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Environmental Assessment of Bacterial Load, Epiphytic Algae, and their Biochemical Contents after and before Flooding in Lake Nasser, Egypt

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ABSTRACT

The current study addressed the variation in epiphytic algae's total protein and lipid contents, as well as bacteria, viz. total coliforms (TC), faecal coliforms (FC), and faecal streptococci (FS) in the aquatic ecosystem during winter and summer. In winter (after flooding), epiphytic algae with 179 species belonging to 6 algal groups were recorded in the five stations of Lake Nasser, Egypt. While in summer (before flooding), the total number of epiphytic algae decreased to 144 species belonging to 5 algal groups. Compared to the value of bacteria after flooding, TC, FC, and FS bacteria possessed greater values before flooding. The high number of those bacteria in water indicates the possibility of pathogen presence. The total protein content of epiphytic algae revealed a large temporal significant change ($p < 0.01$) across seasons; whereas, total lipid showed a very large temporal significant difference ($p < 0.003$). No variances were detected in the space between the stations. After flooding, the total lipid content of the epiphytes was positively affected by total coliform and faecal coliform bacteria ($r = 0.81$ & 0.65, respectively). The results proved that variable environmental conditions (after flooding & before flooding) and seasons, as well as the bacterial load of the water body, affect the epiphytes species and their biochemical composition and diversity in the aquatic ecosystem.

INTRODUCTION

Lake Nasser, for its high reactivity to climate change, like other arid-region lakes, is referred to as an "amplifier lake" **(Street-Perrott & Roberts, 1983)**. During high flood and drought seasons in Lake Nasser, **Abeer *et al.* (2016)** evaluated the variation of chlorophyll a, total protein, carbohydrate, and lipid contents of phytoplankton, as well as the microbial load in water, sediment, plant, and fish in both Dahmit and Tushka Khors. Microorganisms in the aquatic environment play an important role in the turnover of organic matter and the determination of water quality **(Pomeroy & Wiegert, 1981)**. In general, ingesting water polluted with human or animal faeces (including birds) poses the biggest microbiological danger. Pathogenic bacteria, viruses, protozoa, and helminthees can all be found in faeces **(WHO, 2011)**. Microbiological indicators, such as total coliforms (TC), faecal coliforms (FC), and *Enterococcus* spp are used to determine water contamination **(Newall *et al.*, 2015)**. In most situations, epiphytism among algae occurs as a result of competition among various individuals who were unable to find a suitable substratum. Some researchers believe that the epiphytes' unique arrangement on the host is primarily due to the influence of light. Light, according to **Boergesen (1905)**, is responsible for the varied arrangement of epiphytes on the host. Epiphytes development can become so dense and congested that the host plant dies or deteriorates at the end of the season **(Boergesen, 1905)**. Submerged plants, on the other hand, are useful in shallow lake ecosystems for maintaining clear water conditions dominated by macrophytes **(Jackson 2003; Janssen *et al*., 2014)**. In many subsequent studies, variations in the biomass or species composition of epiphytic algae in different host plants were reported **(Kassim *et al*., 1997 & Hadi & AlZubadi, 2001)**. In aquatic environments, epiphytic microalgae are commonly used to identify trophic status, biomass **(Zimba, 1995)**, and as a food supply for higher trophic levels **(Eli *et al*., 2004; Anna *et al*., 2006)**. In addition, they are utilized in primary productivity, water quality and food web interactions **(Lisa & Fred, 2003; Vis *et al*., 2006; Cook, 2007)**. The natural environment's connections between host plants and adherent algae are still poorly understood **(Buczko, 2007)**. The physical properties of most plant substrata, as well as their chemical contribution to connected flora are very dynamic **(Wetzel, 1983)**. **Goher *et al*. (2021)** studied the last few seasons before the water was stored behind the Grand Ethiopian Renaissance Dam (GERD), which began in July 2020. This data could be useful in monitoring future changes in the Lake Nasser environment before and after the GERD reservoir is filled. The current study evaluated how epiphytic algae, their total protein and lipid contents, and the bacterial load varied in 10 areas of Lake Nasser's Main Channel water between December 2020 (after flooding) and June 2021 (before flooding).

MATERIALS AND METHODS

Sites of work

Lake Nasser lies between 22º N and 23º 58` N in Egypt (Fig. 1 & Table 1), and extends southward in Sudan to 20º 27 N` as Lake Nubia. In the primary channel, five sectors were chosen. Water samples were taken from the East and West stations (E & W) in each sector between December 2020 (after flood) and June 2021 (before flood).

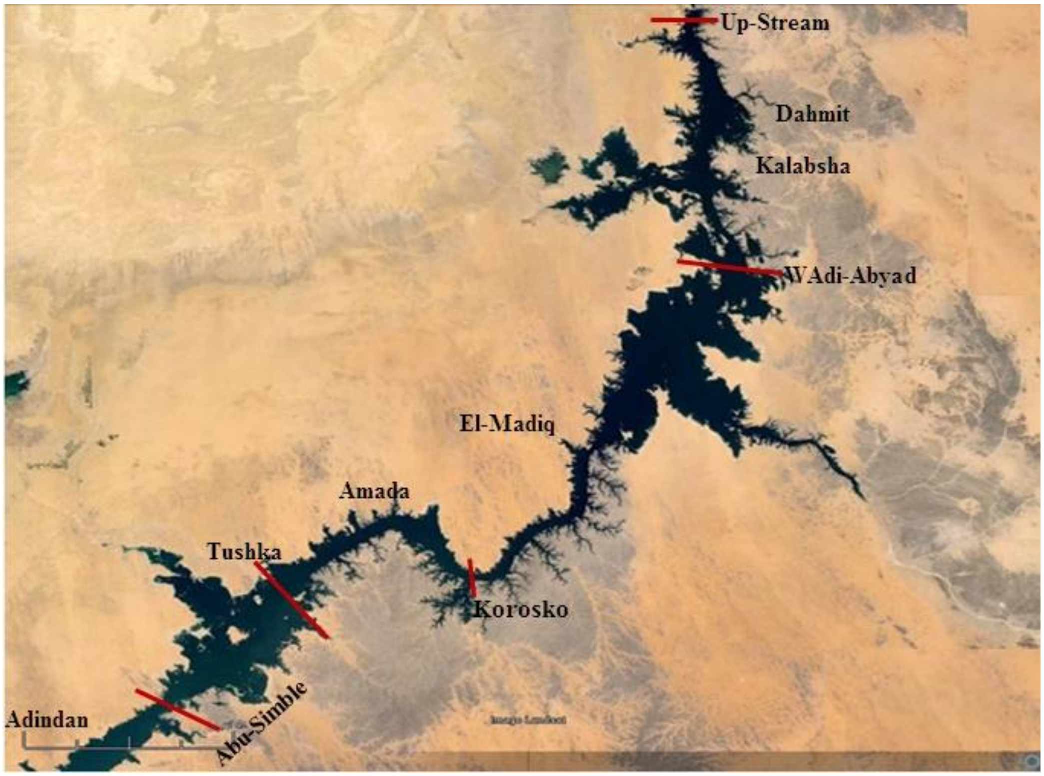


Fig. 1. Main Channel of Lake Nasser (After Goher 2021)

Table 1. Sampling sites in Lake Nasser (Goher 2021)

Section Main Channel	Lat.	Long.
M1-Up-Stream(AHD)	23°56'11.61"N	32°52'7.00"E
M2-Wadi-Abyad	23°20'43.57"N	32°56'9.43"E
M3-El Madiq	22°55'21.23"N	32°36'43.69"E
M4-Tushka	22°36'15.29"N	31°55'16.00"E
M5-Abu-Simble	22°20'21.04"N	31°38'42.52"E

Sample pre-treatment for epiphytic algae

Macrophytes were collected for the analysis of epiphytic algae. Epiphytic algae were collected from submerged parts of widespread macrophytes in water. **Round (1953)** and **Sladeckova (1962)** reported the collection procedure and laboratory evaluation. An inverted optical microscope (Zeiss, Axiovert 25C, Oberkochen, Germany) with a 10 eyepiece and a magnification of 400 was used to count epiphytic algal cells. Methods from earlier investigations were used to identify epiphytic algae species **(Cleve- Euler, 1952 & Mizuno, 1990)**.

Biochemical analysis

The leftover water on the macrophytes was filtered with GF/F filter paper after washing with distilled water, and the fresh weight of the leaves was recorded. Then, materials were stored at a temperature of 80°C for further analysis. The Biuret method was used to determine total protein **(David & Hazel, 1993)**. Total lipid was determined following the method of **Chabrol and Castellano (1961)**.

Microbial analysis

Sterilized glass vials were used to collect samples. During transportation, the obtained samples were transported in an iced insulated container and sent to the analytical laboratory for further analysis. All microbiological investigations on the water sampled were conducted using the approved methodology in the American Public Health Association's standard methods **(APHA, 2005)**.

Total and faecal coliform (TC and FC, respectively test)

The most probable number (MPN) approach was used to determine the number of TC and FC. The results were interpreted using statistical tables to determine the bacteria's MPN. The tubes were then incubated at 37°C for 48 hours for TC and at 44°C (in a water bath) for 24 hours for FC, and the confirmation tests were performed as follows: Positive tubes were streaked over Eosin Methylene Blue (EMB) agar plates with a sterile loop and incubated for 24 hours at 37°C. Microscopic examination was performed to determine that the rods were Gram-negative and did not develop spores.

Fecal Streptococci (FS) test

The MPN of FS, on the other hand, was determined using azide dextrose broth at 37°C for 48 hours, followed by a confirmation test as follows: Dense turbidity indicated a positive tube, which was verified with ethyl violet azide dextrose broth incubated at 37°C for 24 hours. The presence of FS was established by the creation of a purple button at the tube's bottom.

Data analyses

Correlation analysis was used to find connections between protein, lipid, epiphyte standing crop, and bacterial content. When $P < 0.05$, a relationship was declared significant. To compare seasons and stations, a one-way ANOVA was performed.

RESULTS

Bacterial assessment

Coliforms were internationally recognized in assessing the microbiological quality of water. The TC, FC, and FS were estimated by the most probable number (MPN) technique (Table 2). Numbers of TC after flooding are fluctuated between 15 – 460 MPN/100 ml water. While, the numbers of TC before flooding are fluctuated between 0 – 1500 MPN/100 ml water. FC results at Nasser Lake are in the range of 0 – 36 and 0-1100 MPN / 100 ml after and before flooding, respectively. FS numbers after and before flooding, respectively were in the range of 0 – 210 and 0-1200 MPN/ 100 ml.

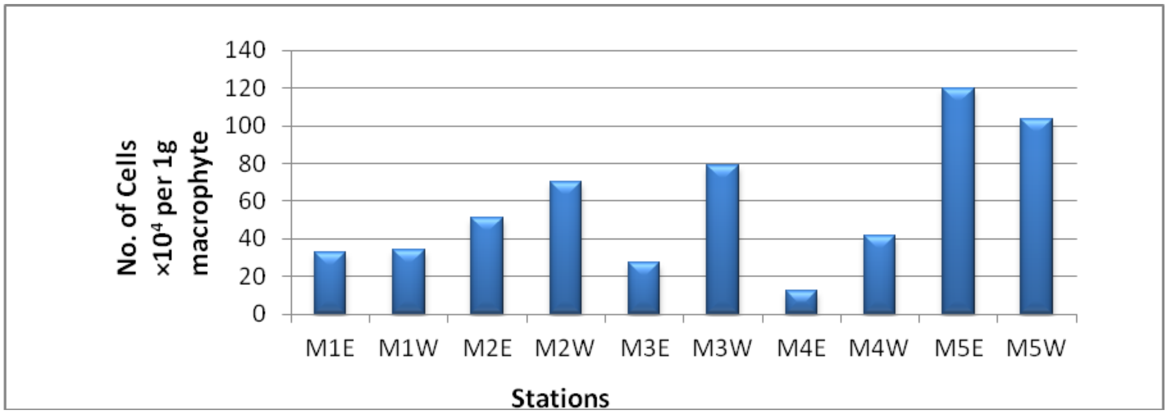
Table 2. Bacterial assessment of main channel at Nasser Lake before and after flooding

Station	Before flooding			After flooding		
	TC MPN /100ml	FC MPN /100ml	FS MPN /100ml	TC MPN /100ml	FC MPN /100ml	FS MPN /100ml
M1E	N.D	N.D	N.D	23	23	210
M1W	4	4	N.D	43	23	6
M2E	1500	1100	43	43	7	44
M2W	240	21	6	23	N.D	N.D
M3E	4	N.D	120	460	28	N.D
M3W	28	15	35	23	21	15
M4E	9	N.D	1200	15	N.D	6
M4W	240	43	20	150	9	N.D
M5E	93	93	43	460	36	150
M5W	43	N.D	75	23	9	9

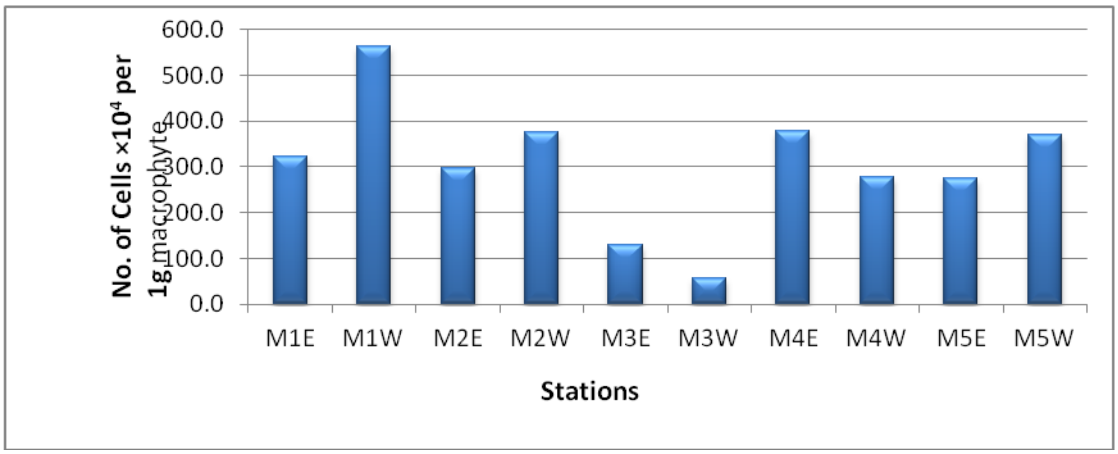
N.D= Not detected

The epiphytic algal species

The obtained results showed that the highest standing crop of epiphytic algal species attached to macrophyte in the main channel after flooding (103.73 cells $\times 10^4$ per 1.0 g of macrophyte) was recorded at Abu-Simble station M5E. While, the lowest one (12.11 cells $\times 10^4$ per 1.0 g of macrophyte) was recorded at Tushka station M4E (Fig.2a). The epiphyte density was higher than it had been in the post-flood period, ranging from 56.8 to 563.5 cells $\times 10^4$ per 1.0 g of macrophyte (Fig.2b).



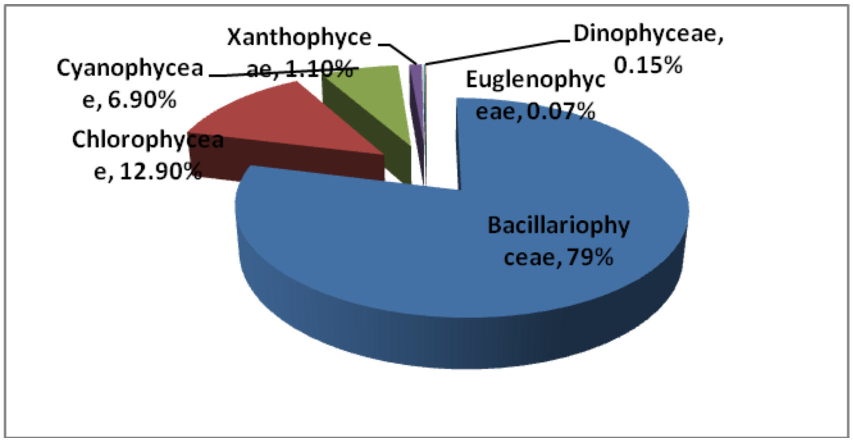
(a)



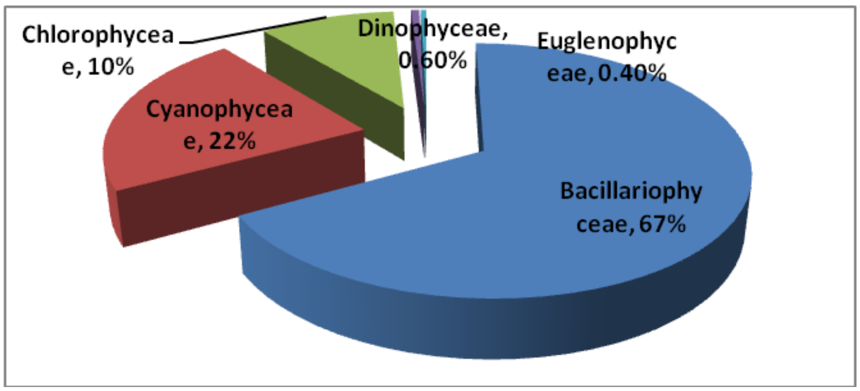
(b)

Fig.2. Variations in the total attached algae (No. of cells $\times 10^4$per 1.0 g of macrophyte): **(a)** After flooding (AF) and **(b)** Before flooding (BF)

Epiphytic algae after flooding in the five stations include 179 species belonging to 6 algal groups. The recorded six algal groups were arranged descendingly as follows: Bacillariophyceae > Chlorophyceae > Cyanophyceae > Xanthophyceae > Dinophyceae > Euglenophyceae as shown in Fig. (3a). Before flooding, epiphytic algae include 144 species belonging to 5 algal groups. The recorded 5 algal groups arranged descendingly as follows: Bacillariophyceae > Cyanophyceae >Chlorophyceae > Dinophyceae > Euglenophyceae as shown in Fig. (3b).



(a)



(b)

Fig. 3. Percentage abundance of the epiphytic algal groups: **(a)** in winter after flooding **(AF)** and **(b)** in summer before flooding **(BF)**

In the after flood period, the dominant species were *Cyclotella ocellata* Pant, *Melosira granulata* (Her.) Ralfs, *Melosira varians* C. A. Agradh, *Syndra ulna* (Nitzsch) Ehr. and *Coelastrum sphaericum* Nägeli. In the pre-flood period, the dominant species were *Navicula gallica* var. *laevissima* (Cleve) Lange-Bertalot, *Cocconies placentula* Ehr., *Cymatopleura solea (*Brébisson) W, *Cymbella tumida* (Brébisson) van Heurck, *Rhopalodia gibba* (Ehrenberg) O.Müller, *Lyngbya limnetica* Lemmermann, *Pseudanabaena galeata* Bocher, and *Stichosiphon sansibaricus* (Hie.) F.E.Dr. & W.A.Daily (Table 3).

Table 3. The most common epiphytic algal species recorded in the main channel (No. of cells $\times 10^4$ per 1.0 g of macrophyte) in the post-flood period (AF) & pre-flood period (BF)

	Stations										
Dominant spp.	M1E	M1W	M2E	M2W	M3E	M3W	M4E	M4W	M5E	M5W	Average
A F											
Melosira varians C. A. Agradh	5	2	16	12	1.6	14.3	0.8	10	20	17	9.9
Syndra ulna (Nitzsch) Ehr.	0.5	5	12	14	5.1	7.5	3	4	12	15	7.8
Cyclotella ocellata Pant	4.3	8	6	10.4	2.3	9.3	2	7	10	12	7.1
Melosira granulata (Her.) Ralfs	1.3	3	0	9	0.8	8.9	0.2	5	12	8	4.8
Coelastrum sphaericum Nägeli	0	0	0	3	0	5	0	0	6	3.9	1.8
B F											
Navicula gallica var. *laevissima* (Cleve) Lange-Bertalot	63.8	213	30	60	50	0	0	50	25	25	51.7
Cocconies placentula Ehr.	85.1	0	100	4	0	0.3	100	50	40	30	40.9
Cymatopleura solea (Brébisson) W.	42.6	21.3	60	40	5	0	50	20	18	15	27.2
Cymbella tumida (Brébisson) van Heurck	42.6	10.6	44	20	2	5	70	10	20	20	24.4
Lyngbya limnetica Lemmermann	3	6.4	5	6	3	1.4	4	100	80	2	21.1
Rhopalodia gibba (Ehrenberg) O.Müller	21.3	42.6	0	50	20	10	30	10	0	0	18.4
Stichosiphon sansibaricus (Hie.) F.E.Dr. & W.A.Daily	0	0	0	0	0	0	0	20	40	50	11
Pseudanabaena galeata Bocher	0	0	0	0	0	0.7	0	0	0	100	10.1

Biochemical contents of the epiphytes

The total biochemical contents of epiphytes in main channel plants investigated in five stations at East [E] and West [W] were shown in Fig. (4). The maximum protein contents of the epiphytes was detected at Aswan HD station (W) after flooding [17.2 mgl^{-1}]. In addition, the maximum lipid contents was detected at Madiq station (E) after flooding [2.0 mgl^{-1}], while the minimum protein contents was detected at Wadi-Abyad and Madiq station (E) before flooding [0.2 mgl^{-1}]. The minimum lipid content was investigated in most stations (E & W) before flooding [0.023 mgl^{-1}]. Total protein exhibited a high temporal significant difference ($p < 0.01$) and total lipid showed a very high temporal significant difference ($p < 0.003$) between seasons, according to ANOVA statistical analysis; whereas, no geographical difference was recorded across the stations. After flooding, the total protein content was negatively affected by *Melosira varians* algae and total TC ($r = -0.38$ & -0.32, respectively), while the total lipid was positively affected by TC and FC ($r = 0.81$ & 0.65, respectively). Before flooding, there was positive correlation between *Scenedesmus bijugatus* epiphytic algae and the total protein content ($r = 0.4$). Also there were negative correlation between the total protein content and TC and FC ($r = -0.39$ & -0.34, respectively). The total lipid content showed positive correlation with *Cocconies placentula* epiphytic algae ($r = 0.32$). *Cocconies placentula* epiphytic algae was positively affected by TC, FC and FS bacteria ($r = 0.47, 0.5$ & 0.48, respectively). Also *Cymatopleura solea* epiphytes was positively affected by TC, FC, and

FS (r = 0.59, 0.57 & 0.36, respectively). *Cymbella tumida* epiphytes showed positive relation with FC and FS (r = 0.3 & 0.72, respectively), while *Rhopalodia gibba* epiphytes show negative relationship with TC and FC (r = -0.33 & - 0.39, respectively). At the same time *Scenedesmus bijugatus* epiphytes was negatively correlated with TC and positively correlated with FC (r = - 0.31 & 0.43, respectively), (Fig. 5).

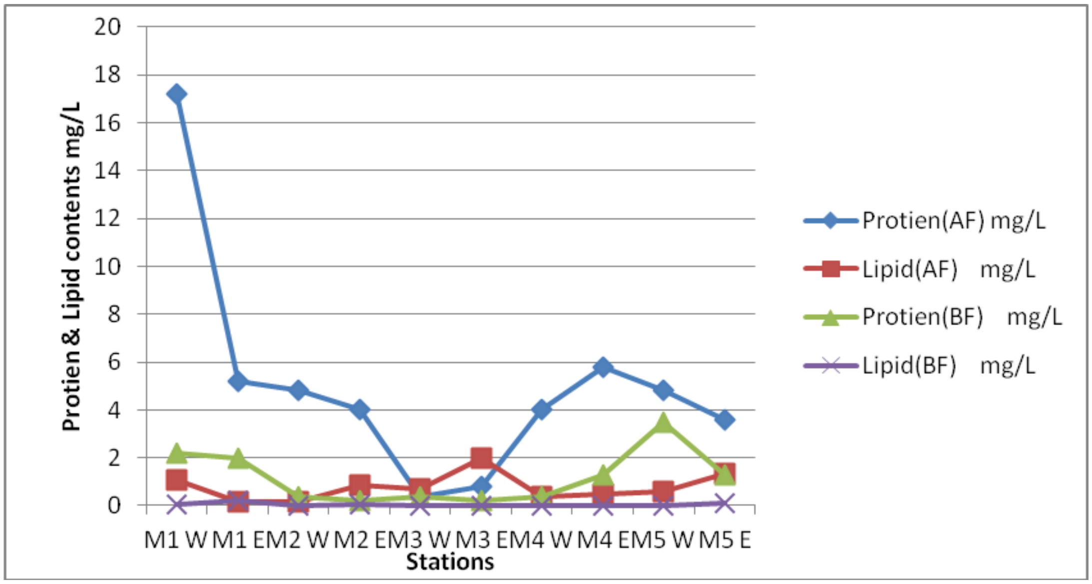


Fig. 4. Biochemical contents of Epiphytes after flooding (AF) and before flooding (BF)

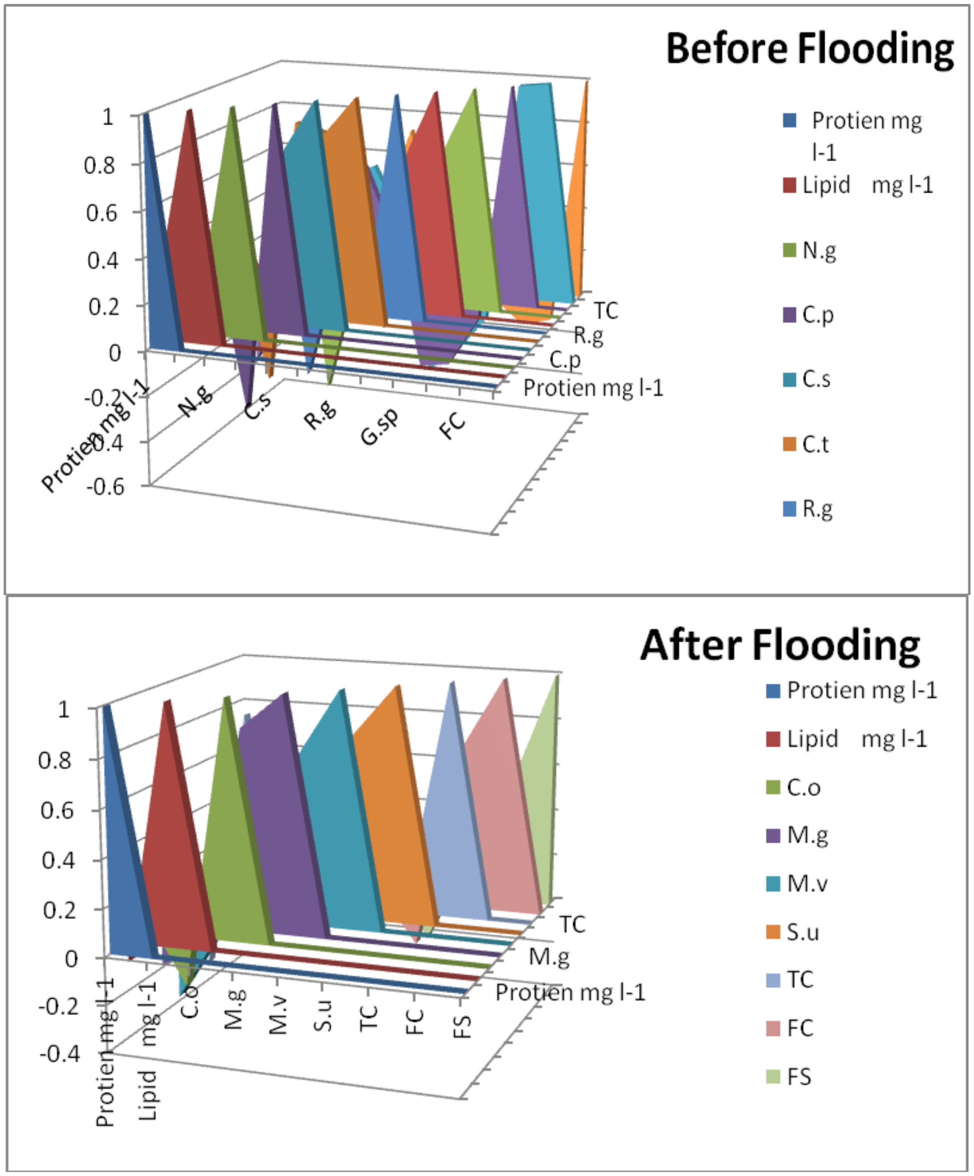


Fig. 5. Correlation between (TC, FC & FS) bacteria; epiphytes and protein & lipid contents after flooding (AF) and before flooding (BF)

DISCUSSION

Water quality is required to support a diverse and healthy fauna and flora in the water system. Water quality refers to the physical, chemical, and biological properties of water in relation to the presence of life, notably human activities. Bacteriological analysis is a critical component in determining water quality; a high level of faecal pollution (bacterial indicators) is directly proportional to faeces contamination, increasing the risk of water-borne diseases **(Shridhar, 2012)**. Faecal coliform counts were utilized as a water quality metric **(Ott, 1987)** and were thought to be good indications of faecal pollution. FC (thermo tolerant) bacteria are a dangerous microbiological contaminant that can cause gastroenteritis, hepatitis A, typhoid fever, dysentery and cholera. As a result, the FC must be completely absent from drinkable water in order to meet the drinking water requirement **(U.S. EPA, 2013)**. FS were used as a method for detecting whether the pollution was from human or animal sources. The presence of the high number of these microorganisms in water indicates the possibility of pathogen presence. In our study all parameters, TC, FC, and FS had higher values before flooding than those after flooding. High values of bacterial indicators may be due to human activities.

Epiphytic communities are one of the most important components in lake environments. In the current study, epiphytic algae abundance was higher in the pre-flood time than in the post-flood period. This observation is consistent with the findings of **Goher (2021)**, who stated that the overall number of epiphytic algae was at its atmost size (the largest) during the hot season and the lowest during the cold season. The organisation of the epiphytic groups differed in both seasons; during the winter and summer seasons, diatoms were the dominant group with the highest percentage of presence. The same result was obtained in the study of **Haroon *et al*. (2020)**. Epiphytic algae can help slowing water flow and providing nutrients to aquatic plants through decomposition processes that stimulate their growth. In contrast, epiphytic algae can also inhibit macrophyte growth **(Carvalho *et al*., 2015)**. Most of the identified algae were originally attached algae, but some were planktonic such as *Cyclotella* spp. *Cocconies* spp., *Melosira* spp., *Syndra* spp., and *Scenedesmus* spp. as pointed out by **Al-Saboonchi and Al-Manshed (2012)**. The dominant species in the summer (before flooding), which have been recorded as associated epiphytic algae communities, are frequent in the rivers that have been studied that far **(Altuner & Garbaz, 1991 & Tahoun *et al*., 2021)**.

The biochemical composition of phytoplankton is used as a biomarker for the aquatic ecosystem. According to ANOVA statistical analysis, total protein has a high temporal significant difference ($P > 0.01$), and total lipid has a very high temporal significant difference between seasons ($P > 0.003$), although there is no geographical difference between the stations. These findings are consistent with those of **Mahmoud (2015)** who stated that, the phytoplankton biochemical compositions in the River Nile vary during the four seasons, depending on physicochemical and environmental variables at various study sites. **Abd El-Karim and Mahmoud (2016)** discovered that the biochemical contents did not considerably differ among various locations of Lake Nasser, but did significantly differ between seasons. High protein and lipid content of phytoplankton reported during the winter season could be regarded as a growth-enhancing ingredient for hydrobionts present in Lake Qarun's aquatic environment (**Flefil & Mahmoud, 2021)**. The biochemical nature of phytoplankton changed substantially between species and locations throughout the seasons, according to principal component analysis **(Sabae & Mahmoud, 2021)**. *Melosira varians* algae and total coliform bacteria both had a negative impact on total protein concentration after flooding ($r = - 0.38$ and $- 0.32$, respectively). Both TC and FC had a beneficial effect on total lipid ($r = 0.81$ and 0.65, respectively). This can be explained by the fact that some epiphytic algae raise the fat content as a kind of protection from harmful bacteria (Fig. 5). *Scenedesmus bijugatus* epiphytic algae and total protein content had a positive connection (r = 0.4) prior to flooding. This is in agreement with the finding of **Mahmoud (2016)** who discovered that total protein makes up the majority of the phytoplankton's biochemical contents, while total lipid makes up the minority. There was also a negative connection between total protein concentration and TC and FC (r = - 0.39 and - 0.34, respectively). **Newall *et al.* (2015)** suggested that the presence of TC and FC bacteria increase the water pollution. This explains why the total protein content of epiphytic algae and these bacteria has a negative correlation. The overall lipid content has a favourable connection with the epiphytic algae *Cocconies placentula* (r = 0.32); this demonstrates that certain algae increase their fat content in order to protect themselves from bacteria. Thus, the present data concluded that some epiphytes increase their lipid content to protect themselves against bacteria, at the same time other epiphytes decrease their protein content due to the presence of some bacteria. The similarity indices and diversity are important in understanding the association and distribution of epiphytic algae species with the host plant **(Fawzy 2016)**. Generally, the distribution, quantity, biochemical contents and variety of epiphytic algal populations may be influenced by bacterial contents and environmental variables (after flooding & before flooding).

CONCLUSION

The distribution, quantity, and variety of epiphytic algal populations and there biochemical contents may be influenced by spatial changes, seasonal and environmental variables; (after flooding & before flooding), including the presence of some bacteria in the water body. The recorded six algal groups are descendingly arranged as follows: Bacillariophyceae > Chlorophyceae > Cyanophyceae > Xanthophyceae > Dinophyceae > Euglenophyceae (after flooding). While, the recorded five algal groups are descendingly arranged as follows: Bacillariophyceae > Cyanophyceae >Chlorophyceae > Dinophyceae > Euglenophyceae (before flooding). To protect themselves against bacteria, some epiphytes increase their lipid content, while others reduce their protein level due to the presence of bacteria. Variable environmental conditions (after flooding & before flooding) and seasons, as well as the bacterial load of the water body should affect the epiphytic algae, composition, behaviour and diversity.

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الملخص العربي

التقييم البيئي للمحتوى البكتيري والطحالب الملتصقة بالنباتات المائية ومحتوياتها البيوكيميائية بعد الفيضانات وقبلها في بحيرة ناصر- مصر

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تمت دراسة تباين النظام البيئي المائي للطحالب الملتصقة بالنباتات المائية، ومحتوياتها الكلية من البروتين والدهون ، وكذلك دراسة البكتيريا القولونية و القولونيات البرازية والمكورات العقدية البرازية خلال الشتاء والصيف. في فصل الشتاء (بعد الفيضان) ، تضم الطحالب الملتصقة بالنباتات المائية في المحطات الخمس (شرقاً وغرباً) ١٧٩ نوعًا تنتمي إلى ٦ مجموعات طحالب ، بينما في الصيف (قبل الفيضان) انخفض العدد الإجمالي للطحالب إلى ١٤٤ نوعًا تنتمي إلى ٥ مجموعات طحالب.

كان لجميع البكتيريا ، بما في ذلك TC و FC و FS ، قيم أكبر قبل الفيضان مقارنة بالفيضان. ويشير وجود عدد كبير من هذه البكتيريا في الماء إلى احتمال وجود العوامل الممرضة. أظهر محتوى البروتين الكلي للطحالب الملتصقة بالنباتات المائية فرقًا نوعيًا زمنيًا مرتفعًا $p< 0.01$ ، وإجمالي الدهن ، أظهر فرقًا نوعيًا زمنيًا مرتفعًا جدًا $p< 0.003$ بين الفصول. كما إنه لا توجد فروق مكانية بين المحطات. وبعد الفيضان تأثر المحتوى الدهني الكلي للطحالب الملتصقة بالنباتات المائية إيجابيا بالبكتيريا القولونية الكلية والبكتيريا القولونية البرازية (r = ٠.٨١ و ٠.٦٥) على التوالي. تشير النتائج إلى أن الظروف البيئية المتغيرة (قبل و بعد الفيضان) والمواسم ، وكذلك المحتوى البكتيري في الجسم المائي تؤثر على أنواع الطحالب الملتصقة بالنباتات المائية وتكوينها الكيميائي الحيوي وتنوعها في النظام البيئي المائي.