JOURNAL OF
ENVIRONMENTAL HYDROLOGY
The Open Access Electronic Journal of the International Association for Environmental Hydrology
On the World Wide Web at http://www.hydroweb.com

VOLUME 23 2015

MONITORING THE TROPHIC STATE AND PHYCOCYANIN PIGMENT OF KARAOUN RESERVOIR, LEBANON

Ali Fadel [1]
Ali Atoui [1,2]
Mirvat Temsah [2]
Maher Matar [1]
Kamal Slim [1,2]

[1]**Laboratory of Microorganisms and Food Irradiation, Lebanese Atomic Energy Commission- CNRS, Beirut, Lebanon**
[2] **Lebanese University, Faculty of Sciences, Hadath, Lebanon**

Eutrophication and toxic cyanobacterial blooms have become a worldwide environmental problem. The Carlson trophic state index (CTSI) is widely used for the classification of trophic conditions of fresh water bodies. The performance of the TriOS microFlu-blue probe in the field, for the measurement of in vivo phycocyanin fluorescence, was compared to laboratory biovolumes. The trophic state of the Karaoun Reservoir, the largest water body in Lebanon, has been poorly studied, as is the case with many freshwater bodies around the Mediterranean Sea. Sampling campaigns were conducted semi-monthly between May 2012 and August 2013 to assess the trophic state and performance of the TriOS microFlu-blue. Karaoun Reservoir was found to be eutrophic to hypereutrophic during 2012-2013. A strong linear relation was found between Trios phycocyanin measurements and the total cyanobacteria biovolumes in Karaoun Reservoir, with a R^2 value of 0.87.

INTRODUCTION

Water quality of freshwater bodies changes over time, at seasonal but also at pluriannual time scales. Continuous changes in lakes and reservoirs hydrology, industrial and agricultural activities in their catchments affect their trophic state. Eutrophication of freshwater bodies can result in toxin producing cyanobacterial blooms that threaten human health.

Carlson trophic and Shannon–Wiener diversity are indices used to identify water quality in freshwater bodies. In the European Union, the phytoplankton community is used as a biological indicator of the ecological status of water bodies monitored in accordance with the Water Framework Directive (European Parliament Council, 2000). In addition, the World Health Organization (WHO) has established guideline values for drinking-water supplies and recreational waters which may contain toxic cyanobacterial populations (Chorus, 2005). Thus, regular monitoring of the trophic state and phytoplankton community in water bodies is critical for assessing the water quality evolution (Jørgensen et al., 2005).

Cyanobacterial blooms can be estimated by measuring phycocyanin pigment. Submersible phycocyanin probes represent a fast and easy method to measure phycocyanin pigment at field. TriOS microFlu-blue is one of these probes used to measure cyanobacteria pigment. Meanwhile the performance of this probe was assessed at the laboratory, little or no studies were performed to asses its reliability at field.

Karaoun reservoir, the largest water body in Lebanon, was built up in 1965, along Litani River (170 km length), the main input to this reservoir. Whereas studies have been carried out on cyanotoxins (Fadel et al., 2014a) and phytoplankton community, and nutrients concentration in the past at Karaoun Reservoir (Fadel et al., 2014b; Slim et al., 2014), there are few documents that describe its trophic status. The aim of this study was therefore to (a) assess the trophic state of Karaoun Reservoir, and (b) to evaluate the performance of TriOS microFlu-blue probe. We assume that the information presented in this paper is highly valuable as they increase the knowledge about phycocyanin monitoring and the trophic state of Karaoun Reservoir.

MATERIAL AND METHODS

Study site

Karaoun Reservoir (33.34° N, 35.41° E), also known as Qaroun, Qaraoun or Qarun, located in the southern part of the Bekaa valley, between the two Lebanese mountains, is the largest freshwater body in Lebanon. It has a surface area of 12 m^2, maximum depth of 60 m and a maximum volume of 224 x 10^6 m^3. This artificial ecosystem was primarily built for agricultural irrigation and hydroelectricity production. It now serves varied purposes such as: commercial fishing, recreation, tourism, and irrigation through canal 900.

Water exits the reservoir through three main outputs: 1) the power generation tunnels located at the bottom of the reservoir, at an altitude of 810 m above sea level 2) two evacuation towers used to supply canal 900 that was constructed for irrigation purpose. 3) the bell-mouth spillway which is located near the dam and used to evacuate the overflow water volume to avoid water over topping and dam damaging (Fadel et al., 2014b).

SAMPLING PROCEDURE

Profiles measurements and sampling for water analysis were performed at the most representative point (S_M), located in the middle of the lake (33° 56’ 55”N, 35° 69’ 47”E . Campaigns were performed

semi-monthly between 11:00 and 13:00 h. Water samples were collected at 0.5 m depth from May to November 2012 and at 0.5, 5 and 10 m depths from March to August 2013. A vertical Niskin bottle of 2.2 L capacity (Wildco, code 1120-D42) was used to get samples at different depths. Samples were stored at 4°C until further processing in the laboratory. Different volumes were sub-sampled at the laboratory from each sample to be used for the identification and counting of cyanobacteria and the analysis of total phosphorus and the chlorophyll-a quantification.

Physical measurements and nutrient analysis

Transparency measurements were performed with a Secchi disk that was lowered through a shaded area. Subsamples used for the analysis of total phosphorus were preserved at 4 °C after addition of 2 mL of 18 M H_2SO_4 per liter (98%). Total phosphorus concentrations were then determined at 880 nm by UV/VIS spectrophotometer using colorimetric ascorbic acid method.

Phytoplankton analysis and phycocyanin pigment

The cyanobacteria from each subsample were determined in the same day according to taxonomic keys based oncell structure and dimensions, colony morphology, and mucilage characteristics (Komárek and Anagnostidis, 1999, 2005). Microscopic identifications and enumeration were carried out under a phase contrast microscope (Nikon TE200, Nikon, Melville, NY, USA). The subsamples used for counting were fixed by formaldehyde (4% formaldehyde of sample volume) and preserved at 4 °C. Phytoplankton counting was carried out under a ×40 objective using Nageotte chamber that accepts 100 µL on 40 bands. The number of bands counted depended on sample concentration. Each subsample was counted on triplicate.

Total biovolumes of each cyanobacteria species was calculated by multiplying the counted number of cells per millilitre by the average biovolume of a cell of that species. Cell biovolumes of each species were calculated according to the most suitable geometric models (Sun and Liu, 2003).

Chlorophyll-a quantification was carried out according to Lorenzen method to calculate Carlson trophic state index (Lorenzen, 1967). A duplicate of each sample was filtered using Whatman GF/C filters that were then kept frozen at -20 ºC for 16 hours. Chlorophyll-a was extracted from these filters in 90 % acetone by ultrasonication and agitation. The extracts were centrifuged at 3500 rpm for 10 min to reduce the turbidity. About 2 mL were used for chlorophyll-a quantification using the Ultraviolet–visible spectrophotometer (LaboTech ThermoSpectronic), then a chlorophyll-a correction was performed by adding 60 µL of 0.1 M HCl to these 2 mL to measure the amount of degradation product, pheophytin-a.

A submersible fluorometer (TriOS microFlu-blue) was used to measure fluorescence profiles of phycocyanin, a pigment specific to cyanobacteria. It is equipped with ultra-bright red LEDs, of an excitation wavelength 620 nm, detection wavelength 655 nm and band-width 10 nm. It gives a linear response to phycocyanin concentration up to 200 µg L^{-1} with an accuracy of 0.02 µg L^{-1}. Measurements were performed every half meter between the surface and the bottom of the reservoir by descending the cable manually at a speed of 5 cm s^{-1} and waiting for values to become stable.

Estimation of Trophic State Index

To classify the trophic state of Karaoun Reservoir, we applied Carlson's Trophic State Index (CTSI) that was calculated according to the following equation (Carlson, 1977):

$$CTSI = (TSI_{(Chl\text{-}a)} + TSI_{(SD)} + TSI_{(TP)}) /3 \quad (1)$$

where:

$TSI_{(Chl\text{-}a)} = 9.8 \ln Chl\text{-}a + 30.6$

$TSI_{(SD)} = 60 - 14.4 \ln SD$

$TSI_{(TP)} = 14.42 \ln TP + 4.15$

Chlorophyll-a (Chl-a) is in µg/L, total phosphorus (TP) in µg/L and Secchi disc depth (SD) in m.

Based on the values of CTSI (Carlson, 1977; Sheela et al., 2011) lakes and reservoirs are classified as oligotrophic (CTSI less than 40), mesotrophic (CTSI between 40 and 50), eutrophic (CTSI between 50 and 70) or hypereutrophic (CTSI greater than 70).

RESULTS AND DISCUSSION

Trophic level of Karaoun Reservoir

The Carlson trophic state index and its attributes for Karaoun Reservoir over a period of two years are presented in Figure 1 and Table 1. CTSI ranged between 52 and 74 and had an average of 61 in 2012, this average increased to 65 in 2013 but the range was narrower, between 59 and 70. TSI (SD) ranged between 50 and 64 and had an average of 57 in 2012, this average stayed constant with 57 in 2013 but the range was wider, between 47 and 65. TSI (CHL) ranged between 48 and 83 and had an average of 68 in 2012, this average decreased to 63 in 2013 and the range was narrower, between 57 and 71. TSI (TP) ranged between 47 and 76 and had an average of 59 in 2012, this average increased to 75 in 2013 and the range was narrower, between 65 and 80. TSI (CHL) was greater than TSI (TP) in 2012 while TSI (TP) was greater TSI (CHL) in 2013. In 2012 and 2013, TSI (CHL) was greater than TSI (SD) except for 20 November 2012. Large positive difference between the index values were encountered during blooms of large phytoplankton species in 16 October 2012 during cyanobacterium *Aphanizomenon ovalisporum* bloom and in 07 November 2013 during dinoflagellate *Ceratium hirundinella* bloom.

Average Carlson's trophic state index values of Karaoun Reservoir in 2012 and 2013 were 61 and 65, respectively. Based on Carlson trophic index classification (Carlson, 1977; Sheela et al., 2011), Karaoun Reservoir is eutrophic. TSI (CHL) was greater than TSI (TP) in 2012 while TSI (TP) was greater TSI (CHL) in 2013. This indicates that P was generally a limiting factor to phytoplankton growth in 2012 and not in 2013. Less algal material is present than expected, based on TP in 2013, and some other factor may be limiting their growth (Havens, 2000). In 2012 and 2013, TSI (CHL) was greater than TSI (SD) except for 20 November 2012. Large positive difference between the index values were encountered during blooms of large phytoplankton species in 16 October 2012 during *Aphanizomenon* ovalisporum bloom and in 07 November 2013 during *Ceratium hirundinella* bloom. Blooms of these large phytoplankton species might have attenuated light and resulted in TSI (CHL) index higher than that of TSI (SD).

Performance of Trios phycocyanin fluorimeter

Cyanobacteria dominated in summer and early autumn in Karaoun Reservoir (Fadel et al., 2014a). Samples taken at 0.5 m, 5 m and 10 m during 2012-2013 for microscopic counting showed that cyanobacteria biovolume ranged between 0.01 and 8.8 mm3 L-1. The major blooming cyanobacteria were Aphanizomenon ovalisporum and Microcystis aeruginosa (Figure 2). Aphanizomenon ovalisporum bloomed on October 2012 and Microcystis aeruginosa on July 2013. The highest cyanobacteria concentrations were recorded at the top 5 m (Fadel et al., 2014a).

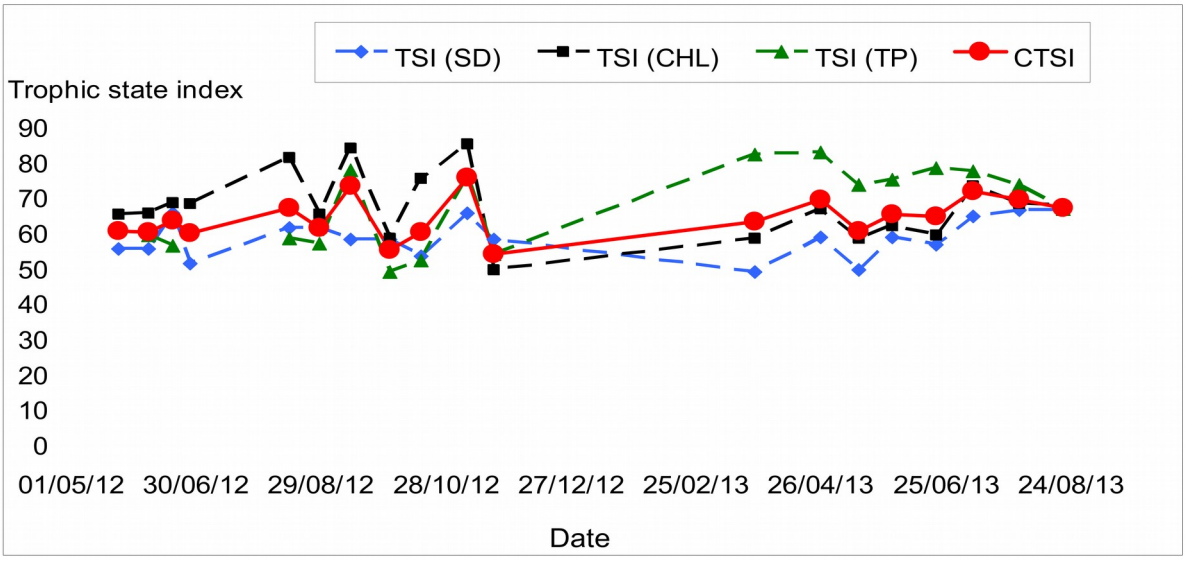

Figure 1. Carlson trophic state index (CSTI) in 2012 and 2013. CTSI classification: Oligotrophic (CTSI less than 40), mesotrophic (CTSI between 40 and 50), eutrophic (CTSI between 50 and 70) or hypereutrophic (CTSI greater than 70).

Table 1. Indices of Carlson trophic state and Shannon diversity of phytoplankton in Karaoun Reservoir in 2012 and 2013

Date	TSI (SD)	TSI (CHL)	TSI (TP)	CSTI
24/05/2012	54.16	63.97	-	59.06
07/06/2012	54.16	64.29	58.05	58.83
19/06/2012	64.14	67.03	55.00	62.06
27/06/2012	50.02	66.79	-	58.40
14/08/2012	60.00	79.75	57.11	65.62
28/08/2012	60.00	63.97	55.68	59.88
12/09/2012	56.79	82.58	76.39	71.92
01/10/2012	56.79	57.17	47.67	53.87
16/10/2012	51.94	73.95	50.74	58.88
07/11/2012	64.14	83.78	74.64	74.19
20/11/2012	56.79	48.18	52.67	52.55
Average 2012	**57.18**	**68.31**	**58.66**	**61.39**
25/03/2013	47.39	57.17	80.70	61.75
26/04/2013	57.37	65.48	81.32	68.06
14/05/2013	48.01	57.17	72.32	59.16
30/05/2013	57.37	60.47	73.66	63.83
20/06/2013	55.15	57.80	76.88	63.28
08/07/2013	63.21	71.99	76.01	70.41
30/07/2013	65.14	66.79	72.19	68.04
20/08/2013	65.14	66.28	65.21	65.54
Average 2013	**57.35**	**62.89**	**74.79**	**65.01**

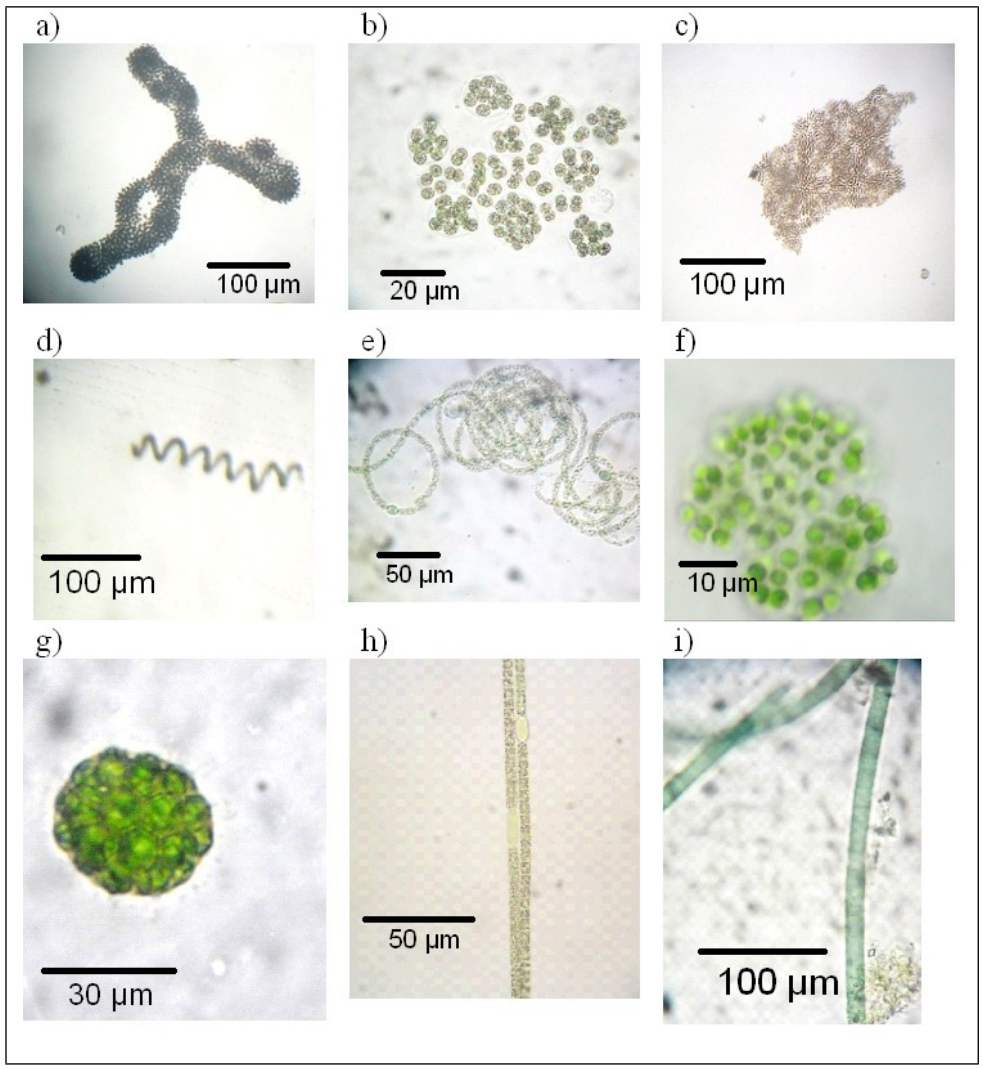

Figure 2. Cyanobacteria species identified in 2012-2013 at Karaoun Reservoir: a) *Microcystis aeruginosa*, b) *Microcystis viridis*, c) *Microcystis ichthyoblabe*, d) *Anabaena spiroides*, e) *Anabaena circinalis*, f) *Radiocystis geminate*, g) *Pilgeria brasiliensis* , h) *Aphanizomenon ovalisporum* and i) *Oscillatoria tenuis*.

Previous studies have demonstrated a very good relationship between the phycocyanin pigment measured by TriOS probe and the cyanobacterial biovolumes determined by microscopy. They showed that the linearity of the TriOS probe determined with a standard solution of PC was highly significant with R^2 = 0.998 (Bastien et al., 2011). Brient et al observed a strong linear correlation between the PC concentration and the number of cyanobacterial cells, with a R^2 value of 0.7296 (Brient et al., 2009). Bastien et al. also observed a strong relationship between the in vivo fluorescence and the total cyanobacterial biovolume, a R^2 value of 0.83 (Bastien et al., 2011). In our study, we counted the cyanobacterial biovolume of 32 samples, taken from Karaoun Reservoir at different dates and depths: 0.5 m, 5 m and 10 m. In all these samples, more than 90 % of the cyanobacterial biovolume consisted either of Aphanizomenon ovalisporum and Microcystis aeruginosa. The comparison of these cyanobacterial biovolumes to their corresponding Trios phycocyanin measurements that were performed at field, at the same depth and date is presented in Figure 2. The results of this comparison show a strong linear relation between Trios phycocyanin concentration and total cyanobacteria biovolumes in Karaoun Reservoir, a R^2 value of 0.87 (Figure 3).

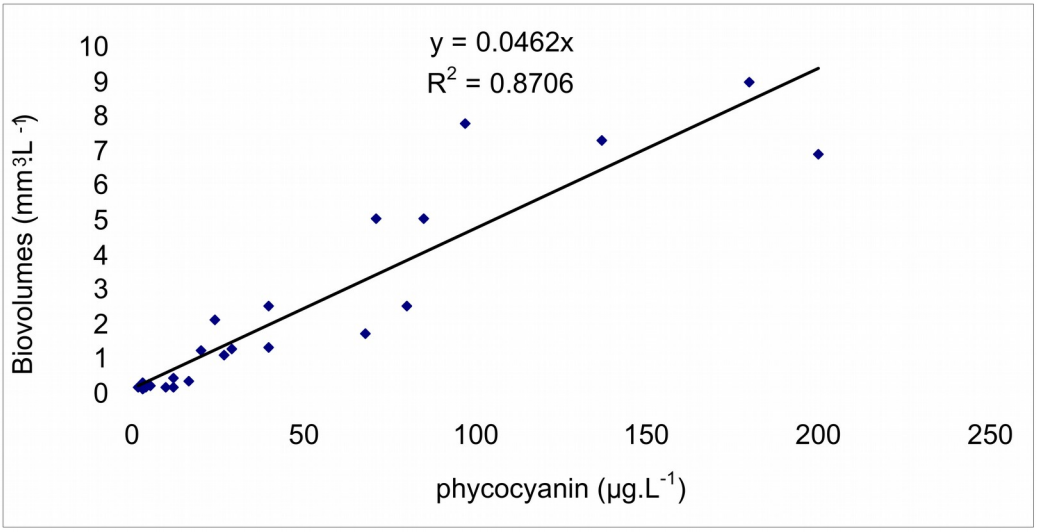

Figure 3. Relationship between cyanobacteria biovolumes and phycocyanin concentrations in Karaoun Reservoir in 2012 and 2013, (n=32).

Beside the limits of Trios microflu probe that was mentioned in previous studies (Brient et al., 2009). After conducting several phycocyanin profiles in 2012 and 2013, additional observations on the limitations of this probe were found. Phycocyanin operates after its connection to a laptop, performed measurements are saved on the computer using MSDA-XE program that comes with the sensor. However, absence of pressure sensors makes these saved profiles useless as it doesn't provide information about the depth at which these measurements were conducted. Measurements are unstable (e.g. at a fixed depth, phycocyanin probe gives a value, then in 2 seconds it would give another values higher or lower by 3 $\mu g.L^{-1}$). Moreover, MSDA-XE is a heavy program that can cause several computer restarts during the measurement process.

CONCLUSIONS

We assess the trophic state of Karaoun Reservoir and the performance of Trios microFlu-blue. Carlson trophic state index applied on Karaoun Reservoir in 2012-2013 has shown that it is eutrophic to hypertrophic. Reservoir managers in Karaoun Reservoir should take actions that reduce the nutrient influx to the reservoir to improve its trophic state.

The Trios microFlu-blue is reliable and interesting probe for monitoring cyanobacterial blooms. A strong linear relation was found between this submersible phycocyanin probe and the total cyanobacterial biovolumes.

ACKNOWLEDGEMENTS

The authors express their gratitude to Dr. Gaby Khalaf from the Lebanese National Council for Scientific Research and Prof. Jalal Halwani from the Faculty of Public Health at the Lebanese University for thoroughly reviewing the manuscript.

REFERENCES

Bastien, C., Cardin, R., Veilleux, E., Deblois, C., Warren, A., Laurion, I., 2011. Performance evaluation of phycocyanin probes for the monitoring of cyanobacteria. Journal of Environmental Monitoring, Vol. 13, pp. 110-118.

Brient, L., Lengronne, M., Bormans, M., Fastner, J., 2009. First occurrence of cylindrospermopsin in freshwater in France. Environmental Toxicology, Vol. 24, pp. 415-420.

Carlson, R.E., 1977. A trophic state index for lakes. Limnology and Oceanography, Vol. 22, pp. 261-369.

Chorus, I., 2005. Water Safety Plans: A better regulatory approach to prevent human exposure to harmful cyanobacteria., in: J. Huisman, H.C.P. Matthijs, Visser, P.M. (Eds.), Harmful Cyanobacteria. Springer.

European Parliament Council, 2000. Directive 2000/ 60/EC of the European Parliament and of the council of 23 October 2000 establishing a framework for community action in the field of water policy. Official Journal of the European Communities L327, 1-72.

Fadel, A., Atoui, A., Lemaire, B., Vinçon-Leite, B., Slim, K., 2014a. Dynamics of the toxin cylindrospermopsin and the cyanobacterium *Chrysosporum* (*Aphanizomenon*) *ovalisporum* in a Mediterranean eutrophic reservoir Toxins, Vol. 6, pp. 3041-3057.

Fadel, A., Lemaire, B.J., Atoui, A., Vinçon-Leite, B., Amacha, N., Slim, K., Tassin, B., 2014b. First assessment of the ecological status of Karaoun Reservoir, Lebanon. Lakes & Reservoirs: Research & Management, Vol. 19, pp. 142–157.

Havens, K.E., 2000. Using Trophic State Index (TSI) values to draw inferences regarding phytoplankton limiting factors and seston composition from routine water quality monitoring data. Korean Journal of Limnology 33, pp.187-196.

Jørgensen, S.E., Löffler, H., Rast, W., Straškraba, M., 2005. Lake and reservoir water uses and abuses, Lake and Reservoir Management. Elsevier.

Komárek, J., Anagnostidis, K., 1999. Cyanoprokaryota 1 Teil: Chroococcales. Ettl, H., Gärtner, G., Heynig, G.H. & Mollenhauer, D. (eds), Süßwasserflora von Mitteleuropa Band 19/1, Spektrum Akademischer Verlag.

Komárek, J., Anagnostidis, K., 2005. Cyanoprokaryota 2 Teil: Oscillatoriales. Büdel, B., Gärtner, G., Krienitz, L. & Schagerl, M (eds), Süßwasserflora von Mitteleuropa Band 19/2, Spektrum Akademischer Verlag (Elsevier).

Lorenzen, C.J., 1967. Determination of chlorophyll and phaeo-pigments: spectrophotometric equations. Limnology and Oceanography Vol. 12, pp. 343-346.

Sheela, A.M., Letha, J., Joseph, S., 2011. Environmental status of a tropical lake system. Environmental Monitoring and Assessment, Vol. 180, pp. 427-449.

Slim, K., Fadel, A., Atoui, A., Lemaire, B.J., Vinçon-Leite, B., Tassin, B., 2014. Global warming as a driving factor for cyanobacterial blooms in Lake Karaoun, Lebanon. Desalination and Water Treatment, Vol. 52, pp. 2094–2101.

ADDRESS FOR CORRESPONDENCE
Kamal Slim
Lebanese University, Faculty of Sciences
Hadath, Lebanon
Email: kslim@cnrs.edu.lb