Factors possibly affecting growth of *Microcystis aeruginosa* and *M. flos aquae* isolated from Wadi El-Raiyan Upper Lake Egypt under laboratory conditions

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ABSTRACT

Experiments were carried out to determine the effects of some environmental factors causing the phenomenon of algal blooming in the upper lake of Wadi El-Raiyan under laboratory conditions. *Microcystis aeruginosa* and *M. flos aquae* were isolated from Wadi El-Raiyan Lakes. Mono-clonal culture of each species was kept in 100-ml BG11 media in the laboratory for 5 days. The algal growth was measured by the determination of chlorophyll *a* and cell count to understand the succession patterns of both species in relation to the tested environmental conditions. The best photoperiod cycle was found to be (14:10 hrs) and the growth of the two species increased with increasing light intensities up to 45µ E m^{-2} s^{-1}. Salinity and the growth of tested species exhibited a significant inverse relationship, increased salinity inhibiting algal growth. Water pH range from 7 to11 was suitable for growth of both species, while a pH below or above this range caused a significant decrease in growth. High nitrogen sources entering into Wadi El-Raiyan Lake may be one of the reasons for the blooming of the tested microalgae. The toxic effects of the bloom-forming species impact the economic fish resources of the lake leading to fish kills.

Keywords: *Microcystis aeruginosa*, *Microcystis flos aquae*,Wadi El-Raiyan, photoperiod, salinity, pH.

INTRODUCTION

Cyanobacteria will periodically grow exuberantly, forming what is known as “blooms.” The reasons for blooms formation are not completely understood, but in some cases they may be related to nutrients added naturally and through man-made sources such as fertilizer runoff or sewage (Ling, 2000; Dionysios, 2010).

Identification of specific environmental factors that promote cyanobacterial blooms has been the quest of many researchers, but no single factor serves as a reliable predictor. The growth of cyanobacteria and the formation of blooms are influenced by physical, chemical and biological factors, which were reviewed by AWWA (1995) and Messineo *et al.* (2009). Cyanobacterial blooms persist in water supplies that contain adequate levels of essential inorganic nutrients such as nitrogen and phosphorus, water temperatures generally between 15 and 30˚C, and pH levels between 6 and 9. Blooms are most common in eutrophic or hypereutrophic bodies of water (WHO, 1998). Nutrients, pH, CO_2, salinity and dissolved oxygen are the main chemical factors that contribute to the development of dominant cyanobactrial blooms (Van Ginkel, 2004; Wangwibulkit *et al.*, 2008). Several studies have shown that cyanobacteria have higher affinity for nitrogen or phosphorus than many other photosynthetic organisms (Kaebernick *et al.*, 2001).

Wadi El-Rayian Lakes were extensively studied for their water quality, physicochemical properties and biological properties (Konsowa, 1996; Taha and Abd El-Monem, 1999; Konsowa and Abd Ellah 2002; Abd El-Karim 2004; Abdel Hameed *et al.*, 2007; Ali *et al.*, 2007). Abd El-Fatah (2010) observed a remarkable algal bloom

of *M.aeruginosa* and *M. flos-aquae* during winter season in the upper lake. The algal bloom causes many problems in the water due to the potential toxicity of *Microcystis* spp. The author indicated deterioration in the physicochemical characters of the studied lakes due to the substantial input of fertilizers and nutrient salts which exacerbates the problem of eutrophication. Khalifa and Abd El-Hady (2010), studied seasonal variations in both phytoplankton biochemical contents and zooplankton density in Wadi El-Raiyan Lakes and concluded that the major chlorophyll *a* peak was recorded in winter season and recorded the phenomenon of Microcystis winter bloom. But toxic effects of the bloom in the lakes and the factors promoting this bloom were not discussed deeply before. Therefore, the phenomenon of harmful *Microcystis* bloom and microcystin production in Wadi El-Raiyan Upper Lake deserves a scientific study as it has a direct effect on economic fishes and human health.

During this study, the phenomenon of algal bloom was recorded from mid December 2008 to the first week of May 2009 and still occurs yearly till now causing adverse toxic effects on economic fishes of the lake. Therefore, the present investigation focuses on the response of the bloom-forming *M. aeruginosa* and *M. flos aquae* to gradual increase in different environmental factors under laboratory conditions. Also, it is an attempt to predict the behavior of the tested algae under different condition of the ecosystem, like changing light duration, light intensity, pH, salinity and nutrients (N&P) under laboratory conditions in an attempt to find a solution for this phenomenon.

MATERIALS AND METHODS

Experimental conditions

The experiments were carried out in the Aquatic Plant Laboratory at El-Kanater El-Khairya Research Station, National Institute of Oceanography and Fisheries. The cyanobacterial species, *Microcystis aeruginosa* and *M. flos aquae* were isolated from the Upper Lake of Wadi El-Rayian. Through a series of laboratory work the isolated species became well adapted and grew very well under laboratory conditions. Subsequently, *M. aeruginosa* and *M. flos aquae* were grown in 100-ml modified BG11 medium(Allen,1973) at room temperature, 25 $\pm$ 1 ^{0}C and light intensity of 30µ$Em^{-2}s^{-1}$ under a day / night program of 14 h light followed by 10 h darkness . The population growth and the pigment estimations were conducted after five days (120hrs) culturing. Three replicates were used for each treatment.

The following experiments were carried out:

Experiment 1: Light duration:

The two species *M. aeruginosa* and *M. flos aquae* were incubated at two light and dark cycles (14:10 hr) and (12:12 hr) in order to determine the best photoperiod cycle.

Experiment 2: Light intensities

The behavior of both species was measured under different light intensities by changing the number of cool white fluorescence tubes 2,4&6 units which provided estimated light intensity of 15,30 & 45 µ E m^{-2} s^{-1}(Munawer and Mazharuddin, 2011).

Experiment 3: Effect of pH

The *Microcystis* spp. were grown under different pH levels of 7, 9, 11 and 12. The concentrations were adjusted by using HCl and NaOH solutions followed by measurements using a pH –meter WTWph91.

Experiment 4: Salinity effect

The *Microcystis* spp. were grown at different salinities adjusted daily using NaCl and distilled water at zero (BG11 medium 0 ppt), 2, 6, 10, 14, 16 and 18 ppt. Salinity of the medium was determined by the direct gravimetric method (APHA, 1992).

Experiment 5: Effect of nitrogen and phosphorus (N&P)

To estimate nutrients requirements, nitrate and phosphate concentration in modified BG 11 medium were altered.

1-The medium was prepared with changed concentrations of nitrogen and phosphorus with constant ratio either by additions or by reductions, nitrogen supplemented (NS), phosphorus supplemented (PS), nitrogen deficient (ND) & phosphorus deficient (PD). In supplemented media those ingredients were increased by (25%, 50% & 75%) at (A, B &C) respectively. In deficient media those ingredients were decreased by (25%, 50% & 75%) at (D, E &F) respectively from the standard formula.

2-The medium was prepared with constant phosphorus concentrations (standard concentration) and changed nitrogen either by increasing or by reducing.

3-The medium was prepared with constant nitrogen concentration (standard concentration) and changed phosphorus either by increasing or reducing.

The growth of *M. aeruginosa* and *M. flos aquae* was measured by the determination of chlorophyll *a* after 120hrs by the Trichromatic Method according to APHA (1992) and by algal cell count using haemocytometer.

Data analysis

Data were statically analyzed using analysis of variance (ANOVA) one &two way using the Minitab (12.1) and the STATISTICA (8.0) computer programs to test for significant differences among different experiments on the chlorophyll *a* biomass and cell count of the tested algae.

RESULTS AND DISCUSSION

1- Effect of Light duration (photoperiod) on growth cultures of *Microcystis aeruginosa* and *M. flos aquae*

A comparative experiment was done in order to observe the difference in growth of *M. aeruginosa* and *M. flos aquae* when incubated at two light and dark cycles (12:12 hrs) (14:10 hrs) and in order to determine the best photoperiod cycle. The results are illustrated in Figs. (1& 2).

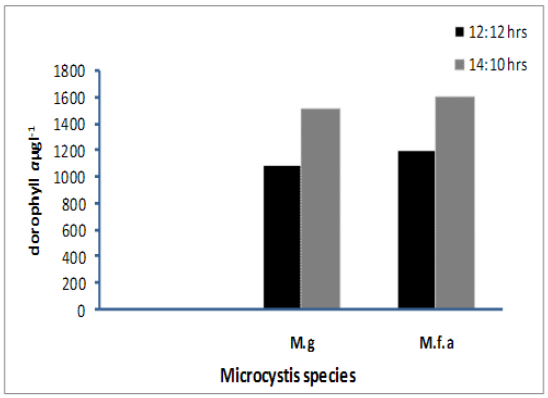


Fig. 1: Chlorophyll *a* values ($\mu g l^{-1}$) of *M. aeruginosa* and *M. flos aquae* cultured at different light duration

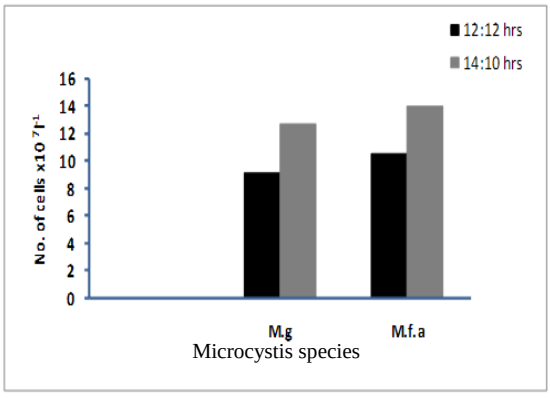


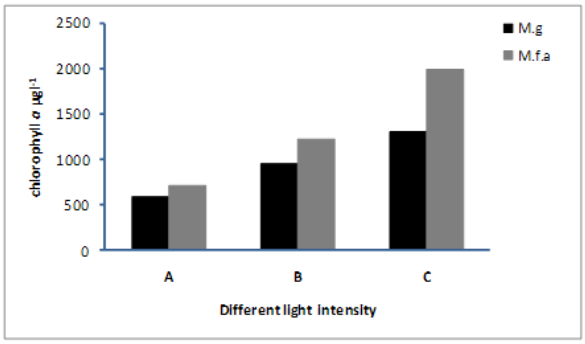
Fig. 2: Cell count (no. of cells x $10^7 l^{-1}$) of *M. aeruginosa* and *M. flos aquae* cultured at different light duration.

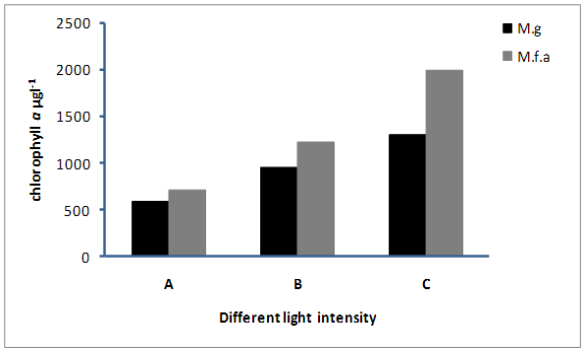
Generally, the data showed higher values of *M. flos aquae* than those of *M. aeruginosa* in chlorophyll *a* and cell counts. Certainly, *M. flos aquae* recorded the highest values of chlorophyll *a* and cell count at 14:10 hrs, (1644.76 µg L^{-1} and 14 x 10^7 L^{-1}respectively), as compared with *M. aeruginosa* which recorded 1506.2 µg L^{-1} and 12.7 x 10^7 L^{-1} respectively at the same photoperiod. Similarly, *M. flos aquae* recorded higher values of chlorophyll *a* and cell count at 12:12 hrs (1273.63 µg L^{-1} and 10.5 x 10^7 L^{-1}respectively) as compared with *M. aeruginosa* (1087.33 µg L^{-1} and 9.16 x10^7 L^{-1}, respectively). Moreover, there was a significant difference ($p < 0.05$) between *M. flos aquae* and *M. aeruginosa* for both chlorophyll *a* and cell count when incubated at 12:12 hrs and 14:10 hrs photoperiod. The culture growth of most *Microcystis* species responded to the long light time as approaching those found under natural conditions at their seasonal bloom. In fact both incubation period and test duration differ from species to another (Gomaa, 1990; Wangwibulk *et al*., 2008; Imai, *et al*., 2009).

From the above mentioned results, it can be concluded that the incubation at a photoperiod 14:10 hrs light and dark cycle was better for the growth of both species than the 12: 12 hrs.

2-Effect of light intensities on growth cultures of *Microcystis aeruginosa* and *M. flos aquae*

In this experiment, the culture growth of isolated *M. aeruginosa* and *M. flos aquae* were tested under various light intensities (15, 30 & 45 µ E m^{-2} s^{-1}), Figures (3&4). Influence of light intensity was investigated in small (100 ml) cultures to reduce the impact of distance from light source to cells, Gomaa, (1990).





A (15 µ E m^{-2} s^{-1}) B (30 µ E m^{-2} s^{-1}) C (45 µ E m^{-2} s^{-1})

Fig. 3: Chlorophyll *a* values (µgl^{-1}) of *Microcystis aeruginosa* and *M. flos aquae* cultured at different light intensities.

Fig. 4: Cell count (no. of cells x $10^7 l^{-1}$) of *Microcystis aeruginosa* and *M. flos aquae* cultured at different light intensities.

The growth of the tested algae represented by chlorophyll *a* content and cell count were significantly higher at the higher light intensities (45&30 µ E m^{-2} s^{-1}) compared to the lower light intensity (15 µ E m^{-2} s^{-1}). *M. aeruginosa* and *M. flos aquae* cell densities increased 1.5 and 1.7 fold at 30 µ E m^{-2} $s^{-1,}$ while they increased by 4.1 and 5.8 folds at 45 µ E m^{-2} s^{-1}respectively. The highest chlorophyll *a* content was at 45 µ E m^{-2} s^{-1}, increased by 119.6% and 176% for *M. aeruginosa* and *M. flos aquae* respectively, as compared to their lowest one at 15 µ E m^{-2} s^{-1}. At the experiments of changes in light intensity, Imai *et al*. (2009) reported that, the cell densities of *M. aeruginosa* and *M. wesenbergii* were higher at 60 and 30 µ mol m^{-2} s^{-1} compared to 0 µ mol m^{-2} s^{-1}. Moreover Amemiya *et al*. (1990) reported that *M. aeruginosa* and *M. wesenbergii* showed higher growth at a high light intensities than *M. viridis* in the laboratory experiments. Hence, growth of *Microcystis* may depend on

multiple factors and the dominant factor may differ depending on species (Imai *et al.*, 2009).The present results go in parallel with the results obtained by Gomaa (1990) who achieved a significant increase in the biomass of *Aphanizomenone flos aquae* as light intensity was increased.

3-Effects of pH on growth cultures of *Microcystis aeruginosa* and *M. flos aquae*.

The results as illustrated in Figures (5&6) indicate that water pH range from 7 to 11 was suitable for growth of both species, while a pH below or above this range caused significant decrease in growth. It was noticed that at pH 11 there was optimal growth for both *M. aeruginosa* and *M. flos aquae* as represented by chlorophyll *a* biomass and cell density and it showed a highly significant value above the control (pH 7). The highest recorded chlorophyll *a* was 98% for *M. aeruginosa* at pH 11 more than at control. While for *M. flos aquae* was 38% only. So, *M. aeruginosa* was more sensitive toward the changes of pH of the medium. Similarly at pH 12 the growth of *M. aeruginosa* dropped severely by 31.4% more than for *M. flos aquae* as appeared by cells count.

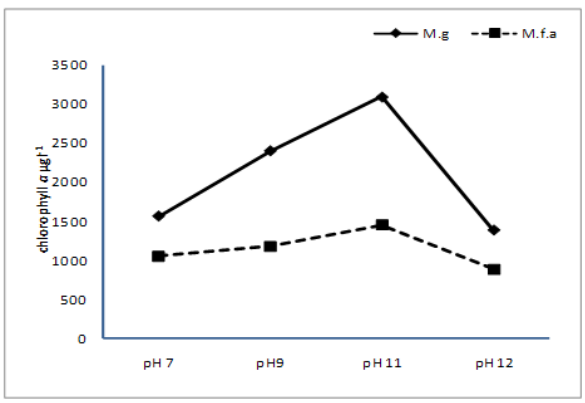


Fig. 5: chlorophyll *a* levels (µgl^{-1}) of *M. aeruginosa* and *M. flos aquae* cultivated at different pH.

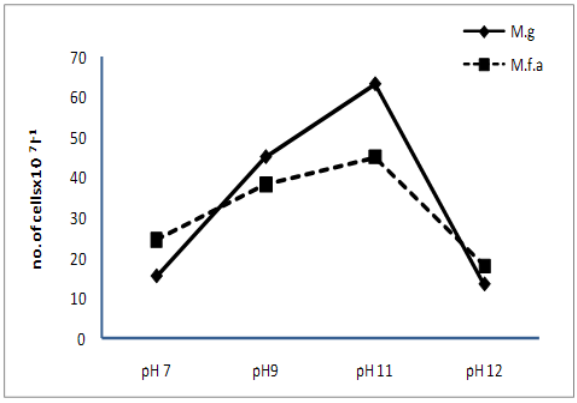


Fig. 6: Cell count (no. of cells x10 ^{7}l^{-1}) of *M. aeruginosa* and *M. flos aquae* cultivated at different pH.

The results of cell count confirmed those of chlorophyll a and show the same trend. The recorded data agree with Wei *et al*. (2001) who stated that the optimal growth of *M. aeruginosa* was found when water pH was between 7.5 and 9 in natural habitat. In fact this range was suitable for the best growth of *M. aeruginosa* and *M. flos aquae* in Wadi El-Raiyan Lake, while under laboratory conditions pH 11 was the best and highly significant.

It was noticed that, the recorded pH values below 7 or above 11 caused significant decrease in growth of these blue green algae. Also, the highest photosynthetic rates for *Oscillatoria rubescens* in natural populations were found between pH 6.5-8.5, while if the pH was less than 6 or greater than 9, the photosynthetic rate was 50% less than that of the optimal pH (Konopka, 1981). Wangwibulkit *et al*. (2008), reported that the filaments of *Oscillatoria* sp. were broken up into smaller filaments when water pH was lower than 6. This indicated that water pH influenced the growth of the blue-green algae, especially when the pH was higher than 9.0 or lower than 6.0. At these levels it could inhibit photosynthesis and adversely affect the morphology of blue green algae. Similar results were reported by Brock (1973) who observed that blue-green algae were not found in natural habitat with a pH less than 5.0.

The pH level was related to nutrients dissolution which caused a change in the species composition and biomass of the phytoplankton (Celekli and Kulkoyluoglu,

2007). At a high pH level, a consistent increase of cell division was differentially regulated in different species of phytoplankton (Alam *et al*., 2001).

4-Effects of salinity on growth cultures of *Microcystis aeruginosa* and *M. flos aquae*.

This experiment was an attempt to predict the response of the bloom-forming *M. aeruginosa* and *M. flos aquae* to the gradual increase of salinity.

The results in Figures (7& 8) show that growth of *M. flos aquae* was slightly higher over that of *M. aeruginosa* and both of them showed a gradual increase from the control (BG11 medium) till they reached the optimum growth at salinity 6ppt for *M. aeruginosa* by 59.5% and at 10ppt for *M. flos aquae* by 92.3% in their chlorophyll *a* contents. In fact, values at salinity 6ppt & 10ppt were highly significant compared with the control of the culture growth of *M. aeruginosa* and *M. flos aquae* respectively. But at these highly optimum culture growths the chlorophyll *a* content per cell was decreased by 62.8% for *M. aeruginosa* and 36.1% for *M. flos aquae.* So, it can be concluded that *M. aeruginosa* has more sensitivity for salinity than *M. flos aquae*. Also, at 10 ppt *M. flos aquae* culture was still good and alive while *M. aeruginosa* culture showed a remarkable decrease in growth.

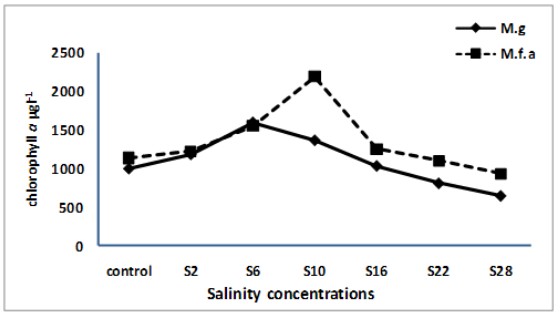


Fig. 7: chlorophyll a levels (µgl^{-1}) of *M. aeruginosa* and *M. flos aquae* cultivated at different salinities

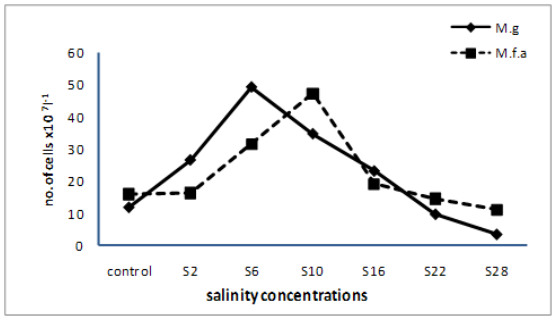


Fig. 8: Cell count (no. of cells x10 ^{7}l^{-1}) of *Microcystis aeruginosa* and *M. flos aquae* cultivated at different salinities

Soeder *et al*. (1967) pointed out that the growth of *Chlorella* cells in media of high salt concentration is more intensely inhibited than is the synthesis of biomass. Chlorophyll *a* of the test algae tended to resist NaCl concentration of 0.2 ppt, where at high doses remarkable decrease in chlorophyll *a* occurred. This is probably because of the interference of high NaCl concentration with the absorption of Mg cation thus impeding its role in chlorophyll formation and other magnesium- dependent metabolic pathways particularly enzyme activity. The chlorophyll content of *Dunaliella* is inhibited at high salinity (Mironyuk and Einor, 1968).

In fact, cell density values of both species confirmed the same results as chlorophyll *a* biomass. The gradual response of the two species to different levels of salinity confirmed the observation of Harding and Paxton (2001), that cyanobacterial genera exhibit a wide range of tolerance to salinity.

The results of this study are in line with those of Wangwibulkit *et al*. (2008) when optimal salinity levels for growth under laboratory conditions ranged from 0 to 10 ppt for *Oscillatoria* sp., and from 0 to 6ppt for *Microcystis* sp. An increase in salinity led to a decreased growth of both species. Also, Liu (2006) recorded that, the tolerance level of the species to salinity is between 0ppt and 10ppt, and as the average salinity of an open estuary ranges between 20ppt to 30ppt, the algal growth would be most likely inhibited and cell lyses would occur. Under microscopic observation of the cultures the same author indicated that cell lyses had occurred in the 10ppt, 15ppt

and 20ppt treatments. There was an indirect relationship (p less than 0.01) between salinity and growth of *M. aeruginosa* cultures.

In the 10-20ppt treatments cell lyses occurred as soon as the cultures were exposed to salinity, along with toxin release. The toxic level was about the same for all treatment because the cell either immediately lyses to release toxin upon reaching a higher salinity, or they were disrupted in the first place. Salinity influences the physiology of blue green algae and could disrupt ion balance or induce nutrients deficiencies (Konopka, 1981).

A decline in photosynthesis under hyperosmotic stresses could change the fine structure of the chloroplasts causing a disruption of energy transfer between the two photo systems (Konopka, 1981).

According the hypothesis of Liu (2006), if the growth rate and toxin production rate of the harmful algal species *M. aeruginosa* depend on salinity tolerance, then salinity would have an indirect relationship with growth and toxin production. Then, it is important to understand the salinity tolerance because the change in concentration of salt in a water system would be a potential way to control the growth of *M. aeruginosa*.

5-Effect of nitrogen and phosphorus (N/P) ratio on the growth of *Microcystis aeruginosa* and *M. flos aquae*.

Nitrogen and phosphorus are the nutrients typically implicated in toxic bloom of cyanophytes. Therefore, experiments were conducted to evaluate the performance of *M. aeruginosa* and *M. flos aquae* when these nutrients were altered in the standard culture modified BG11 medium.

5-1: Different levels of N/ P with constant ratio

In these experiments, cells were cultured in BG11 medium with altered concentrations of nitrogen and phosphorus with constant (N/ P) ratio either by increasing or by reducing. The results are illustrated in Figures (9&10).

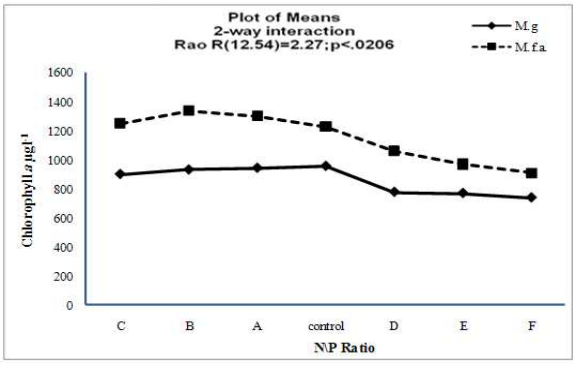


Fig. 9: Chlorophyll *a* of *Microcystis aeruginosa* & *M. flos* aquae related to variable levels of N&P with constant ratio.

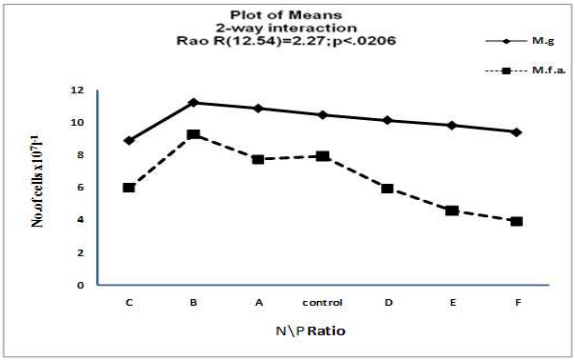


Fig. 10: Cell count of *Microcystis aeruginosa* & *M.* flos aquae related to variable levels of N & Pwith constant ratio.

Chlorophyll *a* contents of the culture of *M. flos aquae* showed a pronounced increase with increasing concentrations of nutrients as represented by (N\P) ratio than that of the control (BG11) by 6%, 9% and 1.8% at A, B & C concentrations respectively. At C concentration the growth begins to decrease, but is still higher than the control. While at (D, E & F) concentrations the growth culture and their chlorophyll *a* contents tended to decrease gradually. Chlorophyll *a* values of (N\P) ratio of *M. aeruginosa* were, more or less, decreased than the control in all treatments (A, B, C, D, E &F). Overall, the control showed highly significant values with all N\P concentrations in both of *M. aeruginosa* & *M. flos aquae*. Also, the (N\P) ratio at the

concentration (D) showed a highly significant value with the control and other concentrations in case of *M. flos aquae* and showed no significant values except with the control in case of *M. aeruginosa*. The concentrations (A, B &C) showed a significant value with other concentrations including the control in case of *M. aeruginosa* and low significance in case of *M. flos aquae.*

Cell count values of both growth cultures of *M. aeruginosa &M. flos aquae* showed approximately the same trend as their chlorophyll *a* contents in all treatments. Cell count of *M. flos aquae* showed a highly significant value with the concentrations (A, B & D) while cell count of *M. aeruginosa* showed no significant values with all the concentrations.

Low nitrogen to phosphorus ratio has also been observed to favour cyanobacteria blooms (Metting and Pyne, 1986; Kaebernick *et al*., 2001). Liu (2006) stated that the N: P ratio is one of the most important factors that affect the growth rate and toxin concentration of the algal species, as the amount of nitrogen in the aquatic system displayed a positive relationship with the toxin production and growth, and the amount of phosphorus displayed a negative one. Investigation of growth and toxin production rates in field blooms and culture trials demonstrated that cyanobacterial dominance and high densities of *M. aeruginosa* were associated with low nitrogen to phosphorus (N: P) ratios (Jacoby, 2000). Downing *et al.* (2005) noted that within a range of moderate N: P atomic ratios, two strains of *M. aeruginosa*, PCC 7806 and UV027 demonstrated a positive and direct relationship between protein concentrations, microcystin production, and growth rate.

5-2: Constant phosphorus concentration with variable nitrogen values.

To explore the effect of phosphorus and nitrogen on the two *Microcystis* species under specific laboratory conditions, the tested organisms were implicatively cultured in the BG11 medium with constant standard phosphorus level and variable nitrogen concentrations. The data are recorded in Figures (11&12).Values of chlorophyll *a* contents at concentrations (A&B) increased above the control by (10.12% &14.13%) and (17.58% & 30.6) for *M. aeruginosa* and *M. flos aquae* respectively.

The two species showed significant values at concentrations (A &B). In other words, when the concentrations of nitrogen were lower than (B conc.) the chlorophyll *a* contents increased with the nitrogen concentration. But when the concentrations of nitrogen were at (C conc.) the chlorophyll *a* contents significantly decreased. In general, *M. flos aquae* response was more strongly affected than *M. aeruginosa* under these specific laboratory conditions. It was noticed that, the concentration (B) showed a significant value with the cell counts of the two species.

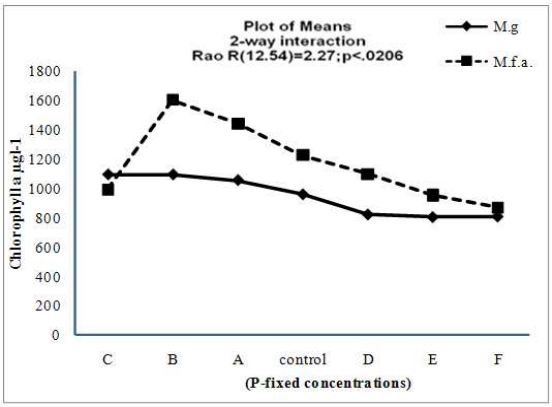


Fig. 11: Chlorophyll *a* of *M. aeruginosa* & *M. flos aquae* related to constant P and variable N level.

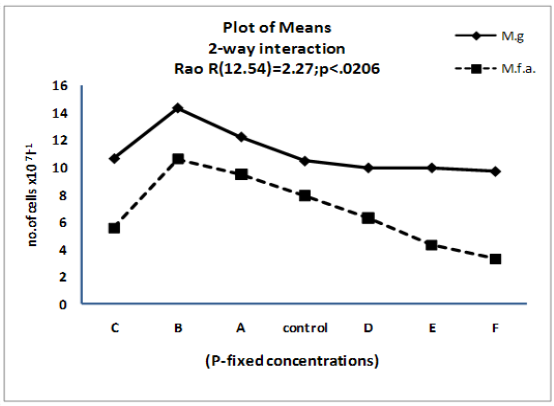


Fig. 12: Cell count of *M. aeruginosa* & *M. flos aquae* related to constant P and variable N level.

The growth of the species might also be linked to the toxin production rate. Accordingly, the growth of *M. aeruginosa* was reduced under a P limitation due to a low C fixation rate, whereas the MC content was higher. Consequently, increases in the MC content per dry weight along with the production of the more toxic form, MC-LR, were observed under more P-limited conditions (Oh *et al*., 2000).

The high nitrogen sources entered into Wadi El-Raiyan Lake may be the reason for the blooming of the tested microalgae. This hypothesis was confirmed by Yoshida *et al*. (2007) who suggested that high nitrate loading may be a significant factor promoting the growth of the microcystin subpopulations within *M. aeruginosa* communities in Lake Mikata. When surface nitrate concentrations increased, there was a rise in the relative abundance of the mcyA subpopulation. This was a positive correlation with the nitrate concentrations whereas temperature and ortho-phosphate had no significant correlation with the presence of mcyA.

5-3: Constant nitrogen concentration with variable phosphorus values.

In these experiments, changes in the total *Microcystis* biomass (as represented by chlorophyll *a* and algal cell count) were investigated at a different N/P ratio from the BG11 medium with constant standard nitrogen level and variable phosphorus concentrations. The results are recorded in Figures (13&14). Chlorophyll *a* concentrations and cell counts of *M. aeruginosa* showed, more or less, slightly higher values at (A conc.) compared to the control by 3.9% and 9.5% respectively. While with gradual decrease in P level there were no effects.

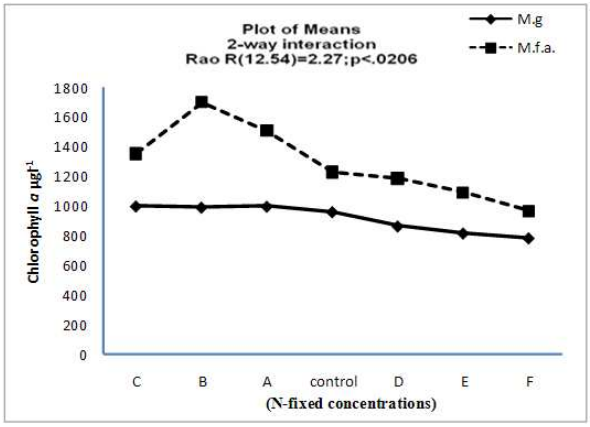


Fig. 13: Chlorophyll *a* of *M. aeruginosa* & *M. flos aquae* related to constant N and variable P level.

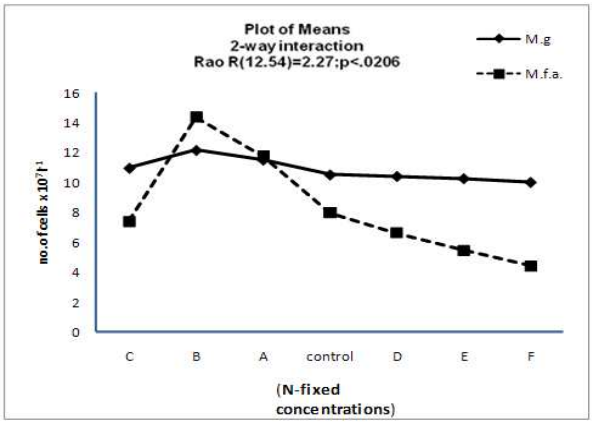


Fig. 14: Cell count of *M. aeruginosa* & *M. flos aquae* related to constant Nand variable P level.

On the other hand, chlorophyll *a* of *M. flos aquae* increased by 23% and 38.5% at A and B concentrations respectively. Also, cell counts showed higher values, 47.9% and 80.9% respectively. Then it followed by noticed inhibition at the concentration (C). On the contrary, there were positive correlations between the decrease in P level and algal biomass.

It was clearly noticed that, constant nitrogen concentrations and variation in phosphorus level either by increasing or decreasing showed highly significant chlorophyll *a* values in both of *Microcystis aeruginosa & M. flos aquae* with few exceptions. While the cell count showed a different trend, since it showed non homogenous pattern of significance. Decreasing the phosphorus level showed a significant value with the cell count of *Microcystis aeruginosa* and sometimes with *M. flos aquae*.

The results of the bioassay experiments are in perfect agreement with the results of the field observations in Wadi El-Raiyan Lake where the growth of *M. aeruginosa* decreased under phosphorus limitation conditions and the bloom was composed mainly of *M. flos aquae* which in turn affect the microcystin producing rate of *M. aeruginosa*. This situation appears clearly, when the toxin was detected only at the beginning of the blooming period while it was not detected from week to another till the end of the blooming period.

The results of the present study go in parallel with those of Kotak *et al*. (1995), when the author noticed that, more P in the culture medium stimulates the growth and microcystin production of *M. aeruginosa* this finding can be extrapolated to *M. aeruginosa* growing under bloom conditions. That is, the reduction of P in eutrophic waters may lower the growth and microcystin-producing rate of *M. aeruginosa*, resulting in the reduction of toxicity.

CONCLUSIONS

In conclusion, the results obtained from this investigation indicate that, there are an inhibitory effect on the growth and survival of *Microcystis* species of Wadi El-Raiyan Upper Lake with increasing in salinity. So, it would be a way to inhibit the bloom. Water pH level from 7-11 were suitable for growth of both species , while a pH lower or higher than this range was associated with a significantly decreased growth of these blue-green algae. Nutrients especially (N&P) implicated greatly in the blooming of algae in the lake so, this study recommended that the water entered the lake through El-Wadi Drain must be increased in volume and adjusted to specific treatment also certain management to the connecting canal must be done in order to increase the water flow to avoid the toxic effects of the bloom forming species affected deeply on the economic fishes inhabiting the lake leading to their death.

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ARABIC SUMMARY

العوامل المحتملة التأثير علي نمو *Microcystis aeruginosa* and *M. flos aquae* في البحيرة العليا لوادي الريان (مصر) تحت ظروف مختبرية

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تم إجراء تجارب معملية لتحديد تأثيربعض العوامل البيئية المسببة لظاهرة الإزدهار الطحلبي في البحيرة العليا لوادي الريان. تم عزل النوعين المشكلين للإزدهار الطحلبي وتحديداً *Microcystis aeruginosa* and *Microcystis flos aquae* وتنقيتهما وزراعتهما في بيئة مناسبة BG11 تحت ظروف مختبرية من أجل التنبؤ بسلوك الطحالب التي تم إختبارها تحت ظروف مختلفة من النظام البيئي. أوضحت النتائج عن طريق قياس كلوروفيل أ وعد الخلايا تحت المجهر أن فترة التعرض للضوء والظلام لـ 14:10 ساعة كانت الأفضل لنمو كلا النوعين عن التعرض لهما لـ 12: 12 ساعة.

أثبتت النتائج أن نمو الطحلبين كانت أعلى بشكل ملموس في شدة الضوء العالية مقارنة مع شدة الضوء المنخفض. كما كانت هناك علاقة عكسية بين نمو الطحالب والملوحة فكلما زادت درجة الملوحة يقل النمو الطحلبي والعكس صحيح. وتجدر الإشارة إلى أن النوع *M. aeruginosa* كان أكثر حساسية للملوحة من النوع *M. flos aquae* بينما كان الأس الهيدروجيني للماء من 7- 11 مناسبا لنمو كلا النوعين من الطحالب.ولكن التركيزات الأعلي أو الأقل تؤدي الى نقص ملحوظ في نمو الطحالب المسببة للإزدهار. كما ان زيادة مصادر النيتروجين التي تدخل البحيرة ربما تكون أحد أهم أسباب ظاهرة إزدهار الطحالب في البحيرة العليا لوادي الريان. إن التأثير السام للإزدهار الطحلبي يؤدي إلى موت الأسماك الإقتصادية في البحيرة.