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RESEARCH ARTICLE

Cyanodiveristy in Some Egyptian Protected Areas

Nahla M. Naguib[1], Adel A. Awad[2], Olfat E. Barakat[3], Aziz M. Higazy[4]

1 Nature Conservation Sector (NCS) – Egyptian Environmental Affairs Agency

2 Microbiology Department, SWERI, ARC

3, 4 Microbiology Department – Faculty of Agriculture – Cairo University

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Corresponding Author

Nahla Naguib
nahla.nagib85@gmail.com

Abstract

Diversity of Cyanobacteria was determined in six protectorates (PAs) in Egypt. These six PAs represent three different habitats *i.e.* marine, fresh water, brackish habitats. A total of 6 isolates were obtained in pure cultures and 44 species were determined belonging to 4 orders, 11 families and 16 genera. RAPD molecular analysis was performed to show phylogenetic relationship among 6 dominant cyanobaterial isolates that reflect different ecological background from where each one was isolated.

INTRODUCTION

The word 'biodiversity' is a contraction of biological diversity. It is most commonly used to replace the more clearly defined and long established terms, species diversity and species richness. Biologists most often define biodiversity as the "the variety of life forms on earth at all levels of biological systems *i.e.* genetic, organisms population, species and ecosystem (Gaston, 2010).

Protectorates (PAs) are a fundamental tool used worldwide for protecting natural resources because of their recognized natural, ecological and/or cultural values, creating a buffer and refuge against a rising tide of human impacts (Dudley, 2008). The first nature reserve in modern history is yellowstone national park in the USA, established in 1872 (Roop and Whittlesey, 2013). There are over 161,000 Protectorates in the world with more added daily, representing between 10 and 15 percent of the world's land surface area (IUCN, 2010; Soutullo, 2010; UN, 2010; UN, 2013). Protectorates act as benchmarks against which we understand human interactions with the natural world (Mora and Sale, 2011). Conservation of critical ecosystem and biodiversity is mandated by regional and international conventions. Convention on Biological Diversity (CBD) obligates Egypt since signed at 1992 to establish and maintain a network of protected areas in order to protect and conserve ecosystems, representative habitats, threatened species, cultural heritage sites, and traditional knowledge. By 2020, Egypt's Protected Area Network should cover 17% of the total land area of Egypt (NCS, 2006).

Cyanobacteria constitute a versatile group of photosynthetic bacteria of immense commercial and ecological importance (Olson, 2006 and Jacquet *et al.*, 2013). They appeared to be a rich source of many useful products and are known to produce a number of bioactive compounds. They are also a rich source of many useful natural products, so they were used as feed, food and fertilizers. They have the distinction of being the oldest known fossils, more than 3.5 billion years old. They can occur as planktonic cells or form phototrophic biofilms in fresh

water and marine environments. A few are endosymbionts in lichens, plants, various sponges and provide energy for the host (Whitton and Potts, 2002).

Existence of cyanobacteria around world are reported everywhere. For example, Harper *et al.* (2003); Hamsisi *et al.* (2004); Doumer *et al.* (2009) and Ramadani *et al.* (2009) studied diversity of cyanobacteria in Africa. Also, Song *et al.* (2006); Nagasathya and Thajuddin (2008); Ionescu *et al.* (2009); Makandar and Bhatnagar (2010); Sivakumar *et al.* (2012) observed diversity across Asia. While, Crosbie and Furnas (2001) and John *et al.* (2009) were interested in diversity of cyanobacteria in Australia. Even in extreme environments, a lot of studies have been done to find out cyanobacteria existence in Antractica and cold ecosystems (Margesin *et al.*, 2008 and Parry & Peacrce, 2014).

In Egypt, a lot of work has been done from 1995 to 2012 (Mohamed, 2002; Hamed, 2005; Hamed *et al.*, 2007; Hamed, 2008; Shehata *et al.*, 2008; Abdelhady & Hussian, 2012 and Hoballah *et al.*, 2013) where qualitatively surveyed, the freshwater habitats were the most productive habitats for blue-green algae where 37 species were recorded, followed by marine (23 taxa), hypersaline (12 taxa) and brackish (11 taxa) aquatic habitats. The widely distributed taxa inhabited the four aquatic habitat types were represented by members of the following species: *Ammatoidea, Anabaena, Aphanocapsa, Calothrix, Chlorogloea, Chroococcus, Cyanobacterium, Cyanosarcina, Cyanothece, Cylindrospermum, Dactylococcopsis, Dichothrix, Entophysalis, Geitlerinema, Gloeocapsa, Gomphosphaeria, Heteroleibleinia, Homoeothrix, Hydrococcus, Jaaginema, Johannesbaptistia, Komvophoron, Leibleinia, Leptolyngbya, Lyngbya, Merismopedia, Microcystis, Nodularia, Nostoc, Oscillatoria, Phormidium, Planktolyngbya, Planktothrix, Plectonema, Porphyrosiphon, Pseudanabaena, Rhabdoderma, Spirulina, Synechococcus, Synechocystis*.

This study was planned to find out the prevailing of cyanobacterial species and their diversity in some protected areas of Egypt in different aquatic ecosystems, *e.g.* Abu Galum (Ag), Wadi El Gemal (WG), Ashtoum El Gamil (Ash), El Omied (Mat), Qaroun (Q), Wadi El-Rayan (WR), Saluga & Ghazal (SG), Dahab Island (DI) protectorates. This might help in establishing a preliminary database of microflora, with special reference to cyanobacteria, to fulfill some of the objectives of the national biodiversity strategic action plan in Egypt.

Materials & Methods

1. Sampling

Samples were collected primarily for the isolation of cyanobacteria from eight Egyptian protected areas 'PAs' (Fig.1) distinguished by their different aquatic ecological features, *e.g.* Abu Galum (Ag), Wadi El Gemal (WG), Ashtoum El Gamil (Ash), El Omied (Mat), Qaroun (Q), Wadi El-Rayan (WR), Saluga & Ghazal (SG), Dahab Island (DI). Samples were taken in four seasons: summer 2011, winter 2012, summer 2012, and winter 2013. All the water samples were collected from surface water with total 96 samples and were subjected to physical, chemical and microbiological analyses.

2. Chemical & physical analyses

Determination of salinity and chemical determinations (ammonia and nitrate, total nitrogen, phosphorous, total soluble salts, COD and BOD.

Temperatures were determined in the sampling site, pH value was measured using JENWAY 3505 pH meter (APHA, 1992), by SensoDirect Con 110 (APHA, 1992).

3. Microbiological analysis

Water samples were examined for presence of some microbial groups. Total fungal counts were counted (cfu/ml) on Rose Bengal chloramphenicol agar medium (Dixon and Formtling, 1995), at 30°C for 5-7 days. For the total viable counts on glucose – yeast extract agar (Postage, 1969). Plates were incubated at 30°C for 24 h. For total spore formers counts, pasteurized dilutions (80°C for 15 min.) from each sample was used for inoculating nutrient agar plates for enumerating the total count of spore forming bacteria. The MPN of both total and feacal coliforms were estimated using MacConkey broth medium incubated at 37°C and 44°C for 24h, respectively. Counts of coliforms were calculated from the statistical tables of Pochon and Tardieux (1962).

4. Isolation, identification and purification of cyanobacteria

a. Culturing and maintenance of cyanobacteria

Liquid enrichment cultures were prepared from water samples. Twenty five ml of each water sample were aseptically added to 250 ml flasks containing 100 ml liquid Allen and Arnon (Allen and Arnon, 1955), ASN III, and BG-11 growth media (Rippka *et al.*, 1979). The enrichment cultures were incubated under continuous illumination with florescent white lamp and incubated at room temperature. Isolates of cyanobacteria which had been previously growing in flasks containing BG11 liquid medium were successively subcultured several times on the same medium and incubated for 3-4 weeks at room temperature until the healthy cultures were obtained.

Cyanobacterial isolats were morphologically identified with a light microscope according to Rippka *et al.* (1979). All isolates obtained were maintained under photoautotrophic growth conditions in BG11 medium.

b. Purification of cyanobacteria

In this experiment, purification process was performed by single filament isolation technique (Vaara *et al.*, 1979 and Barakat *et al.*, 2008). In this respect, BG11 agar medium in Petri dishes were used to examine the ability of cyanobacterial filaments to grow and slides towards a source of light to pick a single filament of cyanobacteria. The cyanobacterium filaments were examined microscopically daily until one single filament moved through the whole plate toward the light source. Once the single filament had moved a sufficient distance into the sterile BG11 agar plate, a piece of agar, containing one single filament of the selected cyanobacterium filament was cut off, and placed into a separate flask containing fresh liquid BG11 medium under sterilized conditions. One month later, the culture was observed to contain only the same cyanobacterium belonging to same species.

c. Identification of cyanobacteria cultures

Identification practices were performed by means of microscopic examinations for purified isolates and by a Drop Method of Lugol's solution, 0.5 µl of the reduced volume was placed in a counting chamber and examined at 10X eyepiece and 40X objective of inverted microscope (APHA, 1992) according to Kofoid (1907), Kofoid & Swez (1921), Geitler (1925) and Prescott (1978).

5. Phylogenetic relationship between prevailed dominant cyanobacteria species

a. DNA extraction and PCR amplification of RAPD

DNA was extracted from 1.5 ml aliquots of cell suspensions of each extracted biofilm sample from 6 cyanobacterial cultures representing 6 PAs according to Morin *et al.* (2010). Five universal primers were used, *i.e.* OPE-B-10, OPE-F-12, OPE-K-10, OPE-09, OPE-P-15. DNA was isolated and purified according to Morin *et al.* (2010). PCR reactions were performed in a total volume of 20 µl containing 10 ng DNA, 200 mM dNTPs, 1 mM of five arbitrary 10- mer primers (Operon Technology, Inc., Alameda, CA, USA), 0.5 units of Red Hot Taq polymerase (AB gene House, UK) and 10-X Taq polymerase buffer (AB gene House, UK). For DNA amplification Biometra thermal cycler (2720) was programmed as follow: 94º C for 5 minute followed by 35 cycles 94 º C for 1 min., 35 º C for 1 min. , 72 ºC for 1 min. and 72 º C for 7 min.

The amplification products were analyzed by electrophoresis in 1% agarose in TAE buffer, stained by ethidium bromide and photographed under UV light. The sequences of the tested primers are presented in Table (1).

RAPD molecular technique was performed for the six pure cultures obtained from six enrichment cultures representing six PAs. The banding patterns generated by RAPD-PCR markers analyses were compared to determine the genetic variation of isolates among 6 PAs. Clear and distinct amplification products were visually examined for presence and absence of bands.

Table (1): Five universal primers sequences used in RAPD-PCR experiment

Primer	Sequence
B-10	5`CTGCTGGGAC3`
K-10	5`GTGCAACGTG3`
F-12	5`ACGGTACCAG3`
P-15	5`GGAAGCCAAC3`
P-09	5`GTGGTCCGCA3`

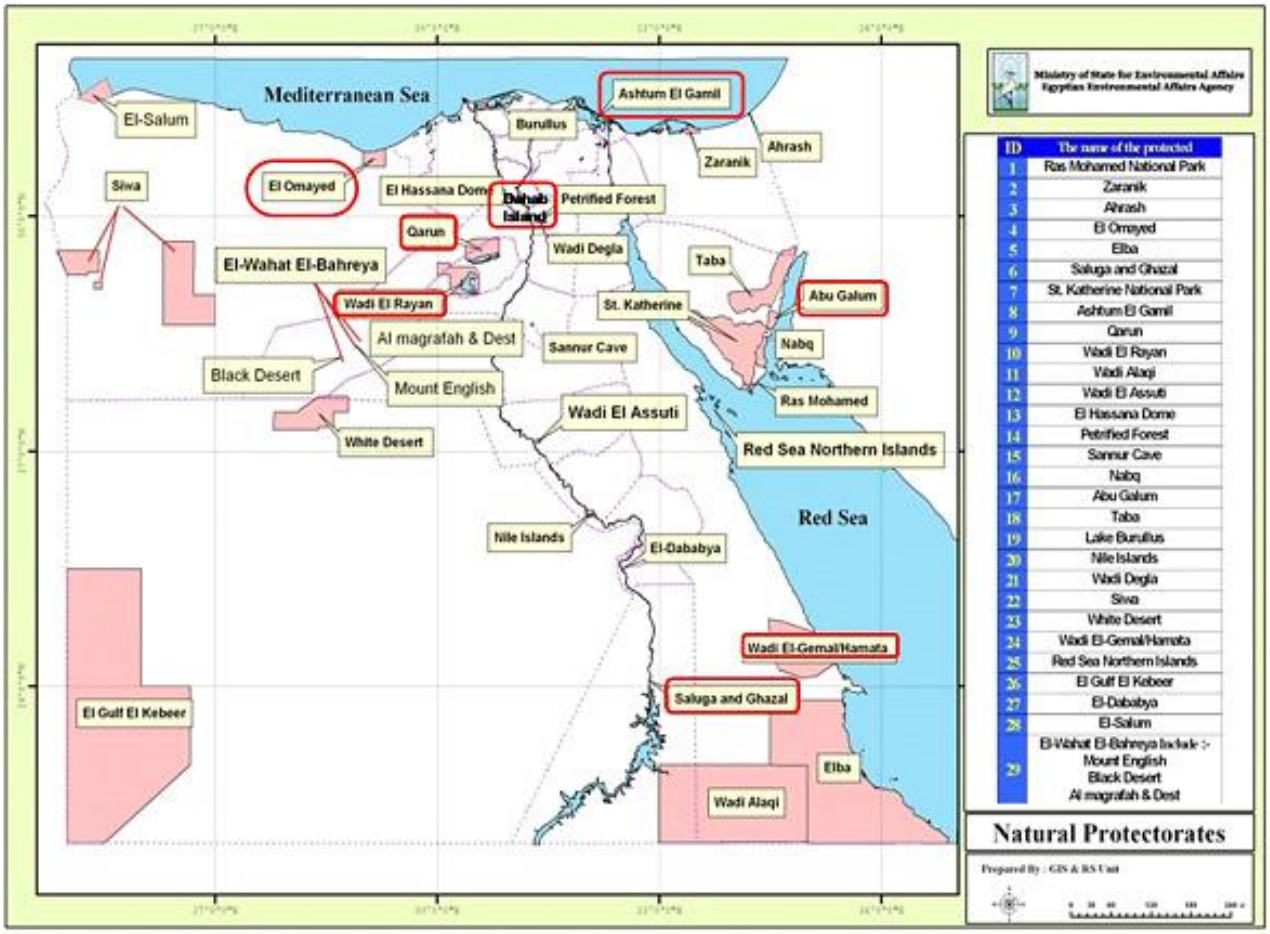

Fig.1. Location of selected protected areas outlined with red colour, during this study

Results and Discussion

1. Chemical and Physical analyses of water samples

pH, temperature and salinity were determined for the representative water samples and the data in Table (2) revealed that pH ranged from 7.8 to 8.7 and temperature ranged from 12°C and 32°C, these were normal values according to Egyptian Environmental Affairs Agency annual reports (EEAA, 2010a, 2010b; EEAA, 2011a, 2011b; EEAA, 2012a, 2010b; EEAA, 2013a, 2013b) which may indicate limited human activities that can alter such water properties.

Salinity values of water samples from Wadi El-Gemal, Abu Galum, Omayed, Dahab Island and Saluga & Ghazal protectorates (Table, 2) were within their normal and expected values as their TSS ranged from 46 to 1 g/l. In this respect, TSS in Ashtum El-Gamil water samples ranged between 2 g/l and 7 g/l, due to the effect of River Nile outlet. Although Qaroun lake protectorate considered as brackish habitat but salinity was slightly higher than expected and ranged between 31 and 43. This is most likely due to wastes discharge from human activities.

Table (2): Physical properties of water samples

Water samples*	pH				Temperature (°C)				Salinity (TSS) (g/l)			
	S** 2011	W 2012	S 2012	W 2013	S 2011	W 2012	S 2012	W 2013	S 2011	W 2012	S 2012	W 2013
Ag	8.2	8.0	7.9	8.2	26	21	25	18	39	36	40	32
WG	8.0	8.2	8.2	8.2	30	26	28	19	46	37	39	38
DI	8.7	7.9	8.1	8.6	29	15	32	14	4	2	1	3
SG	8.1	7.9	7.8	8.0	25	16	24	14	2	1	4	1
Q	8.3	8.4	8.2	8.7	28	18	26	16	33	43	31	32
WR	8.5	8.4	8.6	8.7	28	20	23	15	9	9	9	9
Ash	8.6	8.4	8.1	8.6	22	16	24	17	5	7	2	7
Mat	8.1	8.0	7.8	8.0	23	12	25	18	37	36	37	37

*, Ag, Abu Galum; WG, Wadi El-Gemal; DI, Dahab Island; SG, Saluga & Ghazal; Q, Qaroun Lake; WR, Wadi El-rayan Lake; Ash, Ashtum El-Gamil; Mat, Marsa Matrouh. **, S, Summer; W, Winter.

As illustrated in Fig. (2), average concentration of ammonia in tested water samples was 218 µg/l. The lowest concentration detected in Marsa Matrouh with 4 µg/l and the highest one in Dahab Island with 580 µg/l that could be attributed to human and agricultural activities over there. Average values of nitrate concentration (fig.3) were 422 µg/l. The lowest nitrate concentration was recorded in Abu Galum with 15 and the highest was observed in Qaroun Lake with 1451 µg/l. However, average of total nitrogen values (fig.4) in different water samples 15 mg/l. Meanwhile, the lowest concentrations of total nitrogen were recorded for Wadi El-Rayan samples with 3.35 mg/l and the highest was recorded in Wadi El-Gemal with 43 mg/l. On the other hand, results indicated that average phosphorous concentrations in various water samples (fig.5) were 188 µg/l. In this respect, the lowest concentrations of phosphorous (fig.6) were recorded in Wadi El-Gemal and Abu Galum protectorates with 0.07 µg/l while the highest one was recorded at Ashtum El-Gamil site with 528 µg/l. Similarly, Hussein *et al.* (2008) indicated that total nitrogen content in lake Qaroun was ranged between 1.12 and 7.7 with an average of 3.92 mg/g (0.39%), while the total phosphorous content ranged between 0.77 and 4.74 with average of 2.57 mg/g (0.26%).

Data illustrated in Fig.7 display COD values; with average 29 mg/l. The lowest concentrations recorded in Wadi El-Gemal protectorate with 6.88 mg/l and the highest one in Ashtum El-Gamil protectorate with 89 mg/l. At the same time, BOD average values (fig. 6) was 6 mg/l, the lowest concentration (0.41 mg/l) recorded in Abu Galum and Wadi El-Gemal protectorates, while the highest one in (38 mg/l) Ashtum El-Gamil protectorate.

Regarding, Qaroun and Wadi El-Rayan lakes, results revealed that they exceed the international limits for ammonia, nitrate, phosphorous according to Egyptian Environmental Affairs Agency annual reports (2011b, 2012b, and 2013b). This is most likely due to the huge human impact and waste water discharge in Qaroun lake, and disposal of agricultural waste water in Wadi El-Rayan lake. It is therefore suggested that less concentrations of ammonia, nitrate and phosphorous the less human activities effect on water quality.

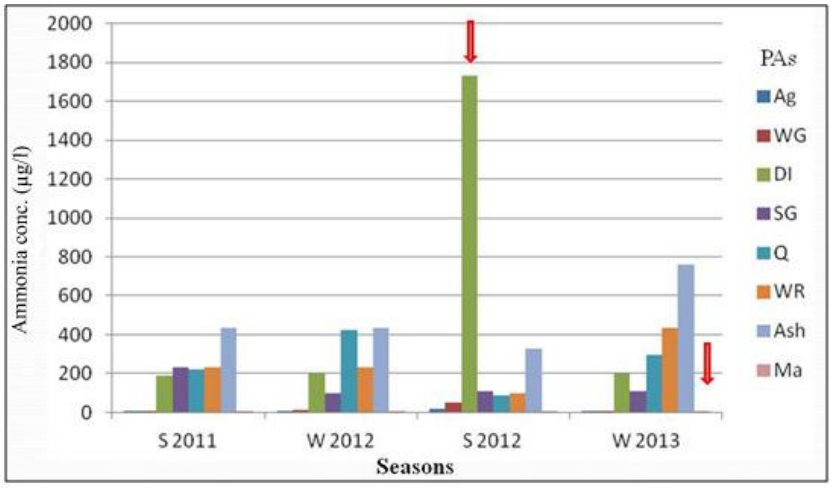

Fig. 2 Ammonia concentrations of the 8 selected PAs; Abu Galum (Ag), Wadi El Gemal (WG), Ashtoum El Gamil (Ash), El Omied (Mat), Qaroun (Q), Wadi El-Rayan (WR), Saluga & Ghazal (SG), Dahb Island (DI) in different seasons.

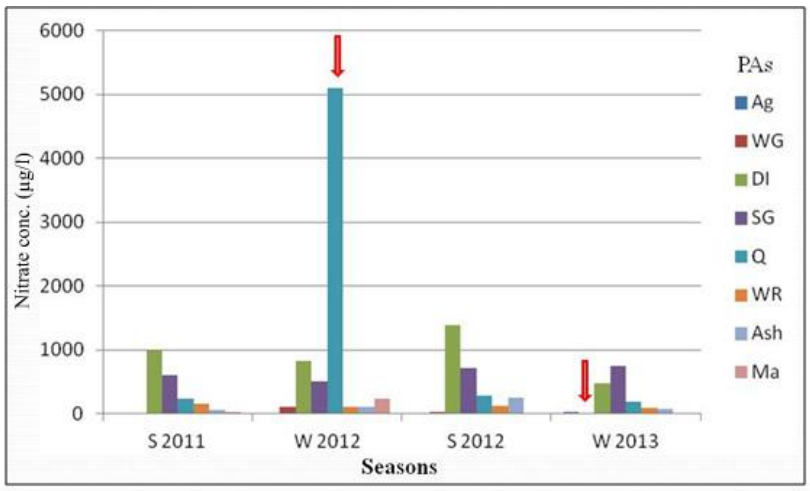

Fig. 3. Nitrate concentrations of the 8 selected PAs; Abu Galum (Ag), Wadi El Gemal (WG), Ashtoum El Gamil (Ash), El Omied (Mat), Qaroun (Q), Wadi El-Rayan (WR), Saluga & Ghazal (SG), Dahb Island (DI) in different seasons.

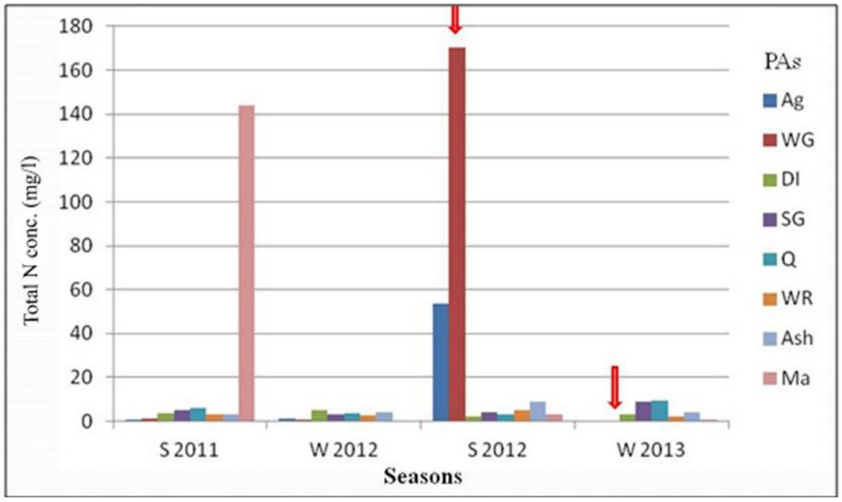

Fig. 4. Total N concentration of the 8 selected PAs; Abu Galum (Ag), Wadi El-Gemal (WG), Ashtoum El-Gamil (Ash), El-Omied (Mat), Qaroun (Q), Wadi El-Rayan (WR), Saluga & Ghazal (SG), Dahb Island (DI) in different seasons.

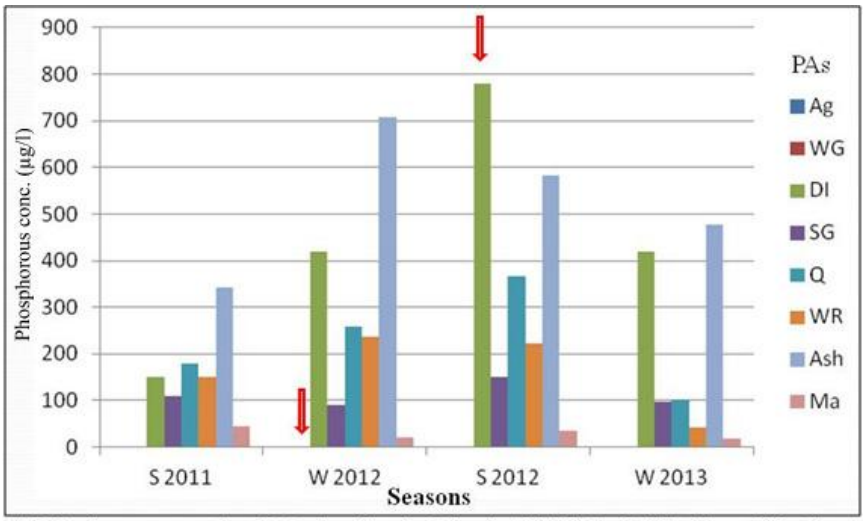

Fig.5. Phosphorous concentration of the 8 selected PAs; Abu Galum (Ag), Wadi El-Gemal (WG), Ashtoum El-Gamil (Ash), El-Omied (Mat), Qaroun (Q), Wadi El-Rayan (WR), Saluga & Ghazal (SG), Dahb Island (DI) in different seasons.

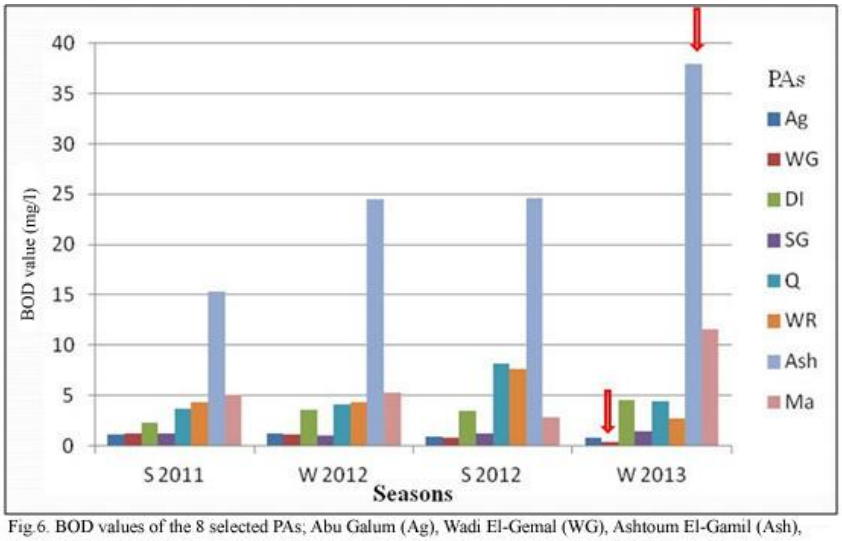

Fig.6. BOD values of the 8 selected PAs; Abu Galum (Ag), Wadi El-Gemal (WG), Ashtoum El-Gamil (Ash), El-Omied (Mat), Qaroun (Q), Wadi El-Rayan (WR), Saluga & Ghazal (SG), Dahab Island (DI) in different seasons.

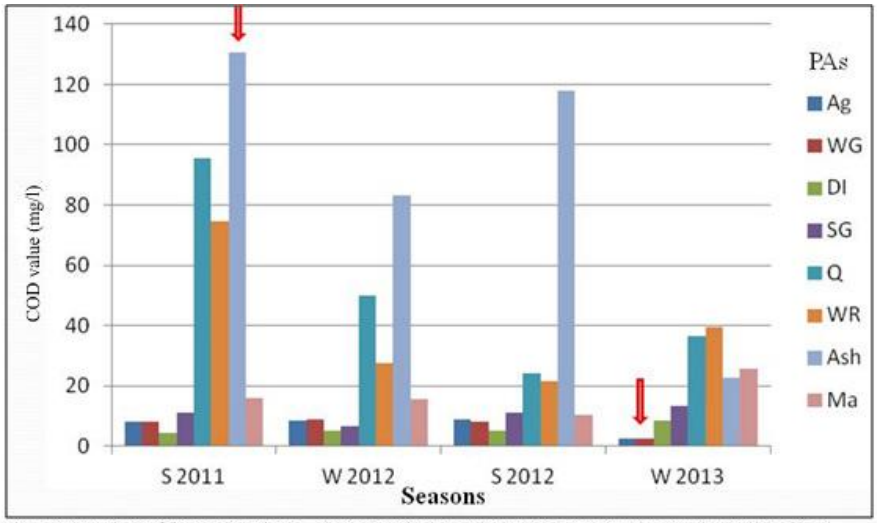

Fig.7. COD values of the 8 selected PAs; Abu Galum (Ag), Wadi El-Gemal (WG), Ashtoum El-Gamil (Ash), El--Omied (Mat), Qaroun (Q), Wadi El-Rayan (WR), Saluga & Ghazal (SG), Dahab Island (DI) in different seasons.

2. Microbiological analyses

During this study, total bacterial counts, total spore-forming bacteria, total fungi and total and feacal coliforms were determined in water samples and the results are presented in Table (3). The data revealed that total bacterial count and total spore-formers ranged between 230-2200 cfu/ml and 100-750 cfu/ml, respectively in summer samples. In winter samples, such counts ranged between 500-1220 cfu/ml and 100-700 cfu/ml, respectively. Similarly, results indicated that fungal counts ranged between 11-92 and 12-92 cfu/ml in summer and winter samples in that order. Regarding the tested protectorates, it was found that the highest total bacterial counts were recorded in Qaroun lake due to discharging of wastes and sewage in it. Meanwhile, total and feacal coliforms were detected, as total coliforms were ranged between 0 and 75 cfu/ml, respectively in summer while they were from 0 to 45 cfu/ml in winter samples. Feacal coliforms densities scored 0 cfu/ml and 45 cfu/ml, respectively in both summer and winter seasons.

Table (3): Total viable counts, total spore-forming bacteria, total fungi total coliforms, and fecal coliforms in water samples of tested Protectorates (PAs)

PAs*	Seasons**	Total counts	Sporeformer	Fungi	Total Coliforms	Fecal Coliforms
		(cfu/ml)			(cells/ml)	
AG	S_2012	800	120	630	140	4
	W_2013	640	120	480	200	0
WG	S_2012	500	100	120	0	0
	W_2013	720	120	110	0	0
DI	S_2012	990	120	920	450	450
	W_2013	640	120	480	450	250
SG	S_2012	230	120	920	0	0
	W_2013	500	100	920	0	0
Q	S_2012	2200	500	120	450	450
	W_2013	1190	500	800	250	250
WR	S_2012	1910	750	110	450	450
	W_2013	1220	700	120	450	450
Ash	S_2012	1930	130	120	750	250
	W_2013	630	350	700	250	200
Mat	S_2012	590	120	200	200	4
	W_2013	500	120	200	70	4

*, Ag, Abu Galum; WG, Wadi El-Gemal; DI, Dahab Island; SG, Saluga & Ghazal; Q, Qaroun Lake; WR, Wadi El-rayan Lake; Ash, Ashtum El-Gamil; Mat, Marsa Matrouh. **, S, Summer; W, Winter.

3. Isolation, identification and purification of cyanobacteria

As presented in Table 4, the relative occurrence of cyanobacteria in summer 2012 and winter 2013 was much greater than summer 2011 and winter 2012. Results also indicated that the most effective medium for selecting cyanobacteria was BG-11 in both marine and fresh water samples. It is indicated that the better growth was observed in Wadi El-Gemal, Saluga & Ghazal, Qaroun Lake, Wadi El-Rayan and Ashtum El-Gamil samples in summer 2012 (Table.4). While, the higher densities of cyanobacteria was detected in Saluga & Ghazal and Ashtum El-Gamil in winter 2013. In addition, microscopic examinations (Fig. 8) were used for the six cultures identification.

During this study 44 species were detected belonging to 4 orders, 11 families and 16 genera in Table(5) *i.e.* in Ashtum El-Gamil there was *Oscillutoria sp.* and *Spiriulina sp.;* in Dahab Island *Phormidium sp.* and *Gloeocapsa sp.* were detected; in Saluga and Ghazal *Pseudoanabaena sp.*; in Qaroun *Anabaena sp.* While, in Wadi El-Rayan *Nostoc sp.*and *Anabaena sp.*, also, in Wadi El-Gemal *Anabaena sp.* was detected. Only six isolates obtained as pure cultures *Oscillutoria sp.* from Ashtum El-Gamil, *Phormidium sp.* from Dahab Island, *Pseudoanabaena sp.* from Saluga and Ghazal and *Anabaena sp.* from Qaroun, Wadi El-Rayan and Wadi El-Gemal.

Table (4): Relative occurrence of cyanobacteria in various enrichment cultures various protectorates (PAs)

PAs	Seasons			
	S_2011	W_2012	S_2012	W_2013
AG	-	-	-	-
WG	-	-	+++	+
DI	-	-	++	+
SG	-	-	+++	+++
Q	+	+	+++	++
WR	+	+	+++	++
Ash	++	+	+++	+++
Mat	-	-	-	-

+++, good; ++, moderate; +, weak; -, no growth

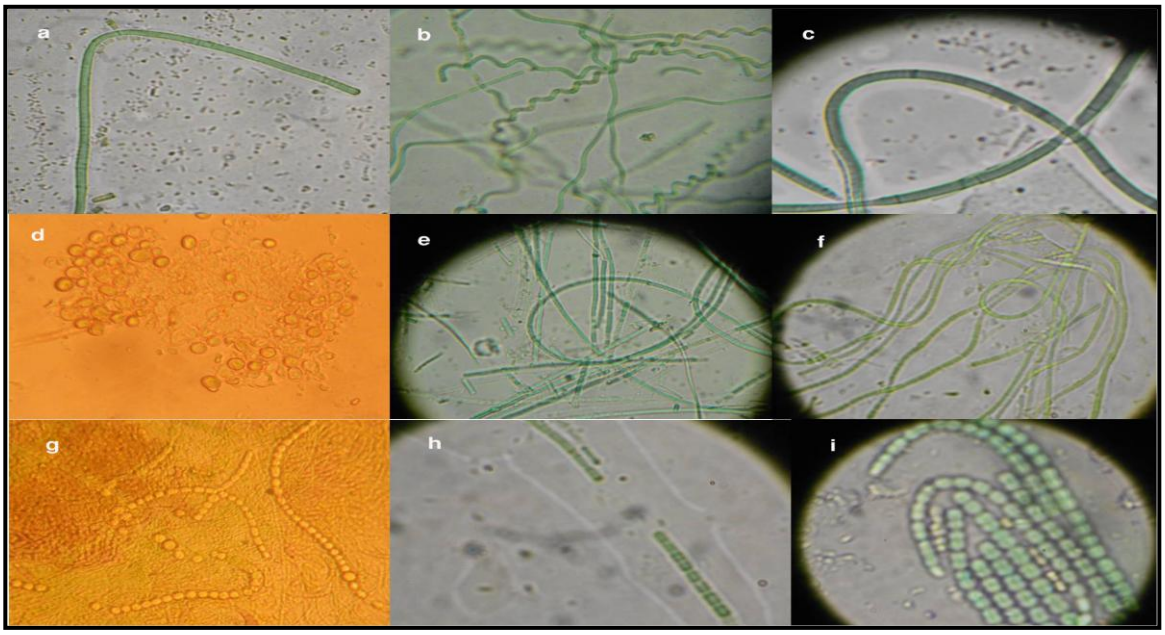

Fig.8. a) Ash, *Oscillutoria sp.*; b) Ash, *Spiriulina sp.*; c) DI, *Phormidium sp.*; d) DI, *Gloeocapsa sp.*; e) SG, *Pseudoanabaena sp.*; f) Q, *Anabaena sp.*; g) WR, *Nostoc sp.*; h) WR, *Anabaena sp.*; i) WG, *Anabaena sp.*

Table (5): Diversity of Cyanobacteria species in tested Egyptian protectorates during this study

Taxa	WR	Q	DI	SG	WG	Ash
Anabaena Bory						
Anabaena circinalis Rabenhorst		+	+	+		
Anabaena flos- aquae Brebisson		+	+	+		
Anabaena inaequalis (Kutzing) Bornet & Flahawt		+	+	+		
Anabaena sp. Bory					+	
Anabaena variabilis Kütz. ex Born. et Flah.	+					
Aphanocapsa Naegeli						
Aphanocapsa koordersi Strom.	+					
Aphanotheca nidulans P. Richter		+	+	+		
Chroococcus Naegeli						
Chroococcus limneticus Lemmermann		+	+	+		
Chroococcus minutus (Kütz.) Näg.	+	+	+	+		
Chroococcus turgidus (Kutzing) Nageli		+	+	+		
Eucapsis Clements and Shanz						
Eucapsis minuta F.E.Fritsch		+	+	+		
Gloeocapsa Kützing						
Gloeocapsa decorticans (A.Br.) Richter	+					
Gloeocapsa sanguinea Kuetzing		+	+	+		
Gomphosphaeria Kützing						
Gomphosphaeria aponina Kütz.	+	+	+	+		
Gomphosphaeria compacta (lemmer.) Strom		+	+	+		
Gomphosphaeria lacustris Chodat		+	+	+		

Table (5): cont.

Taxa	WR	Q	DI	SG	WG	Ash
Lyngbya C. Agardh						
Lyngbya limnetica Lemmermann		+	+	+		
Merismopedia Meyen						
Merismopedia convoluta var.minor (Wille) Tiffany & Ahlstrom		+	+	+		
Merismopedia elegans A.Braun		+	+	+		
Merismopedia glauca (Ehrenberg)Nageli		+	+	+		
Merismopedia major (G.M. Smith) Geitler		+	+	+		
Merismopedia punctata Meyen	+	+	+	+		
Merismopedia tenuissima Lemmermann	+	+	+	+		
Microcystis Kützing						
Microcystis aeruginosa Kütz.	+	+	+	+		
Microcystis flos - aquae (Witlr.) Kirchner	+	+	+	+		
Myxosarcina Printz						
Myxosarcina burmensis Skuja	+					
Nostoc Vaucher						
Nostoc sp. Vaucher						+
Nostoc carneum Ag.		+	+	+		
Nostoc kihlamanii Lemmermann		+	+	+		
Nostoc pruniforme C.A. Agardh		+	+	+		
Nostoc verrucosum (Vaucher) Hist.		+	+	+		
Oscillatoria Vaucher						
Oscillatoria claricentrosa Gardner	+					
Oscillatoria foreoui Frémy	+					
Oscillatoria limosa C. A. Agardh		+	+	+		
Oscillatoria okeni Ag. ex Gomont	+					
Oscillatoria princeps Vaucher		+	+	+		
Oscillatoria sp. Vaucher						+
Oscillatoria tenuis C. A. Agardh		+	+	+		
Phormidium Kützing						
Phormidium angustissimum W. et G. S. West	+					
Phormidium fragile (Meneghini) Gomont	+					
Phormidium laminose (Agardh) Gomont		+	+	+		
Phormidium retzii Gomont		+	+	+		
Radaisia Fremy						
Radaisia violacea Fremy		+	+	+		
Spirulina Turpin						
Spirulina laxissima G.S.West		+	+	+		
Spirulina major Kutz.		+	+	+		
Spirulina platensis (Nordstedi) Geitler		+	+	+		
Spirulina princeps (W & G. S. West) G. S. West		+	+	+		

cont.

Table (5): cont.

Taxa	WR	Q	DI	SG	WG	Ash
Spirulina sp. Turpin						+

WR= Wadi El-Rayan, Q= Qaroun Lake, DI= Dahab Island, SG= Saluga and Ghazal, WG= Wadi El-gemal, Ash= Ashtoum Elgamel; + , present.

Phylogenetic relationship between cyanobacterial isolates

During this study, DNA was isolated and purified according to Morin *et al.* (2010); RAPD molecular technique was performed, the banding patterns generated by RAPD-PCR markers analyses were compared and the genetic variation between 6 isolates were also determined. The used five universal primers resulted in 74 scorable total bands and 72 polymorphic bands which mean 97% polymorphism as appear in Figs. 9-13 and Table (6). Genotype specific RAPD marker -described as the appearance of only one band among different lanes- showed in Table (7). Unique bands that represent genotype specific RAPD marker were 4 for Dahab Island, 4 for Saluga & Ghazal, 1 for Qaroun, 2 for Wady El-Rayan, 3 for Ashtum El-Gamil, and 3 for Wadi El-Gemal.

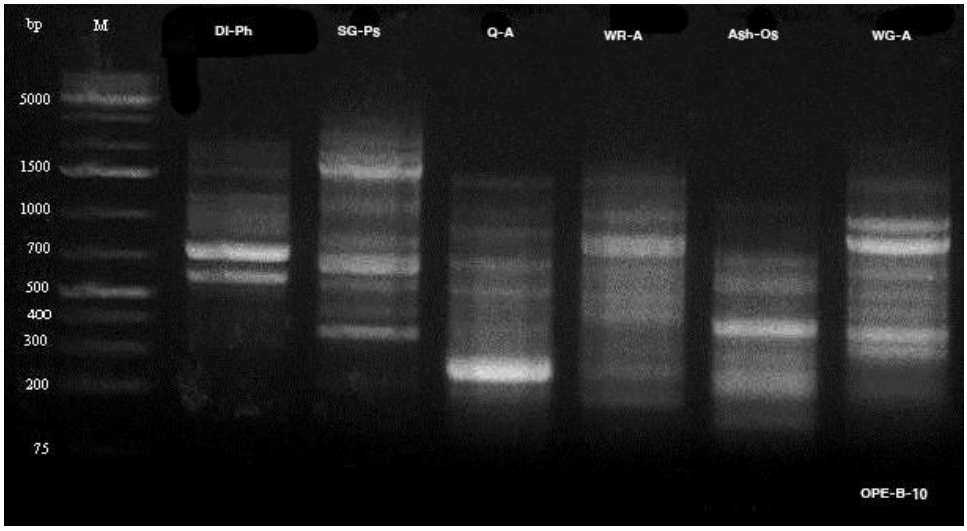

Fig.9. Agarose (0.5%) Gel Electrophoresis, OPE-B-10 RAPD primer pattern of six isolates of cyanobacteria from six examined PAs; Dahb Island (DI), Saluga & Ghazal (SG), Qaroun (Q), Wadi El Gemal (WG), Ashtoum El Gamil (Ash), Wadi El-Rayan (WR). Numbers on the left side correspond to molecular weight

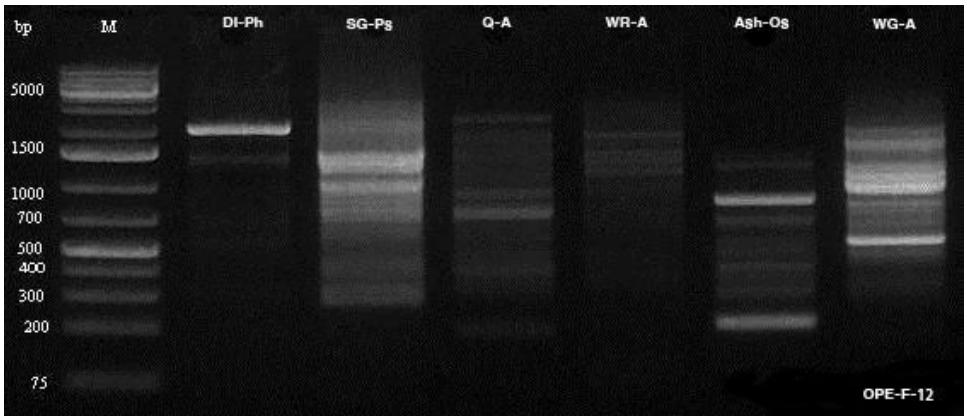

Fig.10. Agarose (0.5%) Gel Electrophoresis, OPE-F-12 RAPD primer pattern of six isolates of cyanobacteria from six examined PAs; Dahb Island (DI), Saluga & Ghazal (SG), Qaroun (Q), Wadi El Gemal (WG), Ashtoum El Gamil (Ash), Wadi El-Rayan (WR). Numbers on the left side correspond to molecular weight

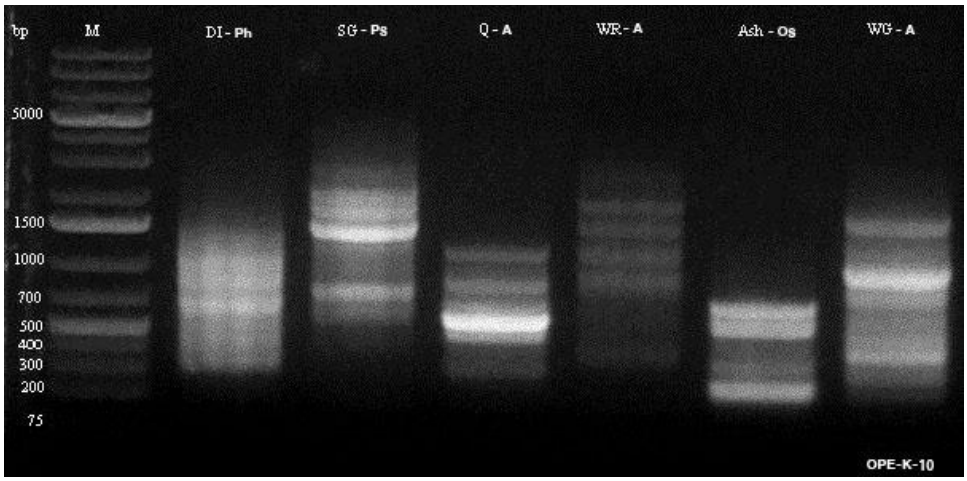

Fig.11. Agarose (0.5%) Gel Electrophoresis, OPE-K-10 RAPD primer pattern of six isolates of cyanobacteria from six examined PAs; Dahb Island (DI), Saluga & Ghazal (SG), Qaroun (Q), Wadi El Gemal (WG), Ashtoum El Gamil (Ash), Wadi El-Rayan (WR). Numbers on the left side correspond to molecular weight

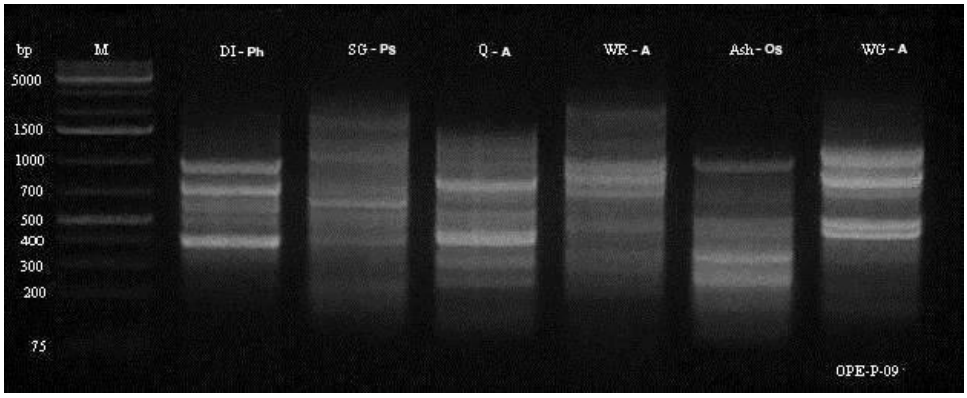

Fig.12. Agarose (0.5%) Gel Electrophoresis, OPE-P-09 RAPD primer pattern of six isolates of cyanobacteria from six examined PAs; Dahb Island (DI), Saluga & Ghazal (SG), Qaroun (Q), Wadi El Gemal (WG), Ashtoum El Gamil (Ash), Wadi El-Rayan (WR). Numbers on the left side correspond to molecular weight

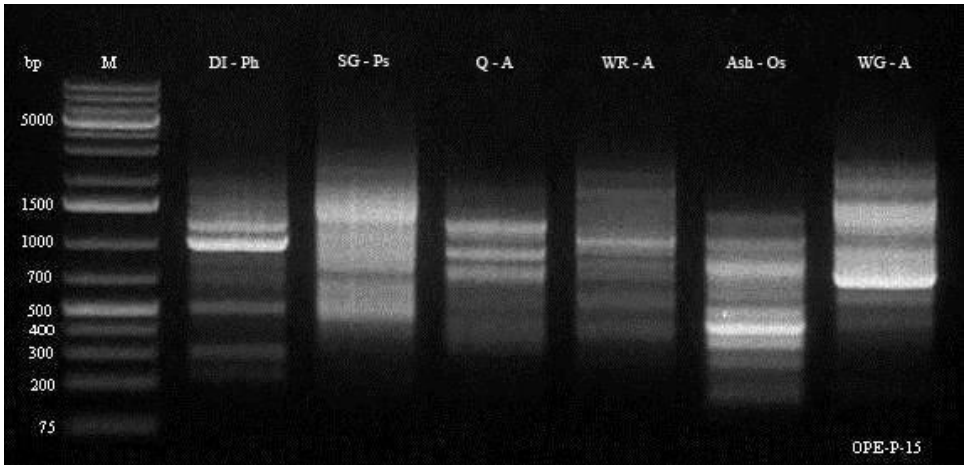

Fig.13. Agarose (0.5%) Gel Electrophoresis, OPE-P-15 RAPD primer pattern of six isolates of cyanobacteria from six examined PAs; Dahb Island (DI), Saluga & Ghazal (SG), Qaroun (Q), Wadi El Gemal (WG), Ashtoum El Gamil (Ash), Wadi El-Rayan (WR). Numbers on the left side correspond to molecular weight

Table (6): Polymorphism percentage between the tested isolates using 5 different primers

Primer name	No of scorable bands	No of Polymorphic bands	Polymorphism %
B-10	16	16	100
F-12	17	17	100
K-10	13	13	100
P-09	14	13	92.8
P-15	14	13	92.8
Total	**74**	**72**	**97.3**

Figure 14 shows the phylogenetic relationship between 6 used cultures from 6 PAs understudy. It is well illustrated that, cluster tree is divided into 3 main branches first one belongs to Ashtum El-Gamil PA, represent a marine ecosystem at Mediterranean, second one refers to Saluga & Ghazal and Dahab Island PAs belongs to a fresh water environment at river Nile, while the last one divided into 2 sub-branches one represents Wadi El-Gemal PA as a marine environment at Red Sea; and the second one represents Wadi El-Rayan and Qaroun PAs as a Brackish habitat at Fayoum. In this respect, it is obviously demonstrated that Wadi El-Rayan and Qaroun PAs have similar ecological features due to discharge of agricultural wastes and waste water, leading to increase the salinity of such lakes. In fact, these results are in accordance with EEAA annual reports (2010-2013). The high percentage of polymorphism (Table 6) may refer to effect of habitats on these isolates (Foster *et al*., 2009) and explains why such isolates may morphologically similar but genetically distinct.

Therefore, it could be concluded that cyanobacteria diversity not only at species level –even they may appear morphologically similar - but also at genetic level, these may refers to habitat from where they were isolated.

Additionally aquatic habitat that suffering from elevated concentration of phosphorous and nitrogen *e.g.* Qaroun Lake that are conducive to algal bloom that have harmful effect for the other organisms under appropriate environmental conditions and as previously indicated by Hussein *et al.* (2008), the water quality should be improved. This may need prior treatment of both domestic and agricultural wastes before discharging.

Table (7): Genotype Specific RAPD markers of each tested cyanobacterial isolates

Isolates	RAPD-Specific marker (bp)	Total
DI - *Ph*	OPE-B-10 (520), OPE-F-12 (2100), OPE-P-15 (300,210)	4
SG - *Ps*	OPE-F-12 (3000), OPE-K-10 (2700), OPE-P-09 (1600, 200)	4
Q - *A*	OPE-F-12 (2500)	1
WR - *A*	OPE-P-09 (3200), OPE-P-15 (2100)	2
Ash - *Os*	OPE-B-10 (150), OPE-P-15 (350, 120)	3
WG - *A*	OPE-F-12 (1600, 1200, 450)	3
Total		**17**

DI-*Ph*, Dahab Island - *Phormidium* ; SG-*Ps*, Saluga and Ghazal – *Pseudoanabaena*; Q-*A*, Qaroun - *Anabaena*, WR-*A*,Wadi El-Rayan – *Anabaena*; Ash-*Os*, Ashtoum Elgamel - *Oscillatoria* ; WG–*A*, Wadi El-gemal-*Anabaena*

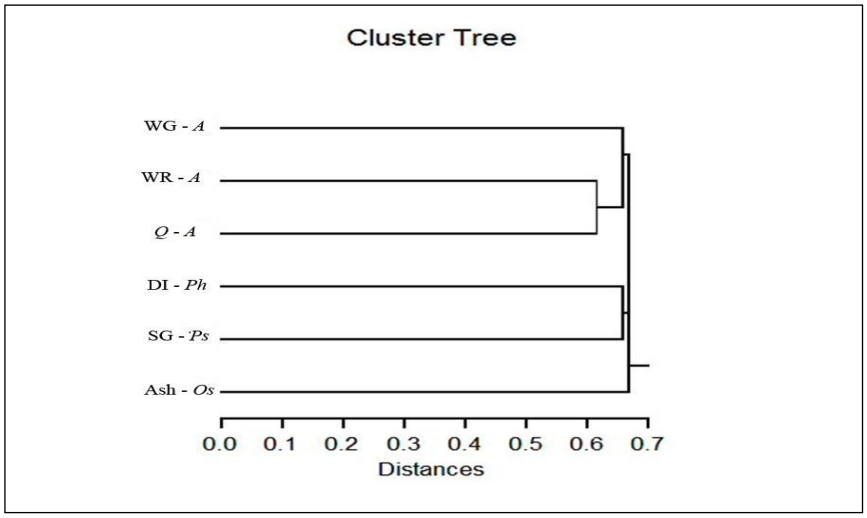

Fig.14. Cluster tree of six isolates representing six protected areas understudy DI-*Ph*, Dahab Island - *Phormidium* ; SG-*Ps*, Saluga and Ghazal – *Pseudoanabaena*; Q-*A*, Qaroun - *Anabaena*, WR- *A*,Wadi El-Rayan – *Anabaena*; Ash-*Os*, Ashtoum Elgamel - *Oscillatoria* ; WG–*A*, Wadi El-gemal-*Anabaena*

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