https://doi.org/10.48047/AFJBS.6.15.2024.11493-11510

AFJBS African Journal of Biological Sciences
Journal homepage: http://www.afjbs.com

Research Paper Open Access

Impact of physico-chemical and biological parameters on the cyanobacteria blooms and Microcystin production in Cheffia DAM North-Est of Algeria

TATA Tahar [1]*, ARIF Salah [2],TICHATI Lazhari [3] ;DAAS Hiba [4],ALLEM Mohcene [5], NASRI Hichem[6],BELLUCCI Stefano[7]

[1,2,3,4,5] Environment and Biodiversity research Division,Environmental Research Center,Alzon Castle,Boughazi Said Street,BP 2024,Annaba,23000,Algeria

[6] Biodiversity and Ecosystem Pollution' research laboratory, Department of Biology Chadli Ben Djedid University, El-Tarf,36000, Algeria

[1,7] INFN-Laboratori Nazionali di Frascati, Frascati,Rome, Italy

Volume 6, Issue 15, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

Published: 25 Sep 2024

doi: 10.48047/AFJBS.6.15.2024.11493-11510

Abstract

Damming eutrophication leads to cyanobacteria blooms degrading drinking water quality, posing a significant challenge to water resource management and public health. The aim of this article is to investigate the spatio-temporal variation and the influence of physico-chemical parameters on *Microcystis* blooms and microcystin content in the Cheffia DAM at El Tarf (north-east Algeria), and to define recommendations for the water quality of this DAM. A bimonthly sampling campaign was conducted from January to June. Samples were collected from five depths (0, -5, -10, -15, -20 meters). At each sampling point, two samples were taken: for physicochemical analysis and for chlorophyll-a and cyanotoxin determination. The enzyme-linked immunosorbent assay (ELISA) was used to determine *microcystin* contents.The results showed that temperature and pH were the main factors positively influencing *Microcystis* proliferation and toxin levels. Orthophosphates were the only nutrient showing a negative correlation with cell density, and Chlorophyll-a was directly correlated with *Microcystis* abundance and toxicity. In conclusion, the results highlight the influence of environmental conditions, especially temperature and pH, on the blooms of *Microcystis* populations and *microcystin* concentrations. Understanding the interactions between these factors is essential for predicting and managing harmful algal blooms in drinking water DAMS and aquatic ecosystems.

Mots clés : *Microcystis*, Microcystin, Eutrophisation, physicochemical parameters,Cheffia DAM,El Tarf Alegria.

The eutrophication of dams for drinking water supply has become a significant problem worldwide (Padedda et al., 2017). This phenomenon, characterized by excessive enrichment of nutrients, especially nitrogen and phosphorus, promotes cyanobacteria proliferation Devlin and Brodie (2023). According to (Zhang et al., 2020) and Ranjbar et al. (2022), an increase in temperature due to climate change exacerbates this issue, providing favorable conditions for the growth of cyanobacteria in freshwater bodies. Cyanobacteria, commonly known as blue-green algae, pose a significant challenge in drinking water dams because of their ability to produce cyanotoxins (Igwaran et al., 2024). Among the cyanotoxins most frequently detected in drinking water reservoirs are microcystins, anatoxins, and cylindrospermopsins, which present significant risks to public health Chorus and Welker (2021).Some cyanobacteria species, such as *Microcystis*, *Dolichospermum*, *Aphanizomenon*, *Planktothrix*, and *Oscillatoria*, are known to produce cyanotoxins and have been associated with water contamination incidents. Other species, although less often implicated, include *Gomphosphaeria*, *Coelosphaerium*, *Gloeotrichia*, *Nodularia*, and *Nostoc* (Buratti et al., 2023); Mokoena (2024).The diversity of toxigenic species underlines the complexity of managing the risks associated with cyanobacteria in aquatic ecosystems. In this regard, (Rodriguez-Mozaz et al., 2019) reported that some cyanotoxins, particularly microcystins, can resist conventional treatment processes. Therefore, advanced treatment techniques such as ozonation and activated carbon adsorption must be developed to remove cyanotoxins effectively. In addition, the importance of managing dam water levels to minimize the risk of cyanotoxin contamination has become more apparent as understanding of the ecological factors influencing cyanotoxin production in dams has developed (Weenink et al., 2021). Finally, Cyanobacteria and cyanotoxins in drinking water dams have a significant socio-economic impact due to the high monitoring, treatment, and prevention costs, emphasizing the importance of long-term preventive measures (Schürmann et al., 2024).

In Algeria, studies on cyanobacteria and their toxins have gained considerable momentum in recent years, reflecting the growing concern about the potential environmental impact of the country's water resources. Investigations into the dynamics of cyanobacteria have focused mainly on water bodies with socio-economic interests, such as dams for drinking water supplies like Mexa (Saoudi et al., 2015), Hammam Debagh (Guellati et al., 2017), Ain Zada (Saoudi et al., 2017; Samia et al., 2019), and Zit Emba (Touati et al., 2019); but also lakes such as Lac des Oiseaux (Bouhaddada et al., 2016) and Lac Oubeira (Arif et al., 2023; Belhaoues et al., 2024).

Our study was performed on water from Cheffia Dam and aims to achieve several goals. We are targeting *Microcystis*, a species with potential toxicity, to assess the temporal variation in its density and to study the spatiotemporal dynamics of microcystin within the dam and investigate the influence of physico-chemical parameters and chlorophyll-a on the proliferation of *Microcystis* and microcystin content. The Cheffia dam, located in the El Tarf region of northeastern Algeria, is a substantial hydraulic structure completed in 1965. Its height is 62 meters, and it has a 171 million cubic meters capacity, and it's used for agricultural irrigation and drinking water supply (Mammeri et al., 2022).

The Cheffia dam ecosystem is known for its biodiversity, especially as a habitat for different bird and fish species (Khanchoul et al., 2019). In addition, the Cheffia ecosystem also benefits from a relatively large catchment area and a significant water storage capacity compared to other dams in Algeria, such as the KoudiatMedouar and Beni Haroun dams, which favors a rich aquatic environment (Bouguerra et al., 2023). However, like many Algerian dams, Cheffia faces similar ecological challenges, notably siltation and eutrophication, which can impact water quality and biodiversity. On the other hand, other dams may have more extensive management programs or restoration efforts, which could lead to healthier ecosystems.

Materials and Methods

Location of the study site

The Cheffia dam is located in the El Tarf region in northeastern Algeria (Geographic coordinates: Latitude: 37.0299, Longitude: 7.25317; 37°1'48''North, 7°15'11'' East) **(Figure 1)**. Its height is 62 meters, and it has a 171 million cubic meters capacity. It is used for both agricultural irrigation and drinking water supply.

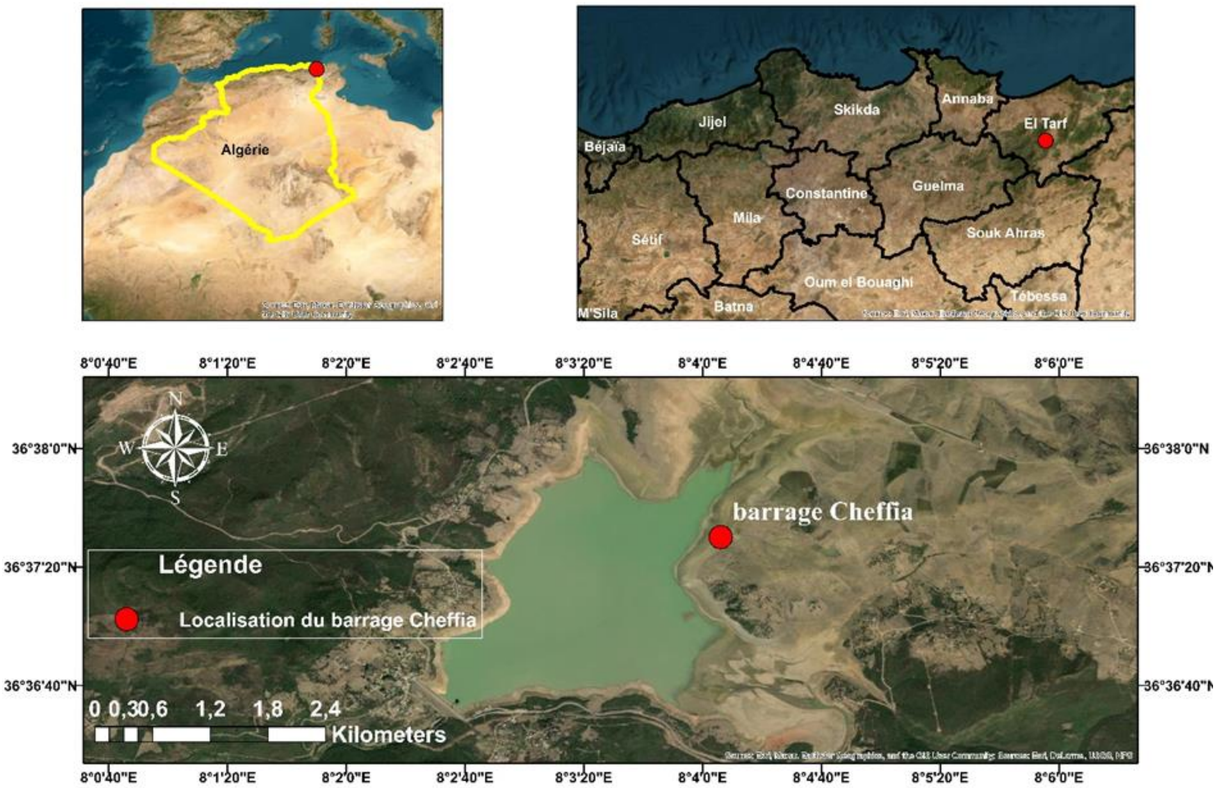

Figure 01 : location of sampling site

Ecological study equipment

A range of specialized equipment was used to carry out our ecological study. The Wildco sampler and multiparameter (WTW. Multi 340i/SET) were used for in situ measurements (T°, pH, and dissolved oxygen). For phytoplankton sampling, a plankton net of 20 µm mesh size was used. Sample collection and storage required a variety of bottles: 125-ml shaded bottles, 1L shaded glass bottles, and 1L plastic bottles. Other essential materials included aluminum foil, a cooler, syringes, beakers, slides and coverslips, and a light microscope for laboratory analysis.

Water and cyanobacteria sampling

Our sampling protocol was carried out fortnightly between January and June at the Cheffia DAM, involving sampling at five depths (0, -5, -10, -15, -20 meters). For each depth, two samples were taken: one for analysis of physico-chemical parameters and the other for extraction of chlorophyll-a and cyanotoxins. Phytoplankton was collected by filtering 50 liters of surface water through a 20 µm plankton net. The obtained filtrate (100 mL) was stored in a shaded bottle with the addition of 5% formaldehyde for preservation, except for *Microcystis* quantitative estimation. In addition, we took two one-liter samples of surface water (0 m), one for nutrient analysis and another for chlorophyll-a and cyanotoxin determination. This method enabled us to collect extensive data on the DAM's water ecosystem.

Physicochemical parameters and chlorophyll-a determination

Nitrate, nitrite, ammonium, orthophosphate, and chlorophyll-a contents were measured spectrophotometrically in the laboratory according to procedures described by the American Public Health Association et al. (1998).

Identification and enumeration of the different *Microcystis* morphotypes

Microcystis was identified using a combined microscopic examination and detailed morphological analysis. The procedure involved depositing a drop of water mounted between a microscope slide and a coverslip. The observation began at the microscope's 10 x objective for an overall view, then at the 40 x and 100 x objectives to observe the details of the cyanobacteria (cell size, filament lengths). This procedure was carried out three times for each sample. Identification was performed using the morphological keys for cyanobacteria as described by (Komárek and Anagnostidis; 2005), (Komárek al., 2014)

Microcystin content determination by enzyme-linked immunosorbent assay (ELISA)

ELISA test was used to determine microcystin content. The process begins by coating a microtiter plate with an antibody specific to the targeted cyanotoxin (microcystin), followed by washing and blocking steps to prepare the surface. Next, the sample will be analyzed, and a series of standard dilutions of the toxin will be added to the plate, allowing the cyanotoxin to bind to the immobilized antibody. After incubation and washing, an enzyme-conjugated detection antibody is introduced, binding specifically to the antibody-cyanotoxin complexes formed. Adding an enzyme substrate triggers a color reaction, the intensity of which is proportional to the amount of cyanotoxin present. The reaction is stopped after a set time, and absorbance is measured using an ELISA reader at a wavelength of 450 nm. The cyanotoxin content in a sample is then calculated by comparing its absorbance to a standard curve established with known toxin content.

Statistical analysis

Spearman's correlation was used to investigate the relationship between physico-chemical parameters, *Microcystis* density and microcystin content.

III. Results

Physico-chemical properties of Cheffia DAM waters

Water temperatures

Water temperatures at Cheffia DAM vary based on depth and time, with mean temperatures at the surface (0 m) ranging from 12.7°C in January to 25.7°C in May. More profound water experiences colder temperatures, with the lowest recorded at -20°C, with a minimum of 10.3°C in January and a maximum of 20°C in June. Analysis of variance reveals a significant difference in water temperature as a function of depth and sampling date **(Figure 2).**

Water pH

As shown in **(Figure 2)**, the water pH for all sampling depths increased between the beginning and the end of the sampling period; the lowest pH values were recorded in January, gradually increasing until May, followed by a slight decrease in June. In terms of depth, thepHof surface water has a higher value.Its value decreases steadily with depth, reaching its lowest value at -20 m. A notable exception occurred in the second sampling (01/29), where the pH increased with depth, rising from 7.38 to 7.54. Analysis of variance indicates a significant difference in pH as a function of depth and sampling period.

Dissolved oxygen

Dissolved oxygen levels decrease gradually with depth from January to June **(Figure 3)**.The lowest oxygen levels were recorded at -15 and -20 m in June (2.22 mg/L). Conversely, we recorded the highest concentrations between January and February at -20 m and during February and March at shallower depths, precisely at 0 m and -5 m. Variance analysis reveals a significant difference in dissolved oxygen levels as a function of depth and sampling period.

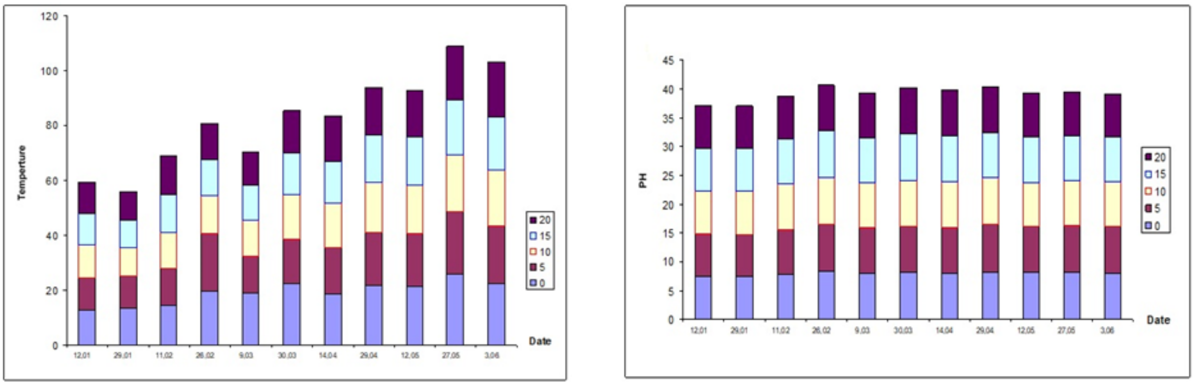

Figure 2: Temperature, pH concentrations fluctuations recorded in the Cheffia DAM between January and June.

Turbidity

The results revealed a Highly heterogeneous turbidity variation pattern in Cheffia DAM waters **(Figure 3).** Turbidity fluctuated significantly, independently of season or depth. An exceptionally high value was recorded in February (11/02) at -20 m depth. The lowest turbidities are recorded at 0 and -10 m depths, with values between 2 and 3 NTU. Variance analysis showed a significant difference in turbidity as a function of depth.

Nitrite

Based on the results presented in **(Figure 3)**, the maximum nitrite level was recorded at all depths during sampling on April 14. The maximum nitrite level was recorded at the surface (0 m) with a value of 6.16 mg/L, followed by a depth of -15 m with a value of 5.81 mg/L. The lowest nitrite levels were recorded at all depths between January and March and in May. Analysis of variance revealed a significant difference in nitrite levels in water samples depending on depth and sampling date.

Nitrate

The nitrate pattern is distinguishable from that of nitrite **(Figure 3).** Nitrate levels changed steadily with sampling period and depth. However, their levels significantly dropped on February 26 and April 14, with concentrations below 2.2 mg/L at all depths. The highest nitrate levels were observed in May at -15 m and -10 m, reaching 12.44 and 8.05 mg/L, respectively. Variance analysis revealed a significant difference in water samples' nitrate levels depending on depth and sampling date.

Ammonium

The ammonium levels in Cheffia DAM water, as presented in **(Figure 3)**, the highest ammonium levels were recorded at -15 and -10 m on March 9, reaching 6.8 and 1.6 mg/L, respectively. The highest concentration at the surface (0 m) was recorded on April 14, with 2.4 mg/L. Analysis of variance reveals a significant difference in ammonium levels depending on depth and sampling period.

Orthophosphate

Orthophosphate levels significantly varied with sampling depth and period **(Figure 3).**The highest levels were found at all depths on January 29 and at -5 m on February 26. The lowest was recorded at all depths on March 30. Analysis of variance showed a significant difference in orthophosphate levels in water samples collected at the Cheffia DAM as a function of depth and sampling period.

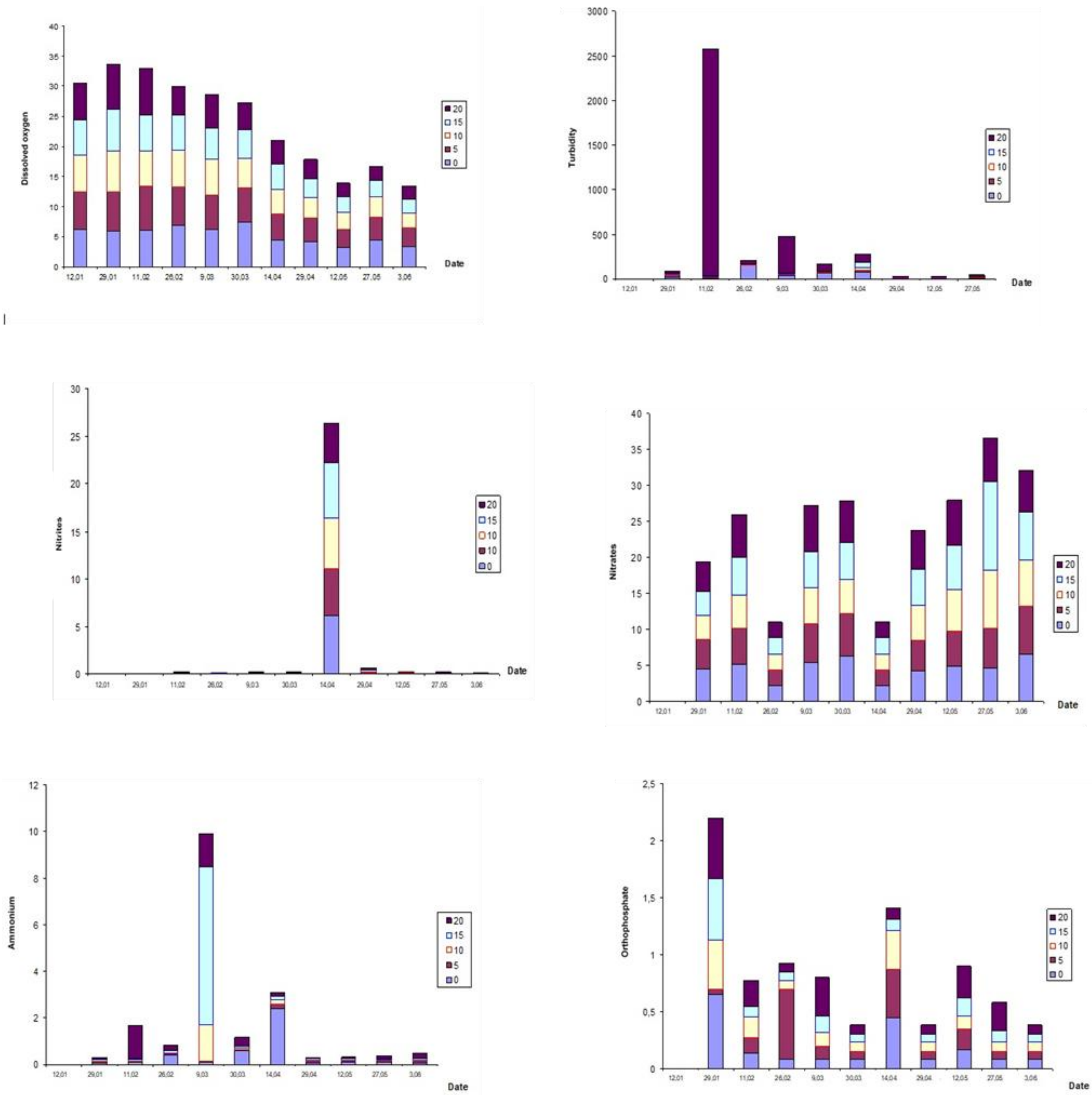

Figure 03: Nutrient concentrations fluctuations recorded in the Cheffia DAM between January and June**.**

Chlorophyll-a contents

Chlorophyll contents varied with the sampling period and depth **(Figure 4)**. The highest chlorophyll contents were recorded at -10 m during the first sampling on January 12, reaching a 23.496 mg/m³ value. However, the highest values are found at the surface (0 m) throughout the study period, with the lowest values recorded on March 9 and April 14. The influence of depth is also apparent, with relatively low chlorophyll content at -20 m, except in June, when a maximum value of 13.05 mg/m³ was found. The analysis of variance revealed a significant difference in the chlorophyll content of the water samples as a function of depth and sampling period.

Identification and enumeration of the different *Microcystis* morphotypes

~~Prevailing *Microcystis* morphotypes detected in water samples from the Cheffia DAM are distinguished by their large, irregularly contoured colonies. These colonies contain several tiny sub-colonies, each formed by cells grouped in a dense mass. The mucilage, itself colorless and very thick, clearly protrudes more than 20 µm beyond the cell cluster. The cells themselves are spherical, measuring between 3 and 4 µm in diameter, and also feature gas vesicles.~~

The dominant morphotypes of the genus Microcystis observed in environmental samples from the Cheffia dam were large colonies. These irregularly outlined colonies are composed of small subcolonies, each consisting of cells grouped in a mass. The slime is colourless, very thick and clearly exceeds the outline of the cell bundle by more than 20 µm. The cells are spherical and small (diameter 3-4 µm) and have gas vesicles. The morphological characteristics of this unusual species are very different from those described in the identification keys for previously known species.

Temporal variation in *Microcystis* density

The enumeration of *Microcystis* cells revealed that its colonies proliferation began on February 26, followed by a decrease until the first fortnight of May **(Figure 4)**. However, the most significant value was recorded on May 27, when it reached its maximum density.

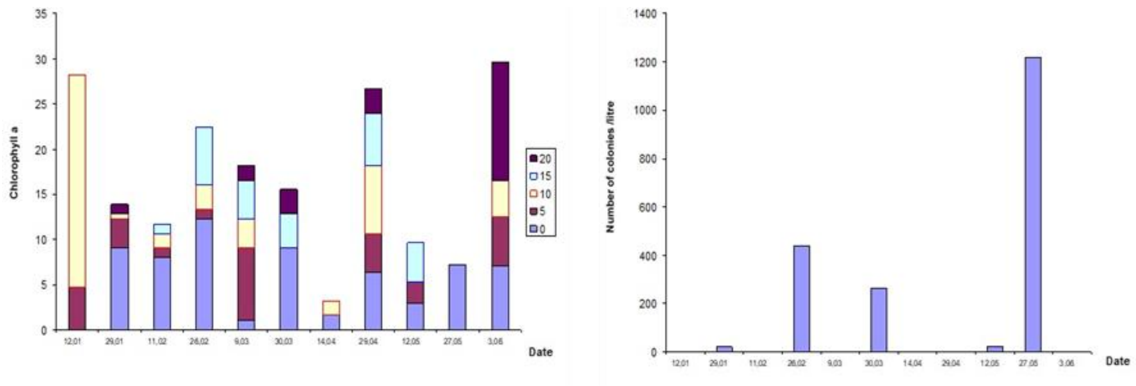

Figure 04: Changes in chlorophyll-a levels, *Microcystis* densities in the Cheffia DAM between January and June.

Temporal variation in microcystin contents

Microcystins were detected only at the surface (0 m) and solely in January, February and May **(Figure 5)**. In particular, two significant peaks in this toxin concentration were recorded on February 26 and May 27. Outside the surface, no microcystins were found at depths of -5, -10 and -15 m, while values at -20 m were very weak, almost nil.

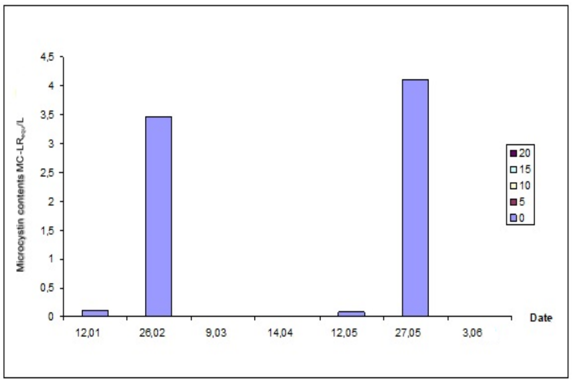

Figure 05: Microcystins contents recorded in the Cheffia DAM between January and June.

Table 1: Correlation analysis between physico-chemical parameters, chlorophyll a, *Microcystis* sp. density and Microcystin.

	Environmental parameter correlation								
	T° (°C)	**pH**	**O_2**(mg/L)	**NO_2^-** (mg/L)	**NO_3^-** (mg /L)	**NH_4^+**(mg/L)	**PO_4**(mg /L)	**Turb** **(**NTU)	**Chl-a** (mg/mg)
Microcystin	r	r	r	r	r	r	r	r	r
(µgMC-LReq/L)	0.369*	0.399*	0.164	-0.100	-0.132	-0.046	-0.171	0.132	0.460*
Density (Cell/L)	0,577*	0,426*	0,037	- 0,158	- 0,089	- 0,103	-0,321*	0,161	0,357*

T°: Temperature; O_2: Dissolved oxygen; NO_2^- : Nitrite; NO_3^- : Nitrate; NH_4^+: Ammonium; PO_4: Orthophosphates; Turb: Turbidity; Chl- a; Chlorophyll- a.

Correlation analyses revealed significant relationships between environmental parameters and the presence of the genus *Microcystis* and its associated toxin ‘microcystin (Table 1). A positive correlation was observed too between *Microcystis* density and two key factors: temperature and pH. The same positive trend was also observed for microcystin content and these same parameters, underlining the potential importance of thermal conditions and acidity levels in these cyanobacteria bloom and microcystin concentration.

Discussion

The prevalence of *Microcystis* species in aquatic ecosystems, especially in drinking water reservoirs, has recently become a significant public health and environmental management issue. Indeed, climate change and increasing eutrophication of water bodies favor *Microcystis* blooms worldwide (Padedda et al., 2017; Van de Waal et al., 2024). *Microcystis* blooms represent a severe threat to water quality. Their proliferation frequently correlates with high nutrient concentrations, higher temperatures, and increased water column stratification (Yang et al., 2023). Such conditions are expected to become widespread with climate change. In this regard, our study found a positive correlation between *Microcystis* genus density, temperature, and pH, a finding that is consistent with previous Mediterranean studies. In their study on Lake Karaoun in Lebanon, (Fadel et al., 2019) found that *Microcystis* blooms were closely associated with high temperatures and alkaline pH. Similarly, the influence of these parameters on the dynamics of cyanobacterial blooms in a Greek lake survey was emphasized by (Gkelis et al., 2017). Additionally, these findings support the suggestion that the Mediterranean climate, characterized by warm summers, promotes the bloom of the *Microcystis* (Li et al., 2016)

Positive correlations were recorded between *Microcystis* genus density, microcystin concentrations, temperature and pH parameters. These results align with those of Xiao (2020), who reported that increasing temperature promotes *Microcystis* growth and microcystin production, especially under high pH conditions. *Microcystis* blooms observed in this study could be explained by optimizing *Microcystis* metabolic processes under these conditions, as evidenced by the positive correlation between temperature and pH. In addition, the predominant influence of temperature and pH on *Microcystis* blooms and toxin production highlights the potential impact of climate change on cyanobacterial bloom dynamics. In this regard, (Visser et al., 2016) suggested that the frequency and intensity of *Microcystis* blooms will increase with global climate change. However, the interpretation of the obtained data must consider the complexity of ecological interactions since microcystin production by *Microcystis* is influenced by many environmental factors that interact in complex ways Boopathi and Ki (2014). Furthermore, (Paerl et al., 2019) and (Lu et al., 2019) suggest that controlling nutrient inputs, especially nitrogen and phosphorus, remains crucial for monitoring cyanobacterial blooms despite the lack of significant correlations in this study. On the other hand, the absence of significant correlations between *Microcystis* density or microcystin concentrations and most other physico-chemical parameters measured is an exciting finding that merits further analysis. This finding contrasts with some Mediterranean studies that found significant correlations with other factors. In this context (Arif et al., 2023), in their study in Lake Oubeira, found a significant correlation between concentrations of nitrogenous elements, temperature, and *Microcystis* proliferation. This discrepancy underlines the complexity of ecological interactions in Mediterranean aquatic ecosystems and the need for a context-specific approach to investigating cyanobacterial blooms.

The negative correlation between *Microcystis* density and orthophosphates (r = -0.321). This relationship has also been reported in another Mediterranean study (Pulina et al., 2019), which can be explained by the fast phosphate consumption by growing cyanobacteria. This finding emphasizes the importance of examining the temporal dynamics of nutrients in investigating cyanobacterial blooms.

The positive correlation between chlorophyll-a concentration and density of the genus *Microcystis* and microcystin confirms the usefulness of chlorophyll-a as an indicator of cyanobacterial biomass and toxic potential. This observation is in line with those of (Bonilla et al., 2023) in their comparative study of Spanish reservoirs, underlining the importance of chlorophyll-a as a proxying factor for estimating cyanobacterial biomass and the toxicity risk.

The health risks associated with *Microcystis* in drinking water are mainly due to their ability to produce microcystins. A meta-analysis by (Zhang et al., 2023) revealed that chronic exposure to low doses of microcystins via drinking water can induce sub-clinical liver damage and increase the risk of liver cancer over the long term.

Conclusion

These results confirm the general trends recorded in the Mediterranean region. However, they highlight certain local peculiarities and underline the influence of environmental conditions, in particular temperature and pH, on Microcystis proliferation and microcystin production. We need to monitor the other parameters (orthophosphates, ammonium, nitrates, nitrites and chlorophyll a) because they are limiting factors in eutrophication and have an impact on bloom production. Understanding the interactions between these factors is essential for predicting and monitoring harmful algal blooms in drinking water DAMS and aquatic ecosystems in general.

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