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Phytoplankton Profile and Toxicity Assessment of Dominant Algal Species from Different Egyptian Aquatic Ecosystems.

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ABSTRACT

A total of 98 species of phytoplankton from 4 different Egyptian sites Kafr El-Zayat, El-Rasua, Wadi El-Rayian Lake and El-Khadra were identified to specify the dominant species. Six dominant species were isolated and purified, four of them (*Oscillatoria brevis*, *O. princeps*, *Microcystis aeruginosa* and *Spirulina platensis*) were belonged to Cyanobacteria and the other two (*Chlorella vulgaris* and *Scenedesmus obliquus*) were belonged to green algae. The toxicity of these species was assessed using brine shrimp and mouse bioassays. The extract of *Oscillatoria brevis* and *Microcystis aeruginosa* exhibited a toxic effect against both bioassays. This result was confirmed by the determination of microcystin-LR using HPLC. High quantities of microcystin-LR were detected in *M. aeruginosa* (1.87µg g^{-1} dry weight) and *O. brevis* (0.81µg g^{-1} dry weight).

Keywords: phytoplankton, brine shrimp, mouse, bioassay, microcystin-LR.

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INTRODUCTION

Phytoplankton are a very important component of aquatic ecosystem. They found in a wide range of different habitats from fresh to marine and hyper-saline environments (Shameel, 2001). Phytoplankton are at the base of aquatic food webs and have global importance for ecosystem function and services. The dynamics of these photosynthetic cells are depended upon annual fluctuations of temperature, water column mixing, nutrients availability and consumption (Field et al., 1998). Climate affects phytoplankton both directly through physiology and indirectly by changing water column stratification and available resource of nutrients and light (Stenseth et al., 2003). Phytoplankton support healthy aquatic ecosystems by fixing carbon dioxide and producing oxygen. Also, phytoplankton may be used as indicator of water quality (Chronakis, 2000).

When the source of nutrients is continuous, especially nitrogen and phosphorus and condition are favorable, phytoplankton growth as a bloom which may impact the ecosystem by reduce the sunlight reaching to aquatic grasses and depletion dissolved oxygen (Saha et al., 2000). A few species of phytoplankton produce toxins which can cause health hazard to humans, domestic animals and wildlife with toxicological effects including neurotoxicity, hepatoxicity, cytotoxicity and dermatoxicity (Carmichael, 2001). The most common freshwater algal toxins include microcystins, homoanatoxin, anatoxin-a and saxitoxins, which are produced by cyanobacteria such as *Microcystis*, *Anabaena*, *Oscillatoria* and *Nostoc* species (Codd, 2000). Human poisoning due to toxins of marine dinoflagellates include paralytic shellfish poisoning, diarrheic shellfish poisoning, neurotoxic shellfish poisoning, and ciguatera fish poisoning (Garcia-Camacho *et al*., 2007). The common dinoflagellates that produce such toxins include *Alexandrium*, *Dinophysis*, *Karenia* and *Gymnodinium* species (He *et al*., 2007).

Several works in Egypt studied the toxicity of specific species or groups of algae (Gomaa *et al.,* 2000; Marrez, 2010 and Zaher, 2012). However, there are no available studies combined both the whole profile of phytoplankton with the toxicity of the dominant species. So, the objectives of this study are to identify the phytoplankton profile in different Egyptian location, isolation of dominant algal species and finally toxicological evaluation of these species using bioassay techniques and analytical method using HPLC.

MATERIALS AND METHODS

Sampling locations

Water and algal samples were collected from four different locationsof Egyptian water resources,Kafr El-Zayat at El-Gharbeia, El-Rasua at Port-Said governorate, Wadi El-Rayian UpperLake at El-Fayioum governorate and El-Khadra Lake at El-Baheira governorate during the period from October 2011 to February 2012 (Fig. 1). These locations were characterized by its highly content of nutrients and low water flow rate, both charaters were required for algal growth.

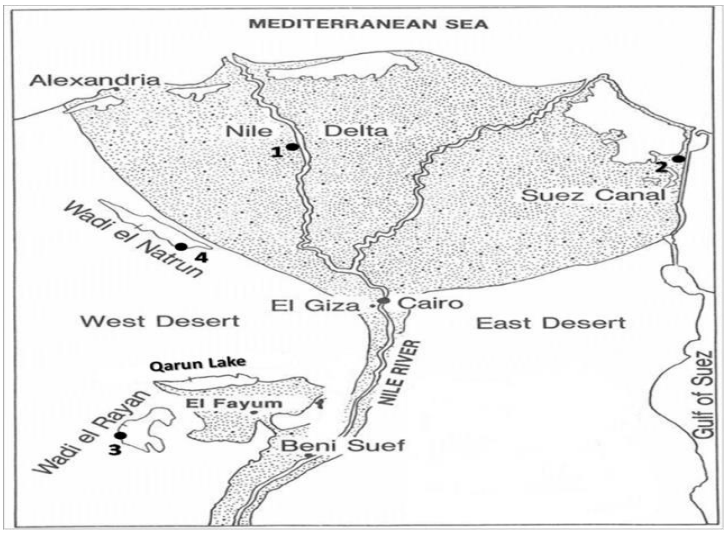

Fig 1: Map of algal sampling locations (1 Kafr El-Zayat, 2 El-Rasua, 3 Wadi El-Rayan lakes, 4 Al-Khadra Lake).

Algae isolation, purification and identification

Collected water samples were divided into two portions, the first one was used for fixation, counting and identification of algal taxa and the second one for isolation, purification, identification and cultivation the dominant species in algal taxa.

Algal counting: Samples were poured into a glass cylinder of 500 ml capacity and 1% Lugol´s solution was added, then left for 5 days. Counting of phytoplankton species was carried out using the Sedgwick-rafter counting chamber, as recommended by APHA (1975). Using a phase contrast microscope, fine random fields were selected, within which the individuals of a species were counted.

Isolation and purification: Fresh samples were obtained by using algal sampling net, they were taken rapidly to the laboratory at 4°C. The algal samples were microscopically examined to detemine the prevailing organisms. The collected microalgae samples were prepared and maintained in BG-11 broth medium (Allen, 1973). Microalgal materials were grown in glass flasks containing 20 ml of sterilized BG-11 medium. Three week old stock cultures were transferred to 250 ml fresh sterilized medium (10% stock + 90% fresh medium), then cultures were maintained at room temperature.

Enrichment culture of microalgal sample was centrifuged (Sigma, 2-15, W. Germany) at 3000 rpm for 15 minutes. After removing the supernatant the cells was suspended in fresh sterile water and mixed using vortex mixture at 1000 to 1500 rpm to get homogeneous suspension. Centrifugation and wash was repeated for six times to expel most of the microorganisms presented in algal sample.

Washed microalgae were streaked through loop in BG-11 agar plates in axenic condition and kept for at least seven days to grow microalgae. Repeated streak plating was carried out to peak up single colony from earlier streaked plates and to make it free from bacteria. The single colonies were picked up by loop and allowed to grow in tubes contain BG-11 medium. The single cell growth and purity of single species was confirmed after observing under microscope. The pure culture of isolated microalgae was maintained in BG-11 medium in tube, vail and volumetric flask in laboratory for further use (Phang and Chu, 1999).

Identification of microalgae species: Species identification was carried out using a phase contrast microscope (Olympus, CX41RF, Phillippines)) accodrding to Desikachary (1959), Prescott´s (1978) and Hindak (1988 and 1990). The characteristics of the species (length, width, thickness and diameter) were measured using an ocular and slide micrometer (Craticules, LTD, Tonbridge, Kent).Micrographs of isolated microalgae species were picked up using Olympus camera (SC100, Münster, Germany) and Olympus soft imaging solution GMBH.

Culture media

Two culture medium were prepared for cultivation of microalgae species, Zarrouk's medium (Zarrouk, 1966) for cultivation of *Spirulina platensis* and BG-11 medium (Rippka *et al*., 1979) for cultivation of *Microcystis aeruginosa*, *Oscillatoria brevis*, *O. princeps*, *Scenedesmus obliquus* and *Chlorella vulgaris*.

Toxicity assessment of isolated microalgal species

Brine shrimp bioassay: Brine shrimp eggs (*Artemia salina* leach) were supplied by Avocet Artemin Inc., Utah, USA. Larvae were used after 24 h of hatching. Five grams biomass of each compact microalgal species, *S. platensis*, *M. aeruginosa*, *O. brevis*, *O. princeps*, *C. vulgaris* and *S. obliquus* were extracted with 10 ml distilled water using ultrasonic cell disrupter equipped with microtip probe of 400 Watt (ULTRASONIC Get 750). Microalgae cells were then centrifuged at 4000 xg for 5 minutes and the supernatant was evaporated to dryness using rotary evaporator (Meyer *et al*., 1982). The dried extracted were dissolved in seawater (36g sea salt in liter) and four concentrations 250, 500, 1000, 1500 and 2000 ppm were prepared in order to assess the toxicity. The number of dead shrimps that was placed in 5 vials (10 shrimps/vial) was counted and percent mortality was calculated.

Mouse bioassay: Compact microalgae biomass was extracted by 1:1 (w/v) 0.1 Molar acetic acid using ultrasonic cell disrupter. Disrupted cells were examined under microscope to ensure the complete rupture of the microalgae cells. Disrupted cells were then centrifuged at 5000 xg for 5 minutes and the supernatant was retained for toxicity test.

To determine the dose response curve, male Albino Swiss mice weighting 20±2 g were obtained from Animal Housing Division, National Research Center, Cairo, Egypt. Potency was expressed as Mouse Units (MU), where 1 MU is defined as the amount of toxins required for killing a 20 g mouse in 15 minutes. The survival time was measured from the completion of the intraperitoneal (i.p.) injection to the last breath (AOAC, 2007). Preliminary toxicity determination was performed using 3 mice for each sample preparation dose. Toxicity was observed and death times were recorded.

HPLC analysis

Standard cyanobacterial toxin microcystin-LR (MC-LR) used throughout the present study was obtained from Sigma, chemical company, USA. Determination of the cyanotoxin MC-LR was carried out according to Amé *et al*. (2003). Twenty mg of dried microalgae cells were placed in Eppendorf tubes, extracted with 1.5 ml of 5% acetic acid and sonicated for 5 min using ultrasonic microtip probe of 400 Watt. The suspension was centrifuged at 4500 xg for 7 min. Supernatant was retained and the pellet re-extracted as before. Combined supernatants were centrifuged at 4500 for 20 min.

Extracted supernatant of sample was applied to a C-18 solid phase extraction cartridge (strata C18, 500 mg/3ml, Phenomenex), which was previously conditioned with 10 ml methanol and 10 ml 5% acetic acid. The cartridge was washed 3 times with 10 ml of 10, 20 and 30% aqueous methanol and toxins were eluted with 10 ml of methanol HPLC grade. The elute was evaporated to dryness at 40˚C and resuspended in 200 µl of methanol prior HPLC analysis. The HPLC system used for MC-LR determination was Perkin-Elmer, series 200 system (USA), equipped with quaternary pump, UV detector set at 238 nm and a C18 column chromatography ODS Phenomenex (250 x 4.6 mm, 5 µm). Mobile phase gradient program at flow rate 1 ml min^{-1} is mentioned in detailed in Table (1).

Table 1: HPLC mobile phase of microsystin-LR

Time (min)	Solution (A) Acetonitrile with 0.05 % TFA	Solution (B) Water with 0.05 % TFA
0	30	70
5	35	65
15	70	30
17	100	0
19	30	70
22	30	70

TFA: Trifluoroacetic acid

RESULTS AND DISCUSSION

Phytoplankton profile in Egyptian aquatic ecosystems

Water and algal samples were collected during the period from October 2011 to February 2012. Samples collection period was chosen based on previous studies (Amin, 2001; Marrez, 2010 and Zaher, 2012), which confirmed that the highest productivity and bloom formation from microalgae species occurred during this period. The phytoplankton profile of different sites and the dominant microalgae species were represented in Table 2 and Figure 2. Seventy-three species of phytoplankton were identified from Kafr El-Zayat site in Rosetta branch of river Nile. The dominant species of algal taxa were *Oscillatoria princeps* and *Oscillatoria brevis*, represented about 55% of the phytoplankton. Fifty-two species of phytoplankton were identified from El-Rasua site in Port Said freshwater canal. *Microcystis aeruginosa* was the dominant species represented 35% of total phytoplankton.

A total of sixty-nine algal taxa were recorded in samples that collected from Upper lake of Wadi El-Rayian lakes. Four species were recorded as the dominant, two species from Cyanophyta, *Microcystis aeruginosa* and *Microcystis flos-aquae* which constituting 30.2% of the total phytoplankton and two species of Chlorophyta *Chlorella vulgaris* and *Scenedesmus obliquus*, which contributed of 14.3% of total algal density. El-Khadra Lake in Wadi El-Natrun region, El-Baheira governorate considered a favorable environment for *Spirulina* sp. growth because its water had a pH ranged from 8.5 to 10.5 and salt concentration of 55g l^{-1} (Aly,

2000). Twenty one species of phytoplankton were identified from the lake, *Spirulina platensis* was the dominant species representing 87.6% of total phytoplankton composition. Although, Bacillariophyta represented one third of the identified algal species, the count of each one was very low(≤102 x 10^2 unit l^{-1}).

Many studies support the present findings. Gomaa et al. (2000) reported that the *Oscillatoria brevis* winter bloom occurred in the freshwater canal of Port Said, Egypt in 1995 and reoccurred for 4 consecutive years. Also, Marrez, (2010) indicated that the major components of winter bloom in both Rosetta branch and Port Said freshwater canal sites were *Oscillatoria princeps*, *Oscillatoria brevis* and *Microcystis aeruginosa*.

Konsowa (1996) reported that the most abundant taxa found in Wadi El-Rayian Upper Lake were *Aphanizomenon*, *Merismopedia*, *Microcystis* and *Lyngbya limetica*. Also, Konsowa and Abd Ellah (2002) identified a total of 65 species of phytoplankton from Upper Lake related to four divisions among of them Cyanophyceae were representive (69.4%) with 16 spp., Chlorophyceae (27.9%) with 25 species, Bacillariophyceae with 21 taxa (2.5%) and Dinophyceae with only three species. Abd El-Fatah (2010) indicated that the main bulk of phytoplankton in Upper Lake was *Microcystis aeruginosa*, *Microcystis flos-aquae*, *Merismopedia* and *Oscillatoria pseudogeminata*.

Six pure dominant species of microalgae were selected for cultivation and production to assess for its toxicity. The light micrographs of these species are illustrated in Figure 3. Four of them belonged to Cyanophyta (*Spirulina platensis*, *Oscillatoria princeps*, *Oscillatoria brevis* and *Microcystis aeruginosa*) and the rest two belonged to Chlorophyta (*Chlorella vulgaris* and *Scenedesmus obliquus*).

Toxicity assessment of isolated microalgae species

Brine shrimp bioassay

The use of aquatic organisms for biomonitoring is an important tool in aquatic ecotoxicology allowing the detection and evaluation of the potential toxicity (USEPA, 1984). Brine shrimp bioassay was used firstly as an easy test to detect any toxicity; however, it is not specific test for algal toxins.

Toxicity assessment of microalgae species by brine shrimp bioassay (Figure4) showed that the highest toxicity (100% mortality) was recorded in both *Oscillatoria brevis* and *Microcystis aeruginosa* at concentration of 1500 ppm, while the brine shrimp bioassay value of other microalgae species were lower than 50% mortality at all concentrations. The obtained results were confirmed by doing the mouse bioassay.

Mouse bioassay

The toxicity of the tested species was calculated using mouse bioassay technique. High toxicity was recorded with both *O. brevis* (1.22 MU) and *M. aeruginosa* (1.03 MU). On the other hand, there were no toxic effect and symptoms in the mice that injected with *S. platensis*, *O. princeps*, *C. vulgaris* and *S. obliquus*.

Campbell *et al*., (1994) reported that hepatotoxic bloom samples and laboratory isolates of cyanobacteria were toxic to brine shrimp larvae. Lee *et al*. (1999) and Marrez (2010) showed that the results of relative toxicity of each cyanobacterial strains assessed using brine shrimp bioassay was almost the same as that of the mouse bioassay. Akin-Oriola (2003) reported that the percentage of brine shrimp mortality increased with increasing dose of toxin or extract and pure microcystin-LR caused 100% mortality of shrimp at 20 µg ml^{-1}.

Azevedo *et al*. (1994) revealed that mouse bioassay with field collected *Microcystis* showed a toxicity close to that (LD_{100}= 31 mg Kg^{-1} mouse, intraperitoneal injection of algal extract). Brooks and Codd, (1987) and Robison *et al*. (1989) reported that the time required for maximal toxin accumulation in liver varied from 1 to 60 min after microcystin-LR administration to mice. Nishiwaki-Matsushima *et al*. (1992) explained the vital effect of the hepatotoxins microcystins in mice by its inhibition the activity of protein phosphatase 1 and 2A. Due to enzyme inhibition, hepatocytes shrink and cause liver damage, followed by internal hemorrhaging. Gomaa *et al*. (2000) proved that the detected toxicity of *O. brevis* was neurotoxic and the mode of action was Na^+ channel blocker.

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Table 2: Phytoplankton profile (x 10^2 unit l^{-1}) in some Egyptian aquatic ecosystems

No	Phytoplankton species	I	II	III	IV	No	Phytoplankton species	I	II	III	IV
Chlorophyta						**50**	*Microcystis aeruginosa* Kutzing	160	900	1200	0
1	*Actinastrum lagerheim*	4	0	100	0	**51**	*M. flos-aquae* (Wittrock) Kirchner	0	0	1120	0
2	*A. hantzschii*	0	22	126	0	**52**	*M. elongata*	12	24	0	0
3	*A. convolutes* (Corda)	6	23	0	0	**53**				3	0
							Nostoc verrucosum (Vaucher) Hist.	22	26		
4	*Chlorella pyrenoidsa*	7	0	123	0	**54**	*N. pruniforme* C.A. Agardh	21	31	0	0
5	*C. variabilis*	12	0	86	0	**55**	*N. carneum* Ag.	2	0	26	0
6	*C. vulgaris*	6	0	352	0	**56**	*Oscillatoria brevis*	775	34	0	3
7	*Coelastrum microporum* Naegeli	9	45	54	0	**57**	*Oscillatoria foromsa* Bory	3	7	0	3
8	*C. quadrata* Morren	4	0	74	0	**58**	*O. limnetica*	0	9	14	32
9	*C. rectangularis* (A.Braun) Gray	45	33	41	0	**59**	*Oscillatoria princeps* Vaucher	890	6	32	0
10	*Micractinium pusillum*	0	0	0	74	**60**	*Phormidium molle* (Kutzing) Gomont	14	77	9	0
11	*O. bergi*	0	0	0	32	**61**	*Phormidium retzii*	4	0	0	0
12	*Oocystis nephrocytioides* Naegeli	6	58	0	92	**62**	*Spirulina jenneri*	0	22	0	66
13	*Pediastrum sturmii* var. *radians* (Lemmer.)	0	8	15	0	**63**	*Spirulina major*	3	9	14	11
14	*P. tetras* (Ehrenberg) Ralfs	23	0	41	0	**64**	*S. platensis* (Nordstedi) Geitler	8	34	33	10160
15	*P. clathratum* (Schroter) Lemmer.	6	0	95	0	**Bacillariophyta**					
16	*P. duplex* Meyen	23	35	0	0	**65**	*Achnanthes flexella* (Kutzing) Brun.	28	19	0	0
17	*P. obtusm* Lucks	22	6	0	0	**66**	*A. lanceollata* (Breb.) Grunow	24	26	0	0
18	*Scenedesmus acuminatus*	0	0	0	109	**67**	*A. inflata*	0	0	0	12
19	*S. quadricauda* (Trup.)de Brebisson	20	54	122	0	**68**	*A. jamalinensis*	0	0	0	23
20	*S. ecornis* (Ehr.)Chodat	30	0	114	0	**69**	*Cocconeis pediculus*	0	0	0	3
21	*S. opliensis* Richter	18	0	33	0	**70**	*Cyclotella meneghiniana* kutz.	34	44	14	0
22	*S. parisiensis* Chodat	2	0	118	0	**71**	*C. operculata* kutz.	8	38	6	0
23	*S. obliquus* (Turpin) Kuetzing	23	138	390	0	**72**	*C. ocellata* Pant	12	37	18	31
24	*S. opoliensis* var. *opoliensis* P. Richter	14	62	23	0	**73**	*C. glomerata* Bachmann	14	0	12	102
25	*S. quadricauda* var. *longispina* Chodat	20	104	124	2	**74**	*C. kutzingiana* Thwaites	9	0	12	0
26	*S. dimorphus* (Turpin) Kuetzing	18	2	0	144	**75**	*C. bodanica* Eulenst	6	0	8	0
27	*S. bibraianum* Reinsch	12	5	0	0	**76**	*C. antiqua* W.Smith	77	4	88	0
28	*S. ellipticus*	0	0	0	155	**77**	*Cymatopleuro solea*	0	0	0	14
29	*S. raciborskii*	0	0	0	122	**78**	*Cymbella enherenbergii*	0	0	0	6
30	*Spirogyra varians*	0	0	0	95	**79**	*C. microcephala*	0	0	0	52
31	*Tetraedron minimum* (A. Br.) Hansgrig	0	44	0	0	**80**	*Fragilaria lyngbya*	14	0	27	0
32	*Volvox tertius* A. Meyer	0	52	0	0	**81**	*F. construens* (Ehr.) Grun	8	72	9	0
Cyanophyta						**82**	*F. gracillima* Mayer	24	6	30	0
33	*Aphanocapsa montana*	0	0	42	0	**83**	*F. brevistriata* Grun	16	0	40	0
34	*Anabaena variabilis* Kuetzing	22	22	0	54	**84**	*Melosira granulata* (Ehr.) Ralfs	24	4	33	0
35	*Anabaena circinalis* Rabenhorst	6	0	0	0	**85**	*M. granulata* var. *angustissma* Muller	45	55	14	0
36	*A. flos- aquae* Brebisson	8	0	0	0	**86**	*Navicula cardinalis* Ehrenberg	0	7	0	0
37	*A. inaequalis* (Kutzing) Bornet & Flahawt	12	0	0	0	**87**	*N. viridula* (Kutz.)	0	3	0	0
38	*Aphanocapsa montana*	14	9	0	0	**88**	*Nitzshchia acicularis* W. Smith	0	15	0	0
39	*Chroococcus limneticus*	0	0	33	0	**89**	*N. obtusa* W. Smith	21	5	0	0
40	*Chroococcus minitus* (Kutzing) Nageli	2	11	18	0	**90**	*Pleurosigma elongatum*	0	0	0	34
41	*C. tenax*	5	8	38	0	**91**	*Stephanodiscus hantzschii* Grun	12	0	0	0
42	*C. turgidus* (Kutzing) Naegeli	14	9	35	0	**92**	*Surirella robusta* Turpin	14	93	0	0
43	*Gloeocapsa polydermatica*	99	0	0	0	**93**	*Synedraulna acus* Kutz.	13	14	18	44
44	*Lyngbya distincta*	8	0	0	0	**94**	*S. affinis* Kutz.	4	0	18	0
45	*Merismopedia elegans* A.Braun	2	4	6	3	**95**	*S. ulna* var. *aequalis* (Kutz.) Hust	8	34	14	0
46	*Merismopedia glauca*	33	7	4	0	**96**	*S. gaillonii* (Bory) Ehr.	14	0	16	0
47	*M. minima*	2	0	0	0	**97**	*S. ulan* var. *oxyrhynchus* (Kutz.)Van Heurck	17	34	6	0
48	*M. major* (G. M. smith) Geitler	0	0	14	0	**98**	*S. actinastroides* Lemmermann	12	32	11	0
49	*M. tenuissima* Lemmermann	33	0	13	0						

I: Kafr El-Zayat, II: El-Rasua, III: Wadi El-Rayian, IV: El-Khadra Lake.

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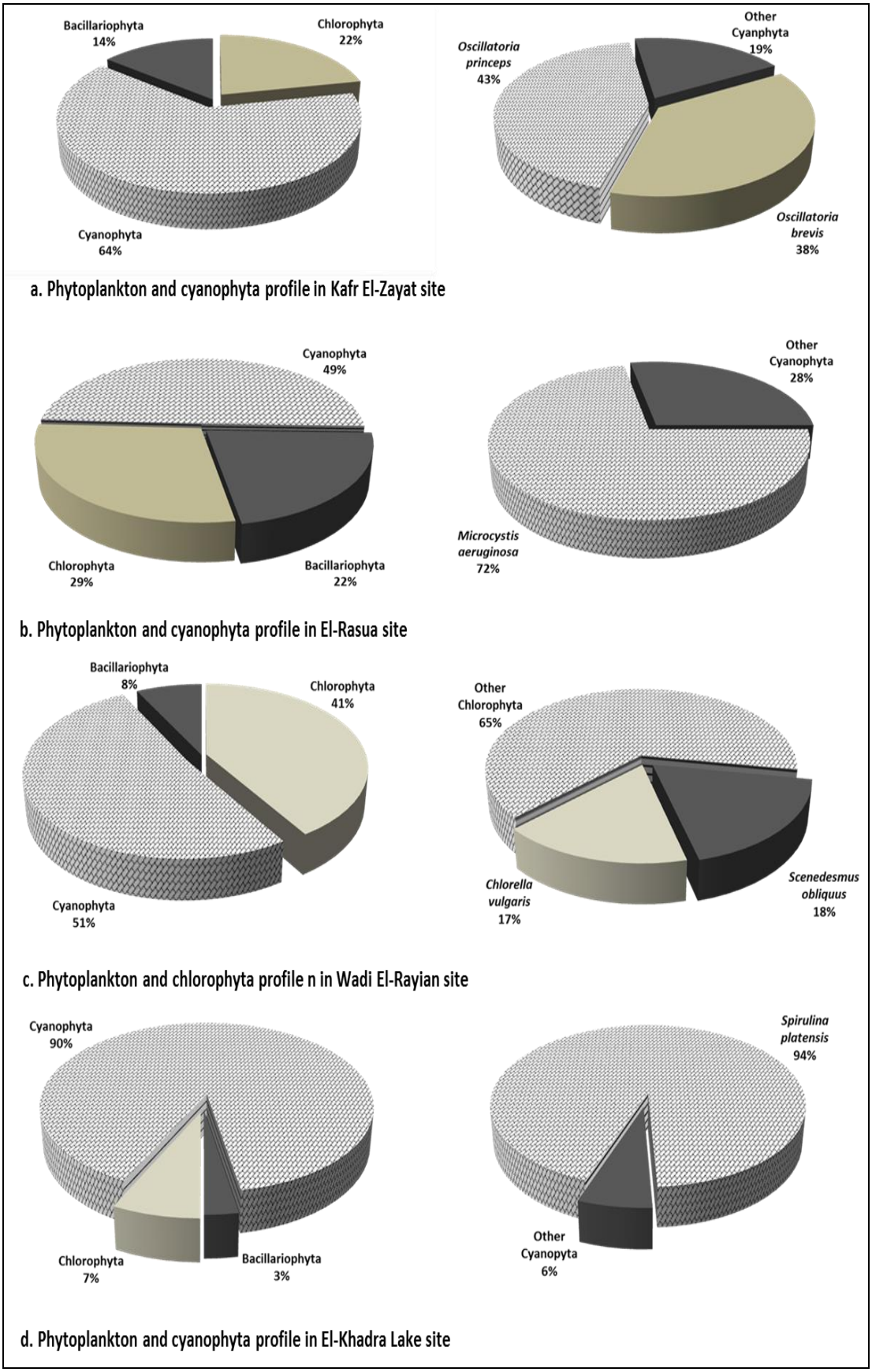

Figure2: Phytoplankton profile recorded in sampling sites.

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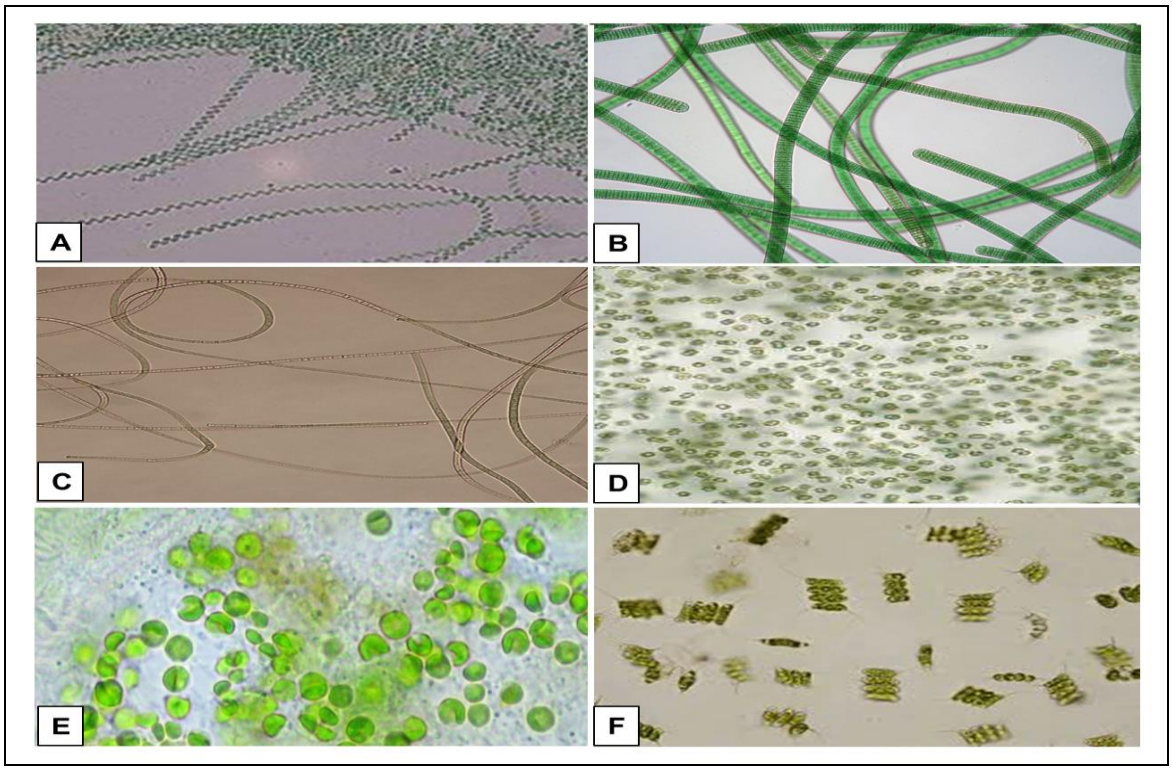

Figure 3: Light micrographs of isolated microalgae species, A. *Spirulina platensis*, B. *Oscillatoria princeps*, C. *O. brevis*, D. *Microcystis aeruginosa*, E. *Chlorella vulgaris*, F. *Scenedesmus obliquus*.

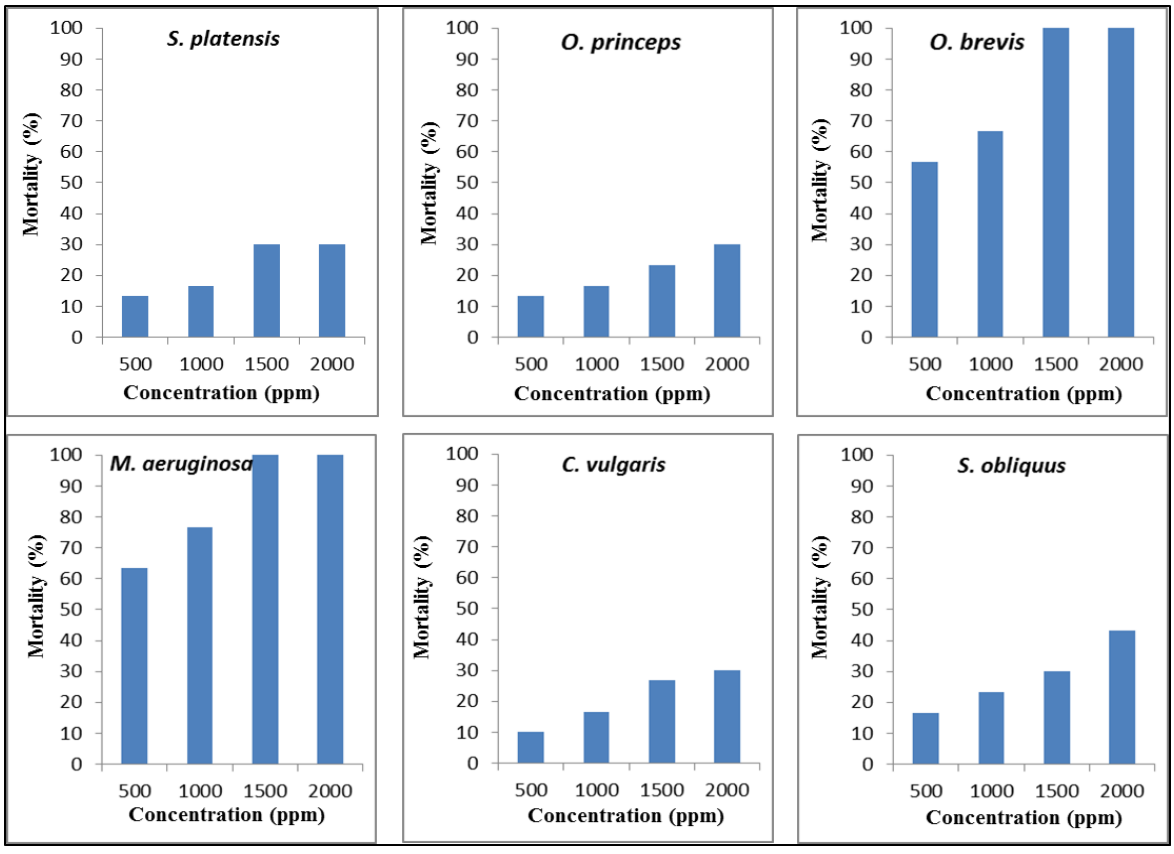

Figure 4: Brine shrimp bioassay of the tested microalgae species.

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Determination of microcystin-LR

The toxic effect of both *M*. *aeruginosa* and *O*. *brevis* was confirmed by determination of microcystin using HPLC (Figure 5). The results indicated that microcystin was detected in *M*. *aeruginosa* (1.87µg g^{-1} dry weight) and *O*. *brevis* (0.81µg g^{-1} dry weight). The both species was recorded toxic species (Carmichael, 1997). Also, Dos *et al*. (2005) reported that microcystin concentration in the surface water containing bloom of *Microcystis* was reached to 1.25µg l^{-1}. Gomaa *et al*. (2010) reported that microcystin concentration in mixed cyanobacterial samples ranged from 0.1 to 6.5 µg g^{-1} dry weight. While, microcystin concentration in pure isolates of *M*. *aeruginosa* was 1.81µg g^{-1} and that of *O*. *brevis* was 0.75µg g^{-1}. The data confirmed that both of *M*. *aeruginosa* and *O*. *brevis* toxicity was due to the presence of microcystin. Zaher (2012) revealed that the hepatotoxins microcystin-LR was detected in mixed bloom from Wadi El-Rayian Lakes as well as in the pure isolate of *Microcystis aeruginosa* and *Microcystis flos-aquae*.

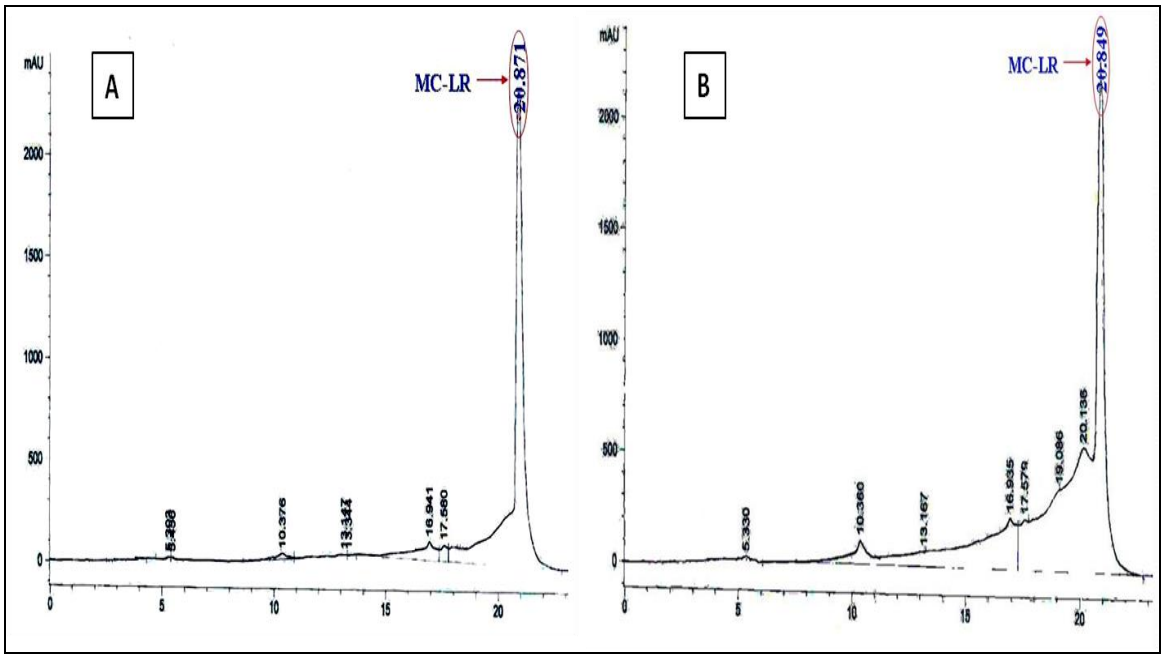

Figure 5: HPLC chromatogram of A. microcystin-LR standard, B. *Microcystis aeruginosa*.

CONCLUSION

Six dominant microalgae species were isolated and purified from four Egyptian aquatic ecosystems. Toxicity assessment of these isolates demonstrated that both *Microcystis aeruginosa* and *Oscillatoria brevis* are capable of produce microcystin-LR at high concentrations which revealed to the importance of controlling them in water as a potential hazard at blooming time.

REFERENCES

[1] Abd El-Fatah, H. Ph.D. Thesis, Fac. Sci., Ain Shams Univ., Egypt, 2010, 417pp.
[2] Akin-Oriola G. Revista Biol. Trop., 2003; 51(1):99–106.
[3] Allen, M. (Ed. Stein, J.), Cambridge University Press, Cambridge, 1973; pp. 56-143.
[4] Aly, M. Egyptian J. Phycol., 2000; 1:203-210.
[5] Amé, M.; Díaz, M. and Wunderlin, D. Eviron. Toxicol., 2003; 18:192-201.
[6] Amin, A. Ph.D. Thesis, Fac. Sci., Suez Canal Unv., Egypt, 2001; 157 p.
[7] AOAC. 18th ed. Washington, D.C., 2007; chapter 49, pp. 102-166.
[8] APHA. American Public Health Association, Washington D.C, 1975; 245p.
[9] Azevedo, S.; Evans, W.; Carmichael, W. and Namikoshi M. J. Appl. Phycol., 1994; 6:262-265.
[10] Brooks, W. and Codd G. Pharmacol. Toxicol., 1987; 60:187-191.
[11] Campbell, D.; Lawton, L.; Beattie, K. and Codd, G. Environ. Toxicol. Water Qual., 1994; 9:71-77.

[12] Carmichael, W. Adv. Bot. Res., 1997; 27:211-256.
[13] Carmichael, W. Hum. Ecol. Risk Assess., 2001; 7:1393-1407.
[14] Chronakis, I. (Eds. Doxastakis, G. and Kiosseoglou, V.), Center for Chemistry and Chemical Engineering, Lund, Sweden, 2000; pp. 39-75.
[15] Codd, A. Ecol. Eng., 2000, 16: 51-60.
[16] Desikachary, V. New Delhi, 1959; pp. 187-198.
[17] Dos, S.; Vieira, J.; Azevedo, M.; Oliveira Azevedo, S.; Honda, R. and Corrêa, B. Toxicon, 2005; 45:901-909.
[18] Field, C., Behrenfeld, M., Randerson, J. and Falkowski, P. Science, 1998; 281: 237-240.
[19] Garcia Camacho, F.; Gallardo-Rodriquez, J.; Sanchez-Miron, A.; Ceron-Gracia, M.; Belarbi, E.; Chisti, Y. and Molina-Grima, E. Biotechnol. Adv., 2007; 25:176-194.
[20] Gomaa, M.; Ayesh, A.; Amin, A.; Daw, Z.; Marrez, D. and Higazy, A. J. Egypt. Soc. Toxicol., 2010; 42:31-38.
[21] Gomaa, M.; El-Manawy, I. and Amin, A. J. Egypt. Soc. Toxicol., 2000; 23:9-15.
[22] He, F.; Yang, Y.; Xu, H.; Zhang, X. and Li X. Neur. Psych. pharm., 2005; 30:1511-1520.
[23] Hindak, F. Publishing hourse of the Slovak Academy of Science VEDA, Bratislava, 1988; 4:599-624.
[24] Hindak, F. Publishing hourse of the Slovak Academy of Science VEDA, Bratislava, 1990; 5:647-671.
[25] Konsowa, A. Ph.D. Thesis, Girls Collage, Ain Shams Univ., 1999; 152pp.
[26] Konsowa, A. and Abd Ellah, R. J. Egypt. Acad. Soc. Environ. Develop., 2002; 3(2): 1-27.
[27] Lee, T.; Chen, Y. and Chou, H. Acta Zoologica Taiwanica., 1999; 10(1):102-109.
[28] Marrez, D. M.Sc. Thesis, Microbiol. Dept., Fac. Agric., Cairo Unv., Egypt, 2010; 126 p.
[29] Meyer, B.; Ferrigni, N.; Putnam, J.; Jacobsen, L.; Nichols, D. and McLaughlin, J. Planta Med., 1982; 45:31-34.
[30] Nishiwaki-Matsushima, R.; Ohta, T.; Nishiwaki, S.; Suganuma, S.; Kohyama, K.; Ishikawa, T.; Carmichael, W. and Fujiki, H. J. Cancer Res. Clin. Oncol., 1992; 118:420-424.
[31] Phang, M. and Chu, L. Kuala Lumpur, University of Malaya, Malaysia, 1999; 77p.
[32] Prescott, A. Third edition, WMC Brown Company Publishers, UK, 1978; 293p.
[33] Rippka, R.; Deruelles, J.; Waterbury, B.; Herdman, M. and Stanier, Y. J. Gen. Microb., 1979; 111:1-61.
[34] Robinson, N.; Miura, G.; Matson, C.; Dinterman R. and Pace, J. Toxicon, 1989; 27: 1035-1042.
[35] Saha, S.; Bhattacharya, S. and Choudhury, A. Environ. Biol., 2000; 21 (1): 9-14.
[36] Shameel, M. Pak J. Mar. Biol., 2001; 7:233-250.
[37] Stenseth, N.; Ottersen, G.; Hurrell, J.; Mysterud, A.; Lima, M.; Chan, K.; Yoccoz, N. Proceedings of the Royal Society of London, Series B: Biological Sciences, 2003; 270:2087-2096.
[38] USEPA. EPA, Washington, DC., 1984; 135p.
[39] Zaher, S. Ph.D. Thesis, Botany Dept., Fac. Sci., Ain Shams Unv., Egypt, 2012; 253 p.
[40] Zarrouk, C. Ph. D. Thesis, Fac. Sci., Univ. Paris, 1966; 146 p.