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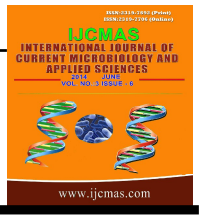

Original Research Article

Ecological evaluation of marine cyanobacteria of El-Khadra lake in Egypt

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A B S T R A C T

El-Khadra lake was ecologically evaluated at four depths (surface layer, 30 cm, 1 meter and sediment water) in September 2011 and February 2012. This was performed in terms of physical, chemical and microbiological properties. Physical analysis of lake water samples revealed the alkaline nature of the lake in all depths. Higher bacterial counts were detected in sediment water samples in both seasons. Fungal counts were higher in September samples at 1meter depth and in sediment water, while in February samples the higher fungal counts were recorded in surface and in 30 cm layers. Total and feacal coliforms counts were also determined in water samples and scored few numbers. Many species of cyanobacteria besides some algae were detected in the lake, they included *Chlorella*, Circular diatom (*Puncticulata spp.),Oscillatoria, Spirulina, Nostoc* and *Anabaena* species. Among them, three representative species were isolated and identified as *Spirulina, Oscillatoria* and *Anabaena*. Their intracellular and extracellular extracts showed no considerable influence on brine shrimp eggs and mice survival. They showed also considerable antimicrobial activities. In this respect, cyanobacterial cultures, filtrates and organic extracts were examined for inhibitory activity against five tested microorganisms *i.e., E.coli, S. aureus, P. aeroginosa, B. subtilis* and *A. niger*. All of the examined microorganisms were inhibited by the culture filtrate of *Oscillatoria spp.,* while the ethanolic extract of *Anabaena* scored a high inhibitory effect against *P. aeroginosa, B. subtilis* and *A. niger*.

Keywords

El-Khadra lake, Ecology, Cyanobacteria, *Spirulina, Oscillatoria, Anabaena,* antimicrobial activity.

Introduction

Inland saline lakes have received attention in recent years due to their sensitivity to climatic changes. Wadi El-Natrun closed lakes are characterized by filtrations of fresh water that comes from river Nile - Rashid branch which is at approximately a 40 km distance from Wadi El- Natrun on both sides. The climate of Wadi El Natrun is arid, resulting in a continuous evaporation of the lakes water, which generates the formation of vast crusts of salt (Awad, 2002). In this respect, Pienitz *et al,* (1992) indicated that changes in evaporation rates and precipitation can affect the physical and chemical characteristics in such lakes.

El-Khadra lake is one of Wadi El-Natrun

closed lakes. It is reaching an area of 162 acre and is located about 16 km from Cairo- Alex desert road and about 3 km from El-Beida lake. Hamed (2008) reported highest electrical conductivity in El-Khadra lake among some Wadi El-Natrun lakes and explained that it was influenced by the geological and climatic conditions. Changes in water chemistry and lake depth, in turn, control the distribution and abundance of its inherent aquatic life.

Cyanobacteria constitute a versatile group of photosynthetic bacteria of immense commercial and ecological importance (Jacquet *et al.,* 2013). They are the most common inhabitants of saline-alkaline lakes in different parts of the world (Grant, 2004). Hamed *et al.* (2007) used the technologies of remote sensing and Geographic Information Systems (GIS) to provide spatial information about Wadi El-Natrun lakes. The obtained GIS-based map monitored a blooming of *Spirulina platensis* inhabiting El-Khadra lake. They also observed some other species of cyanobacteria besides *Spirulina* such as *Gleocapsa*, *Synechococystis*, *Oscillatoria*, *Anabaena* and *Nodularia spp*. inhabiting the lake.

Cyanobacteria are known to produce a great variety of secondary, biologically active, metabolites which cannot be found in other organisms (Arun *et al.,* 2012).

This study was designed to isolate and identify some of the residential species of cyanobacteria in El-Khadra lake, evaluating their antimicrobial activities and exploring their toxicity. This would eventually lead to much better understanding of El-Khadra lake ecosystem.

Materials and Methods

Sampling

Samples were taken primarily for the isolation of cyanobacteria from El-Khadra lake, Wadi El-Natrun, Egypt (Fig.1). Samples were collected in two seasons; the summer samples in September 2011 and the winter samples in February 2012 from the lake centre at four depths (the water surface, 30 cm, 1 meter and the sediment water). Representative samples from each depth were subjected to physical, chemical and microbiological analyses.

Fig.1 Satellite image of El-Khadra lake

a.Physical analysis

Temperatures, pH value and Electrical conductivity (E.C.) of water samples were determined according to APHA (1992), Total Soluble Salts (T.S.S.) were calculated according to the following equation:

$$T.S.S. = E.C. x\ 640$$

b. Chemical analysis

Cations (Na^{+}, K^{+}, Ca^{+2}, Mg^{+2}) and Anions (CO_3^{-2}, HCO_3^{-2}, Cl^{-}, SO_4^{-2}) were determined according to Page *et al.* (1989) at Department of soil science, Faculty of Agriculture, Cairo University.

c. Microbiological analysis

Total bacterial counts and total spore-forming bacteria were determined on nutrient agar medium according to Harrigan and MacCance (1976), total fungi were determined on Rose-Bengal medium (Martin, 1950), total and feacal coliforms were determined using most probable number technique (MPN) according to APHA (1992).

Isolation, purification and identification of cyanobacteria

The enrichment cultures were prepared by aseptical addition of 25 ml of each representative sample to 250 ml flasks containing 100 ml liquid BG-11 medium and were incubated at room temperature under continuous illumination with florescent white lamp with light intensity of 400-500 lux (Ripka *et al.*, 1979).

Microscopic observations were recorded as well as growth appearance during 3-4 weeks of incubation. Several successive transfers were made using the respective medium and cyanobacterial isolates were subjected to a course of isolation and purification. One single filament isolation technique was applied to obtain unialgal cultures (Vaara *et al.*, 1979). Once a single filament had moved a sufficient distance in the BG-11 agar plate, a piece of agar, containing one single filament of the cyanobacterium (Fig. 2), was cut off and cultured in 100 ml fresh liquid BG-11 medium. One month later, the cultures were observed to contain only one single isolate of cyanobacteria. Then, it was diluted and subcultured for further tests and identification.

Morphological identification was done according to Rippka *et al.* (1979) using light and transmission electron microscopes at TEM lab, Cairo University Research Park (CURP), Faculty of Agriculture, Cairo University.

Cultivation and evaluation of Cyanobacterial isolates

The inocula of cyanobacterial isolates; *Oscillatoria* and *Anabaena* were prepared using BG-11 medium. Twenty five ml of each of 30-days old cultures were inoculated into 250 ml Erlenmeyer flasks containing 100 ml BG-11 medium and were incubated at room temperature under continuous illumination.

Spirulina was inoculated into Zarrouk's medium (Zarrouk, 1966). For biomass production, cultures were transferred to larger volumes of 6 liters bottles filled with five liters medium and incubated for 3-4 weeks at room temperature under continuous illumination and continuously aerated with air pump.

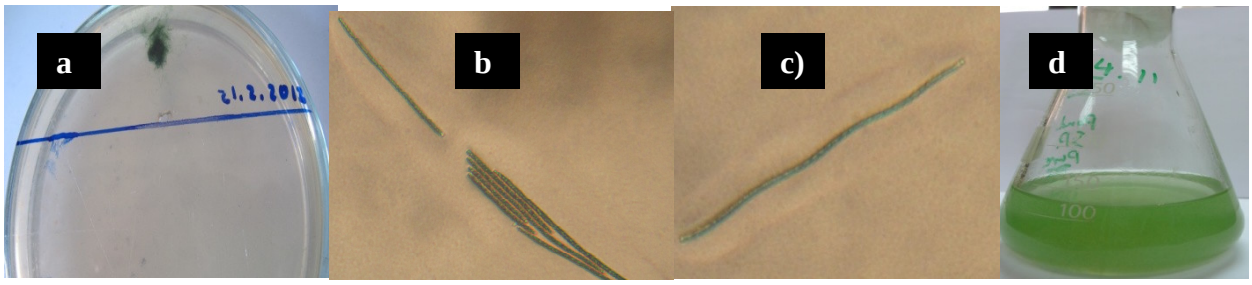


Fig.2:a. Algal mass on BG-11 plate b. *Spirulina* filaments migration through solid BG-11 medium c. One single filament of *Spirulina sp.*, d. Unialgal culture of *Spirulina Sp.*

a. Harvesting the biomass

Wet weight of well-grown cyanobacterial cultures were determined by collecting 20 ml of homogenous algal suspensions and centrifugation at 10000 rpm for 15 min. Dry weights of cyanobacterial cultures were estimated by drying the pellets overnight at 60-70˚C and weighted.

b. Influence of cyanobacterial extracts on brine shrimp eggs and mice survival

Preparation of extracts

The cyanobacterial biomass were collected by centrifugation at 10000 rpm for 5 minutes, the supernatants were used as source of extracellular bioactive metabolites. Five grams of each cyanobacterial strains biomass of each of *Spirulina sp., Anabaena sp., Oscillatoria sp.* and mixed cyanobacterial cultures were extracted with 5% acetic acid using ultrasonic cell disrupter equipped with microtip probe of 400 Watt (ULTRASONIC Gex 750). Disrupted cells were examined under microscope to ensure the complete rupture of the cynobacterial cells and filaments. Disrupted cells were then centrifuged at 5000 rpm for 5 minutes and the supernatants were evaporated to dryness using rotary evaporator and were retained for survival tests by means of brine shrimp eggs and mouse bioassay (Meyer *et al.,* 1982).

Influence of extracts on brine shrimp eggs survival

Brine shrimp eggs (*Artemia salina* leach) were supplied by Avocet Artemin Inc., Utah, USA. Larvae were used within 24 h of hatching. The dried extracts were dissolved in seawater to give four concentrations 500, 1000 and 2000 ppm to evaluate the survival. The number of dead shrimps that were placed in 5 vials with rate of 10 shrimps/vial were counted and percentage of mortality was then calculated.

Influence of extracts on mice survival

Sixteen male Albino Swiss mice weighting 20±2 g were obtained from Animal House, National Cancer Institute, Cairo, Egypt. The survival time was measured from the completion of the intraperitoneal (i.p.) injection to the last breath (AOAC, 2007). One ml containing 500 or 1000 or 2000 ppm of cyanobacterial extracts was intraperitoneally (i.p.) injected into 20±2 g male mice to detect the effect of the crude extract of the collected samples on mice survival (Agrawal *et al*., 2012). Survival was observed and death times were recorded.

c. Antimicrobial activity of cyanobacterial isolates

Cyanobacterial isolates were tested for their inhibitory effects by well diffusion method (Perez *et al.*, 1990) against (*E.coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeurogenosa* ATCC 9027*and Aspergillus niger* NRRL 326.

Preparation of cultures filtrates and extracts

The cultures were harvested by centrifugation at 1000 rpm for 10 min., the supernatants were collected and pellets were suspended in 15 ml ethanol or methanol. The homogenated pellets were then freezed and thawed three times and centrifuged at 10000 rpm for 10 min. The supernatants were harvested and kept at 4˚C till usage.

Antimicrobial assay

Wells were punched in the agar plate, and cultures of each tested organism were swabbed with sterile cotton swabs on the surface of nutrient agar medium. Ten, 50 and 100 µl from each of cyanobacterial cultures, filtrates and extracts were tested against the representative organisms. The plates were then incubated at 37˚C and solvent control was performed in each case. Areas of inhibited microbial growth were observed as clear zone around the well after 24 hours in bacteria and after 48 hours in fungi plate cultures. Antimicrobial activity was measured in cm as diameter of inhibition zone, excluding the well diameter.

Statistical analysis

The data recorded in triplicate and were subjected to ANOVA test. Data analysis was preceded by Assistat software (Silva and Azevedo, 2009).

Results and Discussion

Physical, chemical and biological analysis of lake water samples

a. Physical analysis

pH, E.C., T.S.S. and temperature were determined in the representative water samples. Data in Table (1) revealed that pH was slightly changed according to depth and was ranged from 9.58 to 9.80 in summer samples, and ranged from 9.62 to 9.90 in winter samples. This was accompanied by change in both E.C. and T.S.S. In summer samples, E.C. ranged from 80.2 to 93.4 ds/m while in winter samples they ranged from 29.9 to 30.3 ds/m. On the other hand, temperature differed according to the sampling season as it was 18°C and 29°C in winter and summer, respectively. In this respect, Hamed (2008) found that electrical conductivity of El-Khadra lake water was 62.2 ds/m. The author stated that highest electrical conductivity observed in Wadi El-Natrun lakes including El-Khadra lake was influenced by geological and climate conditions. Similarly, Hamed *et al.* (2007) observed that temperature ranged from 15 to 33°C and total soluble salts ranged from 21.581 to 43.852 g/l which reflect the alkaline nature of the lake.

b. Chemical analysis

Data in Table (1) indicated that the maximum concentration of anions was detected for SO_4^{-2} in summer samples followed by Cl^- and HCO_3^- while SO_4^{-2} was not detected in all depths in winter samples and Cl^- decreased. The prevalent cation in both seasons was Na^+ of which concentrations ranged from 27.124 to 30.569 g/l in summer samples and ranged from 9.235 to 9.734 g/l in winter samples. Generally, cations and anions concentrations increased in summer and decreased in winter, this may be due to the decrease of water level in summer because of high rates of evaporation. In this regard, Hamed (2008) found that sulphate and chloride were the prevalent anions in water samples of Wadi El-Natrun lakes, while sodium was the dominant cation. The author stated that the correlation between conductivity, Na^+ and Cl^- were relatively high, indicating that highest values of conductivity were because of the increased concentrations of these ions.

c. Microbiological analysis

Total bacterial counts, total spore-forming bacteria and total fungi were determined in water samples of El-Khadra lake (Table 2). The data revealed that the maximum total bacterial counts were detected in sed-

Table.1 Physical and chemical properties of El-Khadra lake water samples

Water samples depth	pH		E.C. (ds/m)		T.S.S. (g/l)	
	Sept. 2011	Feb. 2012	Sept. 2011	Feb. 2012	Sept. 2011	Feb. 2012
Surface	9.720	9.900	80.200	30.200	51.328	19.328
30 cm	9.720	9.620	89.100	30.300	57.024	19.392
1 m	9.800	9.890	86.300	30.300	55.232	19.392
Sediment water	9.580	9.860	93.400	29.900	59.776	18.688

Soluble anions (g/l)

	Sept. 2011				Feb. 2012			
	CO_3^{-2}	HCO_3^{-2}	Cl^-	SO_4^{-2}	CO_3^{-2}	HCO_3^{-2}	Cl^-	SO_4^{-2}
Surface	$<10^{-6}$	10.394	15.606	45.120	3.000	3.477	10.906	ND
30 cm	$<10^{-6}$	6.710	18.460	60.000	3.300	3.660	11.928	ND
1 m	$<10^{-6}$	6.137	18.439	54.720	3.240	1.586	10.686	ND
Sediment water	$<10^{-6}$	4.923	21.275	63.360	3.720	1.586	10.906	ND

Soluble cations (g/l)

	Sept. 2011				Feb. 2012			
	Na^+	K^+	Ca^{+2}	Mg^{+2}	Na^+	K^+	Ca^{+2}	Mg^{+2}
Surface	27.124	0.417	3.988	1.927	9.483	0.099	0.020	0.012
30 cm	28.276	0.413	5.186	4.568	9.734	0.135	0.044	0.034
1 m	28.051	0.406	6.792	4.794	9.235	0.092	0.032	0.038
Sediment water	30.569	0.425	7.990	3.126	9.235	0.093	0.040	0.060

ND: Not Detected

imnt water samples in both seasons and the highest counts of sporeformers and fungi were recorded in summer samples. The maximum fungal counts in summer samples were detected at 1 m depth (290 cfu/ml) while the maximum sporeformers densities were detected at surface samples (125 cfu/ml). Ali and Osman (2012) reported that total bacterial counts were increased in El-Khadra lake water samples taken at 1 meter than those at surface and 30 cm-depth. On the other hand, total and feacal coliforms were detected (Fig.3); as total coliforms scored 2 cells/ml at surface water and 4 cells/ml at all other water samples in summer samples while they ranged from 1 to 8 cells/ml in winter samples. On the other hand, the maximum counts of feacal coliforms were 4 cells/ml in summer samples regardless the water sample depth. In contrast, Aly and Osman (2012) found that feacal coliforms were present in surface and subsurface water samples while they were absent in the sediment water samples of El-Khadra lake.

Table.2 Total bacterial counts, total spore-forming bacteria and total fungi.counts (cfu/ml) in water samples of El-Khadra lake at different depths

Microbial counts (CFU/ml)	Sept. 2011				Feb. 2012				LSD 5%
	Surface	30 cm	1 m	Sediment water	Surface	30 cm	1 m	Sediment water	
Total bacterial counts	900	165	500	990	820	80	200	910	361.1^{ns}
Total spore-formers	125	90	50	70	70	30	40	60	55.8^{ns}
Total fungi	100	120	290	200	90	110	70	45	128.3^{ns}

Fig.3 Counts (cells/ml) of total and feacal coliforms in the tested water samples

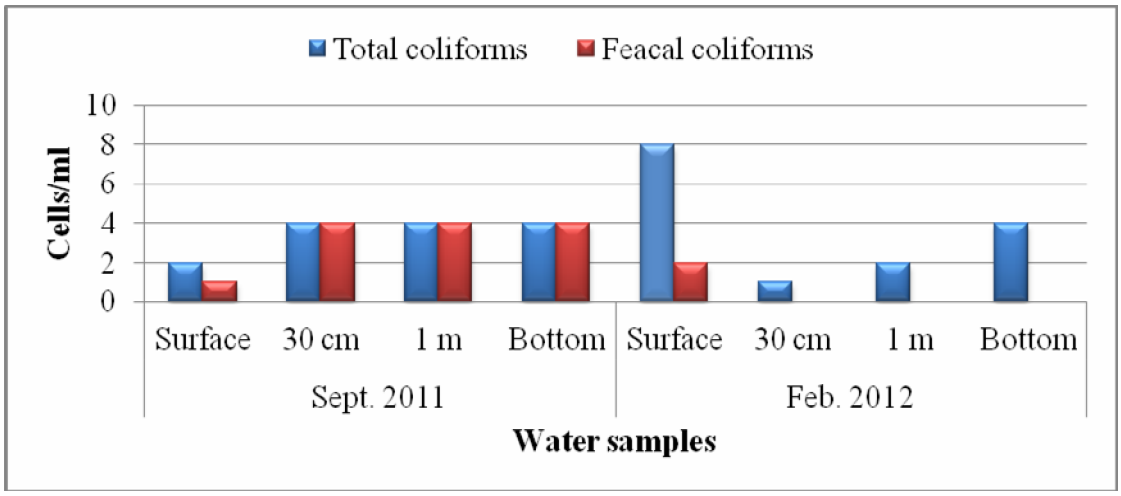


Fig.4 Blooms of cyanobacteria in El-Khadra lake

Isolation, purification and identification of cyanobacteria

Blooms of cyanobacteria appeared in El-Khadra lake (Fig.4) showed low population of cyanobacteria belonging to different species and revealed that spiral cyanobacteria were the most inhabitants of the lake. Hamed et al. (2007) reported that *Spirulina platensis* was the monospecific inhabitant of El-Khadra lake. Microscopic images of the microalgal species observed in this study are shown in Fig (5). Microscopic analysis of the samples allowed preliminary identification of species such as genus *Chlorella, Puncticulata, Spirulina, Oscillatoria, Anabaena* and *Nostoc.* Cyanobacteria were morphologically identified according to Rippka *et al.* (1979), while other algae were identified according to their distinctive characteristics (Carmelo *et al.*, 1996). Table (3) shows the relative occurrence of the phytoplankton in the lake and results indicated that the maximum growth at surface water was due to *Spirulina sp.* followed by circular diatom, *Puncticulata sp. Oscillatoria and Nostoc* recorded considerable growth while *Chlorella* and *Anabaena* showed no growth in surface water samples.

BG-11 medium was used for several successive transfers and enrichment of low cyanobacterial populations in water samples. After enrichment and several successive transfers, cyanobacterial isolates were obtained by single filament isolation technique. Well grown cultures of cyanobacterial isolates were examined under light microscope to ensure their purity from any other species of cyanobacteria. Isolates were identified morphologically using both light and transmission electron microscopes.

Fig. 5 Light micrographs of some phytoplankton inhabitants of El-Khadra Lake a.100x *Chlorella spp.*, b. 10x Circular diatom (*Puncticulata spp*.), c. 100x *Oscillatoria sp.*, d. and e. 40x *Spirulina sp*. f. 40x *Anabaena sp*. g. 40x *Nostoc sp*.

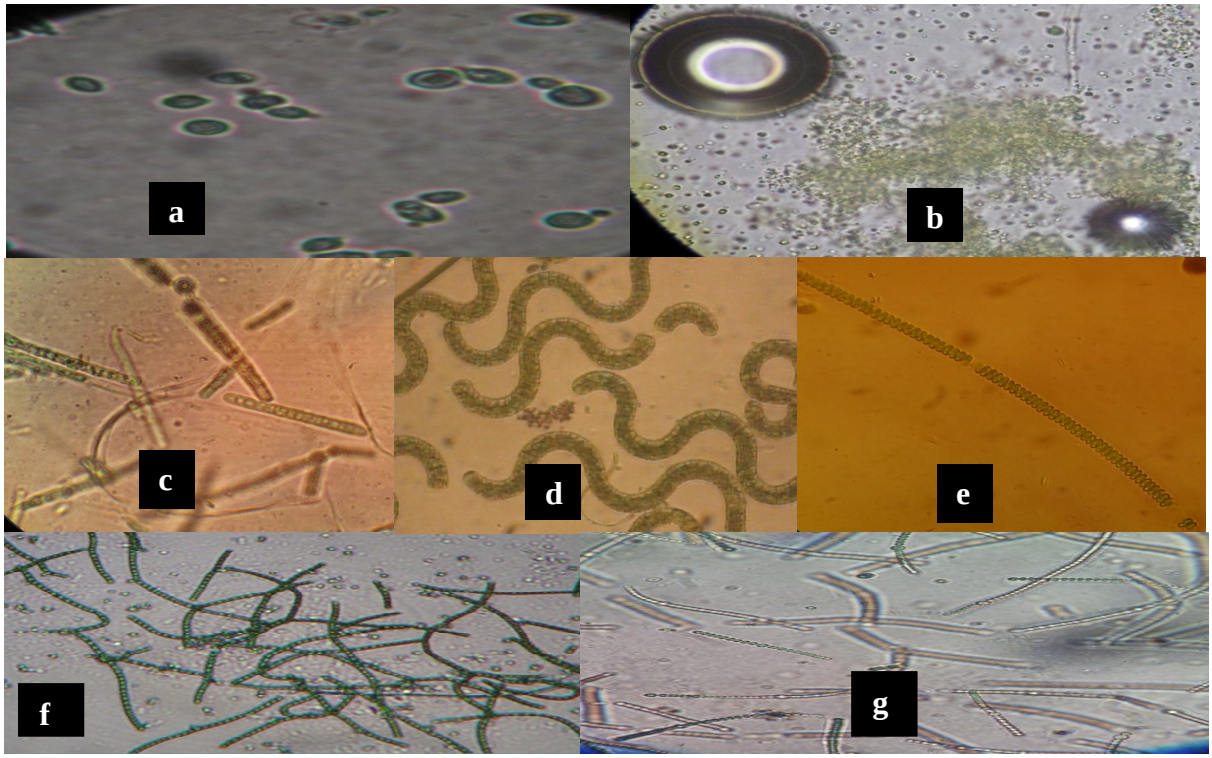


Table.3 Relative occurrence of some phytoplanktons in El-Khadra lake

Samples	*Chlorella sp.*	*Puncticulata sp.*	*Spirulina sp.*	*Oscillatoria sp.*	*Anabaena sp.*	*Nostoc sp.*
Surface	-	++	+++	+	-	+
30 cm	+	++	++	+	-	-
1 m	+	++	+	++	+	-
Sediment water	+	-	+	+++	+	-

(+++), Good; (++), Moderate; (+), Weak; (-), No growth

Spirulina appeared as spiral filaments composed of cylindrical cells arranged in unbranched trichomes (Fig.6a). The diameter of the cells ranged from 4 to 6 um. *Spirulina* do not contain heterocyst and trichomes are motile. Cells had granular cytoplasm containing gas vacuoles and visible thylakoids (Fig. 6b).

Oscillatoria spp. cultures exhibited filaments composed of disc-shaped cells in very long and unbranched trichomes (Fig.6c). Trichomes are slightly constricted at the cross-walls and are thinly sheathed. They do not produce heterocysts or akinetes. Numerous parallel thylakoids run through the cells, occupying most of the cytoplasm volume (Fig.6d). At both trichome ends, the apical cell found with calyptra, which is hemispheric and smaller than the intercalary cells (Fig 6e).

Respecting *Anabaena sp.* cultures, they are filamentous and trichomes usually constricted at the cross walls. Vegetative cells are cylindrical, pale to bright blue-green in color (Fig.6f). Heterocysts are intercalary or solitary. Tyagi (1973) and Bothe *et al.* (2010) found that heterocyst formation depends on availability of carbon intermediates and ATP. Akinetes are solitary or duplicated in row, adjacent to heterocyst or distant, mature akinetes several times are larger than vegetative cells. Thylakoids were arranged peripherally (Fig.6g).

Cultivation and evaluation of cyanobacterial isolates

a. Harvesting biomass

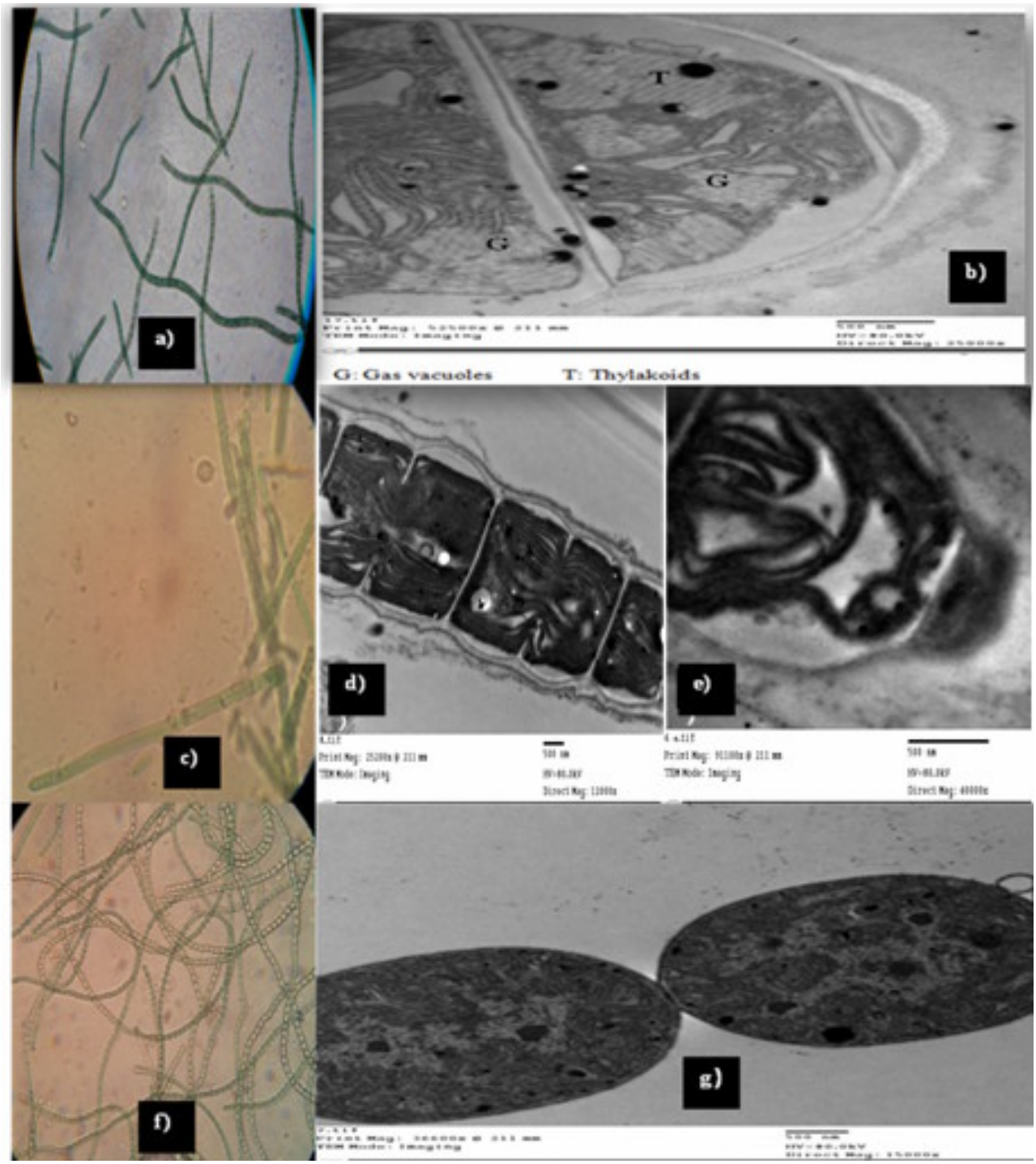
Biomass concentrations (g/l) of the cultures of the isolated cyanobacteria were determined by measuring dry weights. The dry weights of *Spirulina sp.* grown in Zarrouk's medium, *Oscillatoria sp.* and *Anabaena sp.* grown in BG-11 medium were 3.84, 2.45 and 2.38 g/l, respectively.

b. Influence of cyanobacterial extracts on brine shrimp eggs and mice survival

Influence of extracts on brine shrimp eggs

The brine shrimp assay provides a simple and sensitive method for routine monitoring of blooms, particularly in developing countries where sophisticated equipment are not available (Akin-Oriola, 2003). During this study, cyanobacterial samples have been tested for any possible toxicity using Brine shrimp bioassay technique at concentrations of 500, 1000 and 2000 ppm of cyanobacterial extracts.

Fig.6 a) 40x Light micrograph of 30 d old culture of *Spirulina sp.*, b) (25000x) Transmission Electron Micrograph of 30 d old *Spirulina* cell, c) Light micrograph of 100x *Oscillatoria sp.* d) (12000x) Transmission Electron Micrograph of 30 d old *Oscillatoria* cell showing numerous parallel thylakoids inside the cell, e) (40000x) TEM showing an Oscillatoria apical cell with calyptra f) Light micrograph of 40X *Anabaena sp.*, g) (15000x) Transmission Electron Micrograph of 30 d old *Anabaena* cell.



None of the applied extracts had a pronounced influence on survival /mortality % of brine shrimp eggs in mixed culture extracellular extract and *Spirulina* biomass extract at concentration of 500 ppm, but at concentration of 1000 and 2000 ppm, The percentage of survival was 96.7 % and 93.3 %, respectively in mixed culture extracellular extract. In *Spirulina* biomass extract, the percentage of mortality was 16.7 % and 20 % at 1000 and 2000 ppm concentrations respectively.

Similarly, in *Oscillatoria* biomass extract the percentage of survival was 83.3 %, 80 % and 76.7% at concentrations of 500, 1000 and 2000 ppm, in that order, while its extracellular extract showed a percentage of survival 83.3% at concentration of 2000 ppm. Meanwhile, the percentage of survival was 90 %, 90% and 86.7% at concentrations of 500, 1000 and 2000ppm respectively in *Anabaena* biomass extract and extracellular extract (Fig.7).

Influence of extracts on mice survival

The intraperitoneal injection of cyanobacterial extracts showed that none of the used extracts had a pronounced influence on survival/mortality % of mice.

c. Antimicrobial assay of cyanobacterial isolates

The inhibitory effect of cyanobacterial isolates; *Spirulina, Oscillatoria and Anabaena spp.* at various concentrations against five microorganisms showed zones of inhibition at diverse levels (Table 4 and Fig.8). It is clear from Table (4) that the diameter of inhibition zone depends mainly on cyanobacterial isolate, type of the solvent used for extraction, the concentration of the extract per well, concentration of culture and filtrate per well and the tested bacterial and fungal organisms.

Fig.7 Brine shrimp eggs assay of cyanobacterial isolates

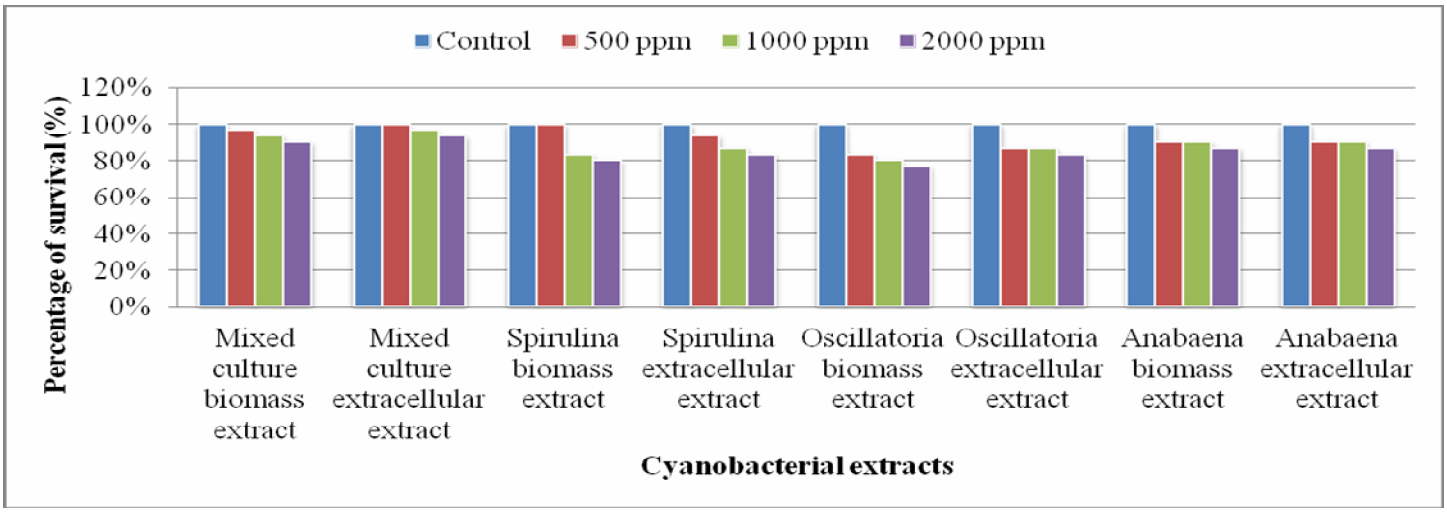


Table.4 Antimicrobial assay of cyanobacterial isolates

Cyanobacterial cultures/ filtrates/ extracts with various concentrations (µl)		Zones of inhibition observed for different microorganisms (cm)				
		E. coli	*S. aureus*	*P. aeroginosa*	*B. subtilis*	*A. niger*
Spirulina						
Culture	10	-	-	-	0.2	0.6
	50	-	-	-	0.7	0.8
	100	-	-	-	0.8	0.9
Filtrate	10	-	-	-	0.7	0.4
	50	-	-	-	0.9	0.6
	100	-	-	-	1.1	0.9
Ethanolic extract	10	-	-	-	-	-
	50	0.4	0.7	0.3	0.2	0.3
	100	0.6	0.9	0.5	0.3	0.9
Methanolic extract	10	-	-	-	-	-
	50	0.7	-	0.2	-	-
	100	0.9	-	0.3	-	1.3
LSD (0.01)				**0.1**		
Oscillatoria						
Culture	10	-	-	-	0.6	0.9
	50	0.5	-	-	1.3	1.0
	100	0.9	0.6	-	2.0	1.3
Filtrate	10	-	-	-	-	0.5
	50	0.4	0.6	0.3	0.2	0.6
	100	1.0	0.8	0.6	0.4	0.8
Ethanolic extract	10	-	0.2	-	-	-
	50	-	0.5	-	0.3	-
	100	-	0.8	0.3	0.8	0.9
Methanolic extract	10	-	0.4	-	-	-
	50	-	1.3	-	0.6	0.7
	100	-	1.4	-	0.8	1.3
LSD (0.01)				**0.3**		
Anabaena						
Culture	10	-	-	-	-	-
	50	0.7	-	-	0.2	-
	100	1.0	-	-	0.5	1.3

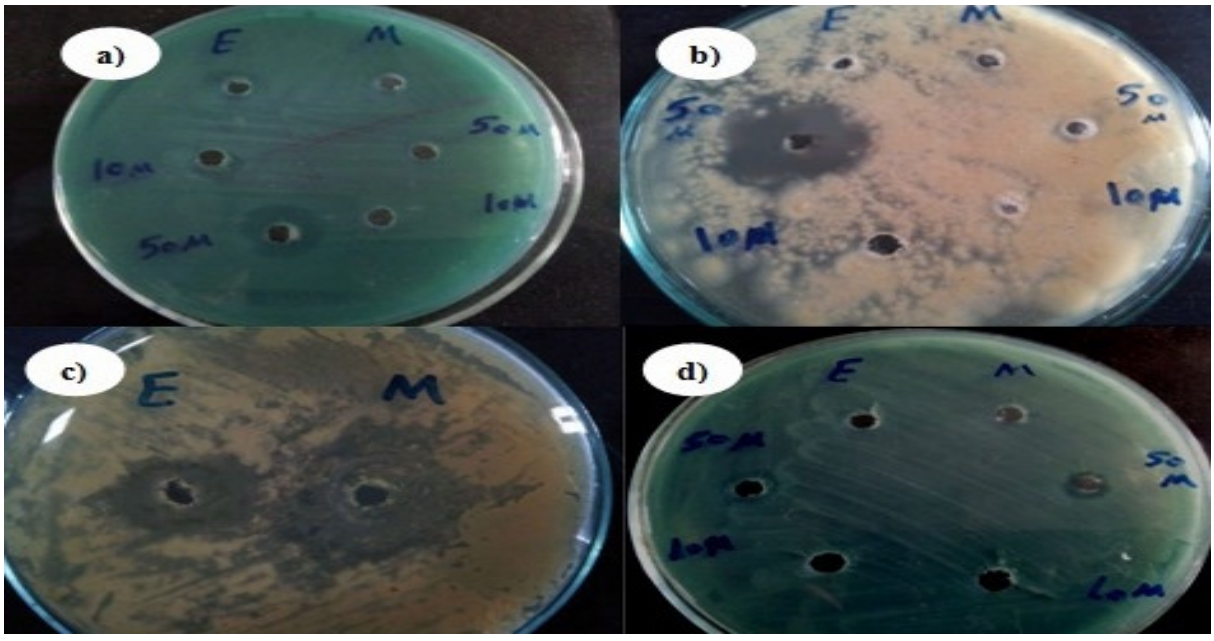
Continued

Table 4. Continued

Cyanobacterial cultures/ filtrates/ extracts with various concentrations (µl)		Zones of inhibition observed for different microorganisms (cm)				
		E. coli	*S. aureus*	*P. aeroginosa*	*B. subtilis*	*A. niger*
Filtrate	10	-	-	-	-	0.5
	50	0.6	-	-	0.5	0.6
	100	0.9	-	-	0.7	0.9
Ethanolic extract	10	-	-	0.2	-	-
	50	0.4	-	1.2	0.8	1.8
	100	0.6	-	1.7	1.5	2.1
Methanolic extract	10	0.1	-	-	0.4	-
	50	0.3	-	-	0.5	0.8
	100	0.4	-	0.2	0.7	0.9
LSD (0.01)			**0.2**			
LSD (0.01)		**0.2**	**0.3**	**0.1**	**0.2**	**0.3**

(-), No detected activity
LSD (0.01) treatments= 0.3

Fig.8 Antimicrobial assay of cyanobacterial isolates. a: inhibition effect of 50 µl ethanolic extract of *Anabaena against P. aeroginosa*, b: inhibition effect of 50 µl ethanolic extract of *Anabaena* against *A. niger*, c : Inhibition effect of 100 µl methanolic and ethanolic extracts of *Oscillatoria* against *A. niger* d: Inhibition effect of 50 µl ethanolic extract of *Spirulina* against *P. aeroginosa.*



Spirulina culture and filtrate showed no inhibitory activity against *E. coli*, *S. aureus* and *P. aeroginosa*, but its filtrate showed significant inhibitory activity against *B. subtilis* at concentration of 100 µl/well. The filtrate of *Oscillatoria* culture showed inhibitory activity against all tested organisms at higher concentrations (50 and 100 µl/well), but showed no inhibitory activity at 10µl/well concentration except in case of *A. niger* as it showed some inhibitory effect (0.5 cm).

At the same time, *Oscillatoria* (100 µl/well) filtrate showed significant inhibitory effect against each of E.*coli*, *P. aeroginosa*, *B. subtilis* and *A. niger*, but showed no significant activity against *S. aureus*. On the other hand, it was observed that *Oscillatoria* culture showed no inhibitory effect against *P. aeroginosa* while it has minimum inhibition effect against *S. aureus* (0.6 cm) only at higher concentration (100 µl/well) and the most significant effect of *Oscillatoria* was at concentration (100 µl/well) culture against *B.subtilis*.

Anabaena filtrate showed no biological activity against *S. aureus* and *P. aeroginosa* while its maximum activity was observed against *A. niger*. Also, *Anabaena* culture showed no inhibitory effect against *S. aureus* and *P. aeroginosa* and showed minimum activity against *B. subtilis* while its significant activity was observed against *A. niger* (1.3 cm) at only (100 µl/well) concentration (Table 4).

Regarding the different solvents used for extraction of bioactive metabolites, Gosh *et al.* (2008) showed that cyanobacterial aqueous extracts are generally less potent in their bioactivity than organic extracts, while Bhattacharyya *et al.* (2013) indicated that the methanolic extract of *Anabaena variabilis* and *Anabaena fertilisima* had high activity against *S. aureus* but their aqueous extract exhibited no inhibitions as compared to the other organic extracts. Therefore, the antimicrobial activity of ethanolic and methanolic extracts of cyanobacterial isolates were applied on the tested microorganisms.

During this study it was found that, the maximum inhibition zone of *Spirulina* was in 100 µl/well methanolic extract against *A. niger* and *E.coli* (1.3 and 0.9 cm, respectively) while the minimum inhibition zone was in 50 µl against *P. aeruginosa* (0.2 cm). The minimum inhibition zone of ethanolic extract was in concentration of 50 µl against *B. subtilis* (0.2 cm). *Spirulina* methanolic extract showed no activity against *B. subtilis* and *S. aureus* at its all concentrations but ethanolic extract 10 µl showed no effect on all tested microorganisms. In contrast, the results obtained by Abedin *et al.* (2008), who found that ethanolic extract of *Spirulina* gave no detected biological activity against *E.coli* and *P. aeroginosa.* On the other hand, they found that ethanolic extract of *Spirulina* had an inhibitory effect on *B. subtilis*, *S. aureus* and *A. niger*. The same authors, reported that *Spirulina* methanolic extract gave no biological activity against *E.coli* and *B. subtilis* but it gave the maximum inhibition zone against *P. aeroginosa* followed by *S. aureus* and *A. niger*. In this respect, these results also are in harmony with those obtained by Arun *et al*. (2012) who found that methanolic extract of *Spirulina* exhibited inhibitory activity against *A. niger* followed by *P. aeuroginosa,* respectively. On the other hand, they reported that *Spirulina* methanolic extract showed the maximum activity against *S. aureus*. However, these

results also go in conflict with Akhtar *et al.* (2012) reported that methanolic extracts of *Spirulina* gave higher inhibition activity against *S. aureus* than that of ethanolic extracts. Akhtar's study reported that *Spirulina* methanolic extracts gave maximum activity against *S. aureus* than *E.coli*.

Maximum inhibition activity of *Oscillatoria* was observed against *S. aureus* and *A.niger* resulting in 1.4 and 1.3 cm inhibition zones, respectively in 100 µl/well methanolic extract but the *Oscillatoria* ethanolic extract gave maximum inhibition zone against *S. aureus* followed by *B. subtilis* and *E.coli*, respectively; while its methanolic extracts gave the maximum activity against *E.coli* followed by *S. aureus* and *B. subtilis*, respectively.

Anabaena showed no detected activity against *S. aureus* in both extracts, but its ethanolic extract (100 µl/well) gave a significant activity against *A. niger* (2.1 cm) followed by *P. aeuroginosa* (1.7 cm). Methanolic extract (10 µl/well) showed the minimum inhibition zone against *E.coli* (0.1 cm), but the higher concentrations showed moderate activity. Generally, the cyanobacterial isolate which showed the significant activity all over other isolates was *Anabaena* that showed significant inhibitory activity against *A. niger* (2.1 cm).

These results are similar to those of Sivakami *et al.* (2013), who found a biological activity against *E.coli* in both extracts. These results disagree with Chauhan *et al.* (2010) where they reported that *Anabaena* methanolic extract gave minimum activity was detected in 10 µl/well ethanolic extract against *S. aureus* (0.4 cm). *Oscillatoria* methanolic extract showed no detected activity against *P. aeuroginosa* while, ethanolic extract (100 µl/well) showed moderate effect (0.3 cm). These results conflict with those of Mathivanan *et al.* (2010); who found that ethanolic extract of *Oscillatoria* showed the maximum inhibition zone against *S. aureus* followed by *p. aeruginosa,* respectively; while the minimum inhibition zone was against *A. niger.* Madhumathi *et al.* (2011) reported that higher inhibitory activity against *B. subtilis* than *E.coli* and had no activity against *P. aeroginosa*, while it had the maximum inhibition zone against *S. aureus*. In addition, Abedin *et al.* (2008) study conflicted with present study results; they found that *Anabaena* ethanolic extract gave the maximum inhibition zone against *P. aeroginosa* and moderate activity against *E.coli, S. aureus* and *A. niger*, but gave lowest activity against *B. subtilis*. Also, they reported that *Anabaena* methanolic extract gave higher inhibitory effect against *P. aeroginosa* and *B. subtilis* and moderate activity against *S. aureus* but it gave lower activity against *E.coli* and *A. niger*.

In conclusion, the results of this study suggested that El-Khadra lake contains several species of marine cyanobacteria that have immense potentials. Results indicated that cultures, cultures filtrates and extracts of some marine cyanobacterial strains isolated from El-Khadra lake showed considerable antimicrobial activity against different microorganisms. Further studies should be made to identify and purify natural products from these cyanobacteria against bacterial and fungal activity. Improving

knowledge of the composition, analysis, and the properties of these cyanobacteria with respect to antimicrobial compounds and non-toxic activity would assist in efforts for different applications among which; pharmaceutical and agricultural applications.

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