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Dynamic of microcystin-LR-producing cyanobacteria in a drinking water supply: Guenitra dam (North-East of Algeria)

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ABSTRACT

The presence of microcystins (MCs) in waters exclusively intended for drinking water production is a major problem for both ecosystems and public health. The current study aimed to identify the environmental factors that explain the spatial and temporal variations in the abundance of cyanobacteria and the concentration of microcystins in the Guenitra Dam, a Mediterranean water body used for drinking water production, irrigation supplies and fisheries. The Guenitra Dam waters are mesotrophic to eutrophic; they shelter nine genera of cyanobacteria in which *Microcystis flosaquae* is dominant in the frequency of occurrence and densities. Cyanobacterial densities in the Guenitra Dam exceed the level of vigilance in January; the alert level 1 was shown during the rest of the year and the alert level 2 was in November, with a peak registration of more than 189 million cells / mL. The highest cell densities were found in surface waters and decreased with depth. In autumn, more than 99% of the overall cyanobacteria density was detected. The MC-LR contents did not exceed 0.5 µg.L-1 except in November (1.02 µg.L-1 in St5) and December (1.5 µg.L-1 in St4). From January to September, the levels did not surpass 0.22 µg.L-1. Statistical analysis revealed the existence of very highly significant ($p < 0.0001$) positive correlation of *M. flosaquae* with microcystins (MC-LR) and highly significant ($p < 0.001$) of *M. flosaquae* with NH_4^+. During the bloom found in November, the presence of MC-LR in the drinking water was recorded, with a concentration of 0.57 µg.L-1, which is clearly higher than that allowed (0.1 µg.L-1) by the WHO. The presence of MC-LR in the drinking water is indicative of a failure in the water treatment process. The presence of cyanobacteria and cyanotoxins in raw and drinking waters of the Guenitra reservoir involves regular monitoring of the cyanobacterial communities and cyanotoxins in raw water.

INTRODUCTION

The preservation of water resources is the greatest challenge in the 21st century. According to **Moilanen *et al.* (2008)**, reservoirs stagnant waters , even with the measures for their development and conservation, are the most threatened, because, with their natural

evolution (filling) an increasingly important anthropogenic pressure is added with respect to the uses of this water (irrigation, transport and energy production).

Paerl and Otten (2013)reported that, the excessive enrichment of freshwater ecosystems with nutrients is mainly associated with a variety of human activities, including: agricultural, urban and industrial activities, which promotes eutrophication and consequently causes an increase in the presence of cyanobacteria. Cyanobacteria are considered the most competitive and pervasive organisms among phytoplankton **(Paerl & Otten, 2013)**. Cyanobacteria development is favored for the nutrient availability, high temperature, solar radiation, the stable water column and the high pH **(Paerl & Huisman, 2008; Carey *et al*., 2012; Paerl & Otten, 2013)**. According to **Komàrek *et al*., (2014),** Cyanobacterial microorganisms have the ability to regulate their position within the water column via gas vacuoles to fix nitrogen gas using specialized cells (heterocysts).In addition they can overcome adverse periods by forming dormant cells (akinetes). Moreover, Cyanobacteria can produce numerous secondary metabolites, which are harmful to human and animal heal, for example the cyanotoxins,**(Codd *et al*., 2017)**. In fresh water, MCs (cyclic heptapeptides) are considered the most common cyanotoxins **(Humbert *et al*., 2017; Du *et al*., 2019)**, and they are found generally in all continents. **Paerl and Huisman (2008)** stated that, due to the hydrological cycling changes, the increased nutrient charges and water temperature, harmful blooms will increase in both frequency and duration. **Erol and Randhir (2012)** considered the Mediterranean region as one of the most vulnerable areas to the impacts of global warming and the precipitation patterns changes in the world.

In Algeria, **Remini *et al*. (2010)** postulated that, water quality and quantity face a real threat combined with the predicted increasing needs of the population (compared to water shortages) due to climate changes. In Algerian reservoirs, several studies reported the presence of various potentially cyanotoxin-producing species. For the Mexa reservoir, **Saoudi *et al*. (2015)** reported the constancy of *Microcystis* and the regularity of *Oscillatoria*; However, *Microcystis* pre dominates with an average density higher than 500 000 cells/ml. In Ain Zada Dam, **Saoudi *et al*. (2017)** noted that concentrations of microcystins ranged from 19.6 µg/L MC-LR in raw water that is equivalent to 6.3 µg/L in the drinking water during the *Planktothrix agardhii* bloom. In Hammam Debagh reservoir, **Guellati *et al*. (2017)** reported that the cyanobacterial community is highly dominated by *Microcystis*. While, in Zit Emba reservoir, **Touati *et al*. (2019)** found that *Microcystis* represent an average proportion of 43% to global cyanobacteria population; this species was followed by *Woronichinia* (21%) and *Planktothrix* (16%).

This study was conducted to fulfill the following targets: i) assessing, for the first time, the trophic status of the Guenitra reservoir, a water body used for drinking water production and irrigation supplies ii) monitoring the spatio-temporal dynamics of cyanobacteria abundance and microcystins (MCs) content and iii) investigating the influence of some environmental parameters on the assemblages of cyanobacteria and MCs (MC-LR eq) production through the application of statistical tests.

MATERIALS AND METHODS

Guenitra "EL baraka" Dam is a hydraulic structure, located in the North-East of Algeria, 50 km south of the wilaya of Skikda (36°42'05.49''N, 6°37'38.39''E). It is fed by five valleys: Fessa valley, Charfa valley, Megramene valley,in addition to the valleys of Mellouh and Essouk. This dam was first built in the watershed of Wadi Guebli **(Mecibah & Zouini, 2016)**. The area of the watershed is 993 Km^2. Its initial capacity is 125 million m^3, with a regulated volume of 48 million m^3 **(ANB, 1984)**.

This dam was first generated in October 1984 to provide drinking water for the citizens of the state of Skikda and cover the water needs of the industrial complex. Moreover, the water originated from this dam is used to irrigate agricultural lands (Saf-Saf valley and Medjez-Edchich plain, i.e. 5.650 ha) **(Mecibah & Zouini, 2016)**.

Samples from the Guenitra Dam water were monthly taken, from January to December 2016, and the following five stations were selected to manage this task (Fig. 1):

- **Station 1 (St1):** located on the surface near the water intake (sheltered from the wind)(36°42'46.80"N, 6°38'1.42"E); **Station 2 (St2):** located on a 12.5 m deep from station 1(36°42'46.80"N, 6°38'1.42"E); **Station 3 (St3):** located on a 20 m deep from station 1(36°42'46.80"N, 6°38'1.42"E); **Station 4 (St4):** located on the surface in the center of the reservoir (exposed to the wind)(36°42'17.60"N, 6°37'19.94"E); **Station 5 (St5)**: located at the bottom of the dike (sheltered from the wind) (36°42'51.65"N, 6°38'6.49"E).

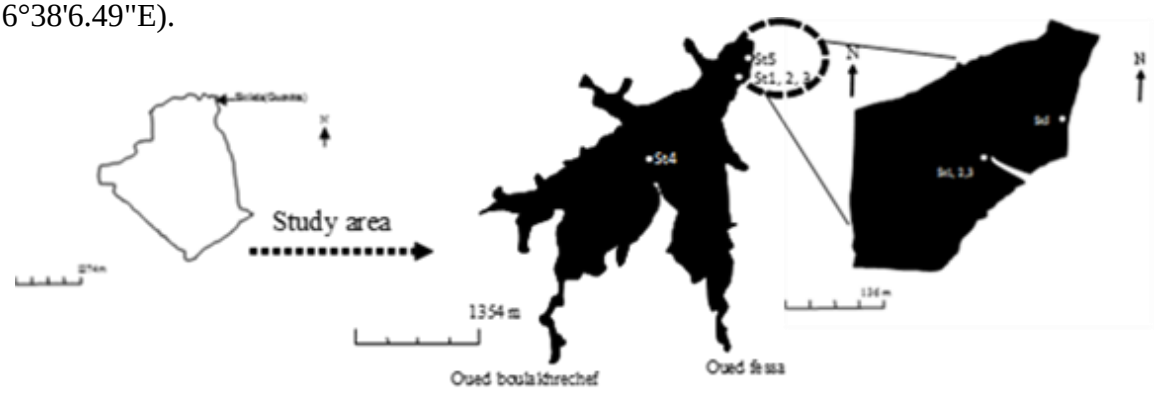


Fig. 1. Localization of Guenitra reservoir and the sampling stations (St1, 2, 3,4 et 5).

Sampling method

For the determination of nutrient salts, chlorophyll (a), microcystins and suspended solids (SS), an amount of 1.5 liter of water was collected, conserved in amber bottles and left in cold conditions until they were transferred to the laboratory for analysis.

Cyanobacteria sampling was performed using plankton filet (Hydro-Bios®), with a size of 20 µm of mesh for the surface stations, while a Van Dorn bottle (Hydro-Bios®)was used for the stations in depth **(Treyture *et al.*, 2007)**. For the enumeration, 1 liter of water was taken from each station using a sampling tube that measures 1m long

and a diameter of 5 cm **(Brient *et al.*, 2001)**. Samples were kept in a cooler and sent to the laboratory.

Abiotic variables, meteorological and chlorophyll (a)

The measurements of temperature (TW), dissolved oxygen (DO), pH, and conductivity (Cond.) were performed *In situ* using a multi-parameter provided with probes (WTW 340i). The transparency of the water concerning stations 1 and 4 was measured using the secchi disk (diameter 25 cm) (EN ISO 7027, 2000).

In the laboratory, the determination of suspended solids (SS) was carried through the differential weighing method of filters **(Aminot & Chaussepied, 1983)**. Nitrites (NO_2^-), ammonium (NH_4^+) and orthophosphate (PO_4^+) dosages were performed according to the colorimetric methods of **Aminot and Kérouel (2004)**. The determination of nitrate (NO_3^-) was carried out using the method of **ISO (1994)**. For the chlorophyll (a) (Chl. (a)) determination, the SCOR UNESCO trichromatic method was applied **(Aminot & Kérouel, 2004)**. The meteorological records (temperature, precipitation and wind speed) were provided free of charge by the management of the dam.

Study of cyanobacteria

The identification of the collected genera was assessed through the microscopic examination of the morpho-anatomical characters and according to the identification keys of **Komàrek *et al*. (2014)**. For the enumeration, the 1 L recuperated with the sampling tube was filtered through a polycarbonate filter; the filtrate is collected with 1ml of water from the sample itself. The cyanobacteria count was defined using the Nageotte hemocytometer cell following the method of **Brient *et al*. (2001)** who used a Carl Zeiss model upright optical microscope (Axiostar plus) equipped with a digital uEye32 camera. The enumeration results were expressed in cells ml^{-1}

Microcystin LR determination

The determination of the intracellular microcystin content was carried out using the enzyme immunoassay ELISA following the protocol recommended by «Abraxis» kit. An amount of 500 ml of raw water was filtered through Whatman GF/C filter for each sample and conserved at -20°C. The filters were extracted in 10 ml of 80% methanol/ water (v/v). The results were expressed as the equivalent of microcystins-LR in $\mu g.l^{-1}$.

Statistical analysis

For the statistical analysis, researchers in the present study used Spearman's non-parametric correlation coefficient (*r*), the non-parametric Kruskal-Wallis test, the Dunn's test and the principal component analysis PCA using R software (3.1.2).

RESULTS

Physico-chemical characterization of Guenitra dam waters

To determine the physico-chemical characteristics of the Guenitra Dam water column, statistical parameters were calculated for each physici-chemical variable, including the arithmetic average and the standard deviation (Sd). The results of this analysis are presented in (Table 1).

Table 1. Statistical description of the spatio-temporal physico-chemical variables of Guenitra Dam water column

Parameter	T°water	pH	Oxyg	Cond	SS	NO_3^+	NO_2^-	NH_4^+	PO_4^+
N	60	60	60	60	60	60	60	60	60
Average	14.43	8.98	7.37	573.93	44.04	5.08	0.11	0.65	0.31
Sd	9.84	0.63	2.41	13.39	69.81	0.61	0.08	0.7	0.28

In Guenitra dam, the water temperature vary from 9.16 to 19.86°C (14.43°C ±9.84), and the dissolved oxygen contents vary from 5.93 to 8.94 mg/L (7.37 mg/L±2.41). The Guenitra Dam waters showed temperatures of more than 15°C from May to October and dissolved oxygen contents of more than 8 mg/l from January to April (Fig. 2A). The pH is alkaline with values ranging from 8.2 to 10.06 (8.98±0.63). However, the pH values of more than 9 were recorded from April to July in addition to December (Fig. 2B). The conductivity values ranged from 561 to 594 µs/cm (573.93µs/cm ±13.39); values of more than 579 µs/cm were detected during the period between September and December. Suspended solids' values ranged between 3.6 mg/l and 158 mg/l (44.04 mg/l ±69.81); Peaks were recorded in October (158 mg/l), November (100.8 mg/l) and December (88.4 mg/l) (Fig. 2E). Transparency (Secchi disc) values ranged from 117.5 to 267.5 cm; values that are less than 200 cm were recorded in the period from January to April in addition to September (Fig. 2E). NO_3^+ showed values that ranged from4.11 to 6.11 mg/l (5.08 mg/l ±0.61); the values higher than 5 mg/l were recorded in January, February, August, October and November (Fig. 2c). The values of NO_2^- ranged from 0.063 and 0.22 mg/l (0.1 mg/l±0.08); the NO_2^- values remained below 0.20 mg/l except in October and December (0.21 and 0.20 mg/L, respectively). Ammonium values (Fig. 2D) ranged between 0.27 and 1.78 mg/l (0.65 mg/l ±0.7); the ammonium content (NH_4^+) did not exceed 1 mg/l except in November and December. Orthophosphate contents (PO_4^+) ranged from 0.08 to 0.75mg/L (0.31 mg/l ±0.28); values of more than 0.40 mg/l were recorded from May to August (Fig. 2D).

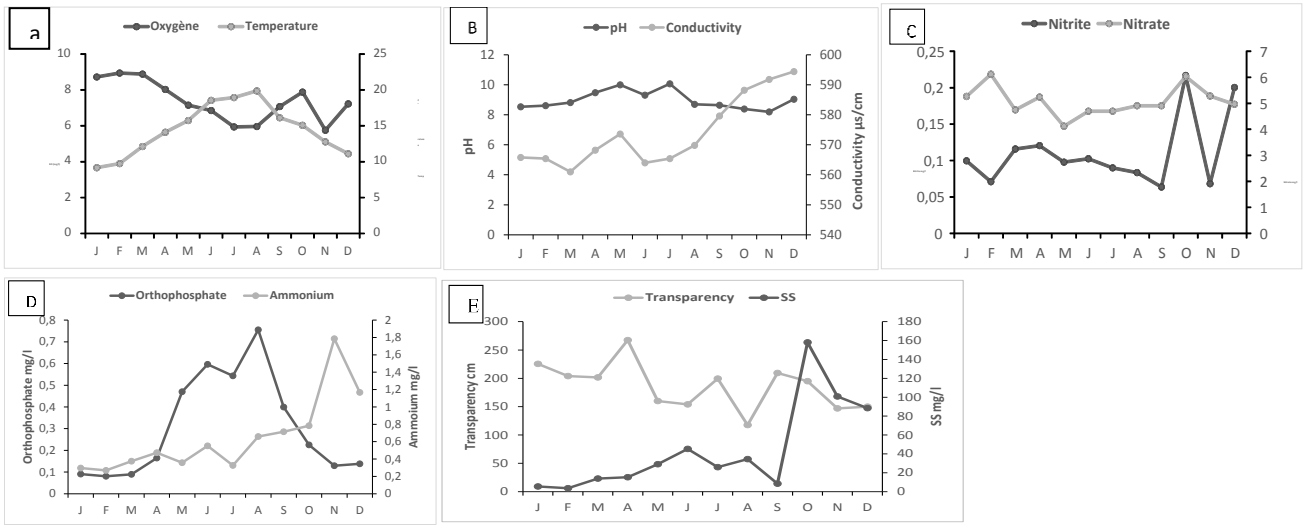


Fig. 2. Monthly variations of temperature,DO (a), conductivity,pH (b), NO_3^+, NO_2^- (c); NH_4^+,PO_4^- (d) and SS-Trans (e) in Guenitra dam.

Diversity and spatiotemporel dynamics of cyanobacteria

The morpho-anatomical characters observation of the cyanobacteria collected from Guenitra Dam water samples allowed us to identify nine genera attached to 4 orders : Chroococcales*(Microcystis,Limnococcus),*Synechococcales*(Coelomoron,Coelospharium, Aphanocapsa,Snowlla),* Oscillatoriales ***(****Planktothrix, Oscillatoria)* and Spirulinales *(Spirulina)*. The calculation of the frequency of occurrence for the identified genus is presented in Table (2) showing that *Microcystis* is frequent, *Limnococcus* is common and *Coelomoron* is occasional. Among the remaining genera, *Coelospharium* and *Oscillatoria* are rare and *Snowlla*, *Aphanocapsa*, *Planktothrix* and *Spirulina* are accidental. In terms of abundance, *Microcystis* is clearly predominant; the latter is followed by the genus *Aphanocapsa* and *Coelospharium* (Table 2).

Table 2. Frequency of occurrence and average density of the cyanobacterial genus collected from Guenitra Dam water samples (2016)

Genus	Frequency of Occurrence (F %)	Observation	Average density Cell. mL
Microcystis	75	Frequent	15826338
Limnococcus	66.67	Common	2
Coelomoron	41.66	Occasional	4
Coelospharium	16.66	Rare	346
Oscillatoria	16.66	Rare	2
Snowella	8.33	Accidental	0.11
Aphanocapsa	8.33	Accidental	361
Planktothrix	8.33	Accidental	3
Spirulina	8.33	Accidentel	0.42

Frequent : FO ≥ 75% ; Common : 75% > FO ≥ 50 % ; Occasional : 50% > FO ≥ 25% ; Rare : 25% > FO ≥ 10% ; Accidental : FO < 10%.

In Guenitra Dam waters, *Microcystis* is represented by the species *M. flosaquae* and *M. aeruginosa*; the evaluation of their monthly density showed the dominance of *M. flosaquae* around the year. The presence of *M. aeruginosa* is illustrated by two peaks in March and September (Fig. 3).

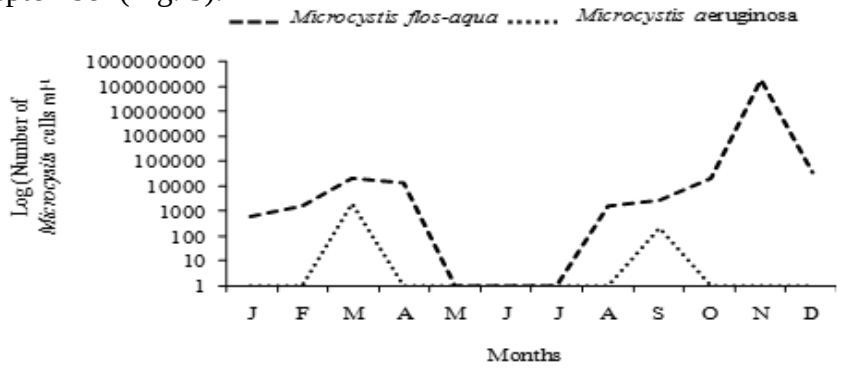


Fig. 3. Monthly fluctuations of *M.flos-aquae* and *M. aeruginosa* **(Guenitra dam, 2016)**

The spatial distribution of cyanobacteria

Cyanobacterial densities pursue the following descending order: St5 ≥ St4 ≥ St1 ≥ St2 ≥ St3. Cyanobacteria are more abundant on the surface; the densities recorded in St1, St2 and St3 represent respectively 93%, 5% and 2% of the density of cyanobacteria present in the water column (Fig. 4). The Kruskal–Wallis test detected significant differences among stations for cyanobacteria.

The temporal dynamics of cyanobacteria

Seasonal distribution

More than 99% of the global cyanobacteria density was detected in autumn; the proportions are in the order of 0.01%, 0.007% and 0.005%, respectively in winter, spring and summer (Fig. 5).

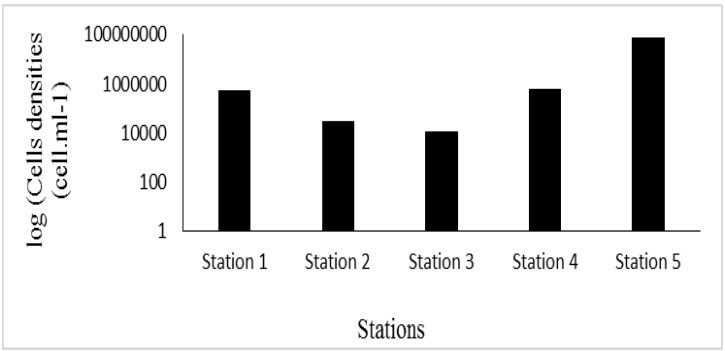


Fig. 4. Spatial distribution of cyanobacteria **(Guenitra, 2016)**

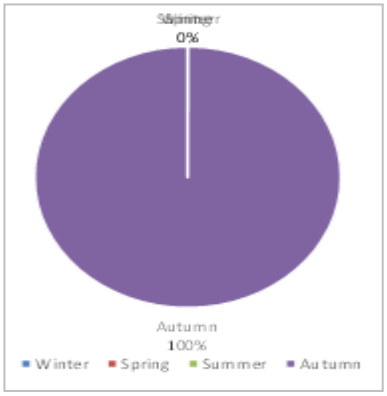


Fig. 5. Seasonal distribution of cyanobacteria **(Guenitra, 2016)**

- **Monthly distribution**

In Guenitra Dam waters, cyanobacteria are at their lowest level (less of 50 cell/mL) in he period from May to July. While, in January, their level exceeds 200 cell/mL and hits 2000 cell/mL for the rest of the year, with a peak of over 189 million cell/mL in November. The evolution of chlorophyll-a is similar to that of cyanobacteria abundance (Fig. 6). The results of the Spearman test showed that the chlorophyll(a) is positively correlated with cyanobacteria (r = 0.45, p=0.0013).

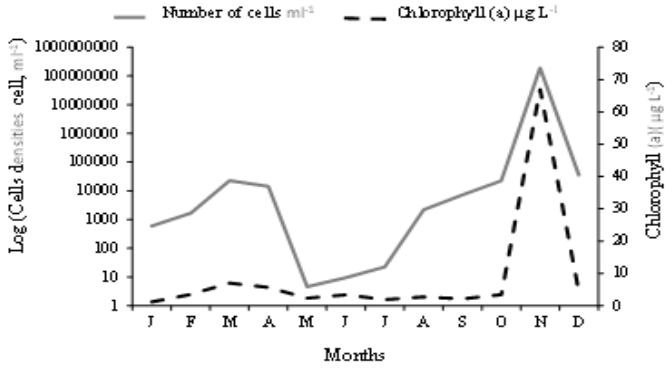


Fig. 6. Monthly fluctuations of the identified cyanobacteria **(Guenitra, 2016)**

MC-LR content

The MC-LR determination showed a high cyanobacterial density; Table (3) exhibits the results of the cyanobacteria enumeration and those of MC-LR assay. Notedly, from January to September, the levels of MC-LR do not exceed 0.022 µg. L^{-1}; they vary from 0.06 to 0.47 µg.L^{-1} in October, from 0.10 to 1.02 µg.L^{-1} in November and from 0.3 to 1.5 µg.L^{-1} in December. The highest values are recorded in the surface water and it is also monitored during the bloom of November, showing the drinking water with a level in the order of 0.57 µg of MC-LR. L^{-1}.

MC-LR toxin shows a highly significant positive correlation with the cyanobacterial densities ($r=0.57$, $p<0.0001$).

Statistical analyses

Inter-stations and inter-months comparison

The Kruskal-Wallis test results relating to the comparison of the medians of each of the twelve variables for the two factors: «station » and « saison » in the five stations under study (S1, S2, S3, S4 and S5) are summarized in Table (4). In addition, the post hoc pairwise comparisons of the Dunn's test are presented by boxplots in Fig. (7) for station factor and Fig. (8) for season factor.

The comparison among stations using the non- parametric Kruskal Wallis test revealed significant differences ($p< 0.05$) for the following variables: TW, dissolved oxygen, chlorophyll (a) and cyanobacteria. The post hoc pairwise comparisons of the Dunn's test indicates that the box diagrams clearly showed this difference in St2 and St3 for TW and

St2, St3 and St5 for DO. For chlorophyll (a), a heterogeneity was noted in stations 1, 4 and 5.

Table 3. Densities of *M.flos-aquae*, *M. aeruginosa* and levels of microcystins-LR recorded during the period from September to December (Guenitra, 2016)

Month	Parameter	Stations				
		Station 1	**Station 2**	**Station 3**	**Station 4**	**Station 5**
Sept.	Cyanobacteria (cells. ml^{-1})	7881	225	5565	14259	8449
	M.flos-aquae(cells. ml^{-1})	1215	0	0	9437	2733
	M. aeruginosa (cells. ml^{-1})	0	0	0	0	1110
	MC-LR ($\mu g.L^{-1}$)	0.018	0	0	0	0.022
Oct.	Cyanobacteria (cells. ml^{-1})	69911	3415	111	14865	24166
	M.flos-aquae (cells. ml^{-1})	69893	775	111	14845	22409
	M. aeruginosa (cells. ml^{-1})	0	0	0	0	0
	MC-LR ($\mu g.L^{-1}$)	0	0	0.067	0.073	0.473
Nov.	Cyanobacteria (cells. ml^{-1})	346853	91806	6729	773857	947 855 384
	M.flos-aquae (cells. ml^{-1})	346853	91806	6729	773857	947 855 384
	M. aeruginosa (cells. ml^{-1})	0	0	0	0	0
	MC-LR ($\mu g.L^{-1}$)	0.117	0.112	0.117	0.105	**1.023**
Dec.	Cyanobacteria (cells. ml^{-1})	8188	6160	399	86105	77489
	M.flos-aquae (cells. ml^{-1})	8188	6160	399	86105	77489
	M. aeruginosa (cells. ml^{-1})	0	0	0	0	0
	MC-LR ($\mu g.L^{-1}$)	0.381	0.429	0.351	**1.50**	0.3

Additionally, cyanobacteria exhibited a heterogeneity in stations 3 and 5. Concerning the season factor. Moreover, the Kruskal Wallis test revealed significant differences for conductivity, SS, NH_4^+, NO_2^-, NO_3^+, $H_3PO_4^+$, pH, cyanobacetria and toxin. The post hoc pairwise comparisons of the Dunn's test indicates that the box diagrams for the conductivity revealed the existence of a heterogeneity for both winter and autumn compared to other seasons. For the box diagram of NH_4^+, this test indicates a clear heterogeneity of summer and autumn compared to other seasons. On the other hand, the box diagram of No_2^- and MCs revealed the existence of homogeneity for all seasons. While, the box diagram of NO_3^+ assessed the existence of heterogeneity for the summer and spring periods compared to those of winter and autumn. With respect to the box diagram of $H_3PO_4^+$, the existence of heterogeneity was detected for the summer season compared to other seasons. Finally, cyanobacteria showed the existence of heterogeneity inwinter and autumn compared to other seasons.

Table 4. The inter-stations and inter-seasons comparison of the median values of physicochemical parameters, chlorophyll (a), toxin and cyanobacteria density in Guenitra reservoir

		Parameter	**T°**	**Cond**	**O_2**	**SS**	**NH_4^+**	**NO_2^-**	**NO_3^+**	**$H_3PO_4^+$**	**pH**	**Chl(a)**	**Cyano**	**Toxins**
Factor	**Station (ddl=4)**	***P*-value**	0.00	0.58	0.00	0.78	0.93	0.31	0.91	0.99	0.82	0.00	0.00	0.69
		Observation	***	Ns	***	Ns	Ns	Ns	Ns	Ns	Ns	***	***	Ns
	Season (ddl=11)	***P*-value**	0.45	0.00	0.13	0.00	0.00	0.01	0.00	0.00	0.00	0.09	0.00	0.00
		Observation	Ns	***	Ns	***	***	**	***	***	***	Ns	***	***
		NB:	*** (*P* ≤0,05),**		**** (*P* ≤0,01),**		***** (*P* ≤0,001),**		**ns (*P* >0,05)**					

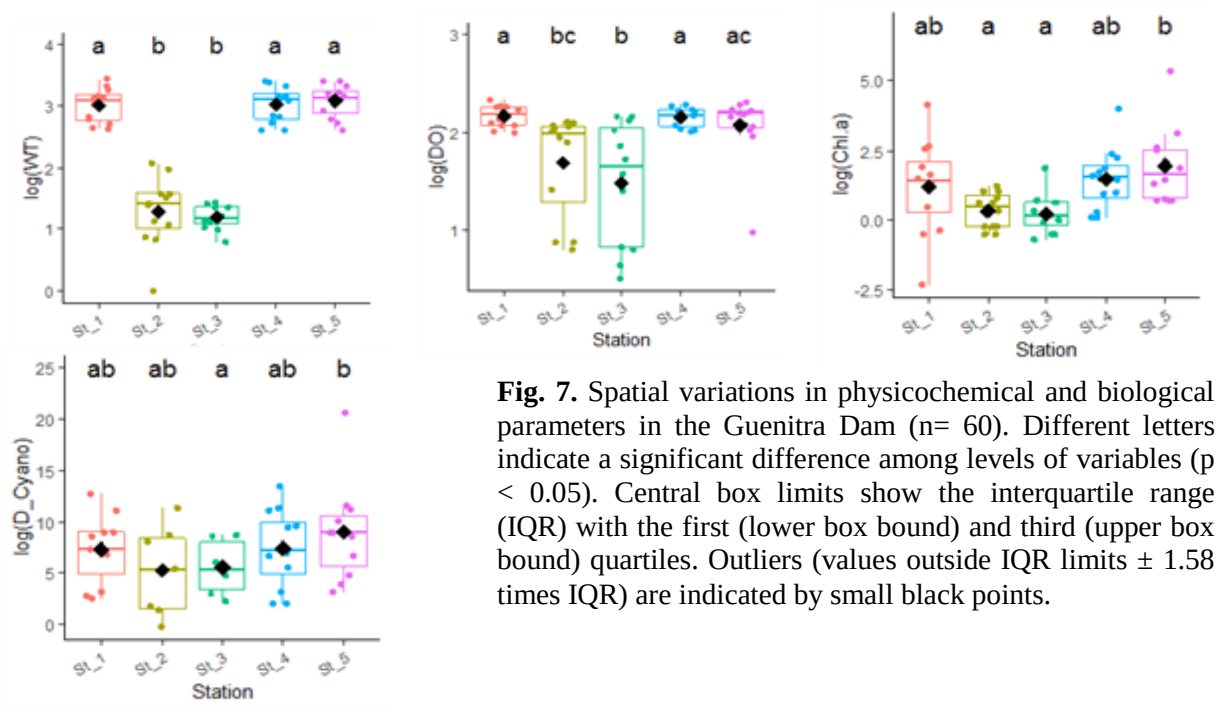
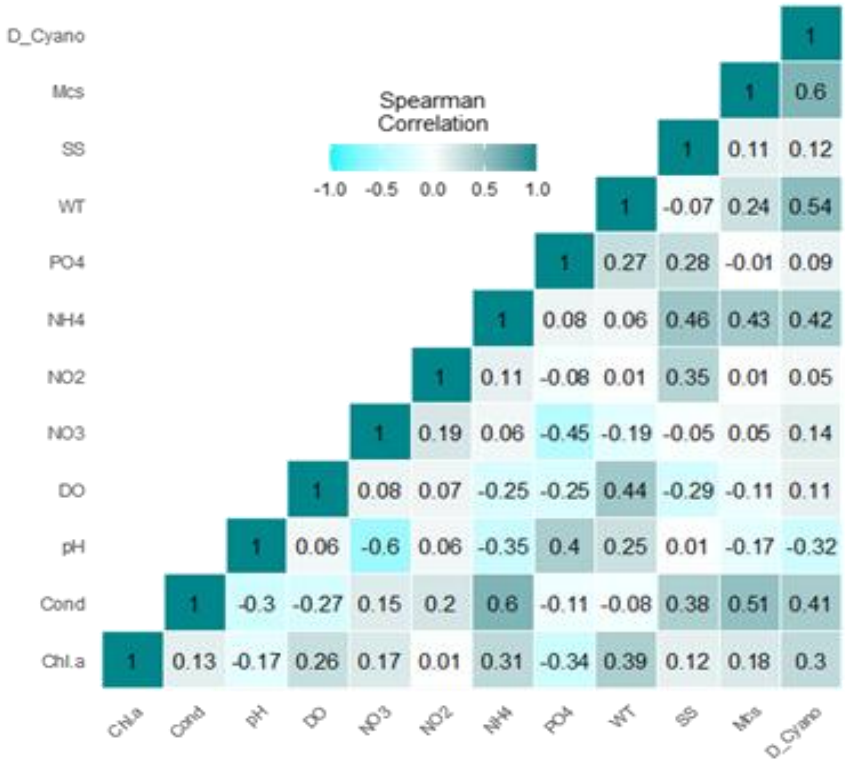


Fig. 7. Spatial variations in physicochemical and biological parameters in the Guenitra Dam (n= 60). Different letters indicate a significant difference among levels of variables ($p < 0.05$). Central box limits show the interquartile range (IQR) with the first (lower box bound) and third (upper box bound) quartiles. Outliers (values outside IQR limits ± 1.58 times IQR) are indicated by small black points.

Table 5. Spearman's rank correlation analysis between cyanobacterial genera and environmental variables WT – water temperature; DO – dissolvedoxygen; Chl-a – Chlorophyll a; Cond – conductivity; pH; NO_3^+ -Nitrates; NO_2^-– Nitrites; NH_4^+ – Ammonium; PO_4^+- Orthophosphate; Trans – Transparency; SS – SuspendedSolids; Mcs –microcystins, cyano- Cyanobacteria;



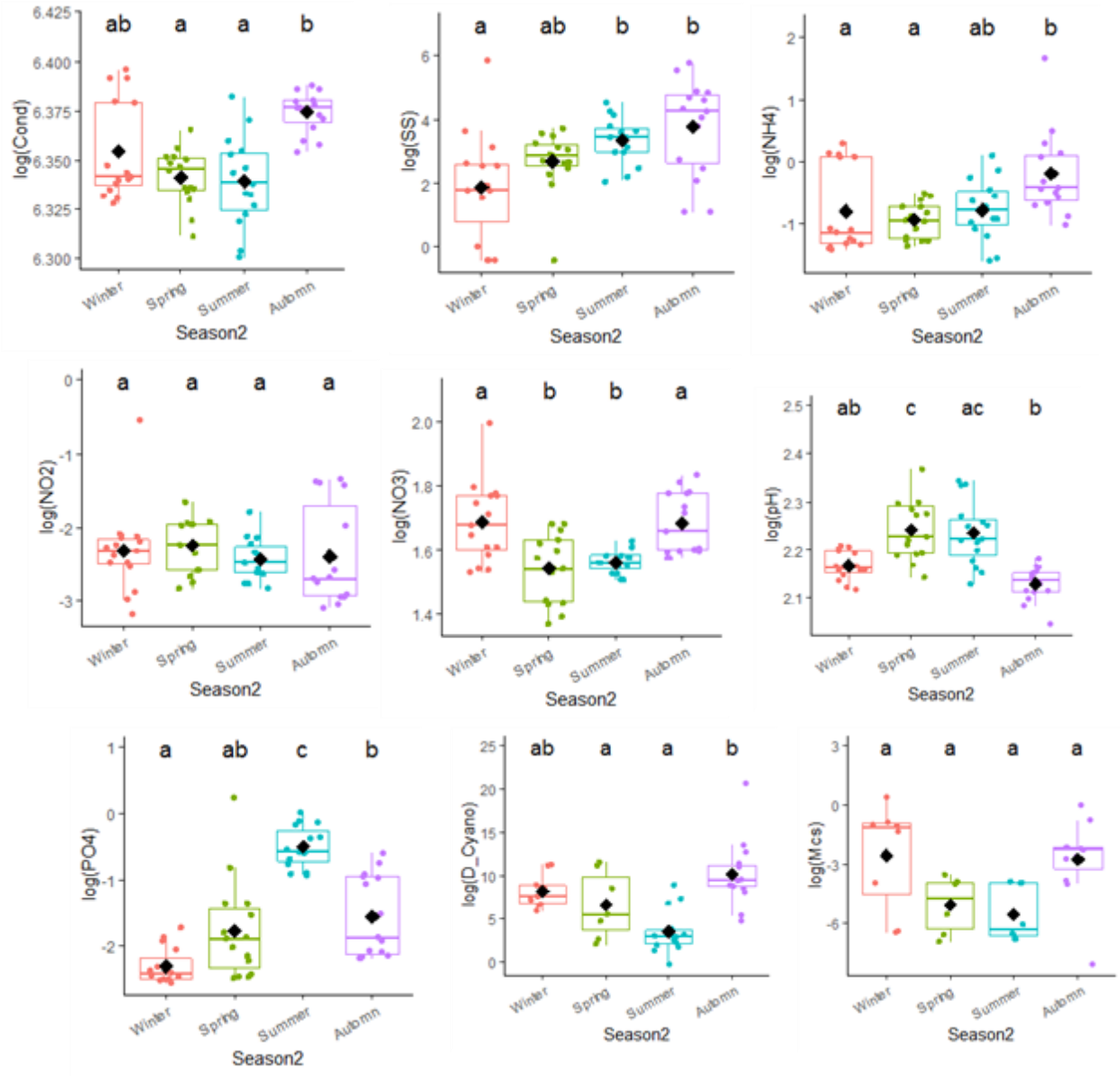


Fig. 8. Seasonal variations in physico-chemical and biological parameters in the Guenitra Dam (n= 60). Different letters indicate a significant difference among levels of variables ($p< 0.05$). Central box limits show the interquartile range (IQR) with the first (lower box bound) and third (upper box bound) quartiles. Outliers (values outside IQR limits ± 1.58 times IQR) are indicated by small black points.

Correlation analysis between environmental parameters and cyanobacetria density

The non-parametric spearman correlation analysis is presented in Table (5) showing a significant positive correlation ($p \geq 0.5$) between MCs and cyanobacteria ($r = +0.6$), WT and cyanobacteria ($r= +0.54$), conductivity and MCs ($r= +0.51$), conductivity and NH_4^+ ($r=$

+0.6). On the other hand, it showed a significant negative correlation between $NO_3^+$and PO_4^+ (r=-0.45) and between pH and NO_3^+ (r=-0.6).

The spatio-temporal variation analysis of both biotic and abiotic parameters distribution in the Guenitra Dam: Principal component analysis (PCA)

Principal component analysis (PCA) shows that the first three factor axes explain 64.12 % of our total variation (Fig. 9).

Axis 1 represents 32.02% of the total variability; on its positive pole, the variables NH_4^+ (r =0.74, cos^2 = 0.55), SS (r = 0.73, cos^2= 0.53), Cond (r = 0.67, cos^2 = 0.41), Chl a (r= 0.62, cos^2 = 0.39), cyano (r= 0.61, cos^2=0.38), toxin (r=0.52, cos^2= 0.27), NO_3^+ (r=0.50, cos^2=0.25) and NO_2^- (r=0.44, cos^2=0.19) are projected; the negative pole of axis 1 projects the pH (r=-0.63, cos^2=0.40).

On the positive pole of axis 1, autumn is projected and distinguished by the strong presence of cyanobacteria, chlorophyll-a and toxin.

Axis 2 represents 17.57% of the variability; on its positive pole. T (r=0.64, cos^2=0.41) and $H_3PO_4^+$ (r = 0.63, cos^2=0.39) are projected ; this is due to the fact that the highest concentrations of $H_3PO_4^+$ appear in summer with the highest water T°. Additionally, it can be noted that the factorial plane 1-2 of the PCA revealed the separation between the depth stations (St2 and St3) and those of the surface (St1, St4 and St5).

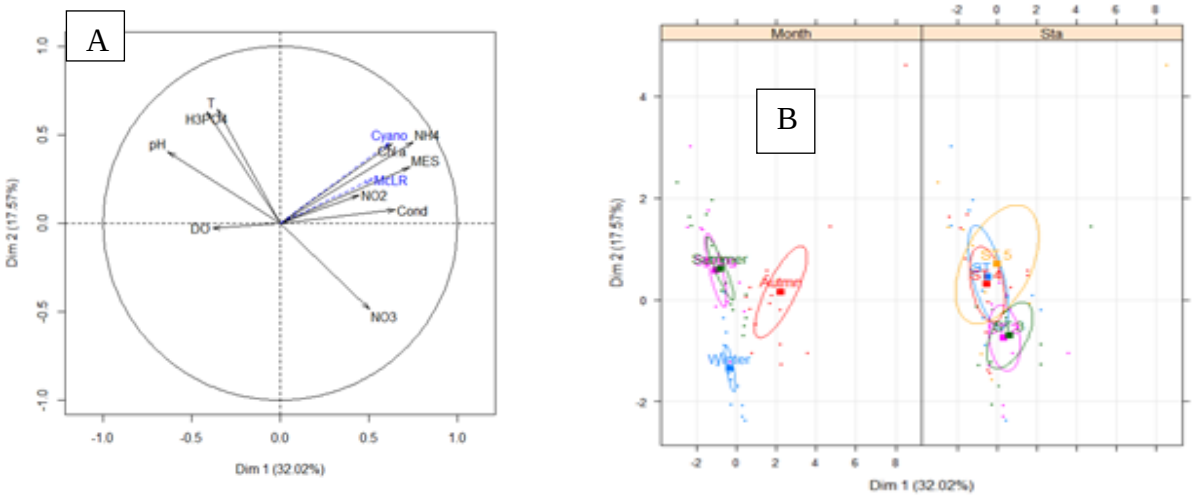


Fig. 9. Principal component analysis based on the spatiotemporal variation in Guenitra Dam. Factorial plane: axis 1: 32.02 %, axis 2: 17.57%. **(A):** Correlation circle of variables assayed with the first two principal axes. **(B):** Projection of stations and seasons on the first two principal axes.

Ascending hierarchical classification

The dendrogram examination, from the AHC based on the inter-station variation of the biotic and abiotic parameters, indicates the existence of three clusters. The first cluster includes stations 2 and 3 located in the depths of the water column, where the lowest cyanobacteria charges are noted. The second cluster includes station 5 located on the

surface, which is characterized by a high charges of cyanobacteria, and the third cluster includes surface stations 1 and 4, which harbor intermediate charges of cyanobacteria. The obtained dendrograms typology by the AHC agrees with the factorial plans of the PCA on the variation of biotic and abiotic parameters and on the structuring of the inter-station variation in the Guenitra Dam (Fig. 10).

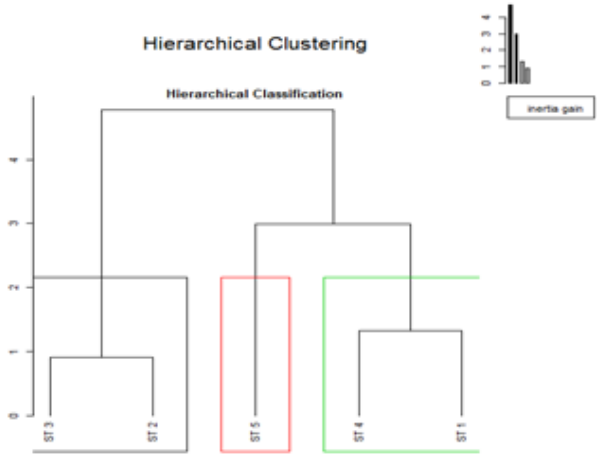


Fig. 10. Hierarchical clustering analysis of five stations of sampling according to the variation of biotic and abiotic parameters

DISCUSSION

The Guenitra Dam waters are alkaline; their pH varies from 8.5 to 10. Temperatures are high in summer (18 ° C) and low in winter (10 ° C). For dissolved oxygen concentrations, they are high (9 mg. L^{-1}) in winter and low (6.95 mg. L^{-1}) in autumn. The conductivity shows its lowest values in winter (564 µs.cm^{-1}) and its highest in autumn (591 µs.cm^{-1}). In addition, it is also in autumn that the highest concentrations of nitrates (5.43 mg. L^{-1}), nitrites (0.16 mg. L^{-1}) and ammonium (1.25 mg. L^{-1}), chlorophyll (a) (25.31 µg. L^{-1}) and SS (115.7 mg. L^{-1}) are recorded. Furthermore, the values of water transparency are lower (164 cm) in autumn. Concerning the orthophosphate contents, the highest values (1mg.L^{-1}) are recorded in the summer period. With an exception in autumn, where the content of Chlorophyll (a) exceeds 25µg. L^{-1}, during the rest of the year; the waters of Guenitra shows levels that vary from 2 to 5.6 µg. L^{-1}.

In autumn, the important water column mixing and its warming by the sun would promote an important micro-algal proliferation illustrated by an average chlorophyll (a) content of about 25 µgL^{-1}. This biomass showed a positive correlation with cyanobacteria density and T° ($p < 0.01$).

The average values of chlorophyll (a) and the transparency observed during the study period indicate that this water body fluctuated between a mesotrophic and eutrophic status, according to the OECD (Organization for Economic Co-operation and Development) classification scheme **(Vollenweider & Kerekes, 1982)**.

Previous studies showed that eutrophic or hypereutrophic water bodies are favorable for the extensive cyanobacterial blooms' occurrence **(Rastogi *et al*., 2014 ; Harke *et al*., 2016)**. This phenomenon leads to a low transparency and a high turbidity induced by the suspended solids that limit light diffusion, and privilege cyanobacteria such as *Microcystis, Planktothrix, Aphanizomenon*. These genera are capable of making vertical movements by regulating their buoyancy in the water column through intracellular gas vacuoles. This mechanism gives an advantage to this group to relocate in the optimum depth in a stable water column to obtain solar radiation during the day in the surface water, and hence absorb enough nutrients in a lower layer at night **(Carey *et al*., 2012)**.

In Guenitra dam waters, nine genera were identified; namely, *Microcystis, Limnococcus, Coelomoron, Coelospharium, Aphanocapsa, Snowella, Planktothrix, Oscillatoria* and *Spirulina.* Among these genera, *Microcystis* is frequent, *Limnococcus* is common and *Coelomoron* is occasional; other genera are rare or accidental. In Mexa, a water body located in the same bioclimatic area (sub humid), **Saoudi *et al*. (2015)** noted the presence of 7 genera, including: *Microcystis, Planktothrix, Oscillatoria, Spirulina, Lyngbya, Pseudanabaena* and *Merismopedia* in which *Microcystis* is dominant in both frequency of occurrence and density.

In the waters of the Guenitra Dam, two species were detected; namely, *Microcystis flosaquae* and *Microcystis aeruginosa*.The former species predominated with an average of 15 million cells.ml^{-1} compared to *M. aeruginosa* that showed less than 200 cells.ml^{-1}. Concerning the other listed genera, their density did not exceed 5 cells.ml^{-1} except those of *Aphanocapsa* and *Coelosphaerium* genera that counted for 361 cell. mL^{-1} and 346 cells. mL^{-1}, respectively. In Guenitra Dam, *Microcystis* is present all along the water column and during the whole year.

The dominance of this genus was also found in other Algerian reservoirs used for drinking water **(Saoudi *et al*., 2015; Guellati *et al*., 2017)**. In Mexa Dam, *Microcystis* predominates with an average density that it is higher than 500 000 cells/ml; the occurrence frequency estimation of the identified genera in Mexa dam shows the constancy of *Microcystis* **(Saoudi *et al*., 2015)**. In Zit Emba Dam, the occurrence frequency estimation shows that from the seven found genera, only *Microcystis* was ubiquitous; this genus represented 43% of the global density of the collected cyanobacteria **(Touati *et al*., 2019)**. *Microcystis* sp.genus has a worldwide distribution **(Harke *et al*., 2016)** and proliferates especially in eutrophic and hypereutrophic ecosystems during the summer season **(Van Wichelen *et al*., 2016)**.

Cyanobacterial densities show highly significant differences (p<0.01) among stations; cell densities decrease with the increasing depth of water column. They represent -50 cm, -12.5 m and -20 m respectively , recording respective percentages of 93, 5 and 2 of the cyanobacteria density present in the water column. In Mexa Dam, **Saoudi *et al*. (2015)** noted that cyanobacterial cells are present in the surface stations of the water column with a percentage of 99, and that their densities decrease as the depth increases. The afore-

mentioned authors also observed the absence of cyanobacteria in the treated water. In Zit Emba Dam, **Touati *et al*. (2019)** postulated that, the cyanobacteria represent the same profile every season, and that the abundances tended to decrease with the increasing depth concerning their vertical distributions.

For the seasonal distribution of cyanobacteria, it was noted that in autumn, more than 99% of the overall densities of cyanobacteria were detected; the proportions are in the order of 0.01%, 0.007% and 0.005% respectively in winter, spring and summer. According to **Saoudi *et al*. (2015)**, in Mexa Dam, the cyanobacteria presented rates of 95,47% of the overall densities in autumn, whereas rates of 3.74 % were detected of the overall densities in summer, while during winter and spring rates did not exceed 0.8%.

The monthly cyanobacterial densities in the Guenitra Dam waters are at their lowest level (less than 50 cell/mL) from May to July, whereas they exceed 200 cell/mL in January and 2000 cell/mL for the rest of the year, with a distinguished peak of over 189 million cell/mL in November. With respect to the guideline values admitted by WHO, in the Guenitra Dam, the cyanobacteria densities exceed the level of vigilance in January; the alert level 1 during the rest of the year and the alert level 2 in November, with a peak of more than 189 million cells / mL **(Affsa/Afsset, 2006)**. In Mexa Dam, **Saoudi *et al*. (2015)** noticed a level of vigilance from January to June, an alert level 1 from July to September and an alert level 2 in October. Only in December, the densities descend under the level of vigilance. Concerning Zit Emba Dam, **Touati *et al*. (2019)** reported that, the densities of cyanobacteria place this water body on alert level 1 during the whole year.

The spearman non-parametric correlation coefficient calculation shows the existence of a positive correlation between the cyanobacterial densities and chlorophyll (a) contents ($p<0.01$). Chlorophyll (a) and cyanobacterial densities show similar fluctuations illustrated by a peak in November, when the pigment and the microalgae are at their optimal values. However, *M. flosaquae* showed a highly significant positive correlation with NH_4^+ ($p<0.001$) ; *Microcystis* has a high affinity for NH_4^+, thus, it is highly competitive for recycled, reduced nitrogen **(Gobler *et al*., 2016)**.

In Guenitra Dam raw waters, the MC-LR concentration revealed values of more than 1 $\mu g.L^{-1}$ in November for St5 (1.02 $\mu g.L^{-1}$) and in December for St4 (1.5 $\mu g.L^{-1}$). During the rest of the year, the contents do not exceed 0.5 $\mu g.L^{-1}$; however, during the bloom formed in November, MC-LR reached a concentration of 0.57 $\mu g.L^{-1}$ in the drinking water; this reveals a failure of the water treatment process.

In Mexa Dam, as in Guenitra dam, the highest MC-LR contents are recorded in surface water and especially in the autumn period (17.36 $\mu g.L^{-1}$ in October); however, the authors reported the absence of this toxin in drinking water **(Saoudi *et al*., 2015)**. In addition, they observed the existence of a positive correlation of MC-LR with cyanobacteria and chlorophyll (a).

In the Guenitra Dam, the microcystins (MC-LR) showed a strong positive correlation with *Microcystis flosaquae* ($p<0.0001$). Therefore, any higher biomass of microcystins

(MCs) producers often leads to a significant level of total MCs contents **(Monchamp *et al.*, 2014; Van de Waal *et al*., 2016; Saoudi *et al*., 2015; Charifi *et al.*, 2019)**. During toxic blooms of *Microcystis* and *Planktothrix*, in the western Lake Erie (USA), MCs concentrations have been controlled by the availability of inorganic nitrogen in the water column **(Horst *et al*., 2014; Davis *et al*., 2015; Harke *et al*., 2016)**. That is showed, in Guenitra reservoir, by the MC-LR positive correlations with NH_4^+ ($p<0.0001$). In many eutrophic ecosystems, the MCs' concentrations have been commonly increased in response to an increasing nitrogen values compared tothe case with the increasing phosphate **(Davis *et al*., 2015)**. Various researchers showed that, high inorganic nitrogen levels are necessary for the N-rich MCs synthesis, and that high levels of exogenous inorganic nitrogen have the ability to promote higher cellular quotas of MCs in the non-diazotrophs *Planktothrix* and *Microcystis* **(Van de Waal *et al*., 2010; Harke & Gobler, 2013)**. According to **Donald *et al*. (2011)**, in the field, NH_4^+ addition compared with NO_3^- addition provokes an increase in MCs concentrations and bloom maintenance for a longer duration. Most studies show that interactive physical, chemical and biotic factors are implicated in the control of the bloom forming cyanobacteria growth and dominance **(Saoudi *et al*., 2015; Thomas & Litchman, 2015; Aguilera *et al*., 2017; Guellati *et al*., 2017)**. In the present study, the relationships between the environmental factors and cyanobacterial communities were investigated by using principal component analysis (PCA) and Spearman's rank correlation. The *Microcystis* omnipresence and its dominance could be explained by its strong correlation with nutrients (NH_4^+, NO_3^- et NO_2^-).

Additionally, it is worthy to note that, the factorial plane 1-2 of the ACP revealed the separation between the depth stations (St2 et St3) and those of the surface (St1, St4 and St5). This agrees with the typology of dendrograms obtained within the CAH on the variation of biotic and abiotic parameters and on the structuring of the inter-station variation at the level of the Guenitra Dam.; The dendrogram indicates the existence of three clusters:The first includes stations 2 and 3 located in the depths, the second includes station 5 located on the surface, which is characterized by high charges of cyanobacteria. The third cluser includes surface stations of 1 and 4, which shelter intermediate charges of cyanobacteria.

CONCLUSION

The present study allowed us to highlight the presence of cyanobacteria and cyanotoxins in raw and drinking waters of the Guenitra reservoir. This requires the establishment of a management and a monitoring program for this water body as well as for other reservoirs intended for drinking water production. These programs should be based on a regular monitoring of the cyanobacterial communities and the cyanotoxins in raw and tap water; as recommended by the WHO, it is necessary to (a) increase the sampling frequency (at least once a week), (b) identify the cyanobacterial genres and species in order to

determine the cyanotoxins that can be produced potentially, (c) evaluate the effectiveness of the drinking water treatment process in eliminating cyanotoxins and plan the use of activated carbon (or other advanced technology) to eliminate cyanotoxins. Thus, a proliferation monitoring is required to determine the return to a normal performance situation. The application of these water body monitoring programs makes it possible to model the parameters responsible for the appearance of cyanobacterial blooms that produce MC-LR significantly, in order to face this global problem, particularly with the accelerated global warming which threatens the reduction of surface water resources on earth and also promote water bodies eutrophication in favor of the development of toxic cyanobacterial blooms.

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