum, Anabaenopsis, Aphanizomenon, Aphanocapsa, Hapalosiphon, Nostoc, Planktothrix, Pseudanabaena, Gloeotrichia, Synechococcus,* and *Synechocystis* are capable of producing MCs, making their hepatotoxic metabolites a global concern. Microcystin-LR (MC-LR) is recognized as the most potent hepatotoxin based on acute toxicity assessments (**Chowdhury *et al.,* 2024**).

A synergistic interaction between increased nutrient levels and climate change is anticipated to exacerbate the occurrence of cyanobacterial blooms, supported by both experimental (**Lürling *et al.,* 2017; Paerl, 2017**) and field studies (**Rigosi *et al.,* 2014**). **Paerl and Huisman (2008)** predicted that harmful blooms will increase in both frequency and duration due to changes in hydrological cycling, elevated nutrient loading, and rising water temperatures. The Mediterranean basin is considered one of the most vulnerable regions to the impacts of global warming and altered precipitation patterns (**Noto *et al.,* 2023**). Understanding the distribution of cyanobacterial toxins across eutrophic lakes is crucial for elucidating the ecological roles of these metabolites, aiding in their remediation, and protecting public health. In Algeria, toxic cyanobacterial blooms dominated by Microcystis were first documented in Lake Oubeira during 2000-2001 (**Nasri *et al.,* 2004**). In 2005, two species of freshwater turtles were found dead during a Microcystis bloom in the same lake (**Nasri *et al.,* 2008**). Additionally, **Amrani *et al.* (2014)** measured microcystin levels in both the water and various organs of the carp and European eel in Lake Oubeira. While studies on environmental parameters affecting cyanobacteria dynamics in Algeria have primarily focused on drinking water supply dams like Mexa (**Saoudi *et al.,* 2015**), Hammam Debagh (**Guellati *et al.,* 2017**), Ain Zada

79

Seasonal Development of Cyanobacteria and Microcystin Production in a Shallow Freshwater Lake (North-Eastern, Algeria)

(Saoudi *et al.,* 2017; Charifi *et al.,* 2019), and Zit Emba **(Touati *et al.,* 2019)**, the underlying factors driving excessive proliferation of cyanobacteria and their bioactive secondary metabolites in Lake Oubeira remain inadequately understood.

This study aimed to i) assess the trophic status of Oubeira Lake; ii) monitor the spatio-temporal dynamics of cyanobacteria abundance and microcystins (MCs) content, and iii) investigate the influence of some environmental parameters on the assemblages of cyanobacteria and microcystins (MC-LR eq) production through the application of statistical tests.

MATERIALS AND METHODS

1. Study site

Lake Oubeira, located in the wetlands of El-Kala National Park in northeastern Algeria (36°50' N, 08°23' E), is the largest freshwater lake in the country, covering an area of 2,200 hectares. This shallow endorheic lake has a maximum depth of approximately 2.40 meters during wet periods and 1.50 meters during dry periods **(Alayat *et al.,* 2013)**. The lake's bottom is covered by a thick mud layer that can reach up to 2 meters in the center. Its hydrological regime is closely tied to weather conditions, primarily receiving surface runoff during the rainy season, with an annual rainfall ranging from 700 to 800mm. Four tributaries feed into Lake Oubeira: the Dey-Legraa River to the East, the Boumerchen River to the Northeast, the Demet Rihana River to the North, and the Messida River to the South. The lake supports diverse flora and fauna, including migratory and resident birds, fish, bivalves, and aquatic plants such as water chestnut (Trapa natans), white water lily (Nymphaea alba), and yellow water lily (Nuphar lutea) **(Samraoui *et al.,* 1998)**. Its waters are primarily used for irrigating peanut crops, aquaculture, and providing drinking water for livestock grazing along its shores.
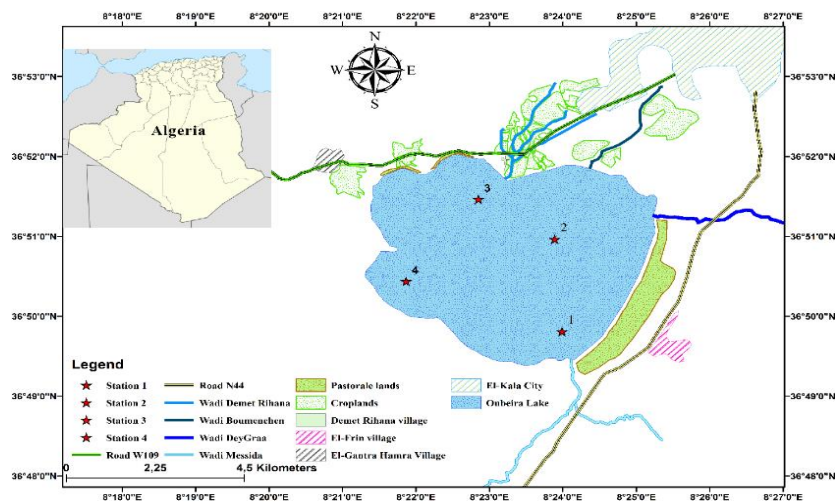
Fig. 1. Geographical location of Lake Oubeira and the distribution of the sampling stations (S1: station 1, S2: station 2, S3: station3, S4: station 4)

2. Sampling protocol

From spring 2016 to winter 2017, sampling was seasonally conducted at four stations. Water samples were collected with a 1m plastic tube at a depth of 1m below water surface, and stored in white plastic bottles, in the cold and dark, until they arrived at the laboratory where they were aliquoted for the various analyses. The water samples for cyanobacterial identification were collected with conical plankton net with a mesh size of 20µm, and the filtrates were immediately conserved in 5% final formaldehyde (v/v) (Fig. 1)

3. Environmental factors

Dissolved oxygen (DO), water temperature (WT), conductivity and pH were measured "*in situ*" using multiparametric probes of type 3420 SET G (WTW, Germany). Water transparency was measured using a 25cm diameter Secchi disc. The nutrient analyses, including nitrites (NO2-N), nitrates (NO3-N), ammonium (NH4-N) and orthophosphate (PO4-P) were performed on filtrates (100ml) using the colorimetric methods as described by **Aminot and Kérouel (2004)**. Suspended solids (SS) concentrations were conducted by the differential weighing method after filtration, a known volume of water sample through a glass microfiber filter (GF/C 47, Whatman) **(Aminot & Chaussepied, 1983)**. Chlorophyll-a concentration was estimated from spectrophotometric measurements after extraction in 90% aqueous acetone **(Parsons, 1966)**.

4. Cyanobacteria analyses

The identification of cyanobacteria was based on microscopic observations of morpho-anatomical characters using taxonomic keys of **Komárek and Anagnostidis (2005)**, **Komárek *et al.* (2014)** and **Komárek (2016)**. The cell count was carried out by a Nageotte cells described in the study of **Brient *et al.* (2008)**. Both the identification and counting of cyanobacteria (cells/mL) were realized by an optical microscope (Carl Zeiss, Germany) equipped with a UI-1240 SE camera (IDS, Germany).

5. Microcystins analysis

The total microcystins (MCs) content in raw water samples was determined by an enzyme-linked immunosorbent assay (ELISA) as follows: After filtration through a glass microfiber filter with a nominal porosity of 0.45µm (47mm diameter, Whatman, Germany), frozen at -20°C and defrosted, as well as treated with an ultrasonic probe, succeeding an extraction of the MCs by using a methanol/water solution (0.8V:0.2V). The samples were analyzed using an ELISA kit (Abraxis, USA) as recommended by the manufacturer's protocol, with an average lower detection level of 0.15µg/ l of MCs. The samples were positively assessed when the concentration of MCs was well above this limit, and the absorbance was read only at one wavelength (450nm), using a microplate ELISA photometer (MindrayMR-96A).

6. Statistical analysis

The statistical analyses were carried out using R software (version 4.1.2). Firstly, the normality of the sample distributions was verified by applying the Shapiro-Wilk test. Then, inter-season comparisons were made using the non-parametric Kruskal-Wallis test, followed by a Dunn's post- hoc test. The seasonal variation of our tested biotic and abiotic variables

81

Seasonal Development of Cyanobacteria and Microcystin Production in a Shallow Freshwater Lake (North-Eastern, Algeria)

was depicted through multiple boxplots using the 'ggplot2' package **(Wickham, 2016)**. Furthermore, the relationships between our datasets were explored through correlation analysis, performed with the "corrplot" package **(Wei & Simko, 2021)**, to calculate Spearman's correlation coefficients and *P*-values. Finally, we conducted a principal component analysis (PCA) on standardized data to characterize the temporal variation of biotic and abiotic variables measured in Lake Oubeira. This analysis was performed using the "FactoMineR" **(Husson *et al.,* 2020)** and "Factoextra" **(Kassambara & Mundt, 2020)** software packages. All statistical analyses were considered significant at *P*< 0.05.

## RESULTS

1. Environmental factors

Table 1. Seasonal mean variations in physico-chemical parameters and chlorophyll-*a* in Lake Oubeira (spring 2016-winter 2017) and WHO standards for recreational water bodies

Season Mean ±sd	WHO Standards	Spring	Summer	Fall	Winter	Inter-season P-value
T (°C)	12–25°C	24.30±2.47	29.20±1.80	17.06±3.28	11.77±0.46	<0.001
pH	6.5–8.5	8.24±0.21	8.94±0.41	8.58±0.26	8.76±0.18	<0.001
DO (mg.L⁻¹)	6.5–8 mg/L	6.99±1.16	6.73±2.04	8.74±1.53	10.55±0.80	<0.001
Cond (µS.cm⁻¹)	<500 µS/cm	431.33±38.18	496.08±9.24	524.41±7.78	537.25±12.89	<0.001
Trans (cm)	>2 m	19.58±3.96	12.50±4.17	9.16±4.17	9.58±3.34	<0.001
NH₄-N (mg.L⁻¹)	<0.5 mg/	2.87±1.60	8.65±7.56	3.60±2.61	2.31±2.04	<0.05
NO₂-N (mg.L⁻¹)	<0.1 mg/l	1.07±0.12	1.15±0.41	1.61±0.38	0.93±0.46	<0.001
NO₃-N (mg.L⁻¹)	<10 mg/L	4.91±2.28	4.82±2.23	4.94±0.55	3.79±0.98	0.001
PO₄-P (mg.L⁻¹)	<0.1 mg/L	1.77±1.09	6.07±9.53	2.53±2.42	1.82±1.55	0.32

SS (mg.L^{-1})	<25mg/L	121.00±81.26	99.00±54.92	123.25±67.01	93.25±70.61	0.35
Chl-a (µg.L^{-1})	<10 µg/L	14.23±6.55	84.36±45.76	87.12±50.83	19.40±14.77	<0.001
Mc-LR (ug.L[-1])	20 µg/L	1.31±0.53	9.57±5.27	10.09±5.49	2.16±2.18	<0.001

SD, standard deviation; p-value – probability value (*P*<0.05).

Inter-season comparisons using the non-parametric Kruskal-Wallis test (Fig. 2) revealed significant differences between seasons for all physico-chemical parameters studied, except for SS and PO4-P. Furthermore, Fig. (2) illustrates the post hoc pairwise comparisons of Dunn's test in box plots for the seasonal factor.
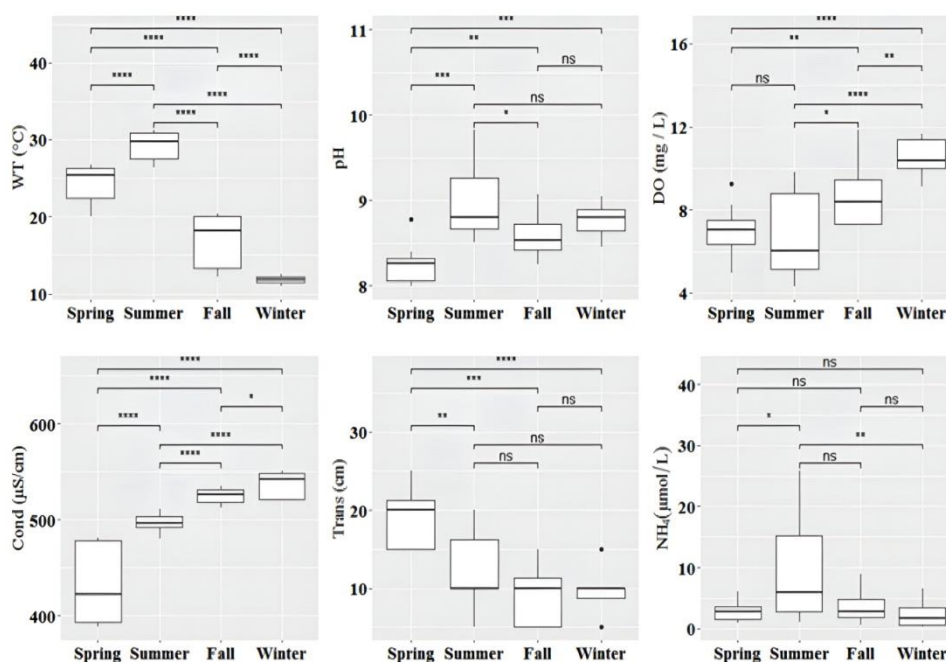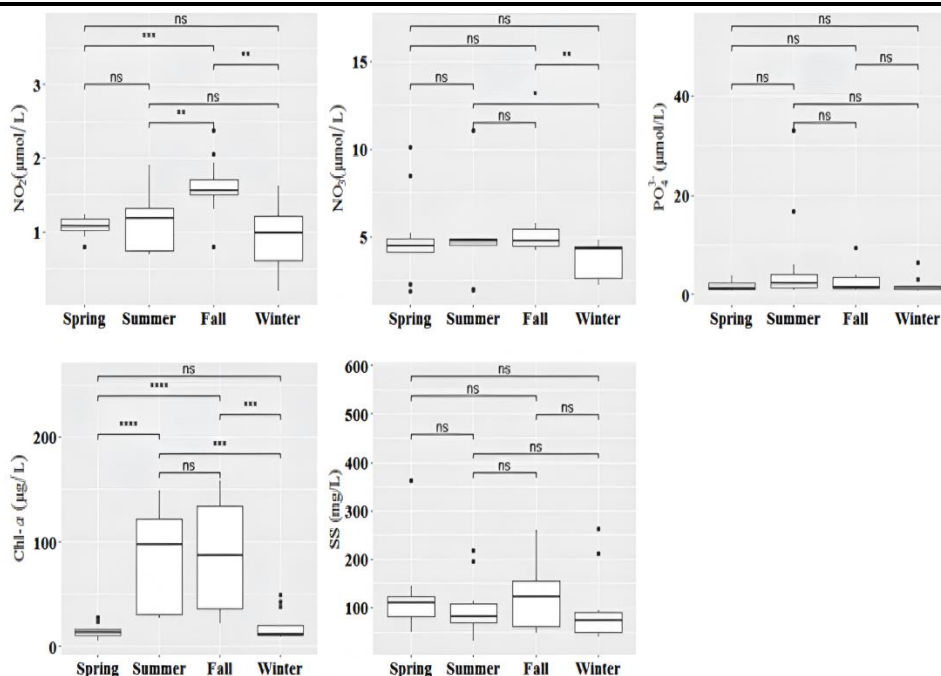
Fig. 2. Box and whisker plots demonstrating seasonal patterns of physicochemical parameters and Chlorophyll-a. The *P*-values from the Kruskal–Wallis test with Dunn's multiple comparison tests are shown as asterisks for each season (ns: not significant; *$P<0.05$, **$P<0.01$, ***$P<0.001$)

2. Generic diversity and occurrence frequency

Microscopic observation of the morpho-anatomical characters of the cyanobacteria collected in the Lake Oubeira waters enabled us to identify twenty-three (23) genera belonging to five orders (Table 2).

**Table 2.** Cyanobacteria genera collected in Lake Oubeira waters (spring 2016-winter 2017)

Order	Genera
Choococcales	*Microcystis, Chroococcus, Gloeocapsa*, Aphanothece**
Oscillatoriales	*Oscillatoria, Planktothrix, Phormidium*, Lyngbya*,*
Nostocales	*Aphanizomenon, Nostoc*, Cylindrospermopsis, Dolichospermum, Cylindrospermum*,*
Synechococales	*Synechococcus, Merismopedia, Pseudanabaena, Woronichinia, Aphanocapsa, Synechocystis*, Limnothrix*, Leptolyngbya*, Planktolyngbya*,*
Spirulinales	*Spirulina*

*Rare Genera.

The occurrence frequency calculation shows that about 50% of the genera recorded in the waters of Lake Oubeira are rare; we also note the omnipresence of *Microcystis*, the constancy of *Pseudanabaena* and the regularity of *Chroococcus* (*Chroo, Dolichospermum, Planktothrix, Synechococcus, Aphanizomenon,* and *Aphanocapsa*). However, the genera *Oscillatoria*, *Merismopedia*, *Cylindrospermopsis*, *Woronichinia*, and *Spirulina* are incidental.

2.1. The most abundant genera

In this study, the genera considered most abundant were those accounting for more than 3% of the overall abundance of collected cyanobacteria (Fig. 3), such as *Microcystis* (44.51%), *Planktothrix* (16.27%), *Pseudanabaena* (8.38%), *Aphanocapsa* (6.06%), *Synechococcus* (3.73%), *Cylindrospermum* (3.98%), *Cylindrospermopsis* (3.93%), *Dolichospermum* (3.17%) and *Aphanizomenon* (3.65%). The nine genera considered account for 93.68% of the overall abundance, while the remaining thirteen genera, grouped under the heading 'others,' make up 6.32% of the total abundance.
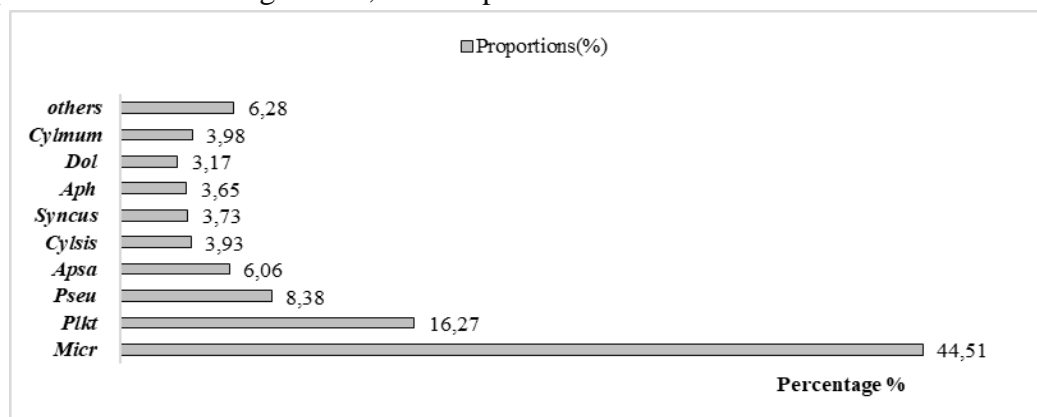
Fig. 3. Relative abundance of the main blooming cyanobacterial genera in Lake Oubeira waters (spring 2016-winter 2017). Abbreviations: *Mic=Microcystis*, *Plank=Planktothrix*, *Pseud=Pseudanabaena*, *Aphsa=Aphanocapsa*, *Syncus=Synechococcus*, *Cylmum=Cylindrospermum*, *Cylsis=Cylindrospermopsis*, *Doli=Dolichospermum*, *Apha=Aphanizomenon*.

2.2. Seasonal pattern of the main genera

The total abundance of cyanobacteria in Lake Oubeira ranged from 184 707 Cell.L^{-1} in spring (representing only 12.34% of the total abundance of the collected cyanobacteria), to 560721 Cell.L^{-1} in summer, corresponding to 37.47% of the total abundance of the sampled cyanobacteria (Fig. 4). The most recurrent periods of increase occurred in summer and autumn (totalling 74.37% of overall abundance), coinciding with the highest peaks. Spring and winter were the periods of lowest abundance (25.61% of total abundance).

Seasonal Development of Cyanobacteria and Microcystin Production in a Shallow Freshwater Lake (North-Eastern, Algeria)

Table 3. Seasonal mean variations in cyanobacteria genera encountered in Lake Oubeira water (spring 2016-winter 2017)

Season Mean ±SD	Spring	Summer	Fall	Winter	Inter-season P-value
Aph	2583.33±3654.43	3297.33±3892.78	22892.33±33809.38	25922.33±14988.23	<0.001
Apha	24653.91±21183.33	18718.33±29760.26	43744.66±49031.32	3627.33±6586.85	<0.05
Cyl	0.00±0.00	13527.33±27012.51	42319.33±49221.16	3003.00±5470.28	<0.001
Cyli	486.66±1685.86	59124.00±80529.98	0.00±0.00	0.00±0.00	<0.001
Doli	1625.66±2992.91	8249.33±7601.47	23275.50±22608.68	14340.33±27746.22	<0.05
Micr	96096.25±45894.97	245798.92±84906.11	232866.75±63411.46	91257.58±45604.99	<0.001
PlanK	1589.50±4049.37	142156.67±76347.35	99700.67±66281.65	0.00±0.00	<0.001
Pseu	11523.83±8208.55	15336.67±8868.46	66683.33±24956.14	31851.33±19008.57	<0.001
Syn	16541.83±23381.98	13493.92±13339.99	14135.00±16520.79	11777.92±15285.39	0.96
Others	29611.00±19683.71	41023.41±24387.51	6579.33±10238.18	16827.41±14634.81	<0.001

SD, standard deviation; *P*-value – probability value (*P*<0.05).
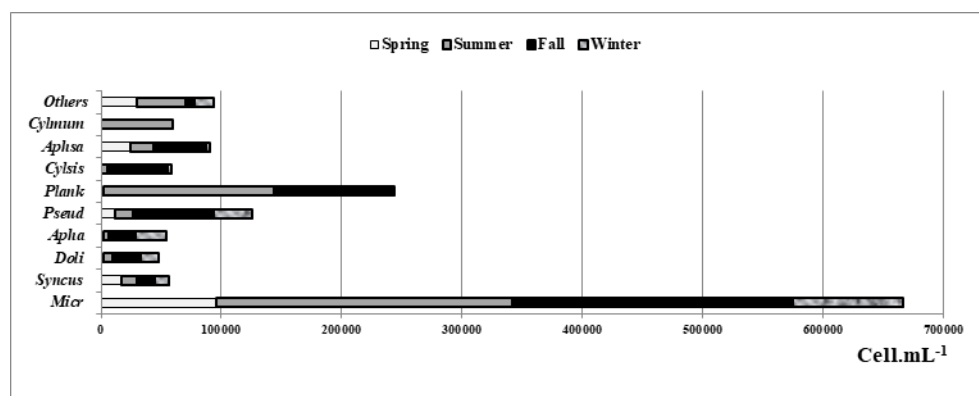
Fig. 4. Densities of the main blooming cyanobacterial genera in Lake Oubeira waters (spring 2016-winter 2017).

Comparisons between seasons, assessed through the non-parametric Kruskal-Wallis test, unveiled significant differences in the abundance of all identified genera for each season, except for *Synechococcus*, (*P*-value 0.96). The post hoc pairwise comparisons from Dunn's test are depicted in the box plots in Fig. (5), providing an illustration of the seasonal variations in genera abundance within Lake Oubeira.

In spring (Fig. 6a), *Microcystis*, *Aphanocapsa*, *Synechococcus* and *Pseudanabaena* mounted mean densities close to 96100, 24650, 16540 and 11520 Cell.L^{-1}, respectively, and they accounted for over 80% of overall seasonal abundance. The genus *Cylindrospermopsis* was absent, and the genera *Aphanizomenon, Planktothrix, Dolichospermum* and *Cylindrospermum* were poorly represented, together accounting for less than 3.5% of the season's abundance. The genera belonging to the group *Others* (16 genera) recorded average densities of over 29600 Cell.L^{-1}, representing 16% of the abundance of cyanobacteria collected in spring.

In summer (Fig. 6b), the genera with average densities above 100,000 cells per liter are *Microcystis* (over 245,800 cells per liter) and *Planktothrix* (over 142,160 cells per liter), together accounting for more than 69% of the overall density. Genera exceeding 40,000 cells per liter include *Cylindrospermum* and the group labeled 'others' (with densities of over 59,100 and 41,000 cells per liter, respectively). Mean densities below 10,000 cells per liter are recorded for *Dolichospermum, Aphanizomenon,* and *Cylindrospermopsis*, while those ranging between 10,000 and 20,000 cells per liter include *Synechococcus, Pseudanabaena,* and *Aphanocapsa*.

The overall abundance values for cyanobacteria collected in summer and autumn (Fig. 6c) are quite close (37.47 and 36.90%, respectively). In autumn, 5 of the 8 genera present recorded mean densities exceeding 40000 Cell.L^{-1} (*Microcystis*, *Planktothrix*, *Pseudanabaena*, *Cylindrospermopsis* and *Aphanocapsa*) accounting for around 88% of overall density. *Cylindrospermum*was absent, and the mean density of the 8 genera belonging to the group "*others*" did not exceed 7,000 Cell.L^{-1}. The mean densities of *Dolichospermum* and *Aphanizomenon* were close to 23,000 Cell.L^{-1}, while that of *Synechococcus* exceeds 14,000 Cell.L^{-1}.

The overall abundance of cyanobacteria collected in winter (Fig. 6d) represents only 35% of that recorded in summer and 36% of that recorded in autumn, due to the absence of *Planktothrix* and *Cylindrospermum,* as well as low densities (close to 3600 Cell.L^{-1}) recorded by *Cylindrospermopsis* and *Aphanocapsa*. In winter, the dominant genera were *Microcystis*, *Pseudanabena* and *Aphanizomenon* (accounting for over 75% of overall density with densities of over 91200, 31800 and 25900 Cell.L^{-1}, respectively), and to a weaker extent *Dolichospermum* and *Synechococcus*.

Seasonal Development of Cyanobacteria and Microcystin Production in a Shallow Freshwater Lake (North-Eastern, Algeria)
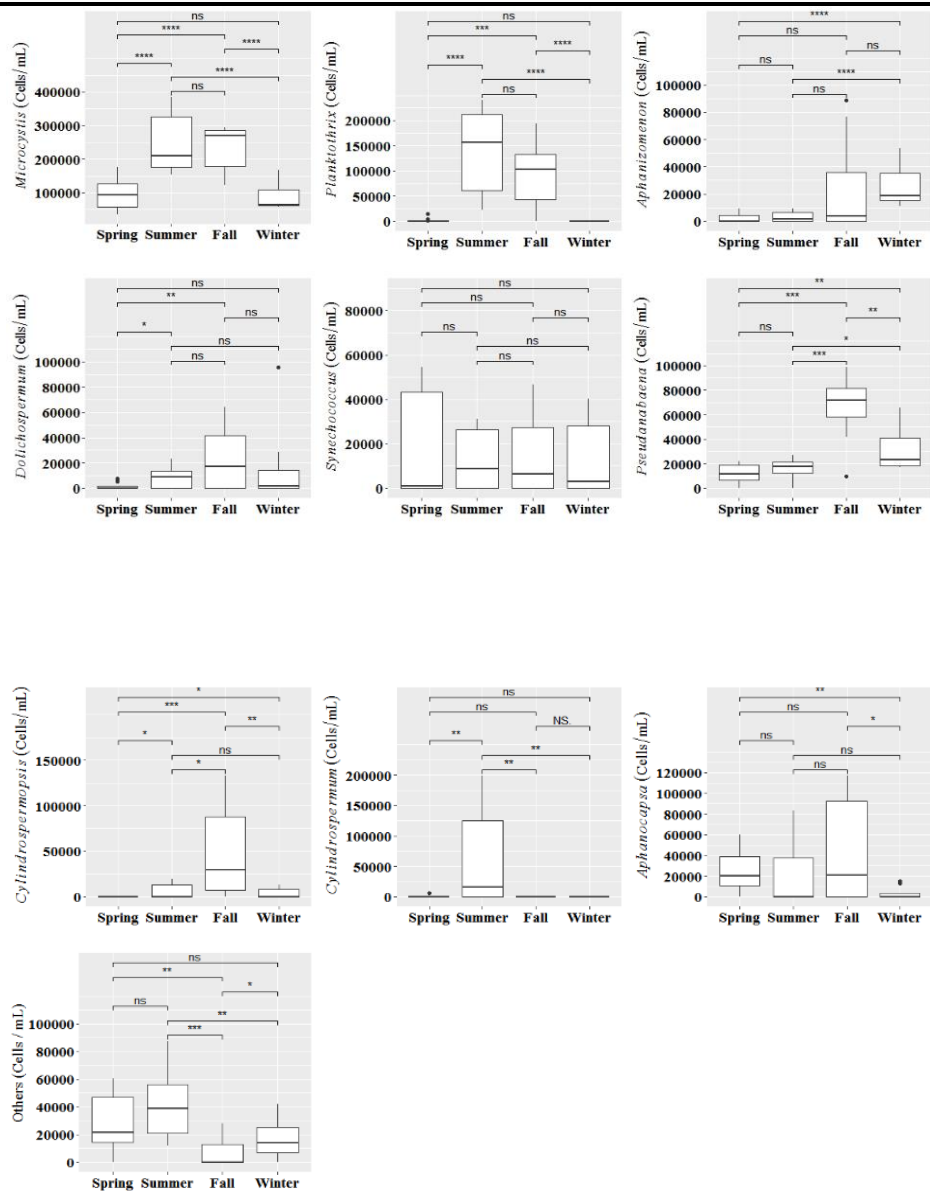
Fig. 5. Box and whisker plots demonstrating seasonal patterns of the main blooming cyanobacteria genera in Lake Oubeira. The *P*-values from a the Kruskal–Wallis test with Dunn's multiple comparison tests are shown as asterisks for each season (ns:not significant ; *$P<0.05$, **$P<0.01$, ***$P<0.001$)
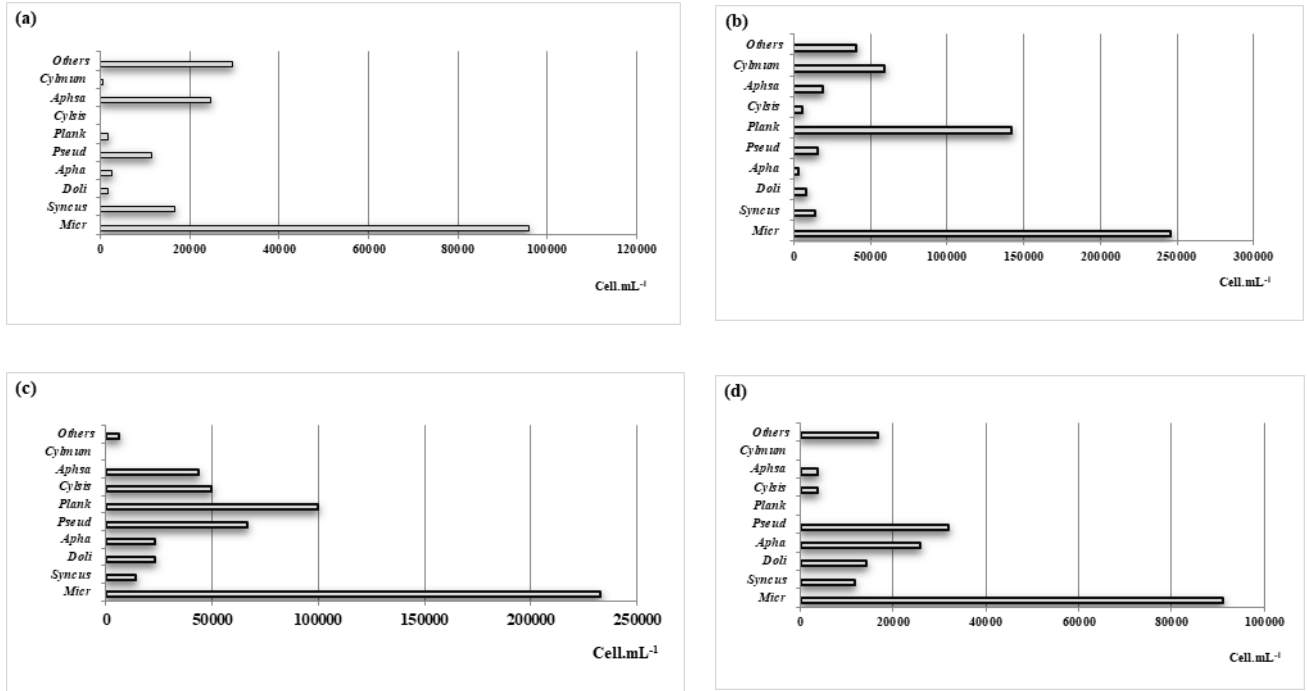
Fig. 6. Assemblage of the main genera collected in each season

3. Intracellular microcystin analysis

Microcystin was found at measurable levels in all the collected samples with contents ranging from 1.31 to 10.09μg eq MC-LR/L in spring and autumn, respectively (Fig. 10).

A peak of 9.57μg eq MC-LR/L appeared in summer and a second one of 10.09μg eq MC-LR/L appeared in autumn; the two peaks appeared during the exponential and steady growth phases of *Microcystis* and *Planktothrix*. This was confirmed by its strong positive correlations with chlorophyll-a and the abundance of *Microcystis* and *Planktothrix*, who themselves are highly correlated with each other (r=0.87, *P*<0.0001), and with chlorophyll-a.

The inter-seasons comparison of microcystin's median values have shown highly significant differences (*P*<0.001) from one season to the other.
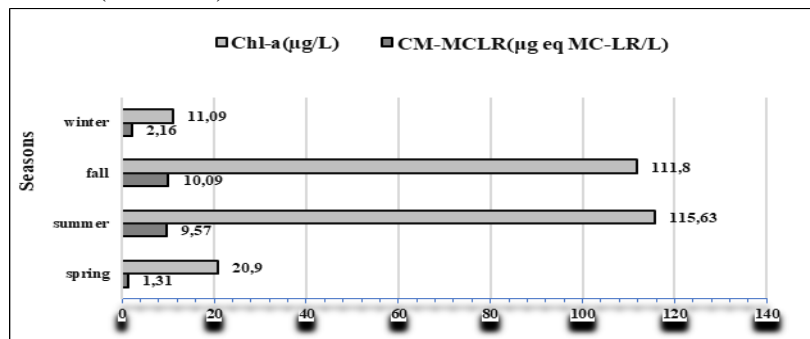

Fig. 7. Seasonal fluctuations in intracellular microcystin (μg eq MC-LR/L) and Chlorophyll a concentrations recorded in Oubeira waters (spring 2016- winter 2017)

89

Seasonal Development of Cyanobacteria and Microcystin Production in a Shallow Freshwater Lake (North-Eastern, Algeria)

4. Statistical analysis

The results of the correlations established by the Spearman non-parametric test are presented in Fig. (8).

In this study, we conducted a comprehensive correlation analysis to explore the relationships between microcystin levels, chlorophyll-a concentrations, and the most abundant cyanobacterial genera in Oubeira Lake. Microcystin exhibited very strong positive correlations with chlorophyll-a ($r=0.99$, $P<0.0001$), *Microcystis* ($r=0.95$, $P<0.0001$), *Planktothrix* ($r=0.84$, $P<0.0001$), *Dolichospermum* ($r=0.53$, $P=0.0001$), *Cylindrospermum* ($r=0.45$, $P=0.001$), and *Pseudanabaena* ($r=0.43$, $P=0.002$). Additionally, microcystin levels showed strong positive correlations with nitrate (NO_3) ($r=0.57$, $P<0.0001$), ammonium (NH_4) ($r=0.53$, $P<0.0001$), and nitrite (NO_2) ($r=0.43$, $P=0.001$), while negatively correlating with transparency (Trans) ($r=-0.40$, $P<0.01$). Similarly, chlorophyll-a demonstrated strong correlations with *Microcystis* ($r=0.95$, $P<0.0001$), *Planktothrix* ($r=0.86$, $P<0.0001$), and *Dolichospermum* ($r=0.53$, $P=0.0001$), as well as with NO_3 ($r=0.57$, $P<0.0001$), NH_4 ($r=0.53$, $P<0.0001$), NO_2 ($r=0.44$, $P<0.001$), and negatively with Trans ($r=-0.40$, $P<0.01$). Additionally, *Microcystis* displayed strong positive correlations with NO_3 ($r=0.56$, $P<0.0001$) and NH_4 ($r=0.55$, $P<0.0001$), along with strong correlations with NO_2 ($r=0.48$, $P<0.001$) and Trans ($r=0.43$, $P<0.01$). *Planktothrix* showed positive correlations with water temperature ($r=0.54$, $P<0.0001$), NH_4 ($r=0.43$, $P<0.001$), and NO_3 ($r=0.40$, $P<0.01$) but had a significant negative correlation with dissolved oxygen (DO) ($r=-0.38$, $P<0.01$). Furthermore, the genus *Dolichospermum* exhibited a significant positive correlation with Trans ($r=0.54$, $P<0.0001$) and significant positive correlations with both conductivity and NO_2 ($r=0.46$, $P<0.001$), while showing a significant negative correlation with temperature ($r=-0.46$, $P<0.001$). Lastly, *Aphanizomenon* was positively correlated with DO ($r=0.63$, $P<0.0001$) and conductivity ($r=0.62$, $P<0.0001$) but exhibited a strong negative correlation with water temperature ($r=-0.66$, $P<0.0001$). Conversely, *Cylindrospermopsis* displayed a positive correlation with water temperature ($r=0.46$, $P<0.001$).
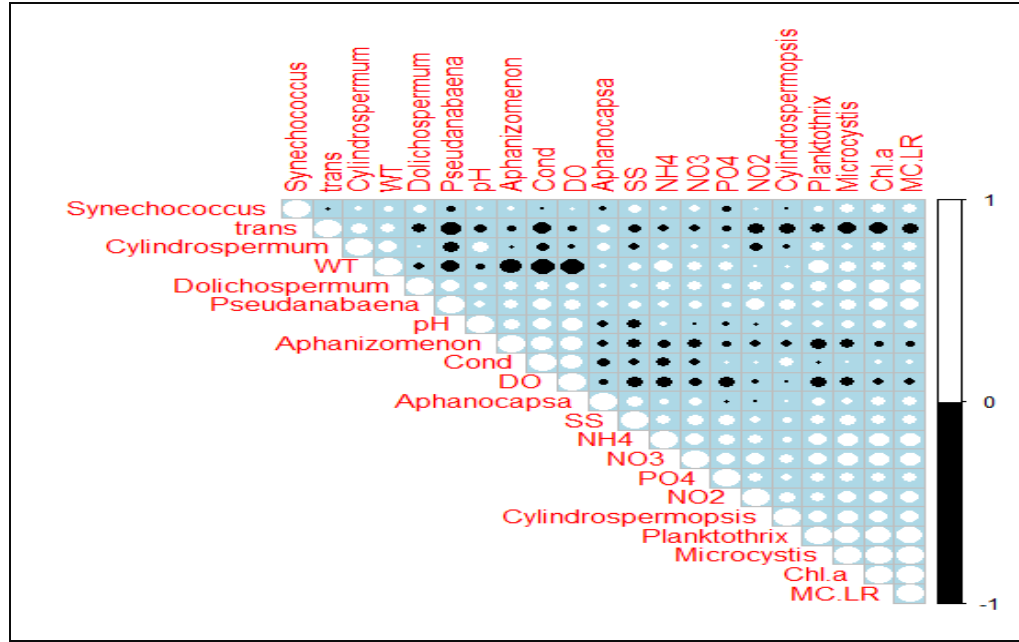
Fig. 8. Correlo-gram of the twenty-one biotic and abiotic variables established by Spearman non-parametric test

The principal component analysis (PCA) results indicate that the eigenvalues of the first two principal components collectively account for approximately 53.89% of the total variance, as shown in Fig. (9).

The first principal component (Dim1) explains 30.61% of the variability. This is primarily influenced by positive loadings from NH4 (r = 0.74), WT (r = 0.72), PO4 (r = 0.71), NO3 (r = 0.67), *Microcystis* (r = 0.64), *Planktothrix* (r = 0.56), SS (r = 0.50), while it is negatively correlated with DO (r = -0.78) and *Aphanizomenon* (r = -0.40).

The second principal component (Dim2) contributes 23.28% of the variability and is positively loaded with Cond (conductivity, r = 0.83), *Pseudanabaena* (r = 0.58), Microcystins (r = 0.54), chlorophyll-a (r = 0.54), and pH (r = 0.53). Conversely, it exhibits a negative correlation with transparency (r = -0.78).
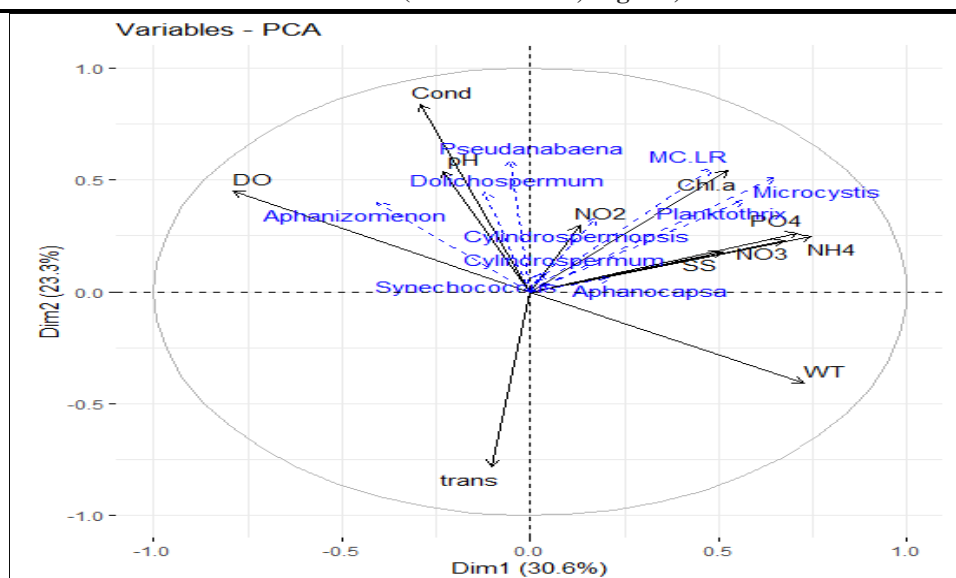
Fig. 9. Principal component analysis (PCA) of the biotic and abiotic variables. Abbreviations for domains: Chlorophyll-a (Chl.a), Microcystins (MC-LR), pH, Conductivity (Cond), dissolved oxygen (DO), ammonium (NH4), nitrite (NO2), nitrate (NO3), orthophosphate (PO4), suspended solids (SS), water temperature (WT), and transparency (Trans)

The representation of seasonal variations (Fig. 10) reveals distinct differences between two main seasons. On the right side of the projection, we observe a warm period, primarily attributed to the summer season. On the left side, the projection reflects a colder period, predominantly associated with the winter season, emphasizing the seasonal gradient of the biotic and abiotic parameters examined at our site.
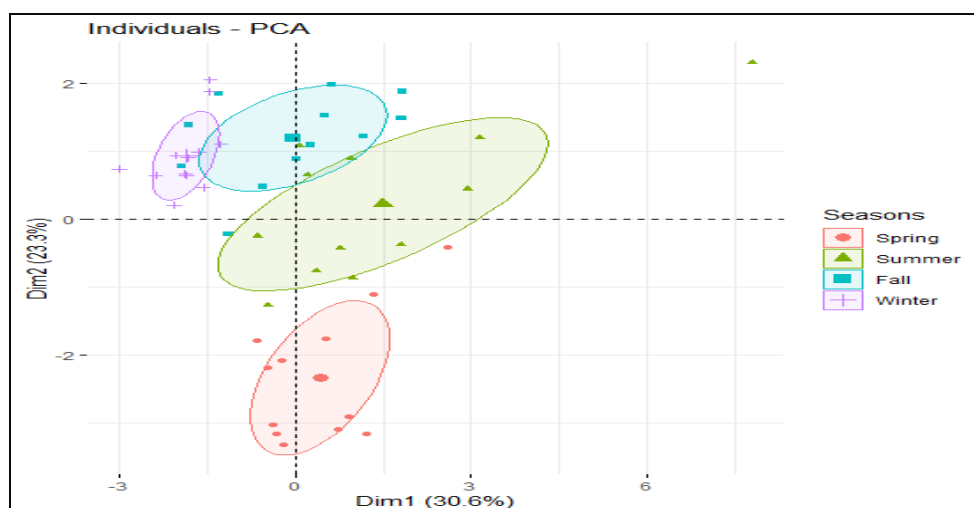
Fig. 10. Projection of seasons variation

DISCUSSION

Lake Oubeira, a shallow endorheic Mediterranean lake, exemplifies the complex interplay between natural processes and human activities. The lake's extended water retention time, coupled with significant anthropogenic influences, has led to substantial organic matter accumulation and high nutrient concentrations **(Arif *et al.,* 2023)**. Recent assessments based on **OECD (1982)** criteria and **WHO (1994)** guidelines reveal a concerning ecological state, with water quality ranging from mediocre to severely polluted. High levels of ammonium, nitrite and phosphate, combined with low water transparency and high chlorophyll-a contents (87.12µg/ L in autumn and 84.36µg/ L in summer), rank the lake as hyper-eutrophic according to the diagnostic grid of **Vollenweider and Kerekes (1982)**. This classification indicates an advanced state of nutrient enrichment and algal growth, underscoring the lake's compromised ecological status due to the combined effects of human activities (agriculture, livestock grazing, fishing, and aquaculture) and natural factors such as wind-driven sediment-water interactions.

Lake Oubeira functions as a natural bioreactor due to its extended water retention time, leading to significant organic matter accumulation in its sediments. **Ghai *et al.* (2012)** described a cyclic process where nutrient enrichment stimulates phytoplankton growth, and subsequent decomposition further increases the lake's trophic status. Chlorophyll-a levels, which **Ramade (1999)** identifies as a reliable eutrophication indicator, fluctuate seasonally in tandem with phytoplankton density, showing lower concentrations in winter and spring and peaking in summer and autumn. This complex interplay of nutrient cycling, phytoplankton dynamics, and organic matter decomposition highlights the intricate ecological processes in Lake Oubeira and underscores the importance of chlorophyll-a measurements in assessing the lake's trophic state and overall ecological health.

Recent research highlights the growing concern of harmful cyanobacteria in eutrophic lakes, exacerbated by summer heat waves and projected to worsen with global warming **(Van de Waal *et al.,* 2024)**. Environmental factors play a crucial role in promoting cyanobacterial dominance and microcystin production **(Monchamp *et al.,* 2014)**. **Kosten *et al.* (2012)** characterize eutrophic water bodies by cyanobacteria-dominated phytoplankton communities, elevated nutrient levels, reduced water clarity, and higher surface temperatures. Studies by **Rastogi *et al.* (2014)** and **Harke *et al.* (2016)** further emphasized that eutrophic or hypereutrophic conditions create favorable environments for extensive cyanobacterial blooms.

The eutrophication of lakes creates conditions of low transparency and high turbidity due to suspended solids, limiting light penetration. This environment favors cyanobacteria equipped with gas vacuoles, such as *Microcystis*, *Planktothrix*, and

93

Seasonal Development of Cyanobacteria and Microcystin Production in a Shallow Freshwater Lake (North-Eastern, Algeria)

Aphanizomenon, which can regulate their buoyancy to access both light for photosynthesis and nutrient-rich sediments (**Carey *et al.,* 2012**). In Lake Oubeira, *Microcystis* dominates, accounting for 44.51% of total cell abundance, attributed to its phenotypic plasticity and competitive nature (**Xiao *et al.,* 2017; Xiao *et al.,* 2018**). Interestingly, *Microcystis* prevails despite being non-diazotrophic and unable to fix atmospheric nitrogen (**Potts *et al.,* 2000**). This dominance is likely due to severe eutrophication from anthropogenic and natural activities, internal nitrogen regeneration, water column stability, lake morphology, and extended water residence time. The prevalence of *Microcystis* in Lake Oubeira exemplifies the complex interactions between nutrient dynamics, physical lake characteristics, and cyanobacterial adaptations in eutrophic ecosystems.

The year-round dominance of *Microcystis* in shallow water bodies can be attributed to its buoyancy, which facilitates nutrient access, particularly in environments with high concentrations of nitrates (NO3), ammonium (NH4), and nitrites (NO2). **Wagner and Adrian (2009)** emphasized that in shallow lakes like Lake Muggelsee, with a maximum depth of less than 8 meters, *Microcystis* can migrate to the hypolimnion, where nutrient levels are elevated. This genus has also been noted for its prevalence in various Algerian lakes (**Amri *et al.,* 2010; Bouhaddada *et al.,* 2016**) and is recognized globally for thriving in eutrophic and hypereutrophic ecosystems, particularly during the summer months (**Mariani *et al.,* 2015; Harke *et al.,* 2016**).

Planktothrix constitutes over 16% of total density and exhibits a strong positive correlation with *Microcystis*, temperature, and nutrients such as ammonium (NH4) and nitrates (NO3). This species thrives in shallow water bodies, leveraging environmental conditions like increased temperatures, particularly above 20°C during late summer and early autumn, which supports its growth (**Kurmayer *et al.,* 2004; Kokociński *et al.,* 2010**). While many eukaryotic taxa show reduced growth rates at temperatures exceeding 20°C, cyanobacterial species, including *Microcystis aeruginosa* and *Planktothrix agardhii*, continue to proliferate (**Paerl & Huisman, 2009**). Both *Planktothrix* and *Microcystis* have adapted buoyancy-regulating mechanisms through intracellular gas vacuoles, allowing them to optimize their vertical positioning in the water column for light absorption during the day and nutrient uptake at night (**Dokulil & Teubner, 2000**). Their ability to store nutrients and thrive in low-light conditions enables *Planktothrix* to compete effectively with *Microcystis*, further reinforcing their positive correlation. Factors such as calm water conditions, which reduce mixing, alongside nutrient enrichment, contribute to their high chlorophyll-a correlations (**Namsaraev *et al.,* 2018**).

Nixdorf *et al.* (2003) classified *Planktothrix* within the S1 functional group of photos- adapting filamentous cyanobacteria, which are prevalent in shallow, wind-exposed lakes characterized by significant sediment-water interactions and low light conditions. During the summer and autumn months, *Microcystis* and *Planktothrix* have

been identified as the primary producers of harmful algal blooms in the Mediterranean water bodies **(Carrasco *et al.,* 2006)**.

The *Anabaena* group, which includes the genus *Dolichospermum*, is notable for its wide morphological variability and ecological success, evidenced by its extensive occurrence. Key adaptations such as gas vesicles, heterocysts, and akinetes enable *Dolichospermum* to access nutrients and light, regulate buoyancy in the water column, and fix atmospheric nitrogen. Although these adaptations provide advantages, *Dolichospermum* blooms primarily occur in eutrophic ecosystems **(Pearl *et al.,* 2014)**. Environmental factors influencing the development of akinetes can be categorized into three main areas: nutrients, light, and temperature. Our findings reveal significant correlations between *Dolichospermum* and nutrients (specifically NO2) as well as environmental factors like transparency and conductivity, suggesting these elements play a crucial role in its dominance.

Eutrophication and climate change have led to an increase in the frequency and intensity of *Dolichospermum* blooms, which now persist longer and dominate more spatially **(Cirés *et al.,* 2016)**. These blooms have been associated with various external environmental factors that promote their proliferation across different water bodies **(Li *et al.,* 2016)**. As conditions such as nutrient enrichment and temperature fluctuations become more prevalent due to these changes, *Dolichospermum* has adapted to thrive, further exacerbating the impacts of harmful algal blooms in aquatic ecosystems.

Zhang *et al.* (2016) identified temperature as a key factor influencing the seasonality of cyanobacteria, noting that *Microcystis* dominates at higher mean temperatures (20-34°C) compared to *Aphanizomenon*, which thrives at lower temperatures (13-25°C). In Oubeira Lake, *Aphanizomenon* was more abundant at temperatures below 20°C during autumn and winter. Eco-physiological studies indicate that only *A. gracile* and *A. flos-aquae* can grow at or below 10°C **(Akcaalan *et al.,* 2014)**, with *A. flos-aquae* showing a competitive advantage over *Microcystis* at temperatures below 15°C **(Wu *et al.,* 2016)**. Additionally, some species within this genus have been observed to bloom in response to conductivity levels starting from 500μS/ cm **(Cirés *et al.,* 2016)**.

According to **Srichomphu *et al.* (2024)**, the densities of *Cylindrospermopsis* are closely linked to nitrogen dynamics, with organic nitrogen, including ammonium, being assimilated and converted into inorganic nutrients. A significant correlation was found between *Cylindrospermopsis* and total nitrogen as well as ammonium levels. In Lake Oubeira, high densities of *Cylindrospermopsis* were observed during autumn when temperatures were elevated. This genus tends to thrive during dry periods with low rainfall **(Chellappa & Costa, 2003)** and high temperatures **(Bouvy *et al.,* 2006)**. The combination of dry conditions and prolonged water retention leads to higher temperatures and extended light exposure, creating favorable conditions for the dominance of *C. raciborskii* **(Bouvy *et al.,* 2000)**.

95

Seasonal Development of Cyanobacteria and Microcystin Production in a Shallow Freshwater Lake (North-Eastern, Algeria)

Various genera of cyanobacteria, including *Microcystis*, *Planktothrix*, *Aphanizomenon*, *Cylindrospermum*, *Dolichospermum* and *Pseudanabaena*, are known to produce microcystins. Indeed, the first five genera showed significant correlations with microcystin concentrations in Lake Oubeira. Microcystins (MCs), a family of cyclic hepatotoxins, present serious health risks to mammals when ingested **(Carmichael, 1994; Chorus & Bartram, 1999)**. Although most studies report total MC concentrations, few have examined the distribution of specific MC congeners in different lakes **(Monchamp *et al.*, 2014)**.

Toxins production is generally correlated positively with cell growth rates **(Briand *et al.*, 2005)**. Consequently, the significant microcystin production observed during the summer and autumn seasons can be attributed to the high densities of cyanobacteria recorded during this period. This correlation is further supported by a strong positive relationship between microcystin concentrations and chlorophyll-a levels, as well as with *Microcystis* and *Planktothrix*, and a significant correlation with *Dolichospermum*.

Monitoring of 30 shallow subtropical lakes in China revealed a significant correlation between microcystin levels and chlorophyll-a, as well as cyanobacterial biomass **(Wu *et al.*, 2006)**. Other studies indicate that microcystin concentrations are expected to rise under eutrophic conditions, particularly with elevated nitrogen levels, turbidity, and cyanobacterial biomass **(Taranu *et al.*, 2017)**. Consequently, higher biomass of suspected microcystin-producing cyanobacteria typically results in increased total microcystin content **(Van de Waal *et al.*, 2009)**.

Nitrogen and phosphorus are critical nutritional drivers for cyanobacterial growth, significantly influencing the formation of toxic blooms **(Sarkar *et al.*, 2021)**. While phosphorus (P) is often viewed as the primary factor driving cyanobacterial biomass, nitrogen (N) availability and its various forms can also impact the overall toxicity of these blooms **(Wagner *et al.*, 2021)**. In our study, we observed a strong positive correlation between total microcystins (MCs, expressed as MC-LR equivalents) and nitrogen compounds such as NO_3 and NH_4^+, along with a significant correlation between MCs and NO_2.

Researches indicate that cyanobacteria, particularly *Microcystis*, demonstrate a competitive advantage over other algal species when utilizing reduced forms of nitrogen, such as ammonium (NH4+) and urea **(McCarthy *et al.*, 2009)**. The speciation of nitrogen significantly affects cyanobacterial growth, persistence, and the production of the hepatotoxin microcystin **(Krausfeldt *et al.*, 2020)**. This suggests that the availability of different nitrogen sources can influence not only the growth dynamics of cyanobacteria but also their potential to produce harmful toxins in aquatic environments.

In many eutrophic systems, microcystin (MC) concentrations increase more frequently in response to high levels of nitrogen than phosphorus **(Davis *et al.*, 2015)**. Studies showed that high levels of inorganic nitrogen are required to synthesize nitrogen-rich microcystins, with an increase in exogenous inorganic nitrogen promoting higher

cellular quotas of these toxins in non-diazotrophic species such as *Microcystis* and *Planktothrix* **(Lee *et al.,* 2000; Van de Waal *et al.,* 2010; Harke & Gobler, 2013)**. In addition, field studies postulated that the addition of ammonium (NH^{4+}) compared to nitrate (NO^{3-}) resulted in higher MC concentrations and prolonged bloom persistence **(Donald *et al.,* 2011)**. In addition, total MC levels were negatively correlated with water transparency, reflecting cyanobacterial growth rates in the water column.

Researches elucidated that toxic cyanobacterial genotypes often possess a competitive advantage over their non-toxic counterparts, particularly in bloom events linked to toxin production. Genera such as *Microcystis*, *Oscillatoria*, *Planktothrix*, *Dolichospermum*, *Cylindrospermopsis*, and *Nostoc* are primarily associated with these harmful algal blooms **(Claudia & Jesus, 2023)**. Additionally, **Teneva *et al.* (2009)** highlight the potential threat posed by species within the genus *Pseudanabaena*, which are known to produce both neurotoxins and hepatotoxins.

CONCLUSION

Lake Oubeira exhibits high chlorophyll-a levels and low transparency, indicating a hyper-eutrophic state. Its shallow depth contributes to frequent sediment disturbance caused by wildlife and wind action, which releases nutrients into the water, significantly increasing internal nutrient loading and eutrophication. Strong correlations between lake trophic status, cyanobacterial abundance and microcystin (MC) levels were found. MC correlates positively with chlorophyll-a and several cyanobacterial genera, including *Microcystis*, *Planktothrix*, *Dolichospermum*, *Cylindrospermum* and *Pseudanabaena*. In addition, MCs were positively correlated with nitrate, ammonium and nitrite levels. The lake regularly harbors potentially toxic cyanobacteria all year round, whatever the season. To protect lake users, continuous monitoring is essential, focusing on the identification and quantification of cyanobacteria and cyanotoxins, while paying attention to seasonal variations. The current state of Lake Oubeira highlights the urgent need for effective management strategies to resolve these water quality problems and restore balance to this unique ecosystem.

REFERENCES

Alayat, H. ; El Khattabi, J. and Lamouroux, C. (2013). EVOLUTION SPATIALE DES CARACTERISTIQUES PHYSICO-CHIMIQUES DES EAUX DU LAC OUBEIRA IMPOSE PAR LES CONDITIONS SEVERES DE LA SECHERESSE (EXTREME NE ALGERIEN). European Scientific Journal, 9(36).

Akcaalan, R.; Köker, L.; Oğuz, A.; Spoof, L.; Meriluoto, J. and Albay, M. (2014). First report of cylindrospermopsin production by two cyanobacteria

97

Seasonal Development of Cyanobacteria and Microcystin Production in a Shallow Freshwater Lake (North-Eastern, Algeria)

(Dolichospermummendotae and Chrysosporumovalisporum) in Lake Iznik, Turkey. Toxins, 6(11), 3173-3186.

Aminot, A. and Chausspied, M. (1983). Manuel des analyses chimiques en milieu marin CNEXO, Brest, 395 pp.

Aminot, A. and Kérouel, R. (2004). Hydrologie des écosystèmesmarins: paramètres et analyses. Editions Quae.

Amrani, A.; Nasri, H.; Azzouz, A.; Kadi, Y. and Bouaïcha, N. (2014). Variation in cyanobacterial hepatotoxin (microcystin) content of water samples and two species of fishes collected from a shallow lake in Algeria. Archives of environmental contamination and toxicology, 66(3), pp.379-389.

Amri, S. ; Branes, Z. and Oudra, B. (2010). Inventaire des cyanobactéries potentiellement toxiques dans la tourbière du lac noir « parc national d'El-Kala » (Algérie). Revue de Microbiologie Industrielle Sanitaire et Environnementale, 4(1), pp.49-68.

Arif, S. ; Djebbari, N. ; Belhaoues, S. ; Touati, H. and Bensouilah, M. (2023). Spatio-temporal dynamics of cyanobacterial abundance and toxicity in a Mediterranean hypereutrophic lake. *Journal of Oceanology and Limnology*, *41*(5), 1834-1848.

Bouhaddada, R. ; Nélieu, S. ; Nasri, H. ; Delarue, G. and Bouaïcha, N. (2016). High diversity of microcystins in a Microcystis bloom from an Algerian lake. Environmental pollution, 216, 836-844.

Bouvy, M. ; Falcão, D. ; Marinho, M. ; Pagano, M. and Moura, A. (2000). Occurrence of Cylindrospermopsis (Cyanobacteria) in 39 Brazilian tropical reservoirs during the 1998 drought. Aquatic microbial ecology, 23(1), 13-27.

Bouvy, M.; Ba, N.; Ka, S.; Sane, S.; Pagano, M. and Arfi, R. (2006). Phytoplankton community structure and species assemblage succession in a shallow tropical lake (Lake Guiers, Senegal). Aquatic Microbial Ecology, 45(2), 147-161.

Briand, J.F. ; Jacquet, S. ; Flinois, C. ; Avois-Jacquet, C. ; Maisonnette, C. ; Leberre, B. and Humbert, J. F. (2005). Variations in the microcystin production of *Planktothrix rubescens* (Cyanobacteria) assessed from a four-year survey of Lac du Bourget (France) and from laboratory experiments. Microbial ecology, 50(3), 418-428.

Brient, L.; Lengronne, M.; Bertrand, E.; Rolland, D.; Sipel, A.; Steinmann, D.; Baudin, I.; Legeas, M.; Le Rouzic, B. and Bormans, M. (2008). Aphycocyanin probe

as a tool for monitoring cyanobacteria in freshwater bodies. Journal of Environmental Monitoring, 10, 248–255.

Carey, C.C.; Ibelings, B.W.; Hoffmann, E.P.; Hamilton, D.P. and Brookes, J.D. (2012). Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. Water research, 46(5), 1394-1407.

Carmichael, W.W. (1994). The toxins of cyanobacteria. Scientific American, 270(1), 78-86.

Carrasco, D.; Moreno, E.; Sanchis, D.; Wörmer, L.; Paniagua, T.; Del Cueto, A. and Quesada, A. (2006). Cyanobacterial abundance and microcystin occurrence in Mediterranean water reservoirs in Central Spain: microcystins in the Madrid area. European Journal of Phycology, 41(3), 281-291.

Charifi, S. ; Merad, T.M.; Guellati, F.Z.; Touati, H. and Bensouilah, M. (2019). Dynamic of Filamentous Cyanobacteria in the Dam Ain Zada (North of Algeria). Journal of Ecological Engineering, 20(5).

Chellappa, N.T. and Costa, M.A.M. (2003). Dominant and co-existing species of Cyanobacteria from a Eutrophicated reservoir of Rio Grande do Norte State, Brazil. *Acta oecologica*, *24*, S3-S10.

Chorus, I. and Bartram, J. (1999) Toxic cyanobacteria in water: A guide to their publichealth consequences, monitoring and management. London: Spon. 416 pp.

Chowdhury, R.R.; Rose, S.; Ezan, F.; Sovadinová, I.; Babica, P. and Langouët, S. (2024). Hepatotoxicity of cyanotoxin microcystin-LR in human: Insights into mechanisms of action in the 3D culture model Hepoid-HepaRG. *Environmental Pollution*, *342*, 123047.

Cirés, S. and Ballot, A. (2016). A review of the phylogeny, ecology and toxin production of bloom-forming Aphanizomenon spp. and related species within the Nostocales (cyanobacteria). Harmful Algae, 54, 21-43.

Claudia, T.L. and Jesus, O.V. (2023). Potentially Toxic Cyanobacteria in a Eutrophic Reservoir in Northern Colombia. Water, 15(20), 3696.

Codd, G.A.; J. Meriluoto and J. S. Metcalf (2017). Introduction: Cyanobacteria, cyanotoxins, their human impact, and risk management, p. 3–8. In Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis, 1st ed.; Meriluoto, J., Spoof, L., Codd, G.A., Eds.; John Wiley & sons, Ltd.: Chichester, UK, 2017

99

Seasonal Development of Cyanobacteria and Microcystin Production in a Shallow Freshwater Lake (North-Eastern, Algeria)

Davis, T.W.; Bullerjahn, G.; Tuttle, T.; McKay, R.M. and Watson, S.B. (2015). Effects of increasing nitrogen and phosphorus concentrations on phytoplankton community growth and toxicity during Planktothrix blooms in Sandusky Bay, Lake Erie. Environ. Sci. Technol. 49, 7197–7207.

Dokulil, M. T. and Teubner, K. (2000). Cyanobacterial dominance in lakes. Hydrobiologia, 438 (1-3), 1-12.

Donald, D.B.; Bogard, M.J.; Finlay, K. and Leavitt, P.R. (2011). Comparative effects of urea, ammonium, and nitrate on phytoplankton abundance, community composition, and toxicity in hypereutrophic freshwaters. Limnol. Oceanogr. 56, 2161–2175.

Du, X. ; Liu, H. ; Yuan, L. ; Wang, Y. ; Ma, Y. ; Wang, R. and Zhang, H. (2019). The diversity of cyanobacterial toxins on structural characterization, distribution and identification: A systematic review. Toxins, *11*(9), 530.

Ghai, R.; Hernandez, C.M.; Picazo, A.; Mizuno, C.M.; Ininbergs, K.; Díez, B. and Rodriguez-Valera, F. (2012). Metagenomes of Mediterranean coastal lagoons. Scientific reports, 2(1), 1-13.

Guellati, F.Z.; Touati, H.; Tambosco, K.; Quiblier, C.; Humbert, J.F. and Bensouilah, M. (2017). Unusual cohabitation and competition between Planktothrixrubescens and Microcystis sp.(cyanobacteria) in a subtropical reservoir (HammamDebagh) located in Algeria. PloS one, *12*(8), p. e0183540.

Harke, M.J.; Steffen, M. M.; Gobler, C. J.; Otten, T. G.; Wilhelm, S.W.; Wood, S. A. and Paerl, H.W. (2016). A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, Microcystis spp. HarmfulAlgae, 54, 4-20.

Harke, M.J. and Gobler, C.J. (2013). Global transcriptional responses of the toxic cyano- bacterium, Microcystis aeruginosa, to nitrogen stress, phosphorus stress, and growth on organic matter. PLOS ONE 8 (7), E69834, http://dx.doi.org/10.1371/ journal. pone. 0069834

Huang, J.; Xu, Q.; Wang, X.; Ji, H.; Quigley, E.J.; Sharbatmaleki, M. and Li, C. (2021). Effects of hydrological and climatic variables on cyanobacterial blooms in four large shallow lakes fed by the Yangtze River. Environmental Science and Ecotechnology, 5, 100069.

Humbert, J.F. and Fastner, J. (2016). Ecology of cyanobacteria. Handbook of cyanobacterial monitoring and cyanotoxin analysis, 9-18.

Husson, F. ; Josse, J. ; Le, S. ; Mazet, J. and Husson, M. F. (2020). Package 'FactoMineR'. Multivariate Exploratory Data Analysis and Data Mining. Available on CRAN: https://cran. r-proje ct. org/web/packages/Facto MineR/Facto MineR. pdf.

Kassambara, A. and Mundt, F. (2020). Factoextra: Extract and visualize the results of multivariate data analyses. v1. 0.7. CRAN. R-project. org/package= factoextra.

Kokociński, M.; Stefaniak, K.; Mankiewicz-Boczek, J.; Izydorczyk, K., and Soininen, J. (2010). The ecology of the invasive cyanobacterium Cylindrospermopsis raciborskii (Nostocales, Cyanophyta) in two hypereutrophic lakes dominated by Planktothrix agardhii (Oscillatoriales, Cyanophyta). Eur. J. Phycol., 45(4), 365-374

Komárek, J. and Anagnostidis, K. (1999). Freshwater Flora of Central Europe Vol. 19/1 Cyanoprokaryota, 1st Part : Chroococcales.

Komárek, J. (2005). Cyanoprokaryota 2. Teil/2nd part: oscillatoriales. Susswasserflora von Mitteleuropa, 19, 1-759.

Komárek, J.; Kaštovský, J.; Mareš, J. and Johansen, J.R. (2014). Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. Preslia, 86(4), 295-335.

Komárek, J. (2016). A polyphasic approach for the taxonomy of cyanobacteria: principles and applications. European Journal of Phycology, 51(3), pp.346-353.

Kosten, S.; Huszar, V.L.; Bécares, E.; Costa, L.S.; Donk, E.; Hansson, L.A.; Jeppesen, E.; Kruk, C.; Lacerot, G.; Mazzeo, N. and Meester, L. (2012). Warmer climates boost cyanobacterial dominance in shallow lakes. Global Change Biology, 18(1), pp.118-126.

Krausfeldt, L. E.; Farmer, A. T.; Castro, H. F.; Boyer, G. L.; Campagna, S. R. and Wilhelm, S.W. (2020). Nitrogen flux into metabolites and microcystins changes in response to different nitrogen sources in Microcystis aeruginosa NIES-843. Environmental microbiology, 22(6), 2419-2431.

Kulabhusan, P. K. and Campbell, K. (2024). Physio-chemical treatments for the removal of cyanotoxins from drinking water: Current challenges and future trends. Science of the Total Environment, 170078.

Kurmayer, R.; Christiansen, G.; Fastner, J. and Börner, T. (2004). Abundance of active and inactive microcystin genotypes in populations of the toxic cyanobacterium Planktothrix spp. Environmental microbiology, 6(8), 831-841.

101

Seasonal Development of Cyanobacteria and Microcystin Production in a Shallow Freshwater Lake (North-Eastern, Algeria)

Lee, S.J.; Jang, M.H.; Kim, H.S.; Yoon, B. D. and Oh, H.M. (2000). Variation on microcystin content of Microcystis aeruginosa relative to medium N:P ratio and growth stage. J. Appl. Microbiol. 89, 323–329.

Li, M.; Peng, Q. and Xiao, M. (2016). Using interval maxima regression (IMR) to determine environmental optima controlling Microcystis spp. growth in Lake Taihu. Environmental Science and Pollution Research, 23(1), 774-784.

Lürling, M.; Van Oosterhout, F. and Faassen, E. (2017). Eutrophication and warming boost cyanobacterial biomass and microcystins. Toxins, *9*(2), 64.

Mariani, M.A.; Padedda, B.M.; Kaštovský, J.; Buscarinu, P.; Sechi, N.; Virdis, T. and Lugliè, A. (2015). Effects of trophic status on microcystin production and the dominance of cyanobacteria in the phytoplankton assemblage of Mediterranean reservoirs. Scientific reports, 5, p. srep17964.

McCarthy, M.J.; James, R.T.; Chen, Y.; East, T. L. and Gardner, W. S. (2009). Nutrient ratios and phytoplankton community structure in the large, shallow, eutrophic, subtropical Lakes Okeechobee (Florida, USA) and Taihu (China). Limnology, 10, 215-227.

Monchamp, M.E.; Pick, F. R.; Beisner, B.E. and Maranger, R. (2014). Nitrogen forms influence microcystin concentration and composition via changes in cyanobacterial community structure. PloS one, 9(1), e85573.

Namsaraev, Z.; Melnikova, A.; Ivanov, V.; Komova, A. and Teslyuk, A. (2018). Cyanobacterial bloom in the world largest freshwater lake Baikal. In *IOP* Conference Series: Earth and Environmental Science (Vol. 121, p. 032039). IOP Publishing.

Nasri, A.B.; Bouaïcha, N. and Fastner, J. (2004). First report of a microcystin-containing bloom of the cyanobacteria Microcystis spp. in lake Oubeira, Eastern Algeria. Archives of environmental contamination and toxicology, 46(2), pp.197-202.

Nasri, H.; Bouaicha, N. and Harche, M.K. (2007). A new morphospecies of Microcystis sp. forming bloom in the Cheffia dam (Algeria): Seasonal variation of microcystin concentrations in raw water and their removal in a full-scale treatment plant. Environmental toxicology, 22(4), pp.347-356.

Nasri, H.; El Herry, S. and Bouaïcha, N. (2008). First reported case of turtle deaths during a toxic Microcystis spp. bloom in Lake Oubeira, Algeria. Ecotoxicology and environmental safety, 71(2), pp.535-544.

Nixdorf B.; Mischke U. and Rücker J. (2003). Phytoplankton assemblages and steady state in deep and shallow eutrophic lakes an approach to differentiate the habitat properties of Oscillatoriales. In Phytoplankton and Equilibrium Concept: The Ecology of Steady-State Assemblages (pp. 111121). Springer, Dordrecht

Noto, L.V.; Cipolla, G.; Pumo, D. and Francipane, A. (2023). Climate change in the Mediterranean Basin (Part II): a review of challenges and uncertainties in climate change modeling and impact analyses. Water Resources Management, *37*(6), 2307-2323.

Olsson, F.; Mackay, E. B.; Barker, P.; Davies, S.; Hall, R. ; Spears, B. and Jones, I. D. (2022). Can reductions in water residence time be used to disrupt seasonal stratification and control internal loading in a eutrophic monomictic lake?. *Journal of Environmental Management*, *304*, 114169.

Paerl, H.W. and Huisman, J. (2008). Blooms like it hot. *Science*, *320*(5872), 57-58.

Paerl, H.W. and Huisman, J. (2009). Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. *Environmental microbiology reports*, *1*(1), 27-37.

Paerl, H.W. and T.G. Otten. (2013). Blooms bite the hand that feeds them. Science 342: 433– 434. doi:10.1126/science.1245276

Paerl, H.W.; Gardner, W. S.; McCarthy, M.J.; Peierls, B. L. and Wilhelm, S.W. (2014). Algal blooms: noteworthy nitrogen. Science, 346(6206), 175-175.

Paerl, H. (2017). The cyanobacterial nitrogen fixation paradox in natural waters. F1000Research, 6.

Parsons, T. R. (1966). Determination of photosynthetic pigments in sea-water. A survey of methods. Monogr. Oceanogr. Method, 1, 21-31.

Potts, M. (2000). Nostoc. The Ecology of Cyanobacteria (Whitton BA & Potts M, eds).

Ramade, F. (1999). Dictionnaire de L'Ecologie. Encyclopedia Universalis, 1023.

Rastogi, R. P.; Sinha, R. P. and Incharoensakdi, A. (2014). The cyanotoxin-microcystins: current overview. Reviews in Environmental Science and Bio/Technology, 13, 215-249.

Rigosi, A.; Carey, C.C.; Ibelings, B. W. and Brookes, J.D. (2014). The interaction between climate warming and eutrophication to promote cyanobacteria is dependent on trophic state and varies among taxa. Limnology and Oceanography, *59*(1), 99-114.

103

Seasonal Development of Cyanobacteria and Microcystin Production in a Shallow Freshwater Lake (North-Eastern, Algeria)

Samraoui, B.; Segers, H.; Maas, S.; Baribwegure, D. and Dumont, H.J. (1998). Rotifera, Cladocera, Copepoda, and Ostracoda from coastal wetlands in northeast Algeria. Hydrobiologia, 386, 183-193.

Saoudi, A.; Barour, C.; Brient, L.; Ouzrout, R. and Bensouilah, M. (2015). Environmental parameters and spatio-temporal dynamics of Cyanobacteria in the reservoir of Mexa (Extreme North-East of Algeria). Adv Environ Biol, 9, pp.109-121.

Saoudi, A.; Brient, L.; Boucetta, S.; Ouzrout, R.; Bormans, M. and Bensouilah, M. (2017). Management of toxic cyanobacteria for drinking water production of Ain Zada Dam. Environmental monitoring and assessment, 189(7), 361.

Sarkar, A.; Rajarathinam, R. and Venkateshan, R.B. (2021). A comparative assessment of growth, pigment and enhanced lipid production by two toxic freshwater cyanobacteria Anabaena circinalis FSS 124 and Cylindrospermopsis raciborskii FSS 127 under various combinations of nitrogen and phosphorous inputs. Environmental Science and Pollution Research, *28*(13), 15923-15933.

Spoof, L. and Catherine, A. (2016). Appendix 3: Tables of microcystins and nodularins. Handbook of cyanobacterial monitoring and cyanotoxin analysis, 526-537.

Srichomphu, M.; Phewnil, O.; Pattamapitoon, T.; Chaichana, R.; Chunkao, K.; Wararam, W. and Maskulrath, P. (2024). Role of Cylindrospermopsis sp. in vertical nitrogen changes observed in tropical oxidation wastewater treatment ponds. Global Journal of Environmental Science and Management, 10(1), 287-300.

Taranu, Z.E.; Gregory-Eaves, I.; Steele, R.J.; Beaulieu, M. and Legendre, P. (2017). Predicting microcystin concentrations in lakes and reservoirs at a continental scale: A new framework for modelling an important health risk factor. Global Ecology and Biogeography, 26(6), 625-637.

Teneva, I.; Mladenov, R. and Dzhambazov, B. (2009). Toxic effects of extracts from Pseudanabaenagaleata (Cyanoprokaryota) in mice and cell cultures in vitro. Nat. Sci. Hum, 12, 237-243.

Touati, H. ; Guellati, F. Z. ; Arif, S. and Bensouilah, M. (2019). Cyanobacteria Dynamics in a Mediterranean Reservoir of the North East of Algeria: Vertical and Seasonal Variability. Journal of Ecological Engineering, 20, 1.

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. Ecology letters, 12(12), 1326-1335.

Van de Waal, D. B.; Ferreruela, G., Tonk, L.; Van Donk, E.; Huisman, J.; Visser, P. M. and Matthijs, H.C. (2010). Pulsed nitrogen supply induces dynamic changes in the amino acid composition and microcystin production of the harmful cyanobacterium Planktothrix agardhii. FEMS microbiology ecology, 74(2), 430-438. Ecol. 74, 430–438.

Van de Waal, D. B.; Gsell, A. S., Harris, T.; Paerl, H.W.; de Senerpont Domis, L.N. and Huisman, J. (2024). Hot summers raise public awareness of toxic cyanobacterial blooms. Water research, 249, 120817.

Vollenweider R.A. and Kerekes J. (1982). Eutrophication of Waters. Monitoring, Assessment and Control. OECD Cooperative Programme on Monitoring of Inland Waters (Eutrophication Control). EnvironmentDirectorate, OECD, Paris, 154 pp.

World Health Organization. (1994). Drinking Water Quality Guidelines: Recommendations (2nd ed). Vol. 1 World Health Organization; 202 p.

Wagner, C. and Adrian, R. (2009). Cyanobacteria dominance: quantifying the effects of climate change. Limnology and Oceanography, 54(6part2), 2460-2468.

Wagner, N.; Prater, C.; Osburn, F. ; Ipek, Y. ; Jeyasingh, P. and Scott, J. (2021). Acclimation to variable nitrogen: phosphorus ratios affects the ionome and physiology of a nitrogen fixing cyanobacteria. In AGU Fall Meeting Abstracts (Vol. 2021, pp. OS52A-02).

Wei, T. and Simko, V. (2021). An introduction to corrplot package. R Package.

Wickham, H. ; Chang, W. and Wickham, M. H. (2016). Package 'ggplot2'. Create elegant data visualisations using the grammar of graphics. Version, 2(1), 1-189.

Wu, S. K. ; Xie, P. ; Liang, G. D. ; Wang, S. B. and Liang, X. M. (2006). Relationships between microcystins and environmental parameters in 30 subtropical shallow lakes along the Yangtze River, China. Freshwater Biology, 51(12), 2309-2319.

Wu, Y.; Li, L., Zheng, L.; Dai, G.; Ma, H.; Shan, K. and Song, L. (2016). Patterns of succession between bloom-forming cyanobacteria Aphanizomenon flos-aquae and Microcystis and related environmental factors in large, shallow Dianchi Lake, China. Hydrobiologia, 765(1), 1-13.

Xiao, M.; Willis, A. and Burford, M. A. (2017). Differences in cyanobacterial strain responses to light and temperature reflect species plasticity. *Harmful Algae*, *62*, 84-93.

Xiao, M.; Li, M. and Reynolds, C. S. (2018). Colony formation in the cyanobacterium Microcystis. Biological Reviews, *93*(3), 1399-1420.

105

Seasonal Development of Cyanobacteria and Microcystin Production in a Shallow Freshwater Lake (North-Eastern, Algeria)

Zhang, M.; Zhang, Y.; Yang, Z.; Wei, L.; Yang, W.; Chen, C. and Kong, F. (2016). Spatial and seasonal shifts in bloom-forming cyanobacteria in L ake C haohu: Patterns and driving factors. Phycological research, 64(1), 44-55.