

# Short-term dynamics and growth parameters of cyanobacteria and microcystins in freshwater from the Sidi-Yacoub dam, North-east of Algeria

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**Abstract.** Boudjellab ZE, Ghannam M, Chaib N, Chekroud Z. 2023. Short-term dynamics and growth parameters of cyanobacteria and microcystins in freshwater from the Sidi-Yacoub dam, North-east of Algeria. *Biodiversitas* 24: 5598-5609. The study aimed to establish the inventory of the blue-green algae species and monitoring the monthly evolution of their specific and total cell biovolume in Sidi-Yacoub dam, located northwest of Algeria from February 2021 to January 2022. At the same time, a monthly assessment of particulate and dissolved microcystin concentrations, chlorophyll pigment concentrations, and certain physicochemical parameters has been conducted. Eight species were listed, cited in descending order of their average relative abundance are: *Microcystis aeruginosa* (54.05%); *Oscillatoria limosa* (23.68%); *Merismopedia* sp. (8.34%); *Anabaena cylindrica* (5.41%); *Aphanizomenon* sp. (2.41%); *Microcystis botrys* (2.66%); *Woronichinia* sp. (2.95%) and *Chroococcoides minuticus* (3.34%). The maximum value of the total biovolume, was recorded during December ( $27.4 \times 10^6 \mu\text{m}^3 \cdot \text{L}^{-1}$ ). The maximum concentration of chlorophyll-a and phycocyanin were respectively 55.26 and 47.46  $\mu\text{g L}^{-1}$ , recorded in September 2021. The microcystins detected were mainly in the cell-bound form, reaching a maximum concentration of 0.134  $\mu\text{g L}^{-1}$  in October 2021. The principal component analysis shows a strong positive correlation between the total monthly cell biovolume, particulate microcystin (MCP) and some physicochemical parameters. *M. aeruginosa* has been identified as the primary producer of microcystins found in the eutrophic waters of the dam, with these microcystins being predominantly maintained in intracellular form throughout most of the year.

**Keywords:** Cell biovolume, cyanobacteria, microcystins, physicochemical parameters, Sidi-Yacoub dam

## INTRODUCTION

Water pollution significantly affects human health worldwide (Al-Jarrah and Islam 2015; Bassem 2020), and continues to be the subject of several recent studies (Nexhdet et al. 2018; Boudeffa et al. 2020; Lambert et al. 2020; Rita et al. 2020; El-Kalliny et al. 2021; Haied et al. 2021; Krumova-valcheva et al. 2021; Boulguerager et al. 2022; Filho et al. 2023). Toxic algae bloom in aquatic environments such as lakes and reservoirs, constitute a real risk to public health, mainly when water bodies are used for many human activities (e.g., drinking water supply, fishing, aquaculture, aquatic leisure, etc.) (Vinçon-Leite et al. 2005; de Lima Pinheiro et al. 2023).

Cyanobacteria are among the most incriminated microalgae in the poisoning of freshwater: They include 150 genera and more than 2,000 species (Bourrelly 1985). Generally, cyanobacteria are aquatic organisms, in planktonic state, letting themselves to be transported by water movements. Other benthic species are fixed on submerged supports (e.g., periphytic organisms) (Prasertsin et al. 2021). Cyanobacteria usually have low growth rates and most prefer high temperatures and low light conditions (Whitton and Potts 2000). They are very efficient in

assimilating and storing nutrients (Wanigatunge et al. 2014; Hassan et al. 2022). They often occur in a colonial form in a mucilaginous gel matrix or filamentous with no cellular nucleus individualized by a membrane (Catherine et al. 2013), which allows them to be weakly subjected to predation by zooplankton. Thus, they can multiply massively when the environmental conditions favor them (Vinçon-Leite et al. 2005). Some species of these microorganisms can produce a diverse range of toxins called cyanotoxins. These species belong to the genera *Anabaena*, *Microcystis*, *Oscillatoria*, *Planktothrix* and *Aphanizomenon* (Ettoumi et al. 2014). These toxins constitute a hazard to human and animal health (Kurmayer et al. 2016; Mazard et al. 2016; Buratti et al. 2017; Humbert et al. 2017; Salmaso et al. 2017; Chorus and Welker 2021; Hendrayanti et al. 2023).

The hepatotoxic microcystins are the most prevalent cyanotoxins of those currently recognized. These cyclic heptapeptides have more than 80 structural variations (Umehara et al. 2017), and are strong targeted inhibitors of protein phosphatases. The most of their activities are hepatotoxic and tumor-promoting, making them one of the most dangerous categories (Li et al. 2017). They can accumulate in various aquatic organisms, and travel up the

food chain to reach organisms at higher trophic levels (Pham and Utsumi 2018).

In this regard, guidelines and recommendations from the World Health Organization (WHO) have existed since 1999 (Chorus and Bartram 1999). It must have a microcystin-LR equivalent concentration of less than  $1\mu\text{g.L}^{-1}$  for distributed drinking water. For chronic exposure, a value of  $0.1\mu\text{g.L}^{-1}$  has been proposed (Vinçon-Leite et al. 2005).

Monitoring includes determining the species present and measuring two biomass indicators, chlorophyll-a and the number of cells. $\text{mL}^{-1}$ . From these parameters, the WHO established recommendation levels for swimming water and drinking water supply (Figure 1).

In Algeria, water is threatened in its quality and quantity due to the increase of industrial, agricultural, and urbanization development, and the urbanization. This has made monitoring and managing phytoplankton growth, especially the presence of potentially harmful species in water sources used for drinking water production, increasingly significant (Amrani et al. 2014). Managing these toxic blooms is based on scientific knowledge of the environmental variables that control them (Vinçon-Leite et al. 2005).

In this context, this study focused, firstly, on the short-term dynamics of the cyanobacteria and their specific and global monthly cell biovolume in the Sidi-Yacoub dam, Algeria. Second, to study the influence of certain environmental parameters on their growth dynamics. Finally, monitor the variations in microcystin levels in the dam's raw by comparing the results obtained with the water quality threshold concentrations set by the WHO.

## MATERIALS AND METHODS

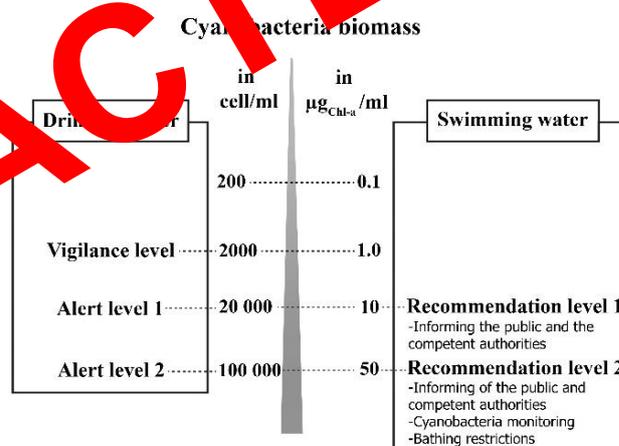
### Study area

The Sidi-Yacoub reservoir is a freshwater reservoir constructed in 1985, situated approximately 20 kilometers south of Oued Bouddag village, with the Chlef city in the north-western region of Algeria. It is built on the Ardjem wadi which feeds the city with an average annual water contribution of  $2.19 \times 10^6 \text{ m}^3$ . This wadi is a tributary of the Chlef wadi flowing into the Mediterranean Sea. The dam was commissioned in 1986 (Chenaoui and Remini 2014) (Figure 2).

This reservoir is located in a region characterized by a semi-arid climate where the average range of temperature recorded from 1975 to 2016 is from  $7.4$  to  $26.4^\circ\text{C}$ . The annual average rainfall was less than 400 mm during these last decades (ONM 2014) and an average annual relative humidity in the watershed region of the reservoir is estimated at around 62%. At the same time, the average daily insolation in the same region is estimated at 7.9 hours/day ( $4.9 \text{ hours}\cdot\text{day}^{-1}$  in December;  $11.0 \text{ hours}\cdot\text{day}^{-1}$  in July and August). This reservoir provides drinking water for the city of Chlef. It is also used to irrigate a perimeter of around 10 thousand ha (ANBT 2005). The main hydro-morphologic features of the Sidi-Yacoub reservoir are summarized in Table 1.

### Sample collection

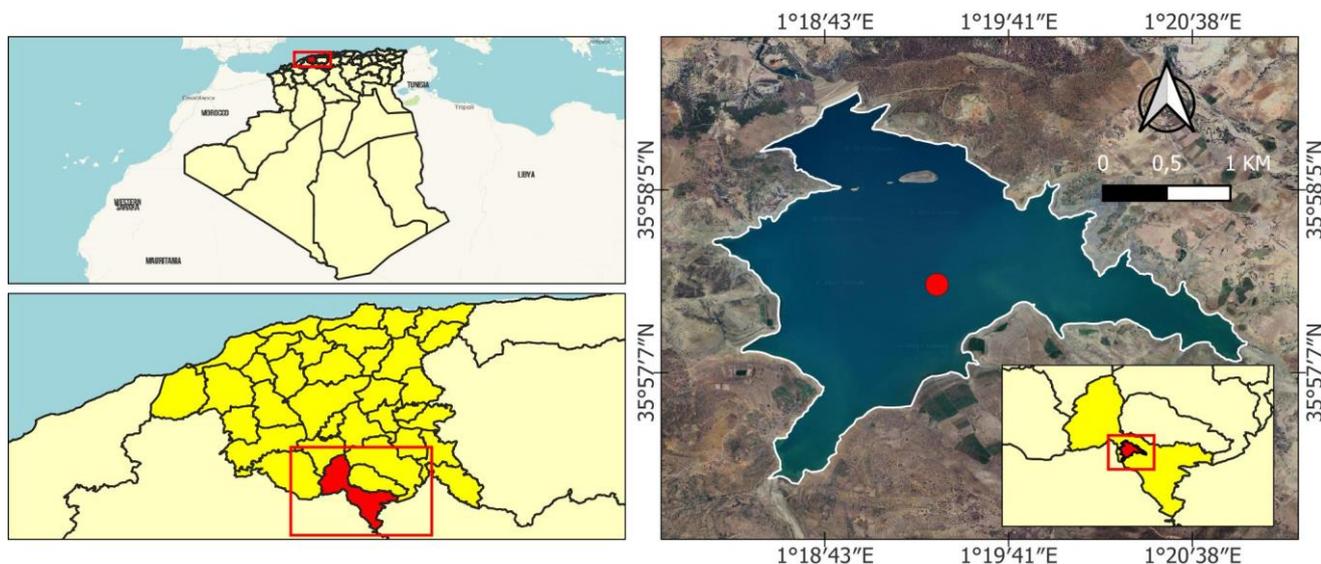
From February 2021 to January 2022, sampling was conducted monthly at the mid-point in the reservoir (Figure 2). Integrated samplers were used to collect whole water grab samples from the euphotic zone. The sampler was used to transfer water into a rinsed 5L container, and this procedure was carried out again until the container was filled. Then, subsamples were obtained from the container to study microcystin, cyanobacteria, and nutrients. A triplicate aliquot from the main integrated raw water sample was kept in 500 mL polyethylene bottles for nutrient analysis. These bottles were rinsed previously for 12 hours in a Nitric acid bath at 10% (v/v) to eliminate all traces of minerals, then completely washed with distilled water and dried. The bottles were rinsed with the reservoir water. Labeled sample bottles were maintained in a coolbox and moved the same day to the lab. Another 1000 mL subsample for phytoplankton was preserved with formaldehyde at a 5% (v/v) final concentration (Beaver et al. 2018). Immediately after collection, a 500 mL subsample for microcystin analysis was frozen. In the laboratory, all samples (except those used to determine cyanobacterial cells) were stored at a freezing temperature of  $-18^\circ\text{C}$  until subsequent analyses.



**Figure 1.** Alert thresholds defined by WHO concerning cyanobacteria biomass in freshwater (Chorus and Bartram 1999)

**Table 1.** Main characteristics of the Sidi-Yacoub reservoir, Algeria (ANBT 2005)

Characteristics	Measurements
Average altitude	245 m
Surface area	8.369 km <sup>2</sup>
Shoreline	24.98 km
Maximum length	5.75 km
Mean depth	24 m
Total capacity	285 Hm <sup>3</sup>
Dead volume	39 Hm <sup>3</sup>
Average annual siltation	0.17 Hm <sup>3</sup>



**Figure 2.** Geographical location of the Sidi-Yacoub reservoir and the sampling station: 35°57'35"N 1°19'18"E, Algeria

### Field measurements

The in situ measurements of the water temperature (Temp, °C), oxygen saturation rate (OSR, mg L<sup>-1</sup>), pH, and electrical conductivity (EC, µs cm<sup>-1</sup>) were monthly measured directly in the reservoir with a portable multi-parameter provided with probes (Multi 340i/WTW®, Germany). Secchi depth (SD, cm) Measurements were taken with a conventional Secchi disk on the boat's shadowed side, and the values were recorded. These values were multiplied by a factor of 2.5 to obtain the depth of the euphotic zone (Borowiak and Borowiak 2016).

### Laboratory analyses

#### *Determination of the nutrient's concentration in fresh water*

For monthly nutrient analyses, collected raw water samples were shaken vigorously and filtered through a GF/C filter (1.2 µm) before being analyzed for dissolved nutrients. Aliquots were taken and for nitrate (NO<sub>3</sub>-N) and nitrite (NO<sub>2</sub>-N) analysis using cadmosalicylic acid and Zambelli reaction, respectively. Orthophosphate concentrations (PO<sub>4</sub><sup>3-</sup>-P) are dosed with Ascorbic acid and total phosphorus (TP) using a spectrophotometer after digestion with persulfate. Kjeldahl nitrogen (KN) was determined using oxidative mineralization with peroxodisulfate methods. The sum of the three forms of Nitrogen concentrations (NO<sub>3</sub>, NO<sub>2</sub> and KN) allowed the calculation of total dissolved nitrogen concentration. Dry residue (DR) was quantified through the evaporation of water samples at 105°C, followed by the weighing of the residues. Magnesium (Mg<sup>2+</sup>) and calcium (Ca<sup>2+</sup>) content were assessed using a titrimetric approach with EDTA. Potassium (K<sup>+</sup>) and sodium (Na<sup>+</sup>) concentrations were measured utilizing an atomic absorption spectrophotometer with a flame (model: Sherwood-410). The sum of total iron (Fe) and ferric ions (Fe<sup>2+</sup> + Fe<sup>3+</sup>) was determined through a spectrophotometric method, involving oxidation by potassium peroxodisulfate and subsequent coloration with

1,10-phenanthroline. Chloride (Cl<sup>-</sup>) levels were assessed using the silver nitrate titration method described by Mohr. Sulfate (SO<sub>4</sub><sup>2-</sup>) concentrations were determined through the gravimetric method involving precipitation with barium salts. These methods are described in Aminot and Chaussepied (1983); Kellier et al. (2009).

#### *Determination of the microcystin concentrations in water*

The quantification of microcystin concentrations is performed in triplicate aliquots of each water sample and analyzed using the PP2A inhibition assay with a commercial ELISA kit (Enviroligix Inc., USA; lower limit detection: 0.1 µg/L) following the manufacturer's recommendations. After three freeze-thaw cycles, total microcystins (MCT) are quantified in water samples to lyse cyanobacteria cells and release toxins (Stefanelli et al. 2017). Meanwhile, the dissolved fraction of microcystins in water (MCD) is quantified on pre-filtered water samples through a 0.45µm porosity membrane. The concentration of toxins associated with cyanobacteria cells and/or adsorbed on particles (MCP) is calculated by subtracting the MCD concentration from the MCT.

#### *Determination of the Cyanobacteria pigment concentrations*

Moreover, to determine phycocyanin pigment (PhC), water samples, in three replicates were filtered one hour after being collected; the filters were then put in 20 mL of 0.05 M phosphate buffer (pH = 6.6) and then frozen for subsequent pigment determination. Phycocyanin content was extracted by 15 seconds of sonication after thawing at 9 ± 1°C in the water bath, causing cell walls to rupture. After acid digestion, samples were filtered through the Whatman GF/C filter to eliminate cellular debris from the extracts. Samples were stored at 4°C in the dark to prevent pigment degradation.

The concentration of PhC was measured by a Shimadzu UV-1601 spectrophotometer (Melco Technorex Co. LTD, Kyoto, Japan) according to equation (1) and expressed in  $\mu\text{g}\cdot\text{L}^{-1}$ :

$$\text{PhC } (\mu\text{g L}^{-1}) = (A_{615} - 0.474 * A_{652})/5.34 \text{ Eq.1 (Lorenzen 1967)}$$

Where,  $A_{615}$  is the absorbance at 615 nm and  $A_{652}$  is the absorbance at 652 nm.

In addition, concentrations of chlorophyll-a were also measured. Water samples were shaken vigorously and an aliquot of 300 mL was filtered through 0.45  $\mu\text{m}$  membrane filters (47 mm diameter, Whatman GF/CTM, Germany). Pigments were extracted in 90% aqueous acetone and measured by spectrophotometry (Shimadzu UV-1601, Japan) according to the Equation (2):

$$\text{Chl-a } (\mu\text{g L}^{-1}) = 27 \frac{v}{l \cdot v} [(A_{0665} - A_{0750}) - (A_{a665} - A_{a750})] \text{ Eq.2 (Lorenzen 1967)}$$

Where,  $A_{0\lambda}$  is the absorbances before acidification;  $A_{a\lambda}$  is the absorbances after acidification. The acidification is carried out directly in the spectrometer tank by adding a few drops of 1 N hydrochloric acid. The spectrophotometer cell is then carefully capped and stirred;  $v$ : the volume (in mL) of acetone used;  $V$ : the volume (in L) of the filtered sample and  $l$ : the length of the optical path of the measuring cell. ( $l = 1 \text{ cm}$ ) (Rodier et al. 2009).

### Quantitative and qualitative analysis of the cyanobacteria

Phytoplankton was examined under inverted light microscopes (model: Olympus® CKX31) at magnification  $\times 400$ , using Utermöhl sedimentation chamber to stain for 8 h. Phytoplankton taxa was then counted at least 40 algal units with intact structure (cells, filaments and colonies) resulting in a counting error of about 10%.

Cyanobacteria identification was performed using recognized and accepted taxonomic keys based essentially on the structure and cell dimension, colony morphology, mucilage and also on the presence of certain specific cellular structures such as akinetes, gas vacuoles and heterocyst (Borrelly 1965; Castenholz 2001; Leitão and Couté 2005).

More than 30 units for each taxon observed, its dimensions were measured ( $\mu\text{m}$ ) using a micrometric eyepiece. These dimensions are used to calculate the specific cell biovolume (SBv) using formulas specific to geometric shapes closely resembling each recorded taxon (Hillebrand et al. 1999).

The total monthly specific cell biovolume (TMSBv) for each taxon was calculated by multiplying mean specific biovolume by its cell abundance (N), according to the following equation:

$$\text{TMSBv } (\mu\text{m}^3) = \text{SBv} \cdot \text{N} \text{ Eq.3}$$

### Statistical analysis of data

A descriptive statistical analysis, allowing for the calculating of the median, the mean, and quartiles (25%, 50%, and 75%), was applied to the results of the monthly physicochemical study conducted on the reservoir water. On the other hand, a Principal Component Analysis (PCA) was applied to the entire dataset, including physicochemical parameters, TMBv, MCT, MCD, MCT, and the monthly biovolume specific to each identified species. This analysis will primarily serve to: (i) Highlight the type of correlation existing between the various parameters and their strength, and (ii) Group the months and establish periods of the year when the results of the different parameters are similar. These statistical analyses using SPSS Statistics 27.0.

## RESULTS AND DISCUSSION

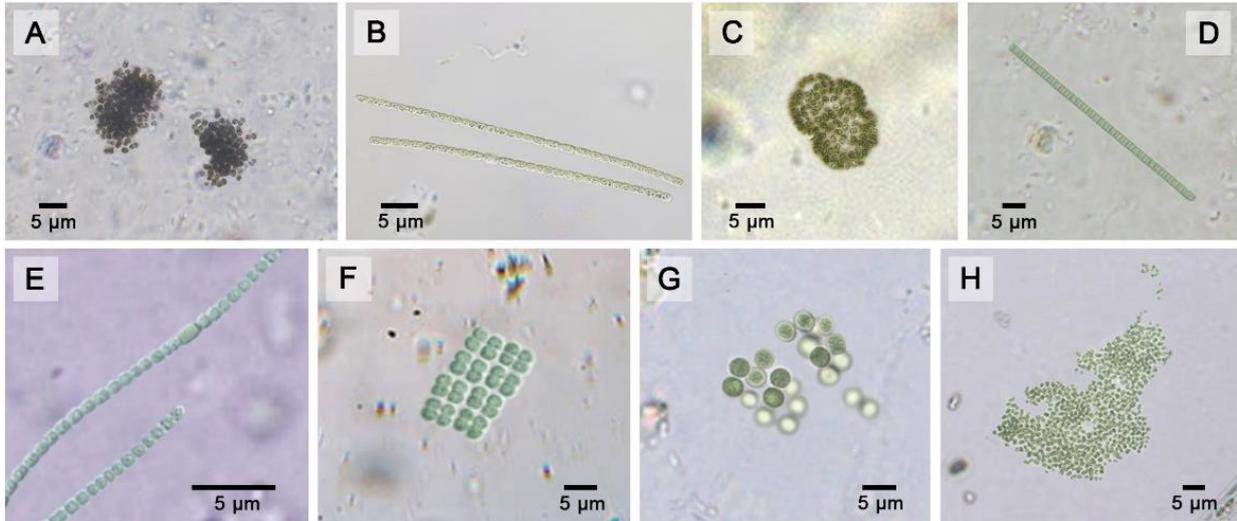
### Temporal variation of cyanobacteria cell density and biovolume

The monthly microscopic water analysis of the Sidi-Yacoub dam showed the presence of 8 species of cyanobacteria (Figure 3) which were omnipresent throughout the study period (Figure 4a). These species are (accompanied by their relative average abundance - RAA): *Microcystis aeruginosa* (Kütz.) Fricz (54%); *Oscillatoria limosa* C. A. G. (23.7%) and *Merismopedia* sp. (8.5%). Other species were identified such as: *Anabaena cylindrica* Lemmermann; *Aphanizomenon* sp.; *Microcystis botrys* (Leitz.) Grunow; *Woronichinia* sp. and *Chroococcus limneticus* Lemmermann, had an RAA not exceeding 3% as shown in Figure 4a. This same figure indicates that the overall cell density of cyanobacteria takes increasingly high values from September to reach a maximum of  $81.75 \times 10^3 \text{ Ind}\cdot\text{L}^{-1}$  in November. Outside this period the cell density did not exceed  $33.14 \times 10^3 \text{ Ind}\cdot\text{L}^{-1}$ .

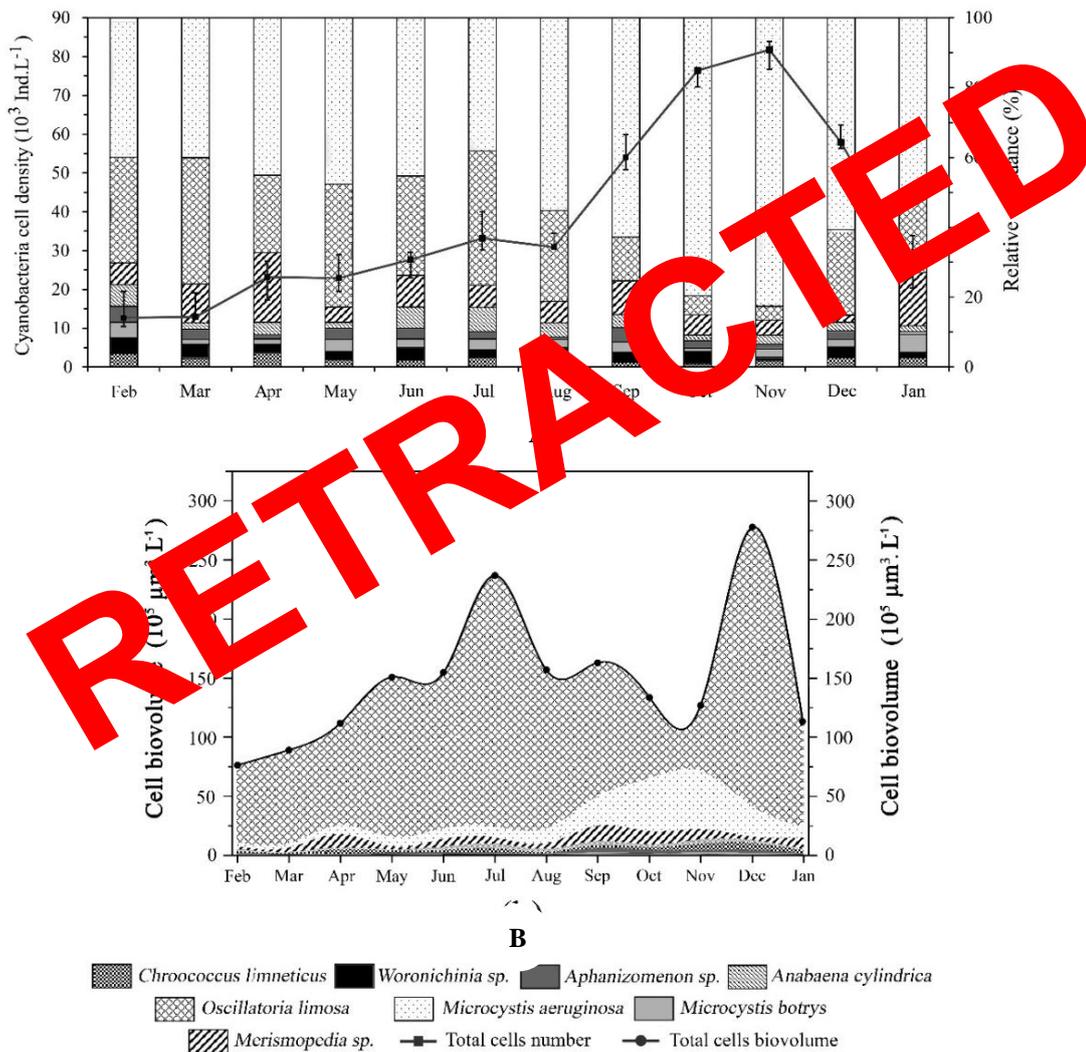
The calculation results of the average specific cell biovolume for each species (SBv) are represented in Figure 5. In contrast, the variation of the total monthly cell biovolume (TMBv), as well as its composition by the TMSBv is represented in Figure 4b from which, we can distinguish the presence of two total cell biovolume peaks, coinciding with July and December ( $23.43 \times 10^6$  and  $27.4 \times 10^6 \mu\text{m}^3\cdot\text{L}^{-1}$ , respectively), with a majority contribution of *Oscillatoria limosa* species in the composition of the TMBv.

### Temporal variation of cyanobacteria pigment concentrations

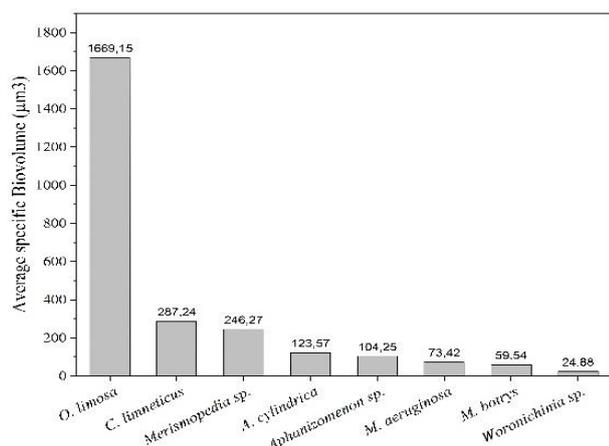
The photometric analysis of phytoplankton pigments in the waters of Sidi-Yacoub Dam reveals that the concentrations of chlorophyll-a vary between 13.65 and  $55.26 \mu\text{g}\cdot\text{L}^{-1}$  (Figure 6). These two limit values are recorded in February and September, respectively. Meanwhile, phycocyanin reached its maximum value of  $47.46 \mu\text{g}\cdot\text{L}^{-1}$  in October, exhibiting a monthly evolution with a similar pattern to that observed for chlorophyll-a (the linear correlation coefficient of Pearson calculated between the two pigments,  $r = 0.802$  with  $p\text{-value} = 0.00167 < 0.05$ ), indicating the significant contribution of cyanobacteria to the composition of the overall phytoplankton community in the dam's water.



**Figure 3.** Photomicrographs of cyanobacteria species found in the waters of the Sidi-Yacoub dam, Algeria: A. *Microcystis botrys*; B. *Aphanizomenon* sp.; C. *Woronichinia* sp; D. *Oscillatoria limosa*; E. *Anabaena cylindrica*; F. *Merismopedia* sp; G. *Chroococcus limneticus*; H. *Microcystis aeruginosa*



**Figure 4.** A. Monthly Cell density variation and the relative abundance of Cyanobacteria species; B. the variation of the total cell biovolume and its composition by the total specific cell biovolume in Sidi-Yacoub dam freshwater, Algeria



**Figure 5.** Specific cell biovolume (SBv) of cyanobacteria species inventoried in the waters of the Sidi-Yakoub dam, Algeria

Regarding the standards applied to pigments described by Chorus and Bartram (1999) (Figure 1), the values obtained for it exceed level 1 of alert and even the second level in August, September, and October. That involves applying the second level of recommendations: informing the public and the competent authorities about the need to monitor cyanobacteria and restrict swimming regularly.

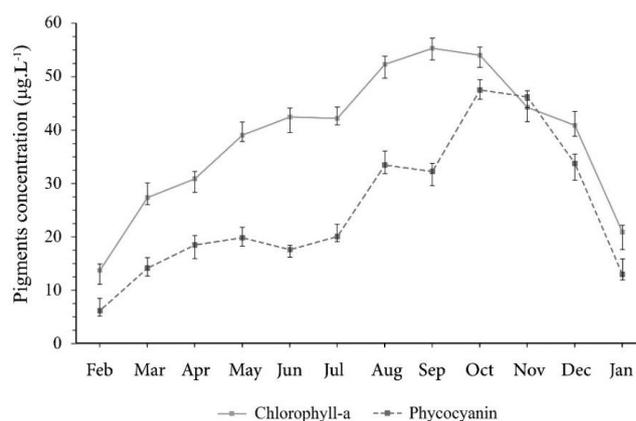
#### Monthly variation of freshwater physicochemical parameters

The results of the monthly physicochemical analysis of the Sidi-Yacoub dam water are represented in a boxplot graph (Figure 7), which displays the maximum and minimum value of each studied parameter, accompanied by the month corresponding to these values, quartile 25, quartile 75, the average and the average values. Compared with the threshold values obtained by Carlson and Simpson (1996), the average values obtained for TP, TN, and SD are 75.92 mg.L<sup>-1</sup>; 11.61 mg.L<sup>-1</sup> and 1.32 m, respectively, indicating an eutrophic state. On another side, the values obtained for the ratio TN/TP (greater than 35) show a limitation of the microalgae growth in particular cyanobacteria by the phosphorus element.

Concerning the aeration in water, very high oxygen saturation rates are recorded during periods of low water temperature in the first months of the year, even reaching 91.6% in February. However, these values drop rapidly in the hot season, reaching a minimum of 40.8% saturation in November. The other studied parameters relating to the water's overall cationic and anionic mineralization, record characteristic values of surface waters, and their seasonal evolution and relations will be discussed later by the principal component analysis (PCA).

#### Monthly fluctuation in microcystin freshwater concentrations

The monthly determination of particulate (intracellular) and dissolved (extracellular) microcystins concentrations (MCP and MCD respectively) in the water samples was carried out by the PP2A inhibition essay. The results obtained are represented in Figure 8, which shows a peak



**Figure 6.** Monthly variation of chlorophyll-a and phycocyanin concentration

in total microcystins level (MCT) recorded in October with a concentration of 0.134 µg.L<sup>-1</sup>. After October, this concentration does not stop decreasing in an almost linear way until reaching its lowest level in January (0.336 µg.L<sup>-1</sup>), while during the period extending from April to September the levels of MCT do not know a significant variation, varying around a value of 0.0685 ± 0.006 µg.L<sup>-1</sup>.

Concerning the composition of MCT over 10 months, it is mainly composed of cell-bound microcystin (MCP) (Figure 8) reaching a maximum MCP/MCD ratio of 8 in July; except in March when the total MCT is shared almost equally between MCP and MCD. The opposite of this situation is observed in February when the proportions are reversed, making the MCD represent the majority fraction found in the dam water, with a rate of 84.56% of the total MCT.

#### Relationship between environmental and biotic parameters

The relationships between physicochemical and biotic parameters were studied using principal component analysis (PCA). This multivariate analysis produced two main axes (factors), F1 and F2, explaining 50.69% and 17.43% of the variation between the different parameters (Figure 9).

The results obtained from this analysis showed that almost the total monthly cell biovolume (TMB<sub>v</sub>) representing the sum of biovolumes calculated for each identified species per month, was positively correlated with axis 1 and MCP.

These two parameters refer to a period between September and November (Figure 9) characterized by relatively high values of the parameters: Temp, TN, TP, NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup> and SD. These parameters are known, precisely, to be stimulants of cyanobacteria growth, and therefore, of other parameters having a direct link, such as TMB<sub>v</sub> and MCP.

The other physicochemical parameters (Ca<sup>2+</sup>, Fe, K<sup>+</sup>, Na<sup>+</sup>, SO<sub>4</sub><sup>2-</sup>, KN, DR and NO<sub>2</sub><sup>-</sup>) showed an increase in concentration, around December and are negatively correlated with the growth of the studied microalgae.

Two other periods of the year are distinctly visible in Figure 9, the first, including January, February, March, and April, representing the year where the dissolved oxygen saturation rate (OSR) shows the highest values which also applies to the concentrations of MCD. This can be explained by the fact that at this period of the year, the cyanobacteria cells release their cellular content of

microcystin, which will therefore end up in a dissolved form in water. This cessation of cyanobacteria growth and cell lysis is caused by a limitation in the nutrients required for their growth. In addition, the lowest values are recorded for the environmental and biotic parameters from May to August.



Figure 7. Boxplot graph of the monthly physicochemical parameter analysis of the Sidi-Yacoub dam freshwater, Algeria

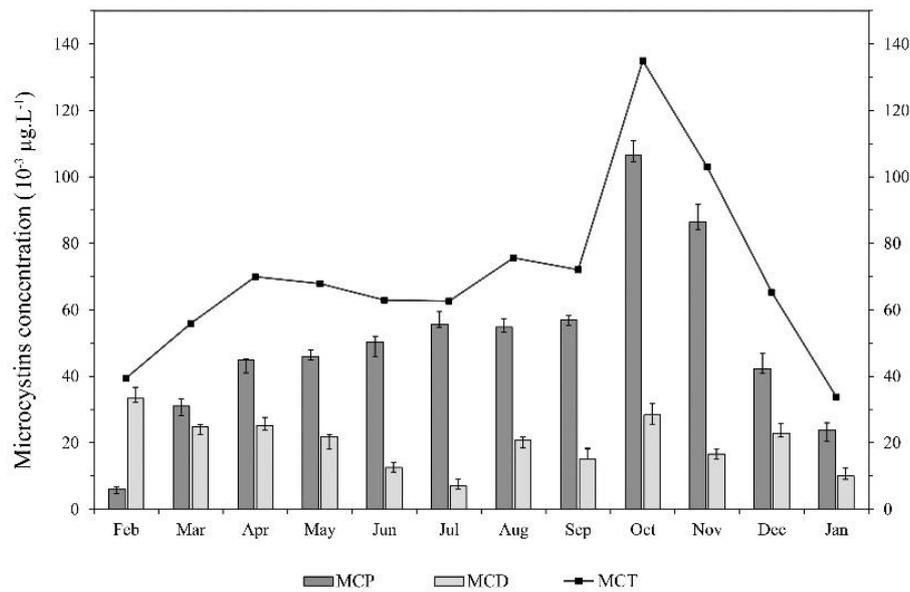


Figure 8. Monthly fluctuation in microcystin concentrations in Sidi-Yacoub freshwater dam, Algeria

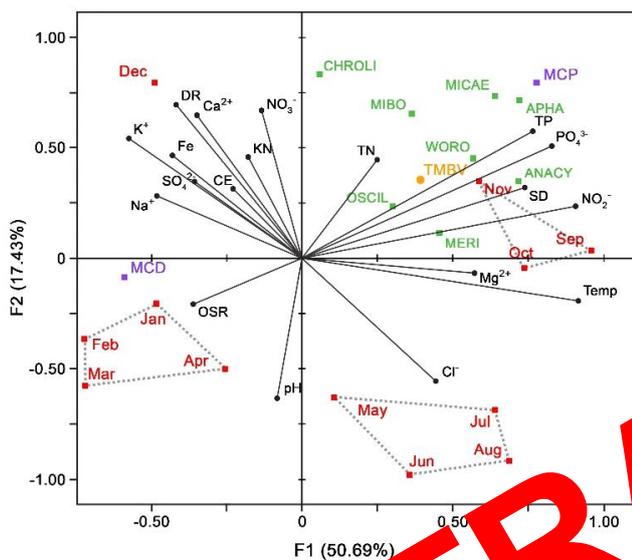


Figure 9. Principal component analysis (PCA) plot illustrating the distribution of two forms of microcystins (in purple) concerning the monthly specific cell biovolume (in green), the monthly total cell biovolume (in yellow), and the physical and chemical parameters (in black) in the waters of Sidi-Yacoub dam

## Discussion

Concerning the sampling point in the middle part of the reservoir, our results indicated that the most abundant species were *M. aeruginosa* and *O. limosa* (54 and 23.7% respectively). Throughout November, *M. aeruginosa* was the most predominant (82.64%) of all microalgae inventoried during our survey. This result is congruent with other studies on numerous Algerian freshwater reservoirs (e.g., Nasri et al. 2007; Guellati et al. 2017; Touati et al. 2019).

It should be noted that extended periods of stable water thermal layering give an advantage for *Microcystis* spp. and some others cyanobacterial species with floating ability due to the presence of gas-vacuol. This genus can move vertically by controlling its buoyancy in the water column (Medrano et al. 2013). This up-and-down migration is linked to changes in colony buoyancy caused by carbohydrate content, this gives to this group the advantage of migrating to the optimal depth within the water column. This enables them to capture solar radiation from the surface water during the day and absorb an adequate amount of nutrients in the lower layer at night, this is due to the variations in colony buoyancy driven by carbohydrate content (Dokuz and Teubner 2000).

The vertical migration capacity of *Microcystis* spp. may explain the difference in cell concentration between the two observed species in the studied dam: *M. aeruginosa* and *Oscillatoria limosa*. Another factor favoring the quantitative dominance of the species *M. aeruginosa* is its increased capacity to absorb nutrients. In a similar study conducted in fish farms in Brazil, *M. aeruginosa* was found to be a constant species, showing the highest cell density among cyanobacteria species, in addition, it bloomed the most, thus generating a high ecotoxicological risks (de Lima Pinheiro et al. 2023).

In fact, according to Kromkamp et al. (1989), *Microcystis* quickly outnumbered *Oscillatoria* sp. in competition tests with a significant input of phosphorus at saturating concentrations due to the assimilation of this nutrient and its more effective usage by the organism.

The monthly calculation of the total specific biovolume (TSBv) showed a strong contribution of *O. limosa* in the composition of the total monthly biovolume (TMBv) even exceeding the total biovolume calculated for the species *M. aeruginosa*; this due, mainly, to the relatively high SBv of *O. limosa* (1669.15 µm<sup>3</sup>) compared to other species, including that of the species *M. aeruginosa* (73,42 µm<sup>3</sup>). It

should be noted that in October and November, the composition of the TMBv is mainly shared between the TMSBv of the species *M. aeruginosa* and *O. limosa*.

According to alert levels defined by WHO, the global biomass of cyanobacteria recorded in the studied waters of Sidi-Yacoub dam has reached only the first level of alert ( $\leq 10^5$  Ind.mL<sup>-1</sup>); this implies the first level of recommendations, which states: "inform the public and competent authorities on the subject of cyanobacteria proliferation in the studied water reservoir".

Wasmund (1997) reported that a massive development of cyanobacteria is marked, coinciding with water temperature values ranging between 15°C and 30°C, with alkaline pH (between 7.5 and 11), an average nitrate (up to 3.805 mg.L<sup>-1</sup>) and orthophosphates (up to 0.0124 mg.L<sup>-1</sup>) concentrations. All these conditions were available in the water of our studied dam. Regarding the pH value, it varied between 7.5 and 7.65, this agreed with the results obtained in several water bodies in Algeria including lakes and dams (Kahoul and Touhami 2014; Draredja et al. 2019; Chabaca et al. 2020; Heramza et al. 2021). It should be noted that the sensitivity of cyanobacteria species and the phytoplankton in general, is well known (Jose and Xavier 2022); therefore are regarded as indicators of changes in the water composition of lakes (Jiang et al. 2014; Poot-Delgado and Okolodkov 2016; Suda et al. 2016) and reflect the trophic state of water bodies (Liu et al. 2014; Taş 2016; Mangoni et al. 2017; Wasmund 2017; de Lima Pinheiro et al. 2023).

The dosage of nutrients was carried out on the upper layer of the water dam; this layer is continually mixed with the runoff recently spilled in the dam. These water courses are loaded with nutrients due to the leaching of the watershed characterized by dense vegetation. The second origin of the nutrients found in the superior layers of the water, come from substances accumulated at the bottom of the dam (sediments); these substances reach the upper layer of the water, in particular the euphotic area, by mechanical movement and mixing of water, this considerably stimulates cyanobacteria growth. These spatial and temporal dynamics of hydrological and physico-chemical parameters cause a heterogeneous distribution of phytoplankton communities in the water body (de Lima Pinheiro et al. 2023).

The recorded values of chlorophyll-a over 20 µg.L<sup>-1</sup> throughout the study period are closely correlated with phycocyanin concentrations, with recorded values of water transparency (below 2 meters throughout the study period). These water parameters indicate, according to Carlson and Simpson (1976) an eutrophic state of water dam with a tendency for cyanobacteria species to dominate the water environment, particularly the genus *Microcystis*, which recorded the highest biomass among all the species of cyanobacteria observed in the Sidi Yakoub dam. The many human activities applied to the waters of the Sidi-Yakoub dam has a negative impact on its quality, exerts a notable influence on the progression of phytoplankton communities (Setyono and Himawan 2018; Namsaraev et al. 2020; Mashkova et al. 2021) and can even cause eutrophication, a natural phenomenon accelerated by

human activity, impacts numerous aquatic ecosystems worldwide and is recognized as a global concern (Ansari and Gill 2014; Ismest'eva et al. 2015; Viaroli et al. 2015; Gao et al. 2020; Pratiwi et al. 2020; Kostryukova et al. 2021), leading to increase in production of phytoplankton biomass (Kostryukova et al. 2018) and its community structure (Schuster et al. 2015; Sharma et al. 2016; Suda et al. 2016; Li et al. 2018; Pratiwi et al. 2018; Song et al. 2019; Wang et al. 2020; Yang et al. 2020; Manamani and Bensouilah 2023), oxygen depletion, reduced light penetration and extensive deterioration of water quality (Rao et al. 2018).

The dominance of cyanophyte species agrees with results obtained by other researchers from reservoirs located in Algeria and a neighboring country with close climatic conditions as in Morocco. Among these climatic conditions is the temperature which is considered the most important determinant of cyanobacteria growth and metabolism of microcystins (Lv et al. 2014; Xue et al. 2016; Pham et al. 2017; Preece et al. 2017; Huang et al. 2022).

Many other studies have demonstrated that it is probably due to a combination of environmental conditions, including nutrient enrichment (Oliver and Ganf 2000), an inorganic carbon limitation (Klemer et al. 1996), a lack of rain with lower turbulence, and a zooplankton grazing (Sharpley et al. 2013).

In our case the maximum cell biovolume per liter of water dam was recorded in December ( $27.4 \times 10^6 \mu\text{m}^3 \cdot \text{L}^{-1}$ ), knowing that the major part of the microcystins is intracellular as shown in Figure 8 and that during the entire study period, this should imply theoretically, maximum values of MCT to be recorded in December. However, the results obtained show that the maximum value of MCT was observed in October and a little less in November, the same period when the species *M. aeruginosa* recorded its maximum biovolume, this suggests that the only or the major responsible for the production of cyanotoxins in the studied dam waters is the species *M. aeruginosa*.

The peak of total microcystins occurs in the months when the highest phycocyanin concentrations are recorded. While the maximum concentrations of chlorophyll-a are recorded outside the period of the maximum MCT, this could be explained that pigment is specific to phytoplankton species in general, while phycocyanin is specific to cyanophyte species. This phytoplankton group contain a wide range of toxigenic strains.

It should be noted that it is important to investigate the identification of phytoplankton beyond the species level, to determine the type of strain incriminated in toxinogenesis, because several studies report that some *M. aeruginosa* strains are far more toxic than others, often by a factor of more than 10 (Bolch et al. 1997).

In our study, the microcystins assay was performed only on samples across the entire water column representing the euphotic zone (Average from 0 to 3.29 m depth). However, Agha et al. (2014) reported that cyanotoxin biosynthesis is mainly located in surface water. Conversely, microcystins and Cyanobacteria cells are usually maintained on the top layer of water (less than 1 m);

this concerns the cell-bound toxins. But these molecules can have other forms: Toxins that are dissolved in water, accumulated in organisms, deposited in sediment, or combined with suspended particles (Song et al. 2015).

During our study period, the intracellular form of microcystins was superior to the dissolved form, only in February when the opposite was observed. This can be explained by the fact that senescence and lysis at the end of blooms cause the intracellular toxins to be liberated into the surrounding water body (extracellular), which causes an increase in the concentration of MCD in the water column (Zhang et al. 2009; de Lima Pinheiro et al. 2023).

Multiple variables, including environmental conditions, dilution, photodegradation, adsorption to solid particles, including living organisms, and biodegradation, influence the ambient concentration of MCD. However, after cell lysis, microcystins could be mixed through the entire depth of the body of water (Song et al. 2015).

In the end, at the cellular level, the species *M. aeruginosa* turned out to be the most abundant. In contrast, in terms of cellular biovolume, the species *O. limosa* records the highest levels in the eutrophic waters of the Sidi-Yakoub reservoir. The dynamics of cyanobacteria do not differ from other reservoirs in Algeria and neighboring Mediterranean countries with similar climatic conditions. This dynamic has led to the conclusion that *M. aeruginosa* is the only or the major responsible species for producing cyanotoxins in the studied dam water. These microcystins reach their highest levels in October followed by November, in the intracellular form and predominantly transform into the dissolved form around February.

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