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Biomonitoring through determination of cyanobacterial genera and assessment of toxicity risks in fresh water: case of Bougous reservoir (Eastern Algeria)

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ABSTRACT

The Bougous dam was built as an option for managing the organoleptic and sanitary quality degradation problems of another dam called Mexa during the harmful algae blooms (HAB) period. The latter is connected to the drinking water production station. Water discharge from Bougous into Mexa allows the strong musty odors, earthy taste and cyanotoxin concentrations released from the waters of Mexa to dilute. The overall objective of this study is the biomonitoring of Bougous waters through: (i) identification and count of the cyanobacterial populations hosted in its waters, (ii) determination of its alert levels according to the WHO recommendations, and (iii) determination of global algal biomass through chlorophyll 'a' dosage and hence evaluation of the trophic state of the dam. For this, Bougous dam was sampled monthly from January to August 2018 in six different stations (S1, S2, S3a, S3b, S3c, S4). Cyanobacteria identification was based on microscopic observation of the morphological characters. Determination of cell densities was done using "Nageotte cell". Microscopic examination results revealed the presence of eight cyanobacterial genera belonging to five orders represented by *Microcystis aeruginosa,* Microcystis novacekii, *Chroococcus limneticus*, *Dolichospermum planctonicum* (*Anabaena*), *Planktothrix isothrix*, *Oscillatoria limosa*, *Spirulina platensis*, *Pseudanabaena limnetica*, *Merismopedia minutissima* and *Limnothrix mirabilis*.Five genera among them are recognized as potentially toxic cyanobacteria. The calculations of the average density showed the dominance of the genus *Microcystis* and that the highest density (6,041 cells/mL) was registered in the station S3b in July. This allowed the classification of the dam in the alert level 1 only in July and August in stations S3b and S2, respectively. The maximum content of Chl 'a' was 13, 22 µg/L recorded in July. Bougous was classified as mesotrophic all along our study period and eutrophic in April and July. In conclusion, the quality of Bougous is satisfying but the presence of potentially toxic cyanobacterial genera can pose health risks for Bougous water users. Therefore, it is necessary to establish a monitoring program for this reservoir as well as for all the other dams used for drinking water production in order to control any risk.

Keywords: Cyanobacteria; Monitoring; Trophic state; Toxicity risks; Bougous dam

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1. Introduction

Currently, drinking water production and water resources preservation are the challenges of the 21st century [1] because of the actual industrial development, agricultural, urban activities, population increase and economic growth. All of these activities require an increasing demand for water [2,3]. Global warming and the accentuated eutrophication of ecosystems have strongly contributed to the increase in the frequency of harmful algae blooms (HAB) in reservoir waters intended for drinking water production all over the world [4]. The production of HAB participates in the modification of the phytoplankton biomass in these reservoirs; hence the need to monitor and assess the trophic state of these water bodies has increased. There are different methods for the evaluation of the trophic state among them the chlorophyll 'a' dosage is used. In recent years, several independent studies have been carried out, noting the importance of Chl 'a' in the classification of the trophic state of water reservoirs [5–7]. Reported that the concept of trophic status monitoring is used in monitoring programs for cyanobacteria in reservoirs intended for the production of drinking water, the HAB represent a serious threat to the public and the environmental health following the release of several cyanotoxins acting on different target organs (liver and nervous system) [8–11]. Many cases of poisoning, intoxication [12] and also animal and human deaths linked to HAB have been recorded worldwide [13–18].

In addition, the high cyanobacterial biomass has often been associated with the green coloration of the water, which has always generated heavy economic losses such as: the organoleptic degradation of drinking water following the production of secondary metabolites (geosmin, 2-methylisoborneol, β-cyclocitral), filters clogging in drinking water treatment plants, excess organic matter and an increased activated carbon bills [19,20]. These disturbances of the organoleptic quality in reservoirs are observed much more during summer when the water level in the reservoirs decreases following the phenomenon of evaporation. Bakker and Hilt [21] explored a less-known management option to reduce cyanobacterial biomass in reservoirs water just by increasing the water levels in them, provided that the incoming water of the contaminated reservoir is in good quality. In Algeria, especially in the North-Eastern region, several studies have confirmed the presence of HAB in reservoirs intended for the drinking water production [22–30].

Bougous dam is a new water body, built in the same region (North-Eastern Algeria) to compensate for the reduction in drinking and industrial water supply capacity of another dam located downstream of Bougous called Mexa. Since only Mexa is connected to the station of drinking water treatment, Bougous was built with the aim of diluting the water of Mexa by a direct water release in order to reduce the musty odors and the earthy taste as well as the heavy loads of cyanotoxins released from the waters of Mexa during the HAB period. As a result, Mexa managers no longer order the stoppage of the water distribution, which has often caused heavy economic losses and dissatisfaction of the consumer.

The goal of this study is the biomonitoring of raw water from Bougous dam through: first of all, the identification and counting of cyanobacteria; second, the determination of alert levels according to the World Health Organization (WHO) recommendations and third, the determination of the global algal biomass through the chlorophyll 'a' (Chl 'a') dosage and the evaluation of the trophic state of the dam.

2. Materials and methods

2.1. Study area and sample collection

Bougous dam is located in the North-Eastern Algeria under a sub-humid climate 20 km east of the Province of El Tarf (36°41′57.97″N/8°25′15.14″E), 6 km upstream of Mexa dam and was constructed on the Oued Bougous (Fig. 1). The construction of this dam was carried out over the period from 2005 to 2010; thus, the first water charge was in February 2010. This water body occupies a surface of 2.26 km² and a height of 71.4 m. The total volume of Bougous reservoir is 66 million/m³ and the nominal water level is about 139 m [31].

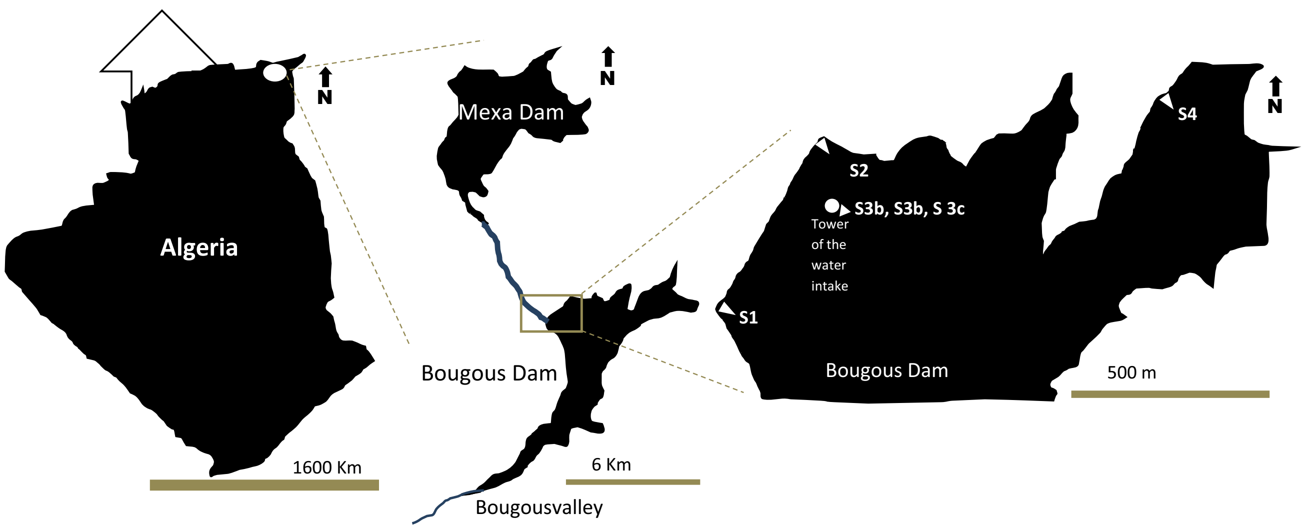

Fig. 1. Geographic localization of Bougous dam area with the position of the sampling stations.

Bougous dam is not connected directly with the water treatment station, it discharges by direct release in the Mexa dam and the latter is the one connected to the treatment station. Bougous dam is used to complete the deficit of Mexa to provide drinking water.

In this study, a monthly follow-up was performed for the first time in this important water body from January to August 2018. Water samples were taken from six different stations (S1, S2, S3a, S3b, S3c and S4) based on the accessibility and the direction of the prevailing winds. Samples S1, S2, S3a and S4 were collected from the sub-surface stations. While samples S3b and S3c were collected from different depths (Fig. 1) according to the protocol by Treyture et al. [32]. The detailed description of characteristics of these sampling stations is explained in Table 1.

2.2. Study of cyanobacteria

Water samples collected for the inventory analyses were examined with an optical microscope (Carl Zeiss model) equipped with a digital uEye32 camera; thus, cyanobacteria identification was done based on the observation of the morphological and the anatomical characters defined based on the literature [33–36]. The quantification of cyanobacteria cells was carried out in a Nageotte chamber according to the Brient et al.'s [37] protocol, where 100 mL of each sample was filtered through a polycarbonate filter. The filter surface was rinsed to collect all the individuals with 1 mL of the same sample. 50 μL of this sub-sample was injected into the Nageotte cell, which presents a grill of 40 bands. The number of bands retained for the calculation of cell densities is that on which there are 40 individuals. The density results were expressed in "cells/mL".

The determination of alert levels is done with reference to the WHO decision standards relating to Alert Levels Frameworks for drinking water [38].

2.3. Trophic state evaluation

The chlorophyll 'a' concentrations provides a strong indication of eutrophication status. This parameter is also a good indicator of the overall algal biomass, so it gives information about the primary production (all phytoplanktons including cyanobacteria) in an aquatic ecosystem [33]. In our study, the Chl 'a' assay was performed for the six sampling stations (S1, S2, S3a, S3b, S3c and S4) using the SCOR/UNESCO trichromatic method described by Aminot and Kerouel [39] based on the extraction and dissolution of the chlorophyll pigments after filtration on a Whatman GF/C filter in 90% acetone. The reading is done at three different wavelengths (663; 645; 630 nm) and the 750 nm wave is for the turbidity control of the test. The Chl 'a' concentration was determined from the average of the results recorded in the six stations and was expressed in μg/L. Evaluation of the trophic state of the Bougous dam during our study period is based on the standards established by Galvez-Cloutier et al. [40], where it defines four classes of trophic state: oligotrophic if [Chla] μg/L < 2.5 μg/L; mesotrophic: 2.5 > [Chla] μg/L < 8 μg/L; eutrophic: 8 > [Chla] μg/L <25 μg/L; hyper-eutrophic: [Chla] μg/L > 25 μg/L.

3. Results and discussion

3.1. Diversity of cyanobacteria

Microscopic examination results revealed the presence of eight cyanobacterial genera belonging to five orders: Chroococcales represented by *Microcystis aeruginosa* (Kützing, 1846), *Microcystis novacekii* (Compère, 1974) and *Chroococcus limneticus* (Lemmermann, 1898), Nostocales represented by the genus *Dolichospermum planctonicum* (*Anabaena*) (Wacklin et al. 2009), Oscillatoriales [*Planktothrix isothrix* (Komarek and Komarokova, 2004) and *Oscillatoria limosa* (Agardh and Gomont, 1892)], Synechococcales [*Pseudanabaena limnetica* (Komárek, 1974), *Merismopedia minutissima* (Lemmermann, 1898), and *Limnothrix mirabilis* (Anagostidis, 2001)] and Spirulinales represented by *Spirulina platensis* (Geitler, 1925) (Tables 2 and 3). According to the literature, five of the eight genera found are recognized as potentially toxic [38], In general, three different forms of cyanobacteria were identified, filamentous forms (*D. planctonicum*, *Planktothrix isothrix*, *O. limosa*, *S. platensis*, *Pseudanabaena limnetica* and *L. mirabilis*) were present during the whole period, while colonial forms (*Microcystis aeruginosa* and *Merismopedia minutissima*) and unicellular form (*C. limneticus*) were present in the period from June to August.

Two orders, Chroococcales and Synechococcales were present during the period from June to July. Nostocales were present in July and August. Meanwhile, Oscillatoriales were present in January, April and July but the order Spirulinales was present only in July. The months of February, March and May were exempt of cyanobacteria.

The results are similar to those reported by Saoudi et al. [22], where they identified the same genera with the

Table 1
Characteristics of the six sampling stations in Bougous dam

Station	Coordinates	Depth	Characteristics
S1	36°42′3.18″N 8°24′56.75E″	Sub-surface	Exposed to the wind with a rocky nature
S2	36°42′18.76″N 8°25′7.34″E	Sub-surface	Sheltered from the wind with a rocky nature
S3a	36°42′12.70″N 8°25′8.39″E	Sub-surface	Exposed to the wind and, in the center of the dam, near to the tower of the water intake
S3b	36°42′12.70″N 8°25′8.39″E	3 m deep	Wind does interfere when it is higher than 30 km/h
S3c	36°42′12.70″N 8°25′8.39″E	6 m deep	Wind does interfere when it is higher than 30 km/h
S4	36°42′22.52″N 8°25′44.06″E	Sub-surface	Exposed to the wind with a clay nature

Table 2
Cyanobacteria taxa identified in Bougous reservoir during the study period

Taxa
Planktothrix isothrix (Komarek et Komarokova, 2004)
Oscillatoria limosa (Agardh et Gomont, 1892)
Merismopedia minutissima (Lemmermann, 1898)
Pseudanabaena limnetica (Komárek, 1974)
Chroococcus limneticus (Lemmermann, 1898)
Microcystis novacekii (Compère, 1974)
Microcystis aeruginosa (Kützing, 1846)
Spirulina platensis (Geitler, 1925)
Limnothrix mirabilis (Anagostidis, 2001)
Dolichospermum planctonicum (Anabaena) (Wacklin et al. 2009)

exception of the genus *Dolichospermum* (*Anabaena*), which was detected for the first time in this area of the Oued Kebir watershed (upstream part of the Mafrag catchment area). The presence of a nitrogen-fixing type testifies the low concentration of this element in the waters of Bougous [41].

The dominance of the order Chroococcales reflects a stable dynamism of the water column of Bougous in summer unlike the Oscillatoriales, which resists turbulence [41–43]. While the presence of Synechococcales, Nostocales and Spirulinales was always associated with a lower water level and high temperatures [44,45].

The five potentially toxic genera identified in Bougous (*Microcystis* sp., *D. planctonicum*, *Planktothrix isothrix*, *O. limosa* and *Pseudanabaena limnetica*) have always been associated with the presence of cyanotoxins in the world [46–50] and in Algeria [22,23,26–28,51].

The highest diversity was recorded in the stations located on the surface (S1, S2, S4) during the months of July and August. In station S1, the following genera were identified *Microcystis aeruginosa*, *Microcystis novacekii*, *C. limneticus*, *Merismopedia minutissima*, *Pseudanabaena limnetica*, *D. planctonicum* and *S. platensis*). In station S2, the identified genera were *Planktothrix*, *Microcystis aeruginosa*, *C. limneticus*, *Pseudanabaena limnetica*, *D. planctonicum* and *S. platensis* while station S4 hosted the genera *Planktothrix isothrix*, *C. limneticus*, *Merismopedia minutissima*, *Pseudanabaena limnetica*, *D. planctonicum* and *L. Mirabilis*. Whereas the lowest revealed diversity was recorded in the station S3c also in July and August (Fig. 2).

The majority of the genera that were identified in this study were reported by other authors in the Mediterranean basin (Spain, Italy, France, etc.) [16]. In the literature review, in Italia, the presence of four genera similar to the one identified in this study was reported [16]: *Microcystis*, *Planktothrix*, *Anabaena* and *Oscillatoria*. Also Mariani et al. [50] in their study on four reservoirs in Northern Sardinia (Italy) highlighted the remarkable seasonal variations of the cyanobacterial community with the presence of some toxic genera, which is in perfect agreement with our results. Our results are also in agreement with what was identified in the Spanish reservoirs where Hurtado et al. [52] noted the presence of *Merismopedia* and *Planktothrix*. Comparing our results with those of Vieira-Lanero et al.'s [53], it was noticed that *Dolichospermum* (*Anabaena*) was more dominant in the Spanish reservoirs (38.49%) than *Microcystis* (34.13%) contrary to our observation in Bougous, where it was *Microcystis aeruginosa*, which dominated.

3.2. Cyanobacteria density

From the quantitative point of view, the monthly variation of the censused orders in Bougous showed the dominance of the order Chrococcales with 2,013 cells/mL (88%) in July followed by the order Nostocales with 141 cells/mL (7%). The Oscillatoriales came in third position with 79 cells/mL (3%) and finally it was found that the Synechococcales and Spirulinales with 35 cells/mL (2%) (Fig. 3).

The average distribution of the identified cyanobacterial genera (Fig. 4) illustrates the clear dominance of the genus *Microcystis aeruginosa* with 314 cells/mL followed by the genus *D. planctonicum* with 18 cells/mL, the genus *Planktothrix isothrix* with 11 cells/mL, *Pseudanabaena limnetica* with 5 cells/mL, *S. platensis* with 4 cells/mL, *C. limneticus* and *Merismopedia minutissima* with 2 cells/mL and finally *L. mirabilis* with 1 cells/mL.

The spatio-temporal variation of all the cyanobacteria identified in Bougous (Fig. 5) shows a fluctuation in the distribution of cyanobacteria from one station to another and from one month to another, the results are in perfect agreement with those of Mariani et al. [50]. The most populated station was S3b (6,061 cells/mL) located at – 3 m followed by, S2 (2,967 cells/mL), station S1 (695 cells/mL), station S4 (222 cells/mL), station S3c at – 6 m (139 cells/mL) and lastly, station S3a (sub-surface) with 61 cells/mL.

According to Fig. 5, the highest density was detected in the months of July and August, while the month of June harbored the lowest density. However, the months of February, March and May were exempt of cyanobacteria.

In reference to the alert levels, standards accepted by the WHO (vigilance level: 100–1.000 cells/mL; alert level 1: 1.000–10.000 cells/mL; alert level 2: 10.000–100.000 cells/mL) for the supply of drinking water [38], the densities noted in this water body correspond to those required for the level of vigilance from January to June 2018. Alert level 1 was reached only in July and August. Alert level 2 was not reached during our study period (January–August 2018).

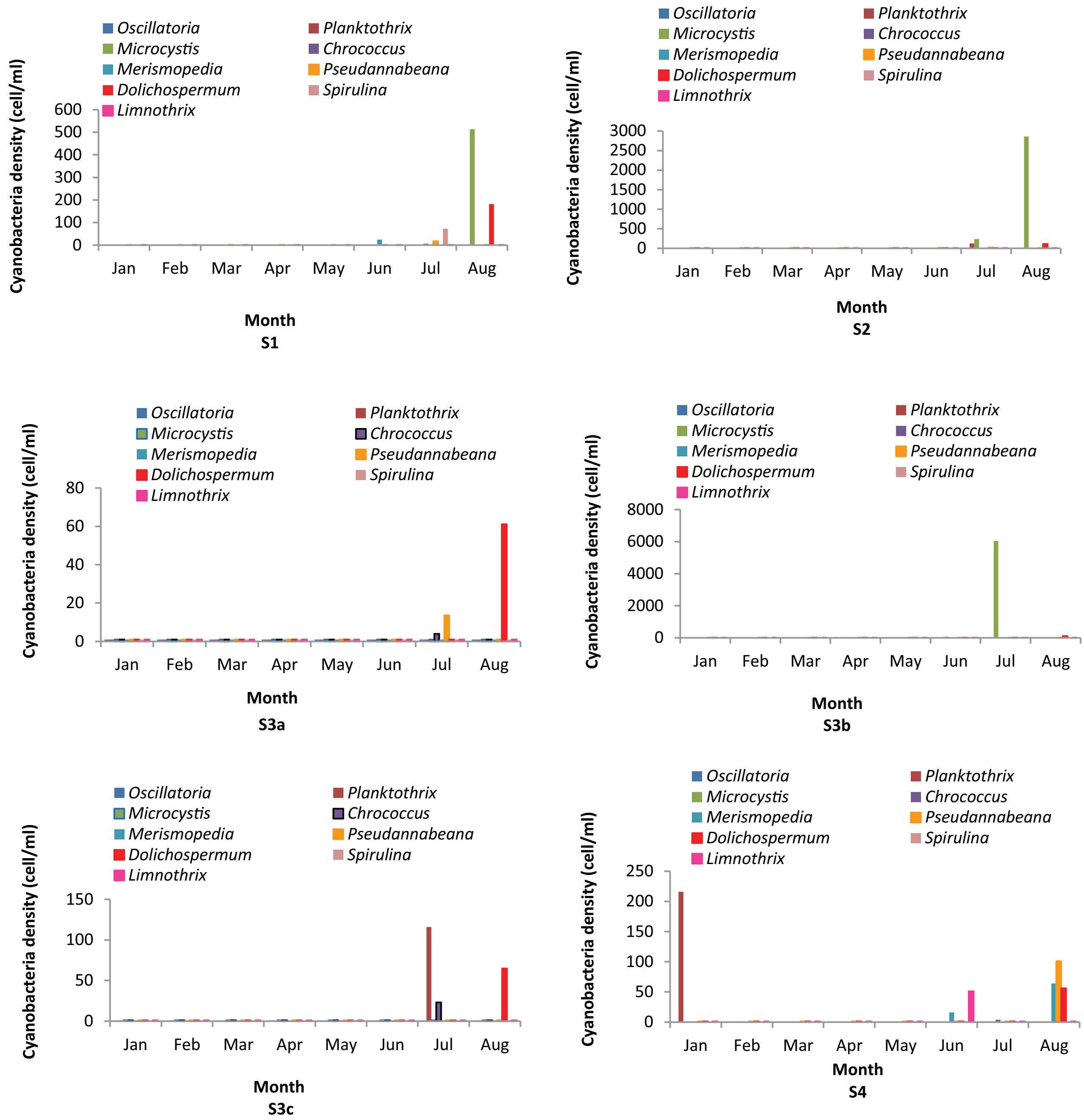

Fig. 2. Spatio-temporal diversity distribution of cyanobacteria in Bougous reservoir 2018.

The results generated are different from those found in Mexa, where Saoudi et al. [22] reported that the level of vigilance exceeded from January until June. The alert level 1 was reached from July to September and the alert level 2 was reached in October. This comparison allows us to conclude that Bougous quality is much better than that of Mexa, so, Bougous waters can be used to dilute (by direct releases) the waters of Mexa during the HAB period [21]. Also the comparison of the results with those of Boufligha et al. [27] carried out on the Guenitra dam located in the same climatic stage (sub-humid) as Bougous confirmed that Bougous was in better quality because Boufligha et al. [27] reported that the level of vigilance was reached in January, alert level 1 from February to December and alert level 2 in November.

Most of the listed genera in Bougous (*Microcystis aeruginosa*, *M. novacekii*, *C. limneticus*, *O. limosa*, *Planktothrix isothrix*, *Merismopedia minutissima*, *Pseudanabaena limnetica*, *L. mirabilis*, *D. planctonicum* and *S. platensis*) are planktonic with gas vesicles, which gives them an advantage over other cyanobacteria, which do not have them. These vesicles allow the cyanobacteria to control their buoyancy in order to have access to the nutrients (N, P, Fe, etc.) from the sediments, and they can store them [54–56]. This

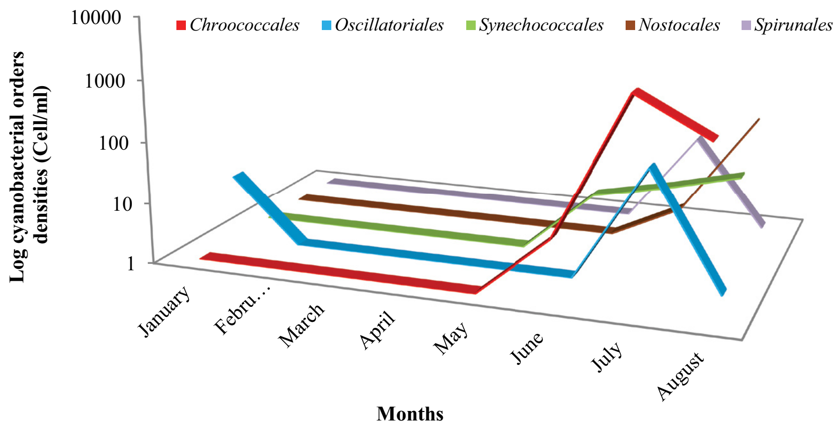

Fig. 3. Distribution of density of cyanobacteria orders during the study period (Bougous dam 2018).

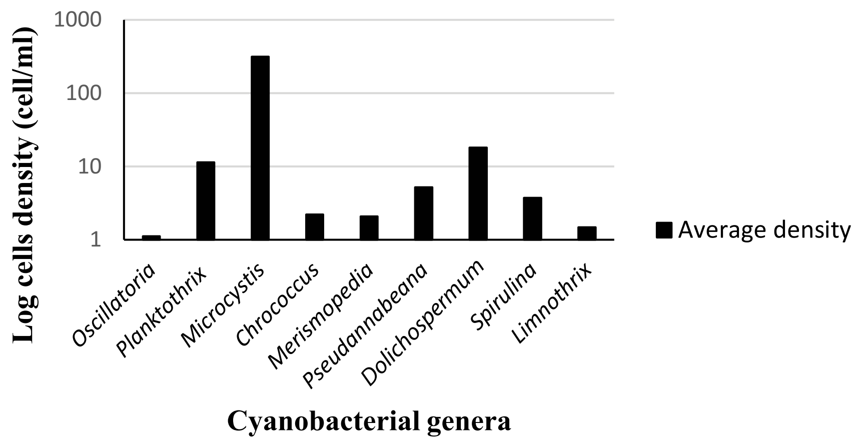

Fig. 4. Distribution of average density of cyanobacteria genera during the study period (Bougous dam 2018).

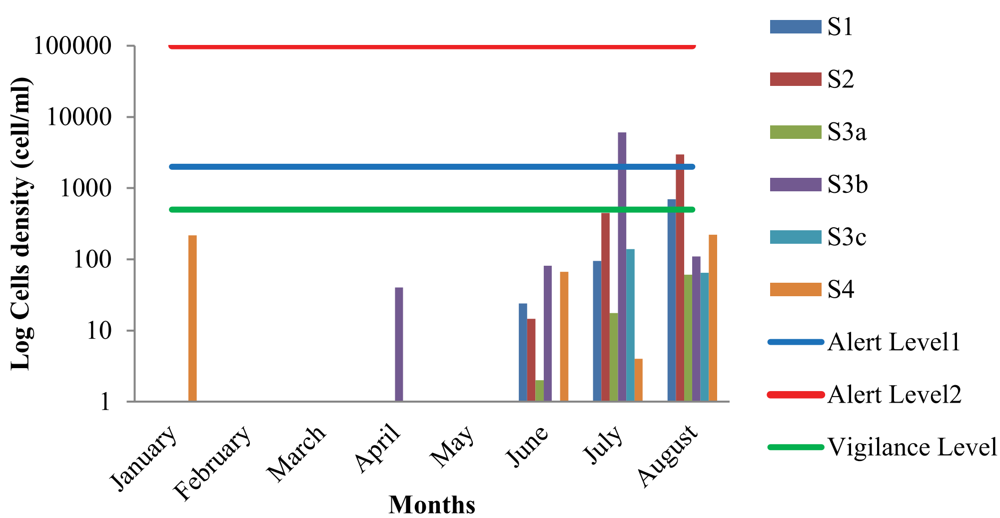

Fig. 5. Spatio-temporal variation of cyanobacteria in Bougous dam during the study period.

interpretation explains the high densities recorded in the S3b station located at –3 m depth, hence the interest of making vertical profiles in a biomonitoring program of a water body. Similar results were reported by authors [22,51,57] who worked on the same study area.

The dominance of *Microcystis aeruginosa* in Bougous waters may be due to environmental factors favorable for the development of this genus as well as to the form of this cyanobacterium, which is characterized by colonies of large cell volumes and a thick mucilaginous sheath, which gives *Microcystis* a great selective power compared with other cyanobacterial genera. This sheath allows *Microcystis* individuals to stick and attach to each other by forming a shield against predators and/or hydrodynamic conditions (wind, precipitation, water currents) [58,59]. The results are in agreement with the majority of works carried out on drinking water production dams in the Mediterranean basin and also in Algeria, which reported the dominance of the genus *Microcystis* [22,24,25,27,28,57,60–63].

Cyanobacteria counting results during the year 2018 confirmed their presence in Bougous waters and that this distribution was seasonal with peaks in the summer period (Fig. 5), these high densities can be explained by the sensitivity of cyanobacteria to environmental variations, in particular to the temperature, which can affect the nutrient dynamics, which provides a favorable situation for the cyanobacterial taxa that are capable of regulating their buoyancy such as *Microcystis*. The stability of the water column also plays a role in the distribution of cyanobacteria in a water body [64–67]. The results are in agreement with several works that have dealt with the influence of environmental parameters on the distribution and the dominance of cyanobacteria in public reservoirs [64,67–70].

3.3. Evaluation of the trophic state

The average content of the Chl 'a' value recorded showed a monthly variation. It oscillated from 1 to 13.22 µg/L with a peak of 13.22 µg/L in July (Fig. 6)

The comparison of cell densities and the chlorophyll 'a' contents (Fig. 6) shows that there is a concordance between these two parameters only in July. This can be explained by the fact that chlorophyll 'a' is an indicator of global algal biomass, so the content recorded in the absence of cyanobacteria (from January to June) may be due to other phytoplanktonic classes such as Chlorophyceae. In addition,

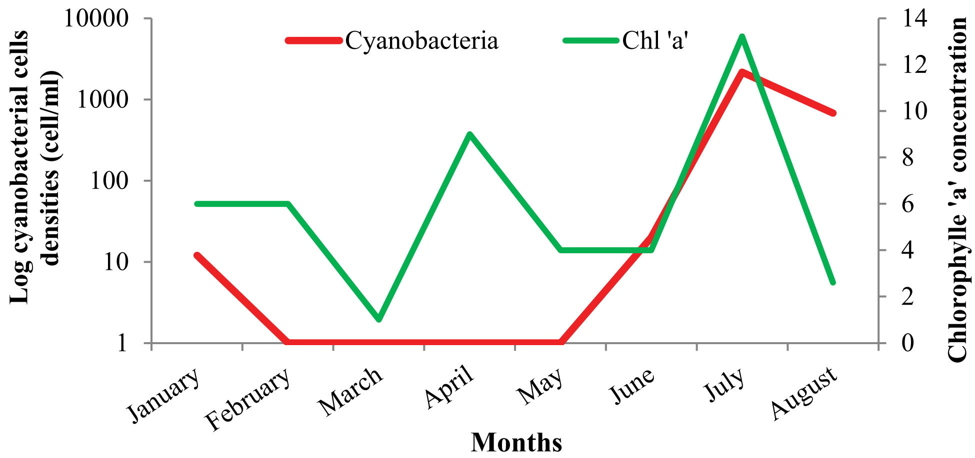

Fig. 6. Cyanobacterial densities variation with the chlorophyll 'a' content during the study period (Bougous dam 2018).

Table 3
Monthly distribution of cyanobacteria

Cyanobacteria orders	Cyanobacteria genus	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
Chroococcales	*Microcystis aeruginosa* *Microcystis novacekii*	–	–	–	–	–	+	+	–
	Chroococcus limneticus	–	–	–	–	–	+	+	–
Oscillatoriales	*Oscillatoria limosa*	–	–	–	+	–	–	–	–
	Planktothrix isothrix	+	–	–	–	–	–	+	–
Synechococcales	*Merismopedia minutissima*	–	–	–	–	–	+	+	–
	Pseudanabaena limnetica	–	–	–	–	–	+	+	+
	Limnothrix mirabilis	–	–	–	–	–	+	–	–
Nostocales	*Dolichospermum planctonicum*	–	–	–	–	–	–	+	+
Spirulinales	*Spirulina platensis*	–	–	–	–	–	–	+	–

Table 4
Trophic status of Bougous dam during the study period

Months	Chl 'a' (μg/L)	TS
January	6	Mesotrophic
February	6	Mesotrophic
March	1	Oligotrophic
April	9	Eutrophic
May	4	Mesotrophic
June	4	Mesotrophic
July	13.22	Eutrophic
August	2.6	Mesotrophic

TS: Trophic status, [Chla] μg/L < 2.5 μg/L oligotrophic, 2.5 > [Chla] μg/L < 8 μg/L, mesotrophic, 8 > [Chla] μg/L < 25 μg/L eutrophic, [Chla] μg/L > 25 μg/L hyper-eutrophic.

Chl 'a' concentrations in association with cyanobacterial densities can be a tool for assessing the trophic status of an aquatic ecosystem [71]. These two parameters can also be used to determine the alert levels in a water body intended for the drinking water production [52].

The results generated are dissimilar with those generated by studies [22,25–27], which showed that there were strong correlations between cyanobacterial densities and Chl 'a'.

According to the chlorophyll 'a' concentration standard revealed by Galvez-Cloutier et al. [40], the trophic profile of Bougous dam was generally mesotrophic (5 months/8) and it was eutrophic only in April and July. The oligotrophic status was only observed in March (Table 4). The passage from the mesotrophic to eutrophic state can be explained by an imbalance resulting from an enrichment of the environment by nutrients, mainly nitrogen and phosphorus [72]. This imbalance can also be favored by climate change, particularly in the southern part of the Mediterranean [50–73]. Several works [62,74–77] have shown that the eutrophic environment favors the development of potentially toxic cyanobacteria, hence the need for annual monitoring of water bodies intended for drinking water production.

4. Conclusion

In conclusion, biomonitoring of Bougous waters has shown that the quality of this water body is satisfying with reference to WHO standards relating to alert levels frameworks for drinking water, the cell density of the potentially toxic cyanobacteria identified and its mesotrophic state. So Bougous waters can be used as an option for management of Mexa waters in order to reduce cyanobacteria biomass and consequently the problems of the organoleptic and sanitary quality degradation of the latter during HAB periods. But the presence of potentially toxic cyanobacteria in the waters of Bougous obliges us to keep it under surveillance by developing management and regular biomonitoring programs. These programs are also recommended for all water bodies intended for the drinking water production.

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