



Association Between the Distributions and Chemical Composition of Aquatic Macrophytes and Bacterial Community Structure in Some Irrigation Canals (Rayahs) of the Nile River, Egypt

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ARTICLE INFO

Article History:

Received: June 25, 2020

Accepted: Aug. 20, 2020

Online: Aug. 23, 2020

Keywords:

Nile River Rayahs;
Macrophytes,
Chemical composition;
Macro-element;
Bacterial
indicator of pollution;
Diazotrophs

ABSTRACT

Variations in distribution, species composition, and chemical constituents of aquatic macrophytes of Al-Rayah El Tawfiky (T) and Al-Rayah El Monoufy (M) in addition to their relations with bacterial communities structure and water variables were studied over the period from spring 2014 to winter 2015. Ten of the aquatic macrophytes species related to 7 families and 9 genera were recorded. *Myriophyllum spicatum* was the most dominant species, the autumn season and Al-Rayah (M) were the richest in species numbers. Protein, lipid, nitrogen, phosphorus, and potassium were detected at higher concentrations in *Ceratophyllum demersum* from Al-Rayah (T), however organic matter and nitrogen-free extracts contents attended their highest values in *Potamogeton nodosus* from Al-Rayah (M).

Microbial analyses indicated the presence of total bacterial counts and the total diazotrophs in high population densities (Up to 10^5). However, total and fecal coliforms populations are exceeding the permissible limits cited by World Health Organization, recorded their highest numbers in spring at the site of Al Rayah (M) which was occupied by *Potamogeton nodose* and *Myriophyllum spicatum*. A significant difference in chemical composition, distribution pattern, the community structure of aquatic macrophytes, and bacteria attributed to the seasons and site differences, in addition to the nutritional potential of the analyzed macrophytes species especially submerged ones were recorded.

INTRODUCTION

Water is the most important natural source for life, being essential for humans and indispensable for all ecosystems (WHO 2011). Surface water in the Nile Delta is of vital importance for domestic, agricultural, and industrial water supply. At Delta Barrage, the Nile River bifurcates into two main branches namely Damietta and Rosetta and four sub-branches (Rayah).

Al Rayah El- Tawfiky and Al Rayah-El Monoufy are two sub-branches canals of Nile River, which divert water to the Eastern and Middle Delta. In recent years the

increases in population together with the agricultural and industrial development have resulted in increasing pollution of water resources that affect life of macrophytes plants and bacterial communities of aquatic system.

Aquatic weeds are unwanted plants grow naturally in lakes, ponds and other water bodies. They are primary producers that have many ecosystem services, like: structure and functioning of aquatic ecosystems (**Zhi *et al.* 2015**), improving of water quality (**Zhang *et al.* 2016**), considered as a base of aquatic food-chains and as good source of food and fodder for humans, aquatic herbivores, fishes and farm animals (**Sharshar and Haroon, 2009**) and as a source of biologically active substances (**Daboor and Haroon, 2012; Haroon and Abdel-Aal, 2016; Haroon and Daboor, 2019**). In contrast, they can cause many ecological losses by adversely affecting, irrigation, navigation, public health as well as the fish production.

The quality of water is typically determined by monitoring bacterial presence especially fecal coliform bacteria, which are used as microbiological indicator of the pollution. Bacteria are the major users of dissolved organic matter and therefore of central importance for carbon cycle in aquatic systems (**They and Marques, 2019**). The availability of fixed inorganic nitrogen often plays a fundamental role in regulating primary production in both aquatic and terrestrial ecosystems. Because biological nitrogen fixation is an important source of nitrogen in waters, particularly if those are to be used for irrigating agricultural lands, the study of N₂-fixing microorganisms is of special concern to our understanding of global nitrogen cycle. Vast array of work has been done on the occurrence and activity of diazotrophs in Egyptian aquatic systems (**Othman and Sabae, 2013 and Othman *et al.* 2016**) and in aquatic world (**Jabir *et al.* 2020**).

The distribution of aquatic plants and microbial species largely depend up on the nutrient status of freshwaters (**Srivastava *et al.* 2017 and Haroon, 2020 a**). In addition, aquatic macrophytes can affect the structure and function of bacterial community in aquatic environment, by releasing allelochemicals substances that inhibit bacteria both directly and indirectly, the latter via inhibition of algae (**El-Sheekh *et al.* 2017**). Therefore, the studying of the factors affecting their distribution and biochemical composition is of paramount important for managing them in useful purposes.

In this study we assessed the seasonal and spatial variations in aquatic macrophytes distribution, community structure and chemical composition of both submerged and floating macrophytes species. In addition, this study has marked structures in bacterial communities which were correlated with various abiotic (seasons, sites and water characteristics of sampling sites) and biotic factors (presence and type of macrophyte) of two different water bodies (Al-Rayah El Tawfiky (T) and Al-Rayah El Monoufy (M)).

MATERIALS AND METHODS

Study area:

Seven sampling sites were selected for each Rayah in addition to one sample before bifurcation of the River Nile (RN) as a control sample for two Rayahs (Figure 1&Table 1).

Plant sampling and preparation:

Different macrophytes life forms were collected seasonally during the period of spring 2014 to winter 2015 from the selected sites. At each site, macrophytes samples were collected by hand, removal to the laboratory, where they were separated into different taxa, identified after **Boulos (2005)**, cleaned, washed with water and dried in shade. A part of each plant species was dried at 50 °C to constant weight, ground to fine powder and stored for estimation of chemical constituents (Including, organic matter, ash, nitrogen free extracts, protein, lipid, nitrogen, potassium and phosphorus contents). The other part was dried separately at 100 °C to constant weight for dry matter determinations.

Plant analysis:

Only submerged and floating macrophytes samples were subjected to the biochemical analysis owing to their importance as a natural food resources for fishes. The plant extract was prepared using sulphuric acid and perchloric acid following Peter (**Burgski, 1968**). Different methods were used for estimation of total ash, protein, lipid, nitrogen, phosphorus, Nitrogen-free extracts (NFE) and potassium (**Egyptian Pharmacopoeia, 1953; Ölberg, 1956; Blight and Dyer, 1959; Black, 1965; Snel and Snel 1967; Pádua et al. 2004 and Umoren et al. 2005 respectively**).

Water sampling and analysis:

Water samples were aseptically collected in sterile brown bottles (500 ml), stored at 4 °C until chemical and bacteriological analysis that completed within 48 h of sampling. In the laboratory, Chemical parameters (Including, pH value, Total nitrogen (TN), Total phosphorus (PO₄), Electrical conductivity (EC), Dissolved oxygen (DO), Biochemical oxygen demand (BOD), Chemical oxygen demand (COD), Nitrite (NO₂), Nitrate (NO₃) and Ammonium (NH₄)) were assessed following APHA (2005) and authors used chemicals data from **Goher (2015)**. Bacteriological analyses was assessed as described below.

Bacteriological analysis:

Autochthonous and allochthonous bacteria were enumerated on plate count agar medium (**APHA, 2005**) at 20 and 35°C incubation temperatures (Autochthonous and allochthonous, respectively). Most probable number (MPN) procedure was followed to determine total coliform (TC) and fecal coliform (FC) using LTB (Lauryl Tryptose Broth) (Lab M limited, UK) (35 °C ± 0.5 °C at 24 ± 2 hours) for total coliform bacteria, EC Broth (Lab M limited, UK) (44.5 °C ± 0.2 °C at 24 ± 2 hours) for fecal coliform bacteria and azide-dextrose broth (Lab M limited, UK) for fecal streptococci (**APHA,**

2005). Total diazotrophs were enumerated using the surface inoculated plate method on N-deficient combined carbon sources agar medium, CCM (Hegazi *et al.* 1998).

Statistical analyses:

One-way ANOVA was applied to assess the significance of variations in chemical composition of macrophytes in relation to the sites of the two water course and the season (SAS, 1985). Microbiology data were statistically analyzed using STATISTICA 10 (StatSoft Inc. 2011). In addition, data were examined with principal component analysis (PCA) in order to indicate the affecting of macrophytes and bacteria with the other abiotic variables, using XL Stat version 2017.

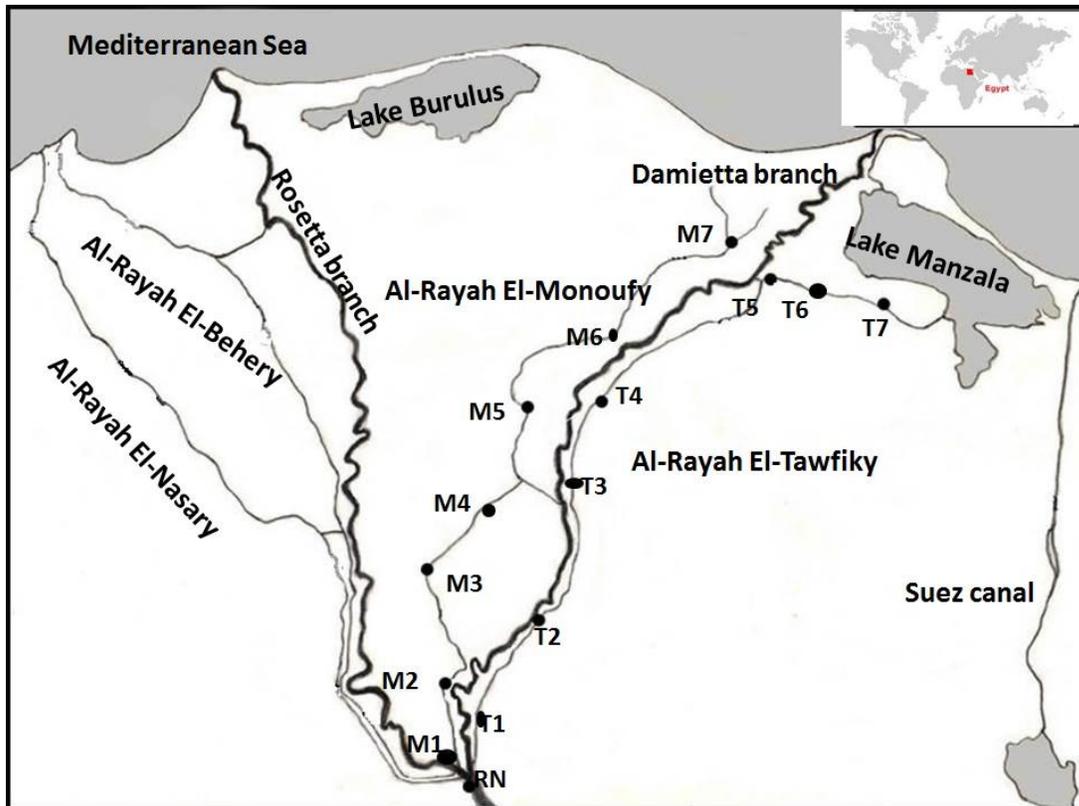


Fig. 1: Map of sampling sites along Al-Rayah El-Monoufy (M) and Al-Rayah El-Tawfiky (T) of the Nile River, Egypt

Table 1: GPS data and description of sampling locations at Al-Rayah El-Tawfiky (T) and Al-Rayah El-Monoufy (M).

Sites codes	Names	Latitude	Longitude	Distance from El Kanater	Description
RN	River Nile	30°10'20.72"	31° 8'23.98"	1.5 km	Upstream El-El-Kanater
T1	El Kanater	30°11'46.58"	31° 7'55.98"	1-1.5 Km	After railway station of El Kanater El Khairia
T2	Banha	30°28'24.0"	31°12'1.04"	40 Km	In the Front of Banha Water plants
T3	Met gamer	30°41'35.3"	31°16'50.2"	70 Km	Between agricultural and residential regions
T4	Agga	30°54'23.12"	31°16'51.88"	100 Km	There are agricultural and residential regions at the two banks
T5	Mansoura	31°04'02.84"	31°25'02.13"	130 Km	Before bifurcation of El-Rayah
T6	Dekerness	31° 5'38.65"	31°37'37.77"	155 Km	Between residential region in the west side and agricultural region in east side
T7	El-Manzalah	31°09'49.9"	31°56'09.5"	185 Km	End of El Rayah
M1	El Kanater	30°11'59.85"	31° 6'44.97"	1Km	Behind fish research station of NIOF
M2	El-Khadra	30°20'15"	31° 02'55"	30Km	Middle of agricultural and residential regions
M3	Shebeen El-Koum	30°32'04.3"	31°00'48.3"	60Km	In Shebeen El-Koum City
M4	El-Santa	30°43'44.50"	31° 7'28.88"	100Km	In El Santa City
M5	Zifta	30°47'26.15"	31° 9'16.12"	130Km	Receives the water from Damietta branch
M6	El-Mahalla	30°56'59.4"	31°09'21.4"	155Km	In El Mahalla City
M7	Belqas	31°07'59.9"	31°22'50.1"	185Km	Middle of agricultural regions and Before bifurcation of El-Rayah to small branches

RESULTS AND DISCUSSION

Floristic composition of the collected macrophytes samples.

Aquatic macrophytes constitute an important components of many freshwater ecosystems, their distribution and community structure is largely controlled by different environmental factors such as, water and sediment chemistry, nutrient availability and allelopathic interaction with other aquatic organisms (**Hansel-Welch *et al.* 2003** and **Del Pozo *et al.* 2011**). During the period of study ten macrophytes species representing the different macrophytes life forms, related to nine genera and seven families were recorded (Table 2). This number of species is lower than that recorded by previous researcher like: **El-Amier *et al.* (2015, 2016)**, **Haroon and Hussian (2017)**, **Haroon (2020b)**; they recorded the presence of 70, 18, 12 and 11 of macrophytes species at Damietta branch, Rosetta Branch, Al Rayah El-Nasery and El-Noubaria Canal and Al- Rayah El Behery of the Nile River Egypt respectively.

The emergent macrophytes acquired a dominant position in the study area represent 60% of the total collected species, followed by submerged and floating species representing 20% for each of them. Throughout the study period Al Rayah El Monoufy showed the richest habitat in species number and autumn was the richest season (8 species).

Table 2. Scientific names, classification and distribution of macrophytes species at different sites and their presence percentage throughout different seasons.

Species	Family	Spring		Summer		Autumn		Winter		TP%
		Sampling sites	P%	Sampling sites	P%	Sampling sites	P%	Sampling sites	P%	
Submerged macrophytes										
<i>Myriophyllum spicatum</i> L	Haloragaceae	T(1-6) and M(2-7)	85.71	T(1,2,4,6) and M(2,4,5,6,7)	64.29	T(1,2,4,5,6,7) and M(2,3,4,5,7)	78.57	T(2,5,6) and M(2,4,6,7)	50	69.64
<i>Ceratophyllum demersum</i> L	Ceratophyllaceae	-	-	M(7)	7.14	T4	7.14	-	-	10.26
Floating macrophytes										
<i>Potamogeton nodosus</i> Poir	Potamogetonaceae	M(1,6)	14.29	-	-	-	-	M(4)	7.14	3.57
<i>Eichhornia crassipes</i> (Mart.) Solms	Pontederiaceae	-	-	T(4) and M(1,7)	21.43	M(6)	7.14	-	-	7.14
Emergent macrophytes										
<i>Saccharum spontaneum</i> (L.) (Per)	Poaceae (Gramineae)	-	-	T(1,4,7) and M(1,5,7)	42.86	T(3) and M(6)	14.29	M(1)	7.14	16.07
<i>Echinochloa stagnina</i> (Retz.) P. Beauv. (Per)	Poaceae (Gramineae)	-	-	-	-	T4 and M5	14.29	M(6)	7.14	3.57
<i>Cyperus alopecuroides</i> Rottb.(Per).	Cyperaceae	-	-	M(2,5)	14.29	T(4) and M(3)	14.29	-	-	7.14
<i>Polygonum tomentosum</i>	Polygonaceae	-	-	M(6)	7.14	T(4)	7.14	-	-	3.57
<i>Polygonum convolvulus</i> L	Polygonaceae	-	-	-	-	M(4,7)	14.29	-	-	3.57
<i>Phragmites australis</i> (Cav.) Trin.	Poaceae (Gramineae)	-	-	T(4,7)	14.29	T(3)	7.14	-	-	5.36

Where: Al-Rayah El Tawfiky =T; Al Rayah El Monoufy =M; Presence percentage in relation to the total survived sites of each season = P% and Total presence percentage in relation to the total survived sites of the year =TP%.

Seasonal and spatial variations in presence percentage of each macrophytes species was observed (Table 2). Among all the macrophytes recorded the submerged macrophyte species *M. spicatum* was found to be the most frequent species as it occurs in 85.71, 64.29, 78.57 and 50% of the sampling sites during spring, summer, autumn and winter respectively. During summer it was followed in presence percentage by *S. spontanium* (42.86%) and *E. crassipes* 21.43%. (Table 2). However, during autumn it was followed in presence percentage by *S. spontanium*, *E. stagnin* and *C. alopecuroides* Rottb. (Per). Only four macrophytes species were recorded during winter, in which *M. spicatum* was the only recorded species at Al-Rayah El Tawofiky, however at Al-Rayah El Monoufy it was found associated with the floating macrophyte species *P. nodosus* and with the two emergent species *S. spontanium* and *E. stagnina*.

Biochemical analysis of the selected macrophytes

Variations in nutritional components in relation to plant species, season and sampling site were observed (Table 3). In general, both floating and submerged macrophytes were characterized by their high water content, which is usually a major deterrent to their harvest and utilization (Edward, 1980). The water content of the macrophytes listed ranged between 88.72 ± 1.24 and 94.12 ± 1.02 % of wet wt with the highest for autumn samples. As shown in results (Table 3) the mean seasonal values of ash and organic matter contents varied significantly ($P < 0.05$) between different seasons, species and sampling sites, with the lowest ash and highest organic matter contents for winter sample of the floating macrophyte species *P. nodosus* from Al Rayah El Monoufy (8.67 ± 0.02 and 91.33 ± 0.02 %DW respectively).

In the two studied canals the nitrogen free extracts (NFE) constitutes the major part of the macrophytes biochemical composition, ranged from 59.40 ± 0.23 %DW for autumn samples of *C. demersum* from Al-Rayah El Tawofiky to 78.57 ± 0.00 % DW for winter samples of *P. nodosus* from Al Rayah El Monoufy for other samples the values fluctuated between 65 to 75% DW.

The protein content of aquatic weeds can vary according to species, seasonal period and geographic area (Ratana-arporn and Anong Chirapart 2006 and Haroon, 2020a, 2020b). In the present investigation samples of *M. spicatum* from Al-Rayah El Tawofiky and Al Rayah El Monoufy attained their lowest protein values during summer and winter being 7.94 ± 0.41 and 7.38 ± 1.23 % DW respectively, however the highest values were detected in autumn being 10.25 ± 2.46 and 11.41 ± 1.65 % DW for the two Rayahs respectively. In contrast samples of *C. demersum* from Al Rayah El Monoufy have the lowest protein content in summer season, but the highest one was detected in their autumn samples from Al-Rayah El Tawofiky (6.50 ± 0.040 and 15.69 ± 0.13 % DW respectively). The total protein content in the studied macrophytes from the two Rayahs were observed to be highest among submerged macrophytes *C. demersum* and *M. spicatum* (15.69 ± 0.13 and 11.41 ± 1.65 % DW respectively) from Al-Rayah El Tawofiky and Al Rayah El Monoufy respectively. The two floating macrophytes species were recorded at Al Rayah El Monoufy and their protein contents fluctuated between 7-9%. The results of the present study are comparable to those reported by Haroon (2008) and Haroon (2020 b), lower than that reported by Haroon (2010) and Abdel Shafy et al. (2016) for different macrophytes species. This may be related to plant species, the part of plant used, time and place of samples collection.

Table 3. Mean values of nutritional components of the studied submerged and floating macrophytes species throughout the period from spring 2014 to winter 2015

Season	Site	WC	Ash	OM.	NFE	Protein	Lipid	N	P*10 ⁻²	K
<i>Myriophyllum spicatum</i>										
Spring	T	88.72±1.24	16.77±5.15	83.24±5.15	70.48±6.44	8.76±1.15	4.00±2.63	1.40±0.17	1.19±0.11	1.92±0.17
	M	90.29±1.13	18.79±7.80	81.21±7.80	67.57±8.41	9.45±1.40	4.19±1.34	1.51±0.29	1.09±0.15	1.95 ±0.09
Summer	T	89.39±1.86	16.70±6.87	83.31±6.87	71.11±7.32	7.94±0.41	4.25±0.80	1.27±0.07	2.34±0.33	3.16±0.47
	M	91.14±2.11	23.33±4.91	76.67±4.91	64.36±1.40	8.22±2.20	4.09±2.07	1.31±0.35	2.98±0.40	3.42±0.67
Autumn	T	92.81±0.67	20.58±6.63	79.42±6.63	62.48±7.06	10.25±2.46	6.69±2.19	1.64±0.39	2.33±1.19	3.70±0.44
	M	92.60±0.37	21.14±0.08	78.86±7.08	64.02±3.25	11.41±1.65	6.03±1.43	1.52±0.24	2.42±1.28	3.70±0.48
Winter	T	91.89±1.22	21.53±10.99	8.47±10.99	63.21±12.78	8.70±0.90	6.57±0.95	1.07±0.14	1.63±0.55	0.13±0.06
	M	91.22±1.28	13.95±5.35	86.05±5.35	73.39±4.70	7.38±1.23	5.28±0.56	1.18±0.19	2.00±2.12	0.68±1.07
Mean annual	T	90.59±2.08	18.88±6.93	81.12±6.93	66.82±7.23	8.91±2.08	5.38±1.97	1.36±0.23	1.89±0.90	2.46±1.32
	M	91.30±1.66	19.66±6.21	80.34±6.21	67.34±5.63	9.12±2.25	4.89±1.78	1.47±0.36	2.12±1.51	2.38±1.32
	T & M	90.95±1.89	19.28±6.50	80.71±6.50	67.08±6.37	9.01±2.15	5.14±1.86	1.42±0.31	2.01±1.29	2.42±1.30
F value	T	12.91	0.64	0.64	1.29	10.41	2.25	4.28	3.05	7.23
P-value	M	0.00	0.60	0.60	0.31	0.00	0.13	0.02	0.06	0.37
F value	M	2.52	1.33	1.33	0.88	3.96	3.83	9.09	4.99	2.34
P-value	M	0.09	0.30	0.30	0.47	0.03	0.03	0.00	0.01	0.01
<i>Ceratophyllum demersum</i>										
Summer	M	90.32±0.21	18.2±0.03	81.8±0.03	71.80±0.11	6.50±0.04	3.50 ± 0.05	1.04±0.04	3.00±0.06	2.50±0.13
Autumn	T	94.12±1.02	17.22±0.51	82.78±0.51	59.40±0.23	15.69±0.13	7.69±0.40	2.38±0.14	1.80±0.15	3.70±0.26
Mean annual	T&M	92.22±2.69	17.71±0.70	82.29±0.70	65.60±8.01	11.09±6.50	5.59±3.09	1.71±1.34	2.40±0.95	3.10±0.85
<i>Potamogeton nodosus</i>										
Spring	M	88.77±1.36	11.76±0.74	88.25±0.74	73.78±2.65	9.29±3.07	5.18±1.17	1.49±0.49	1.11±0.15	2.01±0.27
Winter	M	91.29±0.21	8.67±0.02	91.33±0.02	78.57±0.00	8.27±0.01	4.50 ±0.01	1.32±0.01	2.70±0.21	1.16±0.120
Mean annual	M	90.03±1.78	10.21±2.18	89.79±2.18	76.17±3.39	8.78±0.72	4.84±0.48	1.40±0.12	1.90±1.13	1.58±0.29
<i>Eichhornia crassipes</i>										
Summer	M	92.36±0.47	23.24±5.79	76.76±5.79	60.95±5.10	9.56±1.76	6.25±1.06	1.53±0.28	3.16±1.90	4.11±0.70
Autumn	M	93.23±0.15	11.59±0.06	88.41±0.06	67.35±0.02	7.38±0.05	5.78±0.06	1.61±0.01	2.3±0.07	5.11±0.04
Mean annual	M	92.80±0.62	17.41±8.24	82.59±8.24	64.15±4.52	8.47±1.54	6.01±0.33	1.57±0.06	2.73±0.60	4.61±0.71
F value	(M, C, P, E)	1.15	2.73	2.73	2.65	0.31	0.42	0.31	0.31	3.03
P-value		0.41	0.14	0.14	0.14	0.82	0.74	0.82	0.82	0.12

Abbreviations: Al-Rayah El Tawfiky =T; Al Rayah El Monoufy =M, Water contents (WC), Organic matter (OM), Nitrogen free extracts (NFE), Nitrogen (N), Phosphorus (P), Potassium (K), ± =standard deviation, *Myriophyllum spicatum*, *Ceratophyllum demersum*, *Potamogeton nodosus* and *Eichhornia crassipes* (M, C, P, E.) respectively.

Aquatic plants were recorded to contain in percent dry weight from 1.18 to 5.42% of total lipid content (**Boyd, 1968**). Most of the submerged plants contained less than 4 percent lipid, although there were some exceptions, particularly for oxygen weed. As shown in (Table 3) plant lipids presented highest variations among the studied species, season and sampling site. Among the analyzed macrophytes species, the two submerged species recorded their highest lipid contents in autumn samples from Al-Rayah El Tawofiky being 6.69 ± 2.19 and $7.69 \pm 0.40\%$ DW for *M. spicatum* and *C. demersum* respectively. These differences indicate an acceleration of productive metabolic activity during this period and/or higher consumption of this organic compound during the dry period (**Haroon et al. 2000**). The significant increases in the lipid content (about 5 times) were previously reported for *C. demersum* in the rainy season (**Esteves and Suzuki 2010**), *Potamogeton pectinatus* L. (**Haroon, 2008**) and for *Myriophyllum spicatum* (**Haroon, 2014**). In contrast, the two floating macrophytes species were found to be rich in their lipid contents during hot seasons (spring and summer) (Table 3).

Like other components, the values of macro-elements varied significantly ($p < 0.05$) between different species, sampling sites and seasons (Table 3). As previously recorded by **Haroon (2008)** N, P, and K are very important elements for plant cell as they are inter in protein, fatty acid synthesis and participate in the formation of many enzymes that is important for the chemical reactions inside plant cells, which make their concentrations being affected by plant growth form. This is coming in accord with the present results, where, the three macrophytes species *M. spicatum*, *C. demersum* and *E. crassipes* recorded their highest content of nitrogen (1.64 ± 0.39 , 2.38 ± 0.14 and $1.61 \pm 0.01\%$ DW) and K (3.70 ± 0.44 , 3.70 ± 0.26 and $5.11 \pm 0.04\%$ DW) during autumn (the week growth period), at the same time the highest potassium contents (2.98 ± 0.40 , 3.00 ± 0.06 and $3.17 \pm 1.90 \times 10^{-2}\%$ DW for the three species respectively) were detected in summer. Regarding, the free floating macrophyte species *P. nodosus* the highest N and K content (1.49 ± 0.49 and $2.01 \pm 0.27\%$ DW respectively) were detected during spring.

Bacteriological analyses

Fluctuations in the populations of total bacterial counts developed on either 20 or 35⁰C, presented in Figure (2). ANOVA analysis indicated significant differences attributed to the seasons and the sites. In both canal, total bacteria developed on either 20 or 35⁰C were particularly higher in autumn (up to 10^5 cfu ml⁻¹) and lower values were recorded in winter compared to other seasons. Among sites, in Al Rayah El Tawfiky the higher count developed on 20⁰C were 1.5×10^5 cfu/ml at Site four (There are agricultural and residential regions at the two banks) in Summer which covered by the Submerged macrophytes, *Myriophyllum spicatum*, and emergent macrophytes, *Saccharum spontaneum*. While, in Al Rayah El Monoufy the third site (Shebeen El-Koum City) (unvegetated Sites) gave the lowest value at 20⁰C or 35⁰C in winter. However, **Reitner et al. (1999)** recorded lower bacterial numbers in unvegetated areas compared to vegetated areas covered by the emergent macrophyte, *Phragmites australis*, it is likely that the specific composition of macrophytes may play a role in determining the net effect on bacteria. The bacterial metabolism is constrained by the carbon quality available in the macrophyte-dominated zone (**They and Marques 2019**). Wu et al. 2007, demonstrated that macrophytes may exert direct and indirect influences on the abundance and composition of bacterial communities.

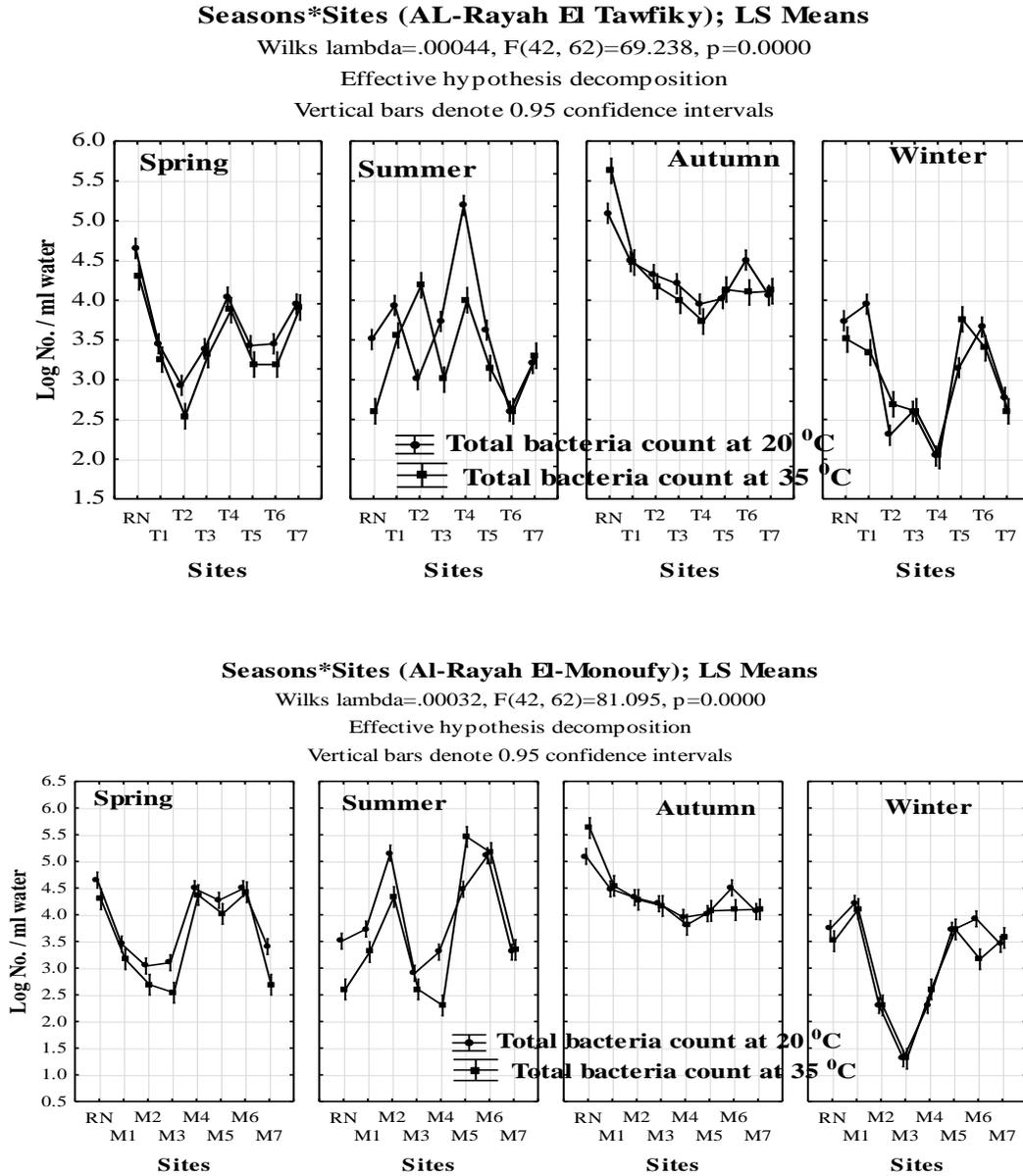


Fig. 2. Spatial and seasonal changes in microbial populations obtained of Al-Rayah El Tawfiky and Al Rayah El Monoufy water samples during the period of spring 2014 to winter 2015 (ANOVA analysis).

The most pathogenic microorganisms responsible for waterborne diseases were originated from a fecal-oral transmission route. Therefore, fecal coli bacteria (FC) are frequently investigated in determining the hygienic quality of water as they present in human and animal feces (**El-Aassar et al. 2018**).

Most probable number (MPN) of indicator bacteria that obtained from Al Rayah El Tawfiky ranged from 90 to 12×10^3 , 3 to 930 and 70 to 11×10^3 MPN/100 ml for total coliforms, fecal coliforms and fecal streptococci, respectively. Particular enrichment of total coliforms was distinguished at the spring season. Results of indicator bacteria ranged in Al Rayah El Monoufy from 90 to 12×10^3 , 4 to 46×10^3 and 70 to 12×10^3 MPN/100 ml for total coliforms, fecal coliforms and fecal streptococci, respectively. ANOVA analysis indicated significant differences attributed to the seasons (Figure 3). In spring season, site one of M1 (Behind fish research station of NIOF) which covered by floating macrophytes *Potamogeton nodosus* (according to Principal Component Analysis shows positive correlation between this plant and FC, $r = 0.943$, $n=4$, $p < 0.05$) and site M6 (In El Mahalla City) which covered also by the floating macrophytes *Potamogeton nodosus*, and submerged macrophytes *Myriophyllum spicatum*, recorded very high fecal coliform number (1500 MPN /100 ml and 4600 MPN /100 ml respectively) which exceeds the recommended level in irrigation water and so it was not suitable to irrigation of any plants or trees. According to WHO Guide (**WHO, 2011**), the recommended level for irrigation of vegetables likely to be eaten uncooked was 200 MPN/ 100ml and irrigation of ornamental fruit trees and fodder crops was 1000 MPN /100ml.

Fecal indicator bacteria such as total coliform, fecal coliform and streptococci are utilized worldwide to measure health hazards, their presence warns of the potential presence of disease causing organisms and should alert the person responsible for the water to take precautionary action (**Sanders et al. 2005**). Sites of Al Rayah El-Tawfiky recorded low number count of FC and within the permissible limit except sites T6 in spring and winter (420 MPN /100 ml and 930 MPN /100 ml respectively), which covered with submerged macrophytes, *Myriophyllum spicatum* in both seasons. The elevated fecal coliform densities may be due to increased nutrient availability as well as protection from UV radiation that are provided by vegetation that act as a secondary habitat for fecal indicator bacteria (*E. coli*) (**Jang et al. 2017 and Mathai 2019**). In addition, the presence of submerged plants in water sources increase the bed and bank roughness, that results in increasing drag, decreasing flow rate and increasing eutrophication of water (**Narasimha and Benarjee, 2016**).

El-Meihy (2018), found the counts mean of total coliform (TC) were ranged between (51×10^2 - 27×10^4 CFU/100 ml) in water samples collected from Rosetta branch during winter and summer. While in Damietta Branch waters were ranged between 29 - 8 000 and 24 - 930/100ml water for TC and FC, respectively. On the other hand, the most probable number of FS varied from 14 to 2900 /100ml water. **MHE (1996) and EC (1998)** accepted the guide values of recreational water up to 500/100 ml water for TC and 100/100ml water for FC and FS (**Khalifa and Sabae, 2012**). WHO considers a water sample non-potable if one or more *E. coli* are present in the water sample. WHO also classifies drinking water into five risk categories according to the *E. coli* count per 100 ml of water sample:(in conformity with WHO guidelines), 1-10 (low risk), 10-100 (intermediate risk), 100-1000 (high risk), >1000 (very high risk) (WHO 2011).

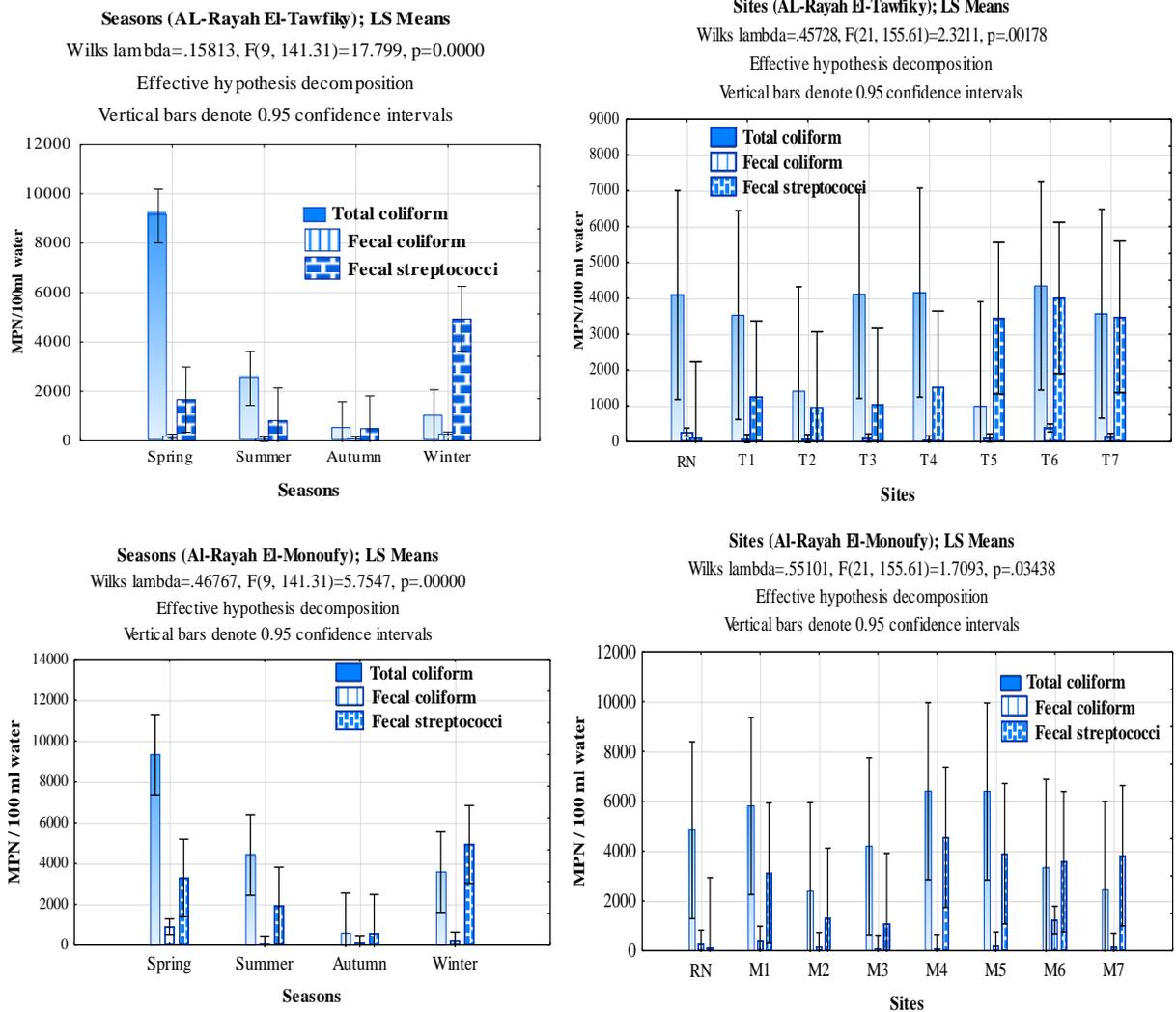


Fig. 3. Combined statistical analysis of bacterial indicators of pollution along the sites of Al Rayah El Tawfiky and Al Rayah El Monoufy water samples during the period of spring 2014 to winter 2015

Fecal coliform can be influenced by land use and increased animal–agriculture system (Pandey *et al.* 2014). In general, livestock and manure application as a fertilizer are reported to be main factors influencing bacterial contamination in agricultural streams. For example, the most contaminated bed sediment was observed in stream with unrestricted cattle access (Bragina *et al.* 2017).

Similar to fecal coli bacteria, fecal streptococci are considered as indicators of water pollution and have been used for many years to determine the quality of water for irrigation and human consumption (**WHO 1989 and Balkhair 2016**). Seasonal fluctuations were significant for all indicators ($P < 0.05$), with the highest count for fecal streptococci were found in winter season in the two canals. The higher flow rates and rainfall during the winter season may be led to higher bacteria counts because of increasing run-off from the informal settlement or re-suspension of bacteria from the river (**Benhalima et al. 2018**). Count of fecal streptococci increased with the increase of Al-Rayah El- Tawfiky course (Figure 3). The increase in FS count may be explained by the introduction of more bacteria by human activities, agricultural, and livestock. Sampling sites are located near agricultural land.

Geldreich and Kenner (1969) suggested that the FC/FS ratio is characteristic for given types of pollution or sources of pollution. The ratio itself is not a quantitative indication of pollution but a qualitative pollution index. Thus, domestic sewage yields FC-FS ratios greater than 4.0, whereas ratios less than 0.7 indicate that the pollution derives from warm-blooded animals other than humans. The low ratios were reported in majority of sample for the two Rayahs indicating the non-human source of pollution except few sites such as M6 in spring season. In the rural areas most of populations have no access to sewer systems or using individual septic tank so, untreated sewage will be released into rivers and this can contribute for fecal contamination (**Al-Badaii and Shuhaimi-Othman, 2015**). Delta drains mainly used for discharge of poorly treated or untreated wastewater (domestic and industrial), and drainage of agricultural areas. Therefore, they contain high amounts of pollutants such as organic matter, nutrients, fecal coliform, heavy metals and pesticides (**Emara et al. 2016 and El-Gohary, 2017**).

The associative nitrogen-fixing bacteria (diazotrophs) were present in high population densities in the canals water (Up to 10^5) (Figure 4) a clear demonstration to the terrestrial supplement to the canals through agricultural drainage waters. ANOVA analysis indicated significant differences attributed to the seasons and sites. Diazotrophs play a vital role in aquatic system, not only as N_2 fixer but also increase the aquatic productivity where diazotrophs can release growth factors beneficial for flora and fauna (**Gonzalez-Bashan et al. 2000 and Othman et al. 2016**).

Spring and winter seasons show low counts of diazotrophs compared to autumn and summer seasons. It is worth noting that the seasons with low populations of diazotrophs have few diversity of vegetation cover. Where one species (*Myriophyllum spicatum*) found in Al Rayah El- Tawfiky, and four species in Al Rayah El Monoufy (*Myriophyllum spicatum*, *Potamogeton nodosus*, *Saccharum spontaneum*, *Echinochloa stagnina*) in these seasons. *Myriophyllum spicatum* was found to spread in almost all sites of the canals. **Anderson and Kalff (1986)**, found that the length and biomass of *M. spicatum* are increasing by addition of nitrogen.

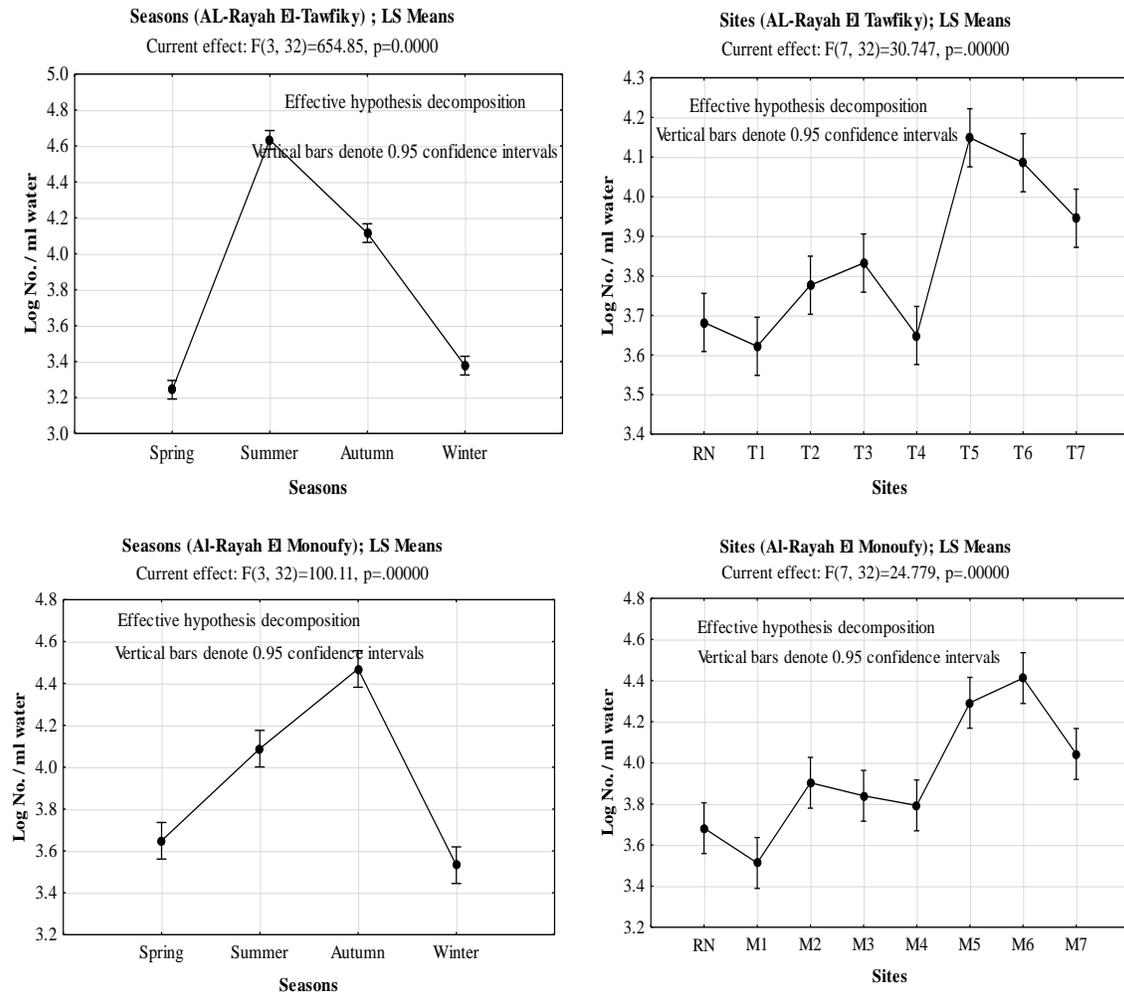


Fig. 4. Spatial and seasonal changes in nitrogen fixing bacteria densities of Al-Rayah El Tawfiky and Al Rayah El Monoufy water samples during the period of spring 2014 to winter 2015 (ANOVA analysis).

Relation between macrophytes, bacteria and water parameters

Different relations between different macrophytes species and the different ecological factors was observed (Figure 5). At Al-Rayah El Tawfiky the distribution of the two submerged macrophytes species *Myriophyllum spicatum* and *Ceratophyllum demersum* show negative but non significance correlation with presence of free floating species *E. crassipes*, which may be attributed partially to the shade caused by this plants, that affect light penetration and lower availability of atmospheric oxygen and carbon dioxide (Sastroutomo, 1985). The same trend of correlation was observed between *Eichhornia crassipes* and the three emergent macrophytes species *Echinochloa stagnina*, *Cyperus alopecuroides* and *Polygonum tomentosum* that explain the absence of this plant species from sites occupied by these species. *Ceratophyllum demersum* was significantly correlated with the emergent species *Echinochloa stagnina* and *Cyperus alopecuroides* ($r = 1$ at $P < 0.05$). In addition a strong positive relations between *Saccharum spontaneum* and *Phragmites australis* ($r = 0.985$ at $P < 0.05$) and between *Echinochloa stagnina*,

C. alopecuroides and *Polygonum tomentosum* ($r = 1$ at $P < 0.05$) were detected. Similar relations were recorded by **Shaltout et al. (2009)** and **Haroon and Hussian (2017)**.

In contrast at Al Rayah El Monoufy the free floating species *E. crassipes* was positively correlated with the three emergent macrophytes species *Saccharum spontaneum*, *Cyperus alopecuroides* and *Polygonum tomentosum* ($r = 0.943, 1.00$ and 0.870 at $P < 0.05$). *Ceratophyllum demersum* showed negative but non significance relation with *Potamogeton nodosus* and *Echinochloa stagnina*, however it have a significance positive relation with *Polygonum tomentosum* ($r = 1.00$ at $P < 0.05$), This variation could be related to the different environmental factors as well as the allelopathic interaction between different macrophytes species and other aquatic organisms, beside human impact and effect of grazing animals. The distribution and abundance of aquatic macrophyte were found to be affected by water and sediment characteristics (**Alahuhta et al. 2012; Hussian and Haroon 2019 and Haroon, 2020b**). This is coming in accord with the present study, where the distribution of most recorded macrophytes species was negatively correlated with water DO, COD, BOD, PO₄ and positively correlated with water NO₂, NO₃, Temp, and pH values.

Simple linear correlation coefficient showed a significant different relations between some water characteristics and macrophytes biochemical composition except for plant lipids. In the two studied Rayahs water temperature was positively correlated with plant potassium (K) at $P < 0.05$, $P < 0.01$ ($r = 0.545$ and 0.457 respectively). For samples from Al Rayah El-Tawfiky PH value was positively correlated with protein, NFE, N and phosphorus at $P < 0.05$ ($r = 0.401, 0.351, 0.421$ and 0.394 respectively) while it was correlated with K at $P < 0.01$, $r = 0.568$, however DO was only correlated with K at $P < 0.01$, $r = -0.528$. However, NO₂ was positively correlated with K ($r = 0.389$ $P < 0.05$). Similar relation was observed between NO₃ and protein and K ($r = 0.388$ and 0.417 at $P < 0.05$ respectively). For samples from Al Rayah El Monoufy, pH was positively correlated with phosphorus, DO and BOD were negatively correlated with K at $P < 0.05$. Some researcher like **Rather and Nazir (2015); Haroon et al. (2018); Haroon (2020a, 2020b)** confirmed the relation between water nutrients and plant components however other researcher (**Nichols and Keeney, 1976 and Tucker and Debusk, 1981**) mentioned the absence of this relation.

Different relations were also detected among different bacterial groups, plants components and chemical water parameters, for example at Al-Rayah El Tawofiky, TBC at 20°C was significant correlated with plant K content ($r = 0.494$ $n = 28$ at $P < 0.05$), while TBC at 35°C was significant correlated with plant N, lipid, ash water content, NO₂ and NO₃ ($r = 0.415, 0.445, 0.414, 0.376, 0.385, 0.383$ respectively $n = 28$ at $P < 0.05$) and with protein and potassium (0.485 and 0.583 respectively $n = 28$ at $P < 0.01$), also fecal streptococci was significant correlated with BOD ($r = 0.469$ $n = 28$ at $P < 0.05$). At Al-Rayah El Monoufy, total diazotrophs was significant correlated with DO ($r = -0.451$, $n = 28$ at $P < 0.05$) fecal streptococci was only correlated with plant lipids ($r = 0.367$, $n = 28$ at $P < 0.05$), TBC at 20°C and Total coliform was negatively correlated with lipid and phosphorus ($r = -0.329$ and -0.349 , $n = 28$ at $P < 0.05$ respectively).

Principal Component Analysis (PCA) shows negative correlation between dissolved oxygen and total diazotrophs, in two canals ($r = 0.584$ & 0.888 , $n = 4$, $p < 0.05$), as expected due to this bacterial group is belong to a facultative anaerobic bacteria. While it

shows significant positive correlation with *Phragmites australis* and *Saccharum spontaneum* ($r= 0.967$ & 0.959 , $n=4$, $p<0.05$) in Al Rayah El Tawfiky (Figure 5).

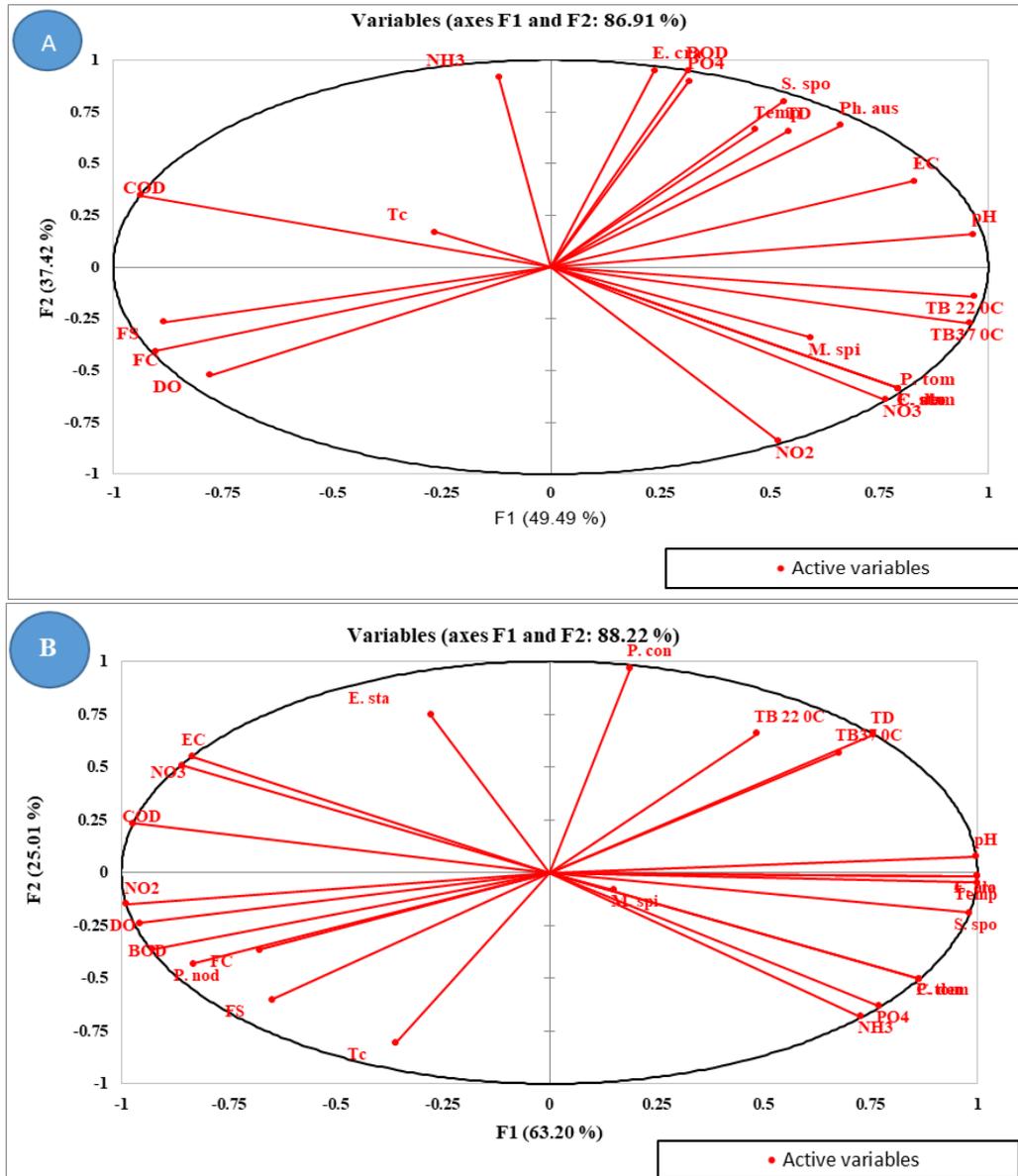


Fig. 5: The Principal Component Analysis (PCA) diagram of physicochemical parameters, macrophytes species and bacterial groups. (A) Al-Rayah El Tawfiky, (B) Al Rayah El Monoufy

Abbreviations: *Myriophyllum spicatum* L, (*M. spi*), *Ceratophyllum demersum* (*C. dem*), *Potamogeton nodosus* Poir, (*P. nod*), *Eichhornia crassipes* (Mart.) Solm (*E. cra*), *Saccharum spontaneum* (L.) (*S. spo*), *Echinochloa stagnina* (Retz.) P. Beauv. (*Per*) (*E. sta*), *Polygonum tomentosum*, (*P. tom*), *Polygonum convolvulus* L (*P. con*), *Cyperus alopecuroides* Rottb (*Per*) (*C. ale*), *Phragmites australis* (Cav.) Trin. (*Ph. aus*), total bacterial count (TB) at 22, 37 °C, total coliform (TC), fecal coliform (FC), faecal streptococci (FS) Water temperature (Temp), Total nitrogen (TN), Total phosphorus (PO₄), Electrical conductivity (EC), Dissolved oxygen (DO), Biochemical oxygen demand (BOD), Chemical oxygen demand (COD), Nitrite (NO₂), Nitrate (NO₃) and Ammonium (NH₄).

CONCLUSION

A significant differences in distribution, community structure and chemical composition of aquatic macrophytes and bacterial groups attributed to the seasons and sites differences was observed. In comparison, autumn season and sites of Al- Rayah El Monoufy were found to be the richest in species numbers. The submerged macrophyte species *Myriophyllum spicatum* was the most dominant and widely distributed species and *Ceratophyllum demersum* was the richest species in nutritional components. Bacterial analysis indicated the presence of indicator species of pollution in higher densities that exceeding the permissible limits cited by world health organization (WHO) especially at Al- Rayah El Monoufy. In addition, the results confirmed the nutritional potential of studied macrophyte species (especially submerged ones), beside their relation with different bacterial groups and different environmental variables that could be benefit in purposes of nutrition and water purification.

ACKNOWLEDGMENT

The present study was done in the framework of project plan of National Institute of Oceanography and Fisheries “The environmental status of the Nile Rayahs”. The authors wish to express their sincere thanks to prof. Mohamed El-Sherif Goher, Head of Chemistry laboratory, National Institute of Oceanography and Fisheries, for providing chemicals data.

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