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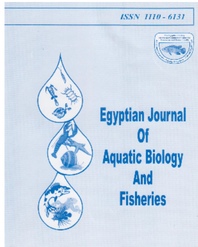

Phytoplankton blooming in Lake Qarun in relation to chlorophyll *a* measured by fluorometric and spectrophotometric techniques

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ABSTRACT
Lake Qarun is a closed saline lake in the northern part of El- Fayoum Depression of Egypt. The objective of this work is to give a complete picture of lake biotic factors to alarm on dinoflagellates blooming phenomenon and to compare between chlorophyll *a* values measured by Fluorometer and Spectrophotometer. Monthly patterns in Chlorophyll *a* concentrations were measured during the period from May 2015 to April 2016, using two different methods; by fluorometric and spectrophotometric techniques. Phytoplankton species in Lake Qarun are heterogeneously distributed all over the year; dinoflagellates were the main dominant group, chlorophyll *a* frequently accumulated as distinct peak (336 $\mu g l^{-1}$) in July 2015. The phenomenon of dinoflagellated blooming of *Prorocentrum micans, Gymnodinium lantzschii* and *Exuviaell aapora* that turning the lake water to brown in colour, is recorded annually. The two methods used in detection of chlorophyll *a* during this work are accurate and simplified bring the picture in Lake Qarun. Actually, results of fluorometer are more sensitive and higher than those of spectrophotometer. In fact, high salinity, agricultural drainage water from drains and aquaculture activities turned the lake to variable, sensitive aquatic ecosystem, affecting significantly on phytoplankton structure and blooming dynamics determination. There is no record of toxicity effects or mass fish mortality in the lake due to this phenomenon.

INTRODUCTION

Lake Qarun is a closed saline basin among the inland water lakes of Egypt, originated from the old lake Moreis (freshwater Lake). It is located in the western desert in El-Fayoum depression, 90 Km southwest of Cairo.

Eutrophications of estuarine and coastal waters are increasing, and so it must developed rapidly detectable indicators of these changes. Phytoplankton species are good indicators of ecological changes. They are easily detected; their identification and quantification are relatively easy. They conduct a great share of primary production and they are sensitive to diverse ecological stressors (Paerl *et al*., 2007).

The lake receives agricultural waste water from cultivated lands (Abdel-Satar *et al*., 2003). Also high load of nutrients salts from aquaculture in the southern part of the lake. The high evaporation rate of water (16.78 cm month $^{-1)}$, gradual increase of its salinity, trace metals and pollution affect greatly on its water quality and on its food web including phytoplankton, zooplankton and fish.Therefore, physico-chemical and biological parameters affect deeply on supereutrophic lake Qarun (Abdel-Monem and Konswa, 2001).

The change in composition and biomass of phytoplankton, were recorded as a result of increasing salinity, noting the complete disappearance of some species nevertheless, some others were able to withstand the high salinity. Some studies deal with phytoplankton composition and biomass in Lake Qarun; (El-awamri, 1982) identified 173 benthic diatoms taxa near the estuaries drains, 156 taxa away from the estuaries drains and 47 phytoplankton taxa in the water of Lake Qarun. ((Abdel-Moniem, 1991) identified 119 taxa where he recorded that the phytoplankton community inhabiting the Lake is mainely formed of Bacillariophyceae (64%) followed by Dinoflagellates (27%) whereas (Kobbia *et al*., 1992) recorded 64 taxa and 50 taxa were recorded in a seasonal investigation by (Abdel-Moniem, 2001). (Fathi *et al*., 2001) studied the phytoplankton communities of Lake Qarun among group of North African Lakes. They reported that the phytoplankton communities of most of these lakes are from Mediterranean origin. (Fathi and Flower, 2005) recorded 49 phytoplankton species, Bacillariophyceae dominated the phytoplankton (between 80% - 94%), The lake blooming of dinoflagellates mainly occur in winter & spring seasons (Konsowa, 2007); (Abou El-Gheit *et al*., 2012). (Abd El-Karim, 2012)identified 197 species, diatoms formed 89 species and 26 species from dinoflagellates. (Hussian *et al.*, 2014) identified 92 microphytobenthic species the Bacillariophyceae were most diverse with 63 species, comprised 71.4% of all recorded taxa, the species of *Melosira granulate* and *Melosira varians* were common and abundant in summer and winter season. So, phytoplankton in Lake Qarun is mainly formed of diatoms and dinoflagellates despite the variation in composition and percentage abundance .The highest density of phytoplankton and the peak of chlorophyll *a* content were recorded in the eastern site of the lake(Napiórkowska-Krzebietke *et al*., 2016).

The most dramatic effect of dinoflagellates on aquatic environment occurs during the warmer season, usually mid to late summer, which may colour the water golden or red, and this is called a "red tide". Blooming of algae in Lake Qarun is a phenomenon need extensive study in order to give data base for optimal condition for algal blooming in the lake, although there is no record of its toxicity or fish mortality.

The pattern that become apparent is the acceleration of changes in Lake Qarun ecosystem, especially its physico-chemical and biological characters; is due to climate change, massive agricultural drainage water, caused high variability in the main nutrients concentrations (Shadrin *et al*., 2016). These patterns are clearly observed all over the world (Shadrin and Anufriieva, 2013).

Chlorophyll *a* are simple method to discuss the response of increased nutrient concentrations, fluctuation in climate conditions, high salinity on perturbation in Lake Qarun, Egypt , and this is the question of this study which aims to give complete picture of lake biotic factors for twelve successive months and to alarm on the blooming phenomenon which recorded for several years ago in Lake Qarun.The study also aim to compare between chlorophyll *a* values measured by Fluorometer and those measured by Spectrophotometer.

MATERIALS AND METHODS

Area of the study

Lake Qarun is a closed saline basin at El-Fayoum depression located between longitudes 30˚ 34" and 30˚ 49" E and latitudes 29˚ 25" and 29˚ 34" N. The lake has elongated rectangular shape (its length is about 45 Km from east to west with about 6.7 Km mean width. It covers an area of about 243 Km^2 and with a total water volume of 924 million m^3. At the middle region of the lake, there is a sandy island known as El- Qarn which has an area of about 2.3 Km^2. The water level of the lake fluctuates between 44 and 45 m below sea level. The lake is relatively shallow, the shallowest region lies at the eastern most part of the lake near El- Bats Drain and its average depth is about 4 m. The deepest region of the lake lies at the northwest of El-Qarn Island (8.2 meters). The northern shore of the lake is surrounded by desert as it marks the beginning of the Western Desert of Egypt. The southern shore is surrounded by agriculture lands which are naturally irrigated by Nile water through Bahr Youssef Canal. The lake evaporation rate ranged from 4.1cm month $^{-1}$ to 28.4 cm month $^{-1}$ with an annual average of 16.78 cm month $^{-1}$(Ibrahim, 1998).

During the present study, four stations were selected East, middle, west and in front of NIOF station at El-Fayoum , this selection was to cover the area of study in order to give complete picture of its biotic factors for twelve successive months.

Sampling program

Sampling program was carried out on monthly basis from May 2015 to April 2016 (twelve successive months). Intensive sampling of biotic factors was concentrated on four main sites, east, west, middle and in front of National Institute of Oceanography and Fisheries (NIOF) station to represent different habitats of the lake Figure (1).

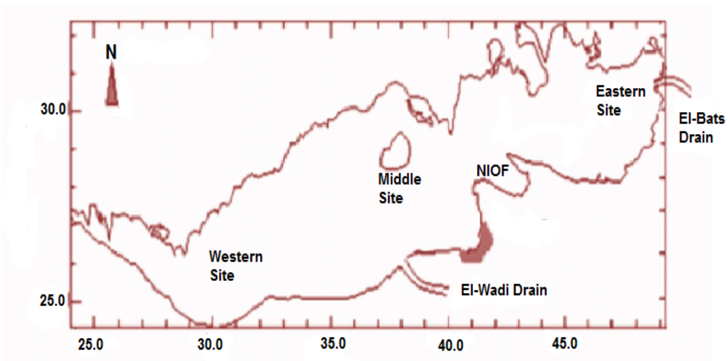

Fig. 1: A map of Lake Qarun showing the sampling locations

Chlorophyll *a* determination

A known volume of water samples was filtered in situ on glass microfiber filter GF/F, using filtration unit (Sartorius). The filter paper containing the filtrate was foiled in aluminum foil and preserved in dark ice box. In laboratory, chlorophyll *a* was measured by two different methods in order to differentiate between the accuracy of both of them.

By using spectrophotometer, chlorophyll *a* was extracted by socking the filter in 5 ml acetone (90%) and preserved in dark at 20ºC overnight. The samples were shacked well and centrifuged; the clear acetone extract was siphoned carefully then measured with spectrophotometer using 90% acetone as blank. Chlorophyll *a* was estimated using Kontron 930 UV visible Spectrophotometer. The concentrations of chlorophyll *a* were calculated according to the trichromatic equation(Apha, 1995).

By using fluorometer, chlorophyll *a* was measured according to the method developed by Dr. Nicholas A. weischmeyer of Moss Landing Marine Laboratories, flurometric analysis of chlorophyll *a* in the presence of chlorophyll b and pheopigments, (1994).Chlorophyll *a* estimated using digital fluorometermodel 10-AU equipped with 1.13mm cuvette holder; 2 optical filter kit PN10-040 or 10-040R (Apha, 1995).

Statistical analysis of data

Canonical Correspondence Analysis (CCA)

The data of Chlorophyll and physico-chemical variables were drawn up in the form of one matrix and were analyzed by canonical correspondence analysis (CCA) using Brodgar Program, version 2.4.8 (Highland Statistics, 2005). CCA is an unlinear technique used to relate variation in biotic properties to measured variation of the environment. The constrained ordination axes correspond to the directions of the greatest variability of thedata set that can be explained by the variables (Lepš and Šmilauer, 1999), XL State 2001 program was used to analyze correlation and regression analysis.

Data of physico-chemical and biological parameters used in statistics cited from (Mosad, 2018).

RESULTS

During the present study at Qarun Lake, four stations were selected east, middle, west and in front of NIOF station at El-Fayoum , this selection aims to compare between chlorophyll *a* values measured by Fluorometer and those measured by Spectrophotometer. Also, the study give special interest to the dinoflagellates blooming dynamics phenomenon observed during different months in Lake Qarun.

Chlorophyll *a* concentrations were determined on monthly basis from May 2015 to April 2016. Chlorophyll *a* measured by fluorometer were heterogeneously distributed attained its lowest values (8.82 μgl^{-1}) at the middle station (Figure 3) of the lake during March 2016.While, chlorophyll *a* frequently accumulated as distinct peaks (336 μgl^{-1}) in front of NIOF station (Figure 5) during July 2015.

At the east station of the lake (Figure 2) chlorophyll *a* values ranged from17.6 μgl^{-1}in October 2015 to187.8 μgl^{-1} in March2016.

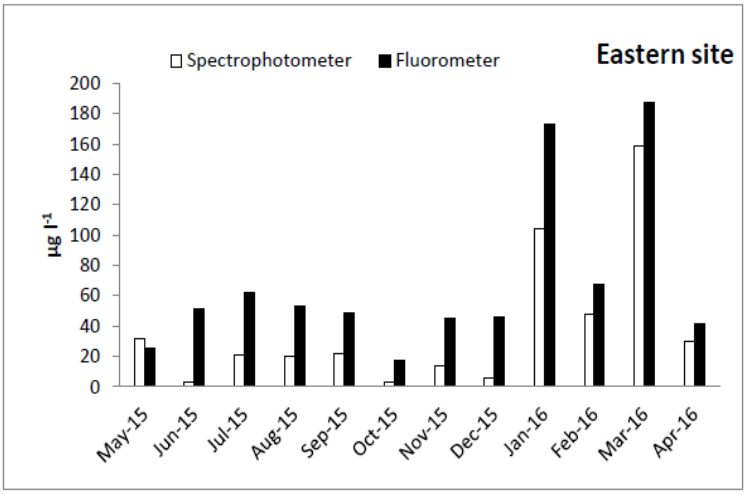

Fig. 2: Chlorophyll "*a*" Concentrations (μgl^{-1}) at the Eastern site of Lake Qarun.

The middle of the lake attained its highest value (77.6 μgl^{-1}) in August while the lowest value was (8.82 μgl^{-1}) in March. Chlorophyll *a* concentrations in the west station (Figure 4) of the lake varied from 9.54 μgl^{-1}to39.8 μgl^{-1} during October and March respectively. With regard to the NIOF station (Figure 5) chlorophyll *a* recorded a minimum of 9.72 μgl^{-1} in March and maximum of 336μgl^{-1} in July. On the other hand, chlorophyll *a* was measured by spectrophotometer at the same stations of lake, at the east station (Figure 2) chlorophyll *a* showed two major peaks during January and March (172.9 &158.4 μgl^{-1}) respectively, while the lowest value of (3.1 μgl^{-1}) was recorded in October.

Chlorophyll *a* at the middle of the Lake (Figure 3) ranged from (0.3to125.9 μgl^{-1}) during July & August respectively. Values of chlorophyll *a* in west of the lake (Figure 4) attained its lowest value (0.8 μgl^{-1}) during July while the highest one (40.6 μgl^{-1}) recorded in August. With regard to the NIOF station (Figure 5) chlorophyll *a* recorded a minimum of 2.3μgl^{-1} in October and maximum of 111.9μgl^{-1} in February.

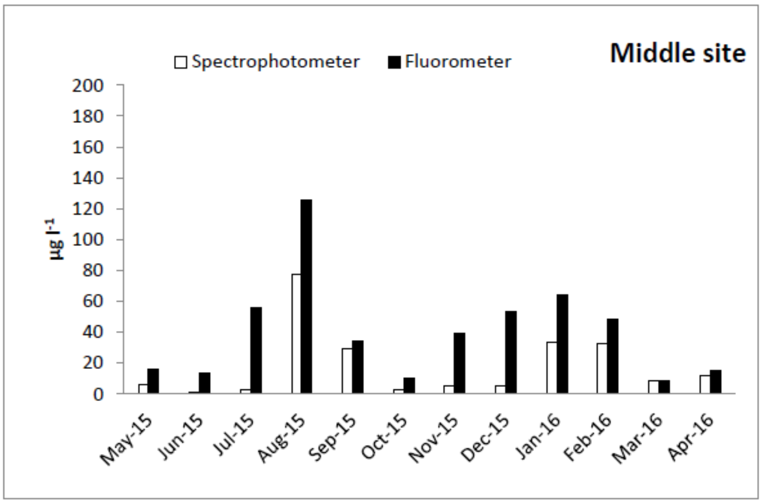

Fig. 3: Chlorophyll " *a*" Concentrations (μgl^{-1}) at the Middle site of Lake Qarun.

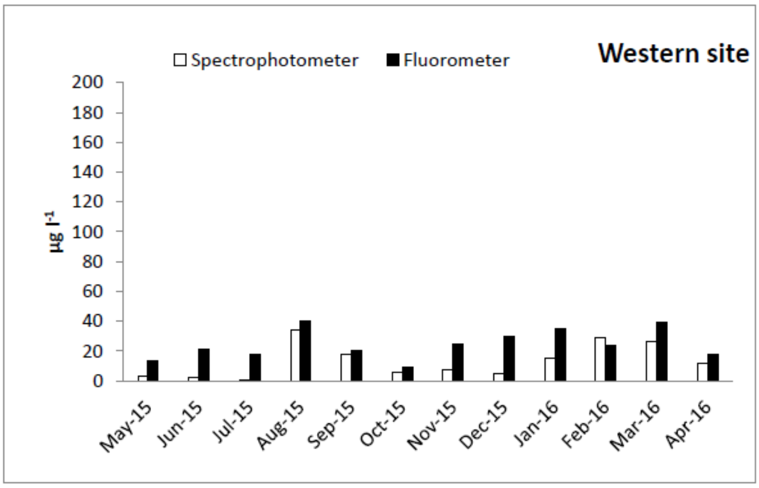

Fig. 4: Chlorophyll " *a*" Concentrations (μgl^{-1}) at the Western site of Lake Qarun.

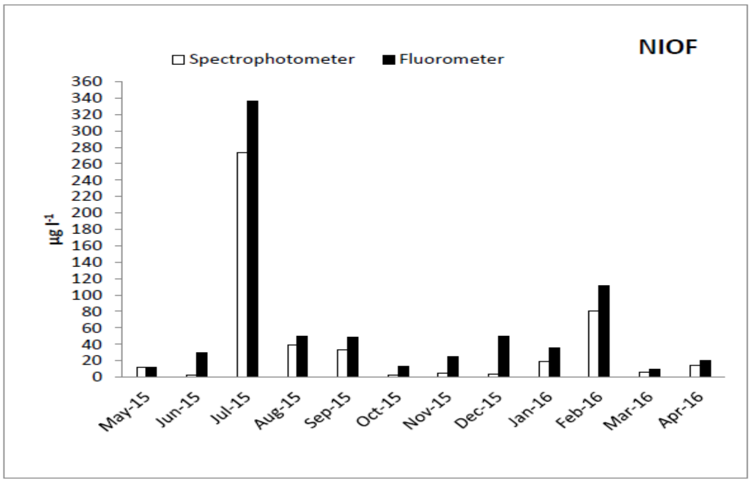

Fig. 5: Chlorophyll " *a*" Concentrations (µgl^{-1}) at the NIOF site of Lake Qarun.

Generally, chlorophyll *a* values detected by the Flourometer were higher than those measured by Spectrophotometer, in order to compare between the two techniques used for chlorophyll determination.

The relation between Chlorophyll *a*, b &c and the bloom forming class of phytoplankton (Dinoflagellates) with some physico-chemical parameters at Qarun Lake:

During July when the bloom forming species of *Gymnodinium lantzschii* noticed Canonical Correspondence Analysis (CCA) is carried out for analyzing 10environmental variables and 7 biotic variables as shown in (Figure 6)

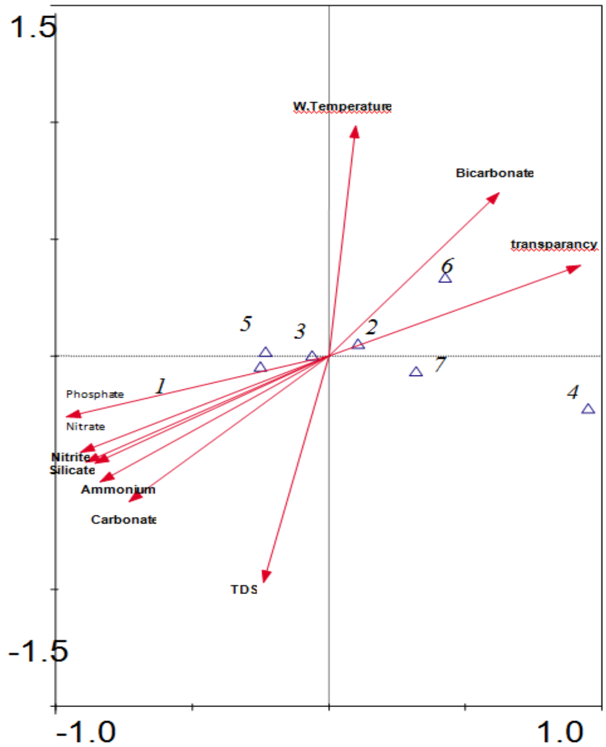

Fig. 6: Ordination a biplot diagram based on canonical correspondence analysis (CCA) of chlorophyll and the most common phytoplankton classes with environmental variables at Qarun Lake during July.

Sum of all canonical eigenvalues =0.135

Key :1 Chlorophyll *a* measured by spectrophotometer, 2 Chlorophyll *a* measured by Fluorometer, 3 Chlorophyll b ,4 Chlorophyll c, 5 Dinophyceae, 6 Cyanophyceae, 7 Bacillariophyceae

The CCA biplot diagram showed a positive correlation between NH_4, NO_2, NO_3, PO_4, SiO_3, CO_3 and TDS with total dinoflagellates and chlorophyll *a* ,indicating the bloom forming species affected deeply by these parameters.

Typically, the chlorophyll *a* peaks were coincided with enough growth limiting nutrients especially, ammonia, nitrite, nitrate and phosphate.

During January, Canonical Correspondence Analysis (CCA) is carried out for analyzing 10 environmental variables and 7 biotic variables as shown in (Figure 7). At the east station of Qarun Lake there were two major peaks where diatoms and dinoflagellates were flourishing and *Gymnodinium lantzschii Prorocentrum micans*, *Exuviaella apora Cyclotella. meneghiniana*, *C. operculata*, C. glomerata and *Nitzschi. acicularis* are the most common species.CCA indicated that, the most important factors affecting on phytoplankton species are alkalinity, water temperature, silicate and transparency.

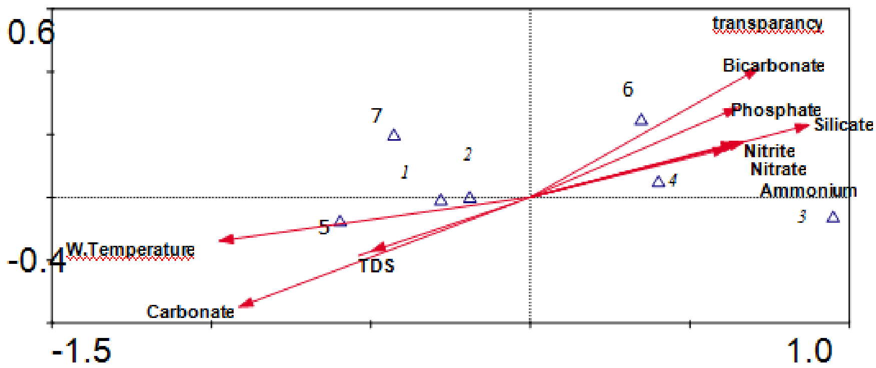

Fig. 7: Ordination a biplot diagram based on canonical correspondence analysis (CCA) of chlorophyll and the most common phytoplankton classes with environmental variables at Qarun Lake during January.
Sum of all canonical eigenvalues =0.152
Key : 1 Chlorophyll *a* measured by spectrophotometer, 2 Chlorophyll *a* measured by Fluorometer, 3 Chlorophyll b 4 Chlorophyll c, 5 Dinophyceae, 6 Cyanophyceae, 7 Bacillariophyceae

During March, Canonical Correspondence Analysis (CCA) is carried out for analyzing 10 environmental variables and 7 biotic variables as shown in (Figure 8).

Another peak in values of chlorophyll *a* was detected due to high flourishing of dinflagellates at the east station of the lake. CCA biplot diagram indicated the most important factors affecting on phytoplankton species were NH_4, NO_2, NO_3, DO and bicarbonate alkalinity. While carbonate alkalinity, silicate, phosphate and water temperature have no effect on phytoplankton flourishing during this month.

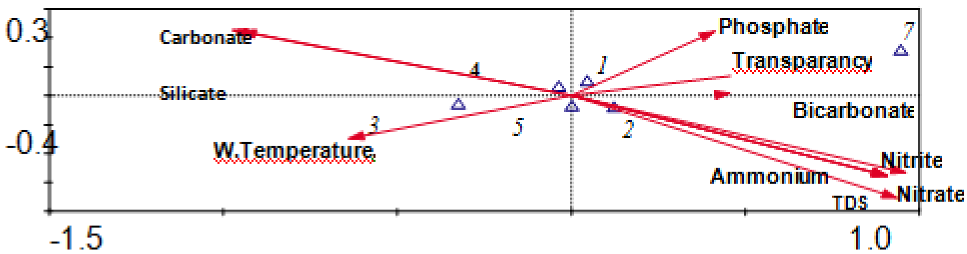

Fig. 8: Ordination a biplot diagram based on canonical correspondence analysis (CCA) of chlorophyll and the most common phytoplankton classes with environmental variables at Qarun Lake during March.
Sum of all canonical eigenvalues =0.0223
Key :1 Chlorophyll *a* measured by spectrophotometer, 2 Chlorophyll *a* measured by Fluorometer, 3 Chlorophyll b 4 Chlorophyll c, 5 Dinophyceae, 6 Cyanophyceae, 7 Bacillariophyceae

DISCUSSION

Assessment and monitoring of lakes water quality to study the ecosystem biotic, a biotic variables and pollution effect are basic factors in aquatic ecosystems. Generally, as shown from chlorophyll *a* data, Lake Qarun can be designed as supereutrophic lake, it was subjected to different kinds of pollutions including sewage, agricultural and aquaculture drainage water.

The maximum distinct peak of chlorophyll *a* (336 μgl^{-1}) was recorded in July; this highest value was attributed to the blooming of *Gymnodinium lantzschii*as observed from phytoplankton samples collected from the same sites and the same period. Ibrahim *et al*., 1998 mentioned that the maximum chlorophyll *a* values in July. Actually the reason for algal blooming in the lake are not completely known but it may be related to the high amount of agricultural, aquaculture drainage and sewage wastes enter the lake via the drains (Zaher, 2012).

At the eastern station of the lake chlorophyll *a* maximum value of 187.8μgl^{-1} recorded in March was due to the high flourishing of *Exuviaella apora* and *Prorocentrum micans* which are armoured, marine, planktonic, bloom-forming dinoflagellates. Several studies recorded dinoflagellate blooming of phytoplankton in the lake (Ibrahim, 1998, Hussian *et al*., 2014, Abd El-Karim, 2012, Fathi and Flower, 2005 and Napiórkowska-Krzebietke *et al*., 2016).

A bloom of these dinoflagellates result in a visible coloration of lake water colloquially known as red tide, but no record of shellfish poisoning in the lake. Actually the bloom forming species in the lake are usually considered harmless (Granéli *et al*., 1990).It might excrete substances which inhibit diatoms, but apparently these substances do not affect zooplanktons and fish at higher trophic levels(Uchida, 1981). The toxicity of *P. micans* need confirm and only few reports recorded problems of shellfish kills, in South Africa (Horstman, 1981) .

The distribution of chlorophyll *a* concentrations is deeply influenced by the interacting effects of waste water discharge, nutrient availability, and the morphometry of the aquatic ecosystem. Input of nutrients and simultaneously influences the accumulation and transport of biomass through variable flushing rates (Paerl *et al*., 2007).

During the present study there were strong negative correlation between chlorophyll *a* values and transparency values in all months and the highest value was (r= -0.8) in the blooming period, except in August and September when the positive correlation appeared (r=0.95, 0.66).The gradual decrease in transparency values in the lake during years indicates the eutrophication development (Shadrin *et al*., 2016) due to the agricultural and sewage drainage water as well as the waste of fish farms arises around the lake. As expected, the blooming period of phytoplankton coincided with the maximum ratio of chlorophyll *a* in July, the transparency were low (18cm). Transparency value at this month showed a negative correlation with Dinoflagellates. (Fathi and Flower, 2005) recorded only 5cm transparency values during the blooming months when phytoplankton standing crops reached peak biomass in Qarun Lake. Transparency of water was affected by phytoplankton blooms (Kobbia *et al*., 1992); (Mansour and Sidky, 2003); (Abdel-Satar *et al*., 2010).

Strong negative correlation appeared between chlorophyll *a* and the main nutrients (NH_4- NO_2-NO_3-PO_4 -HCO_3) during September indicating the high amount of nutrients enter the lake via the major drains beside aquaculture activities.

Canonical Correspondence Analysis (CCA) is carried out during the three months representing the blooming period in the lake, the CCA biplot diagram during

July, showed a positive correlation between NH_4, NO_2,NO_3 ,PO_4,SiO_3,CO_3 and TDS with total dinoflagellates and chlorophyll *a*, indicating the bloom forming species affected deeply by these parameters.

Typically, the chlorophyll *a* peaks were coincided with enough growth limiting nutrients especially, ammonia, nitrite, nitrate and phosphate (Paerl *et al*., 2007) because the flourishing of phytoplankton forming the bloom is consuming the limiting nutrients, this coincided with (Pinckney *et al*., 1998); (Paerl *et al*., 1998) who attributed this to phytoplankton uptake. The idea that nitrate and phosphate are the primary limiting nutrient in lake Qarun was discussed by (Ibrahim, 1998),who attributed the increased phytoplankton growth to phosphate and nitrate (strong positive correlation) enter the lake from some external sources.

During January, another blooming phenomenon was observed with dominance of dinoflagellates and diatoms. CCA indicated that, the most important factors affecting on phytoplankton density are alkalinity, water temperature, silicate and transparency. The negative correlation between water temperature ,TDS and transparency was agree with those obtained by (Fathi and Flower, 2005).Also the negative correlation with silicate can explain abundance of diatoms (Konsowa, 2007). The dominant classes showed a positive correlation with water temperature and carbonate alkalinity and a negative correlation with the major nutrients, bicarbonate and transparency.

During March another peak in values of chlorophyll *a* was detected due to high flourishing of dinoflagellates at the east station of the lake. CCA biplot diagram indicated the most important factors affecting on phytoplankton species wereNH_4,NO_2, NO_3,DO and bicarbonate alkalinity, these relations indicated that the bloom resulted from the high amount of organic matter enter the lake via drains (Zaher, 2012) where agricultural drainage, sewage discharge and anthropogenic activities ,result in low biodiversity in the aquatic system especially plankton diversity (Khalifa *et al*., 2016). Also the analysis indicated that, these parameters showed high positive correlation with dinoflagellates. Higher values of nitrate-N recorded in the lake probably reflected the direct influence of agricultural runoff (Kobbia *et al.*, 1992). While lower values of nitrate-N occurred during enhanced growth of phytoplankton (Mansour and Sidky, 2003) but neither N or P appeared to be strongly depleted during the phytoplankton peak.

Actually, this study emphasis that Lake Qarun is supereutrophic lake, in addition the blooming occurred in the lake due to high amount of nutrients discharged into the lake from the two main drains (El-Bats and El-Wadi) beside other aquaculture activities around the lake. Although some blooming is due to dinoflagellates but there is no toxic effect on the inhabiting fish and there is no record for any fish mortality in the lake. On the other side, the two methods used in detection of chlorophyll *a* during this work are accurate and simplified bring the picture in Lake Qarun. The method used for detection of chlorophyll *a* will influence the results considerably (Nehring *et al*., 1969). Actually, fluorometer is more sensitive than spectrophotometer as the first reflect direct measurement of chlorophyll *a* without interference with chl. b and c , while spectrophotometer depend on the trichromatic equation causes transfusion with Chl. b and c.

CONCLUSION

Comparing the two techniques used for detection of chlorophyll *a*, it was proved that analyses with fluorometer was higher and sensitive than those of spectrophotometer. Chlorophyll *a* concentrations in the lake and the flourishing of some species during certain months indicating that phytoplankton in the lake are heterogeneously distributed. Although the regular occurrence of the bloom forming species in the lake but there is no record of its toxicity or fish mortality, but it may occur any time in the future at favorable condition especially with increased nutrients supply from drains and fish farms.

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ARABIC SUMMARY

العوالق النباتية المزهرة فى بحيرة قارون وعلاقتها ب كلوروفيل أ والتى تم قياسها بتقنيات الفلوروميتر وسبكتروفوتميتر

شيماء صبرى زاهر و عزت عواض ابراهيم

تقع بحيرة قارون فى شمال منخفض الفيوم ، مصروهى بحيرة مغلقة مالحة. تهدف هذه الدراسة الى إظهار صورة كاملة للعوامل البيولوجية فى البحيرة وإلقاء الضوء على ظاهرة الإزدهار الطحلبى من الدينوفلاجلات والمقارنة بين نتائج كلوروفيل أ بواسطة جهازى فلوروميتر و سبكتروفوتميتر.

تم قياس عنصر الكلوروفيل أ شهريا بدءا من مايو٢٠١٥ وحتى أبريل ٢٠١٦ بطريقتين مختلفتين بواسطةجهازى فلوروميتر و سبكتروفوتميتر.

يختلف توزيع العوالق النباتية فى بحيرة قارون طوال العام وتعد مجموعة دينوفيسى هى المجموعة السائدة من الطحالب. يكون أعلى مستوى من كلوروفيل أ فى شهر يوليو ٢٠١٥ حيث يبلغ(٣٣٦ ميكروجرام/ لتر). كما تولى الدراسة إهتماما خاصا لظاهرة الإزدهار الطحلبى من الدينوفلاجلات
لثلاثة أنواع من الطحالب وهى

Prorocentrum micans, Gymnodinium lantzschii and *Exuviaella apora*

حيث يتحول لون مياه البحيرة الى اللون البنى وذلك بصفة دورية كل عام.

إن الطريقتين المستخدمتان لقياس الكلوروفيل دقيقة وبسيطة وتعطى صورة كاملة لبحيرة قارون. إن نتائج الكلوروفيل التى تم قياسها بواسطة الفلوروميتردقيقة وأعلى من نظيرتها فى جهاز سبكتروفوتميتر.

إن الملوحة الزائدة ومياه الصرف الزراعى من المصارف والمزارع السمكية حولت البحيرة إلى نظام بيئى مختلف له تأثير كبير على تركيب العوالق النباتية والإزدهار الطحلبى فى البحيرة. الدراسة لم تسجل أى تأثير لسموم طحلبية أو موت مفاجئ للأسماك فى بحيرة قارون.