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Seasonal biodiversity and ecological studies on the epiphytic microalgae communities in polluted and unpolluted aquatic ecosystem at Assiut, Egypt

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ABSTRACT

A qualitative and quantitative study on epiphytic microalgae was carried out seasonally from November 2015 to August 2016 to follow up their community structures on aquatic macrophytes related to some physico-chemical properties of two polluted and unpolluted water bodies at Assiut, Egypt. A total of 169 species related to 64 genera of epiphytic microalgae were recorded. The most dominant algal group was Bacillariophyceae (43.2%), followed by Chlorophyceae (34.91%), Cyanophyceae (20.71%) and Euglenophyceae (1.18%). The total number of epiphytic algae fluctuated between 11.1×10^4 ind.g^{-1} plant dry wt. on *Phragmites australis* in summer at Nazlet Abdellah (polluted site) and 10.02×10^7 ind.g^{-1} plant dry wt. on *Myriophyllum spicatum* in winter at El-Wasta (unpolluted site). Some epiphytic microalgae were dominant as P*seudanabaena limnetica, Calothrix braunii, Scenedesmus acutus, and Ulnaria ulna. Others were specific on certain macrophytes as Aphanocapsa thermalis* and *Ulothrix sp.*, which grow on Phragmites australis, while Synechocystis minuscula attached itself on Myriophyllum spicatum. Analysis of PERMANOVA showed that the most important factors that induced the variation in epiphytic microalgae were the temporal variation and host plant. Water temperature, pH, nitrate, chloride, phosphate and total dissolved salts were the highest abiotic factors correlated with the variation in composition of epiphytic microalgae.

KEYWORDS

Epiphytic microalgae, macrophytes, biodiversity, PERMANOVA

INTRODUCTION

Aquatic plants are major components in the spatial heterogeneity; they consist of important centres for the conservation of aquatic biodiversity (Thomaz et al. 2008; Mormul et al. 2010). Aquatic macrophytes form the specific environment by either suppressing or stimulating aquatic organisms, as well as by enhancing the availability of a large area suitable for colonialism and the attached epiphytic community (Algarte et al. 2009).

Epiphytic algae are organisms that attach themselves directly on aquatic macrophytes by secreting jelly, while others are in loose association with the aquatic vegetation. They are colonizing on aquatic macrophyte and sediment surfaces, and constitute heavy carbonate accumulations on plant stems (Gaiser et al. 2011). Epiphytes are part of a very heterogeneously structured community, which contains primary producers and consumers as well as decomposers. Epiphytic microalgae usually found in running water, and play a prime role in the environmental balance between different groups of aquatic plants and their environment (Hassan et al. 2014).

In ecosystems, epiphytic community is considered as one of the basic parts of food webs (Abe et al. 2007); also, the productivity of epiphytic algae may equalize or even exceed the productivity of phytoplankton and macrophyte (Wetzel 1993). Furthermore, interactions between benthic microalgae, phytoplankton and the host plant determine the entire ecosystem character and the ecosystem responses to the changing environmental conditions (Liboriussen & Jeppesen 2003). Epiphytes may be useful to macrophytes by reducing water movement; also, the dead and decomposing epiphytic algae material becomes a source of nutrients for macrophyte growth (Borowitzka et al. 1990). On the other hand, an increase in epiphytic algae may reduce the growth and production of macrophytes due to their shading and reduce the diffusion of nutrient from water to macrophytes. In return, aquatic plants release allelopathic compounds, which reduces epiphyte (Al-Saboonchi & Al-Manshad 2012). Biomass of epiphytic microalgae may be influenced by many environmental factors such as temperature, location, light, pH, water level, seasonal changes (Verhulst 2013; Demir et al. 2014), morphology of macrophytes especially stem or leaf length (Messyasz et al. 2006), and chemical factor such as nutrients (Kormas et al. 2006). There is little information on the epiphytic microalgal communities in the Egyptian water bodies (El-Karim et al. 2009; Fawzy 2016). The objective of the current research was to study the qualitative and quantitative composition of the epiphytic microalgae on some aquatic macrophytes in polluted and unpolluted aquatic sites at Assiut, Egypt, as well as to study the effect of water quality on the diversity of epiphytic microalgae.

1. MATERIALS AND METHODS

1.1. Study area and sampling

Six species of aquatic macrophytes were recorded and collected from two aquatic sites at Assiut, Egypt, polluted site 1 (Nazlet Abdellah, 27˚9′54.5″N, 31˚12′43.2″E) and unpolluted site 2 (El-Wasta, 27˚10′22.8″N, 31˚12′55.3″E), during November 2015 to August 2016. The macrophytes were *Phragmites australis*, *Myriophyllum spicatum, Echinochloa stagnina, Potamogeton nodosus, Potamogeton perfoliatus* and *Persicaria salicifolia.* These macrophytes were defined and deposited in the herbarium of Botany and Microbiology Department, Faculty of Science, Assiut University.

1.2. Epiphytic algae sampling

Epiphytic microalgae were collected from the stems of the macrophytes. A number of stems reached 10–50 per m^2 spaces at the sites of sampling. Water depth at sites ranged from 0.5 m to 1 m. The stems were cut and placed in polyethylene bags. A collection of epiphytic microalgae was performed by scraping the surfaces of stems into a definite volume of distilled water (100 ml) by using a soft brush and then placed in a reciprocating shaker for 30 minutes. The counting of epiphytic microalgae was performed using a Sedwick-Rafter cell (Ganf 1974) and the number of individuals per gram of dry weight of the macrophyte was expressed using a binocular microscope. The biomass of epiphytic microalgae was represented as chlorophyll a and estimated according to Metzner et al. (1965). The epiphytic algal species were identified according to Kramer and Lange-Bertalot (1991); Lund and Canter-Lund (1995) and http://www.algaebase.org. Relative abundance of each taxon is estimated as follows: Rare, present in < 25% of the examined algae. Common, present in 25–49% of the examined algae. Abundantly, present in > 50% of the examined algae.

1.3. Water analysis

Two water samples of the study sites were collected seasonally, and the parameters were estimated in triplicate for each sample. Water temperature was measured in situ by a Celsius thermometer. pH was measured by using a digital pH meter (pH Pen Jenco Electronics, U.S.A) and electrical conductivity (E.Cµmhh.cm^{-1}) was measured by EC Meters (YSI Model 35 yellow springs, OH, USA). The total dissolved solids were determined according to Jackson (1958). Nitrate- nitrogen was determined by Bulgariu and Bulgariu (2012). The method described by Dewis and Freitas (1970) was used for the determination of orthophosphate. Sulphate was determined by Sheen et al. (1935). Chlorides were estimated according to Jackson (1960). Na^+ and K^+ were detected by a flame photometric technique according to Williams and Twine (1960) using (Dr Lange Flame Photometer M 71 D type Nr/ LPG 075). Ca^{+2} and Mg^{+2} were estimated by the Versene method according to Schwarzenbeck and Bederman (1948).

1.4. Data analysis

The contribution of the plant and geographical variation to the epiphytic microalgal assemblages were studied by using the PERMANOVA analysis. The PERMANOVA design contained three factors: site (S) with two levels as fixed factors, plant (Pl) with six levels and season (Se) with four levels as random factors. The PERMANOVA test was conducted based on Bray Curtis resemblance using the permutation of residuals under a reduced model (999 permutations). When the PERMANOVA presented significant differences ($p < 0.05$), a pairwise comparison (999 permutations) was done to explore the variations between pairs of all levels of the selected factor. A distance-based redundancy analysis (dbRDA) plot allowed the visualization of the samples' ordination based on the assemblage of epiphytic algae and the correlation to environmental factors (McArdle and Anderson 2001). The analysis was based on the Bray Curtis similarity between the biological data and Euclidean distance for normalized environmental variables. The analyses of PERMANOVA and dbRDA were carried out by PERMANOVA+ in PRIMER v6 software (Anderson et al. 2008).

2. RESULTS

The physico-chemical analyses of the water samples were shown in Table 1. The water temperature ranged between 18—28°C. The highest pH values were recorded during winter (9.52) and the lowest (7.37) during autumn at site 1. The polluted site recorded the highest values in electrical conductivity, total soluble salts, chloride and potassium in summer, as well as nitrate showed the maximum content during winter (0.42 mg/L) at the same site. Phosphate fluctuated within 0.02 mg/L to 0.22 mg/L at site 2 in spring and autumn, respectively. Sulphate concentration ranged between 0.18 mg/L during summer at site 1 and 2.23 mg/L during winter at site 2. While the highest value of sodium was recorded at site 2 (37.9 mg/L) in summer, calcium level seasonally ranged from 80 mg/L to 53.3 mg/L, whereas the magnesium concentrations recorded the maximum value (114 mg/L) at site 2.

Relatively high variability in the epiphytic algal biomass as represented by chl a was detected (Figure 1). Gen-

Table 1. Physico-chemical characters of the investigated water samples seasonally collected from various polluted and unpolluted fresh water at Assiut- Egypt.

Sites and seasons / Parameter	Autumn		Winter		Spring		Summer	
	polluted	unpollu-ted	polluted	unpollu-ted	polluted	unpollu-ted	polluted	unpollu-ted
	S1	S2	S1	S2	S1	S2	S1	S2
Temperature°C	22	22	18	19	28	28	28	28
pH	7.37 ±0.01	7.78 ±0.01	9.52 ±0.01	8.62 ±0.01	9.22 ±0.01	9.01 ±0.06	7.81 ±0.01	7.90 ±0.01
Electrical conductivity (µmho.cm^{-1})	187.5 ±0.64	174.0 ±0.58	161.1 ±2.66	185.1 ±1.28	147.7 ±1.29	149.6 ±1.67	192.9 ±0.79	185.9 ±1.84
Total dissolved solids (T.D.S.) mg/L	120.0 ±0.4	111.4 ±0.4	103.1 ±1.7	118.5 ±0.8	94.5 ±0.82	95.8 ±1.07	123.5 ± 0.50	119.0 ±1.18
NO3-N (mg/L)	0.38 ±0.02	0.17 ±0.00	0.42 ±0.01	0.38 ±0.01	0.09 ±0.01	0.07 ±0.01	0.37 ±0.03	0.29 ±0.02
PO4-P (mg/L)	0.15 ±0.01	0.22 ±0.00	0.09 ±0.00	0.11 ±0.00	0.14 ±0.00	0.02 ±0.00	0.08 ±0.00	0.09 ±0.00
SO4-S (mg/L)	2.08 ±0.00	1.60 ±0.01	0.50 ±0.02	2.23 ±0.01	0.71 ±0.00	0.58 ±0.001	0.18 ±0.01	0.37 ±0.02
Chloride (mg/L)	39.7 ±0.09	36.2 ±0.03	37.3 ±0.12	30.3 ±0.09	46.7 ±0.09	40.83 ±0.07	51.3 ±0.03	42 ±0.06
Sodium (mg/L)	31.55 ±0.61	31 ±0.29	35.2 ±0.29	32.6 ±0.00	32.55 ±0.03	29.95 ±0.32	37.8 ±0.00	37.9 ±0.03
Potassium (mg/L)	3.94 ±0.02	3.25 ±0.03	2.87 ±0.02	2.93 ±0.02	3.74 ±0.01	3.54 ±0.02	5.45 ±0.01	5.39 ±0.01
Calcium (mg/L)	53.3 ±0.03	60 ±0.06	76.7 ±0.09	80 ±0.06	70 ±0.06	56.6 ±0.03	70 ±0.06	53.3 ±0.03
Magnesium (mg/L)	54 ±0.23	16 ±0.03	28 ±0.15	20 ±0.03	26 ±0.03	56 ±0.06	90 ±0.06	114 ±0.07

S1: Nazlet Abdellah, S2: El-Wasta

erally, the highest algal biomass was recorded seasonally on *Myriophyllum spicatum* at Nazlet Abdellah site in autumn (721.95 µg/g^{-1} plant dry wt.); on the other hand, the lowest algal biomass was recorded on *Persicaria salicifolia* at El-Wasta site in winter (4.85 µg/g^{-1} plant dry wt.) (Figure 1).

In general, 169 of algal species were identified from the investigated macrophytes, of which 73 species (22 genera) belong to Bacillariophyceae, 59 species (23 genera) belong to Chlorophyceae, 35 species (17 genera) to Cyanophyceae, and 2 species (2 genera) to Euglenophyceae. The dominant algal group was Bacillariophyceae (43.2%), followed by Chlorophyceae (34.91%), Cyanophyceae (20.71%) and Euglenophyceae (1.18%). The rare algal taxon was present in < 25% of the examined algae and was represented by 128 species. Only 31 species represented as common species were present in 25—49% of the examined algae. 10 species had a high occurrence and were abundantly present in > 50% of the examined algae.

The highest diversity of algal species was observed in winter and autumn (Table 2). *Pseudanabaena limnetica*, *Lyngbya lagerheimii*, *Scenedesmus acutus*, *Scenedesmus quadricauda*, *Ulnaria ulna*, *Cyclotella meneghiniana* and *Fragilaria capucina* were the dominant algal species. *Aphanocapsa thermalis*, *Synechocystis minuscula*, *Ulothrix* sp., *Actinastrum hantzschii*, *Surirella* sp. and *Encyonema* sp. were some of the rare occurrence species endemic to a specific host plant. The highest numbers of epiphytic microalgal species (52 species) were observed on *Myriophyllum spicatum* and *Phragmites australis* in autumn and winter, respectively at Nazlet Abdellah (Table 2). The lowest number were on *Echinochloa stagnina* (10 species) in summer at El-Wasta (unpolluted). Highest total algal counts expressed as individuals were 10.02 ×10^{7} ind g^{-1} plant dry wt. on the stem of *M. spicatum* in winter at site 2 (Figure 2).

Three-way PERMANOVA on epiphytic microalgal assemblage between the three studied factors (site, season, and plant) revealed that, the temporal variation, as well as the host plant were the most important factors that induced variation in epiphytic algal assemblage (season, $P = 0.008$; host plant, $P = 0.044$). However, the sites were unable to show variation between the associated epiphytic microalgal genera ($P > 0.05$). Similarly, the interactions among site, plant and seasons have no effect on the presence/absence of epiphytic algae ($P > 0.05$, Table 3).

Temporal variation in epiphytic algal composition was related to the physico-chemical properties of water (Figure 3). Water temperature, pH, phosphate, nitrate, chloride and total dissolved salts were the highest abiotic factors correlated to

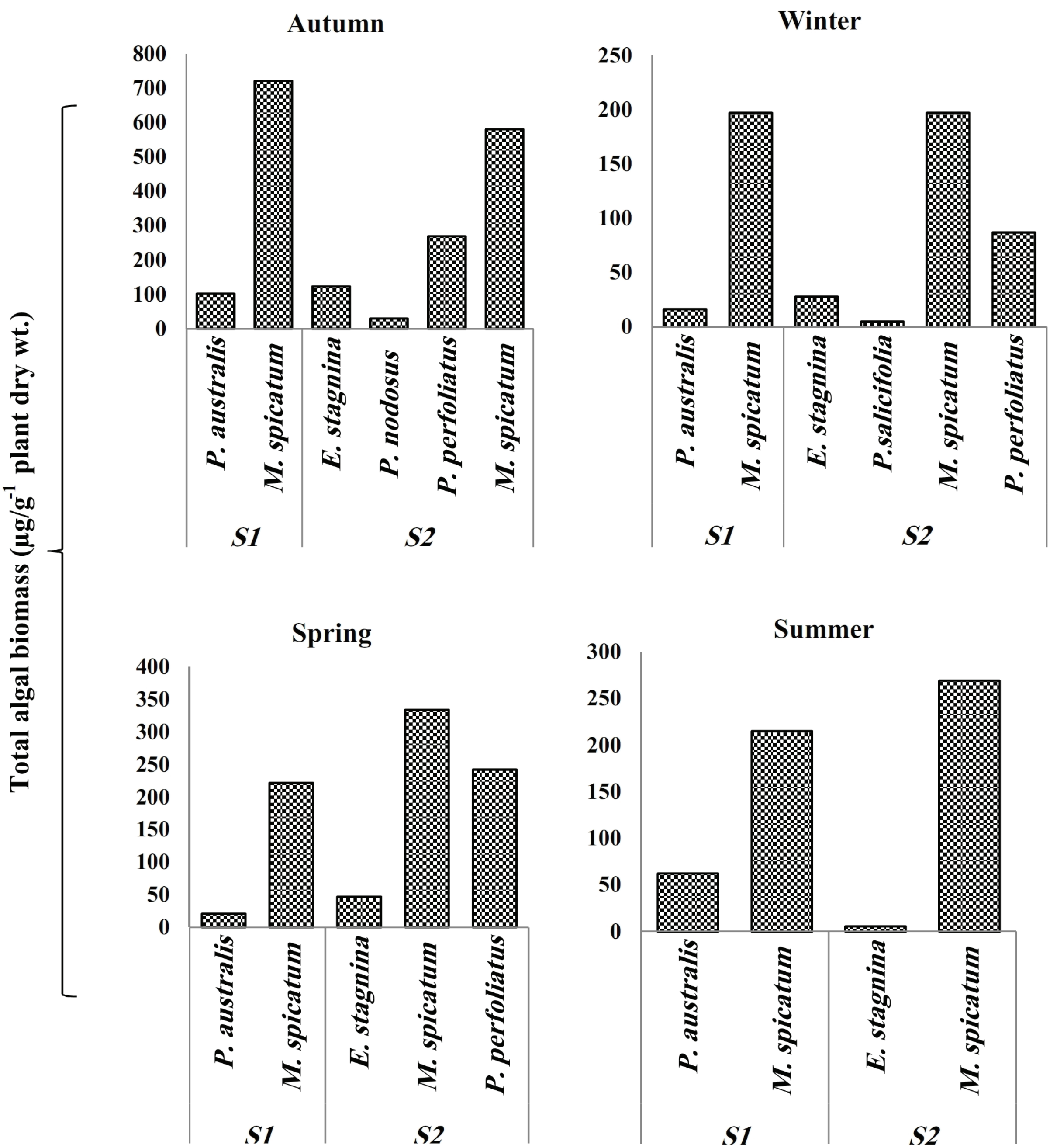

Figure 1. Total algal biomass of epiphytic algae present on the investigated macrophytes in polluted and unpolluted freshwater sites, at Assiut, Egypt.

the variation in epiphytic algal composition. For example, pH showed higher positive correlation to the epiphytic algal community associated with host plants collected in spring, while nitrate and total dissolved salts showed higher positive correlation to the epiphytic algal community associated with host plants collected in cold and hot seasons (winter and summer), along with water temperature, phosphate and chloride.

3. DISCUSSION

Epiphytic algae have a considerable role to play in energy, nutrient conversion as well as being a bioindicator of pollution (Davies 2009). The biomass of epiphytic algae varied according to the host plants, sites and season. Generally, the high value of epiphytic algae biomass was recorded in all seasons on *Myriophyllum spicatum*, while, the lowest was on the stem of *Persicaria salicifolia* in winter at site 2. Epiphytic algal biomass was enhanced when the level of nitrogen and phosphorus concentration was elevated in the water body and

Table 2: Occurrence remark of epiphytic algal species recorded on the investigated macrophytes present in polluted and unpolluted freshwater sites at Assiut- Egypt

Sites and Seasons	Autumn						Winter						Spring					Summer				O.R
	Polluted		Unpolluted				Polluted		Unpolluted				Polluted		Unpolluted			Polluted		Unpolluted		
	S1		S2				S1		S2				S1		S2			S1		S2		
Plants / Algal taxa	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Potamogeton nodosus	Potamogeton perfoliatus	Myriophyllum spicatum	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Persicaria salicifolia	Myriophyllum spicatum	Potamogeton perfoliatus	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Myriophyllum spicatum	Potamogeton perfoliatus	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Myriophyllum spicatum	O.R
Cyanophyta																						
Anabaena iyengarii Bharadwaja	+							+											+			R
A. naviculoides F.E.Fritsch	+	+				+																R
A. sp.							+															R
Aphanothece bullosa (Meneghini) Rabenhorst																				+		R
Aphanocapsa thermalis Brügger																		+				R
Arthospira platensis Gomont									+	+					+			+				R
Calothrix braunii Bornet & Flahault	+	+					+	+	+	+				+	+							L
Chroococcus minor (Kützing) Nägeli	+	+	+																			R
Ch. turgidus (Kützing) Nägeli.		+			+														+			R
Cylindrospermum catenatum Ralfs ex Bornet & Flahault	+																					R
C. sp.																		+	+			R
Lyngbya lagerheimii Gomont ex Gomont	+	+				+	+		+		+		+					+				L
L. sp.		+	+			+																R
Merismopedia convolute Brébisson	+																					R
M. tenuissima Lemmermann	+	+	+	+			+	+					+						+			L
Microcystis flos-aquae (Wittrock) Kirchner			+	+																		R
Nostoc sp.							+			+												R
Oscillatoria brevis Kützing ex Gomont	+																					R
O. formosa Bory ex Gomont						+																R
O. limosa C.Agardh ex Gomont	+				+													+				R
O. margaritifera Kützing ex Gomont		+																				R
O. subbrevis Schmidle							+						+									R
Phormidium articulatum (N.L.Gardner) Anagnostidis & Komárek	+	+																				R
Ph. chlorinum (Kützing ex Gomont). Umezaki & Watanabe															+			+				R
Ph. corallinae (Gomont ex Gomont) Anagnostidis & Komárek							+		+				+		+					+		R
Ph. favosum Bory	+						+															R
Ph. gardneri Muzafarov										+												R
Ph. Insigne (Skuja) Anagnostidis										+											+	R
Ph. laetevirens (P.Crouan & H.Crouan ex Gomont) Anagnostidis & Komárek								+						+						+		R

continued Table 2: Occurrence remark of epiphytic algal species recorded on the investigated macrophytes present in polluted and unpolluted freshwater sites at Assiut- Egypt

Sites and Seasons	Autumn						Winter						Spring					Summer				O.R
	Pollu-ted		Unpolluted				Pollu-ted		Unpolluted				Pollu-ted		Unpollu-ted			Pollu-ted		Unpol-luted		
	S1		S2				S1		S2				S1		S2			S1		S2		
Plants / Algal taxa	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Potamogeton nodosus	Potamogeton perfoliatus	Myriophyllum spicatum	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Persicaria salicifolia	Myriophyllum spicatum	Potamogeton perfoliatus	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Myriophyllum spicatum	Potamogeton perfoliatus	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Myriophyllum spicatum	O.R
Ph. molle Gomont								+								+		+	+			R
Ph. sp.		+				+				+		+									+	R
Pseudanabaena limnetica (Lemmerman) Komárek	+	+	+				+	+	+	+	+	+	+	+		+						H
Schizothrix fasciculate Gomont ex Gomont			+			+																R
Spirulina laxa G.M Smith			+			+	+	+			+		+									L
Synechocystis minuscula Woronichin						+																R
No. of Cyanophyta	13	11	7	2	2	8	10	7	5	7	3	2	6	3	4	2		7	5	3	2	
Chlorophyta																						
Actinastrum hantzschii Lagerheim																			+			R
Ankistrodesmus acicularis (Braun) Korshikov					+			+	+									+			+	R
A. angustus C.Bernard				+						+												R
A. falcatus (Corda) Ralfs.		+				+		+										+	+	+		L
Acutodesmus acutiformis (Schröder) Tsarenko & D.M.John	+		+			+			+	+								+				L
Bulbochaete sp.													+									R
Chlamydomonas reinhardtii P.A.Dangeard		+	+			+			+		+											R
Chlorella vulgaris Beyerinck (Beijerinck)		+	+				+		+		+							+	+			L
Chlorococcum echinozygotum Starr							+	+					+	+			+	+	+	+		L
Coelastrum microporum Nägeli		+													+					+		R
Cosmarium angulosum Brébisson								+					+									R
C. granatum (var). Nordstedtii Hansgirg.	+	+					+	+	+		+		+	+	+				+			L
C. subprotumidum Nordstedt	+	+				+	+															R
C. subtumidum Nordstedt		+																		+		R
Desmodesmus serratus (Corda) S.S.An, Friedl & E.Hegewald	+	+	+			+	+	+		+	+		+	+								L
D. flavescens (Chodat) E.Hegewald								+														R
Dictyosphaerium ehrenbergianum Nägeli		+																				R
Kirchneriella contorta (Schmidle) Bohlin							+															R
Microspora amoena (Kütz.) Rabenh													+									R

continued Table 2: Occurrence remark of epiphytic algal species recorded on the investigated macrophytes present in polluted and unpolluted freshwater sites at Assiut- Egypt

Sites and Seasons	Autumn						Winter						Spring					Summer				O.R
	Polluted		Unpolluted				Polluted		Unpolluted				Polluted		Unpolluted			Polluted		Unpolluted		
	S1		S2				S1		S2				S1		S2			S1		S2		
Plants / Algal taxa	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Potamogeton nodosus	Potamogeton perfoliatus	Myriophyllum spicatum	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Persicaria salicifolia	Myriophyllum spicatum	Potamogeton perfoliatus	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Myriophyllum spicatum	Potamogeton perfoliatus	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Myriophyllum spicatum	
M. pachyderma (Wille) Lagerheim.								+														R
M. sp.							+						+									R
*Monactinus simplex (*Meyen) Corda	+	+		+	+	+	+						+					+				L
Monoraphidium arcuatum (Korshikov) Hindák							+															R
M. contortum (Thuret) Komárková--Legnerová						+	+	+		+												R
M. circinale (Nygaard) Nygaard	+	+									+	+						+				R
M. griffithi (Berkeley) Komárková--Legnerová	+		+																			R
Mougeotia sp.1	+	+		+		+	+		+						+			+				L
M. sp.2																			+			R
Oedogonium calliandrum L.R.Hoffman													+									R
O. curvum Pringsheim ex Hirn							+						+									R
O. sp.1		+		+																	+	R
O. sp.2	+												+	+			+	+	+			L
O. sp.3																			+			R
Oocystis lacustris Chodat				+													+					R
Pediastrum boryanum (Turpin) Meneghini						+																R
P. duplex Meyen		+					+	+											+			R
S. acuminatus (Lagerheim) Chodat										+	+											R
S. acutus Meyen	+	+		+	+	+	+		+		+							+	+	+	+	**H**
S. armatus (Chodat) Chodat								+					+									R
S. bijuga (Turpin) Lagerheim.		+						+							+							R
S. ellipticus Corda				+			+															R
S. incrassatulus Bohlin	+	+	+															+	+			R
S. javanesis Chodat																			+	+		R
S. microspina Chodat								+			+											R
S. naegelii Brébisson													+									R
S. opoliensis P.G.Richter		+	+			+									+		+					R
S. quadricauda Turpin		+		+		+	+	+		+	+				+	+		+	+			H
Spirogyra communis (Hassall) Kuetzing														+								R
Stauridium tetras (Ehrenberg) E.Hegewald	+	+					+	+											+		+	L

continued Table 2: Occurrence remark of epiphytic algal species recorded on the investigated macrophytes present in polluted and unpolluted freshwater sites at Assiut- Egypt

Sites and Seasons	Autumn						Winter						Spring					Summer				O.R
	Polluted		Unpolluted				Polluted		Unpolluted				Polluted		Unpolluted			Polluted		Unpolluted		
	S1		S2				S1		S2				S1		S2			S1		S2		
Algal taxa / Plants	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Potamogeton nodosus	Potamogeton perfoliatus	Myriophyllum spicatum	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Persicaria salicifolia	Myriophyllum spicatum	Potamogeton perfoliatus	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Myriophyllum spicatum	Potamogeton perfoliatus	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Myriophyllum spicatum	
S. sp.													+	+								R
Staurastrum anatinum Cooke & Wills	+	+		+		+													+			R
S. johnsoni West & G.S.West						+																R
S. planctonicum Teiling		+																				R
Stigoclonium helveticum Vischer											+											R
S. tenue (C.Agardh) Kützing.																		+		+		R
S. farctum Berthold																+		+				R
Tetraedron minimum (A.Braun) Hansgirg		+					+	+											+			R
Ulothrix sp.													+									R
Westella botryoides (West) De Wildeman.							+															R
No. of Chlorophyta	13	23	7	9	3	14	19	16	7	6	10	1	15	6	6	2	4	14	16	7	4	
Bacillariophyta																						
Achnanthidium minutissimum (Kützing) Czarnecki							+	+			+		+	+		+			+		+	L
A. intermedia Kützin								+		+		+			+							R
A. sp.1																			+		+	R
A. sp.2							+	+			+	+	+	+		+	+					L
Amphora inariensis Krammer	+			+			+															R
A. ovalis Kützing								+	+		+		+	+	+	+		+	+		+	L
Aulacoseira granulata (Ehrenberg) Simonsen			+	+			+				+	+			+	+		+	+		+	L
A. italica (Ehrenberg) Simonsen					+				+													R
A. sp.		+	+									+			+		+		+			L
Bacillaria paradoxa J.F.Gmelin, nom. illeg.															+							R
Cocconeis placentula Ehrenberg								+		+	+	+		+	+	+		+	+		+	L
Cyclotella meneghiniana Kützing		+	+	+			+	+	+	+	+	+	+		+	+	+	+			+	H
C. caspia Grunow															+							R
C. quillensis L.W. Bailey			+	+					+	+	+											R
C. striata Kützing				+	+							+										R
C. sp.																	+					R
Cymbella affinis Kutzing	+	+			+		+	+	+		+	+	+	+	+	+	+		+		+	H
C. naviculiformis Auerswald ex Heiberg		+	+						+		+											R

continued Table 2: Occurrence remark of epiphytic algal species recorded on the investigated macrophytes present in polluted and unpolluted freshwater sites at Asiut- Egypt

Sites and Seasons	Autumn						Winter						Spring					Summer				O.R
	Pollu-ted		Unpolluted				Pollu-ted		Unpolluted				Pollu-ted		Unpollu-ted			Pollu-ted		Unpol-luted		
	S1		S2				S1		S2				S1		S2			S1		S2		
Plants / Algal taxa	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Potamogeton nodosus	Potamogeton perfoliatus	Myriophyllum spicatum	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Persicaria salicifolia	Myriophyllum spicatum	Potamogeton perfoliatus	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Myriophyllum spicatum	Potamogeton perfoliatus	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Myriophyllum spicatum	
C. tumida Brébisson											+											R
Encyonema sp.														+								R
Epithemia sorex Kutzing							+	+			+	+	+	+	+		+	+	+		+	H
Fragilaria capucina Desmazières	+	+	+	+	+			+	+	+	+	+		+	+	+	+		+		+	H
F. sp.1															+	+		+	+		+	R
F. sp.2	+														+	+						R
F. sp.3	+								+	+			+			+	+	+	+		+	L
Gomphonema angustum C.Agardh	+	+	+				+	+											+			L
G. clevei Fricke					+		+			+												R
G. intracatum Kützing							+				+		+	+				+				R
G. olivaceum (Hornemann) Brébisson							+								+	+	+	+			+	L
G. minutum (C.Agardh) C.Agardh															+			+				R
G. truncatum Ehrenberg																					+	R
G. sp.1				+												+					+	R
G. sp.2				+										+								R
Hantzschia amphioxys (Ehrenberg) Grunow							+				+			+			+					R
H. sp.										+	+											R
Meridion circulare (Greville) C.Agardh	+	+		+			+	+										+	+		+	L
Navicula absoluta Hustedt												+										R
N. clementis Grunow													+									R
N. cryptocephala Kützing					+		+					+									+	R
N. cryptotenella Lange-Bertalot	+		+				+		+		+				+							L
N. distans (W.Smith) Ralfs		+																				R
N. gregaria Donkin	+	+					+	+					+					+				L
N. radiosa Kützing				+											+	+					+	R
N. salinarum Grunow								+											+			R
N. secreta Pantocsek								+	+	+		+			+				+			L
N. transitans Cleve											+											R
N. trivialis Lange-Bertalot	+	+	+	+			+	+	+	+	+		+			+	+	+				H
N. sp.	+									+	+	+				+		+				L
Nitzschia clausii Hantzsch	+	+											+	+	+			+	+			L
N. dissipata (Kützing) Rabenhorst		+								+												R

continued Table 2: Occurrence remark of epiphytic algal species recorded on the investigated macrophytes present in polluted and unpolluted freshwater sites at Asiut- Egypt

Sites and Seasons	Autumn						Winter						Spring					Summer				O.R
	Pollu-ted		Unpolluted				Pollu-ted		Unpolluted				Pollu-ted		Unpollu-ted			Pollu-ted		Unpol-luted		
	S1		S2				S1		S2				S1		S2			S1		S2		
Plants / Algal taxa	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Potamogeton nodosus	Potamogeton perfoliatus	Myriophyllum spicatum	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Persicaria salicifolia	Myriophyllum spicatum	Potamogeton perfoliatus	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Myriophyllum spicatum	Potamogeton perfoliatus	Phragmites australis	Myriophyllum spicatum	Echinochloa stagnina	Myriophyllum spicatum	O.R
N. homburgiensis Lange-Bertalot											+											R
N. digitoradiata (W.Gregory) Ralfs	+																					R
N. linearis W.Smith															+		+	+			+	R
N. paleacea (Grunow) Grunow																	+					R
N. palea (Kützing) W.Smith	+	+	+	+	+		+	+	+	+								+				L
N. radicula Hustedt								+							+		+					R
N. sigmoidea (Nitzsch) W.Smith							+															R
N. vermicularis (Kützing) Hantzsch			+						+				+									R
N. umbonata (Ehrenberg) Lange--Bertalot									+		+							+				R
Pinnularia divergens W.Smith														+								R
P. streptoraphe Cleve	+	+									+											R
P. sp.	+	+												+								R
Rhoicosphenia curvata (Kützing) Grunow																		+				R
Rhopalodia gibba (Ehrenberg) Otto Müller	+			+			+	+					+	+					+			L
Staurosirella leptostauron (Ehrenberg) D.M.Williams & Round							+		+			+						+				R
Stephanodiscus sp.			+		+																	R
Surirella sp.				+																		R
Synedra acus Kützing			+													+	+				+	R
S. sp.		+	+	+	+				+	+	+			+	+	+			+		+	H
Tryblionella angustata W.Smith					+										+	+			+		+	R
Tryblionella apiculata W.Gregory	+	+																				R
Ulnaria delicatissima (W.Smith) Aboal & P.C.Silva											+											R
U. ulna (Nitzsch) Compère	+	+	+	+	+		+		+	+	+	+	+		+	+	+	+	+		+	H
No. of Bacillariophyta	19	18	15	16	11		22	18	17	15	25	16	15	16	24	20	16	21	20		22	
Euglenophyta																						
Euglena sp.							+															R
Phacus acuminatus Stokes	+																					R
No. of Euglenophyta	1						1															
Total no. of species	46	52	29	27	16	22	52	41	29	28	38	19	36	25	34	24	20	42	41	10	28	

S1: Nazlet Abdellah, S2: El-Wasta, O.R = Occurrence remake, R=1-24%, L=25-49 %, H= 50-100%

Table 3. Results of three-way PERMANOVA tests (with the site [Si] as a fixed factor and Plant [Pl] and season (se) as random factor)

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
Si	1	3224.6	3224.6	2.2308	0.078	577
Pl	5	12686	2537.2	1.4829	0.044	994
Se	3	11536	3845.2	1.9729	0.008	999
SixPl	4	7642.5	1910.6	1.1925	0.194	997
SixSe	3	4336.5	445.5	1.2365	0.184	997
PlxSe	8	14618	1741.9	1.0872	0.298	999
Total	24	53204				

df, degrees of freedom; SS, sum of squares; MS, mean squares; Res, residuals

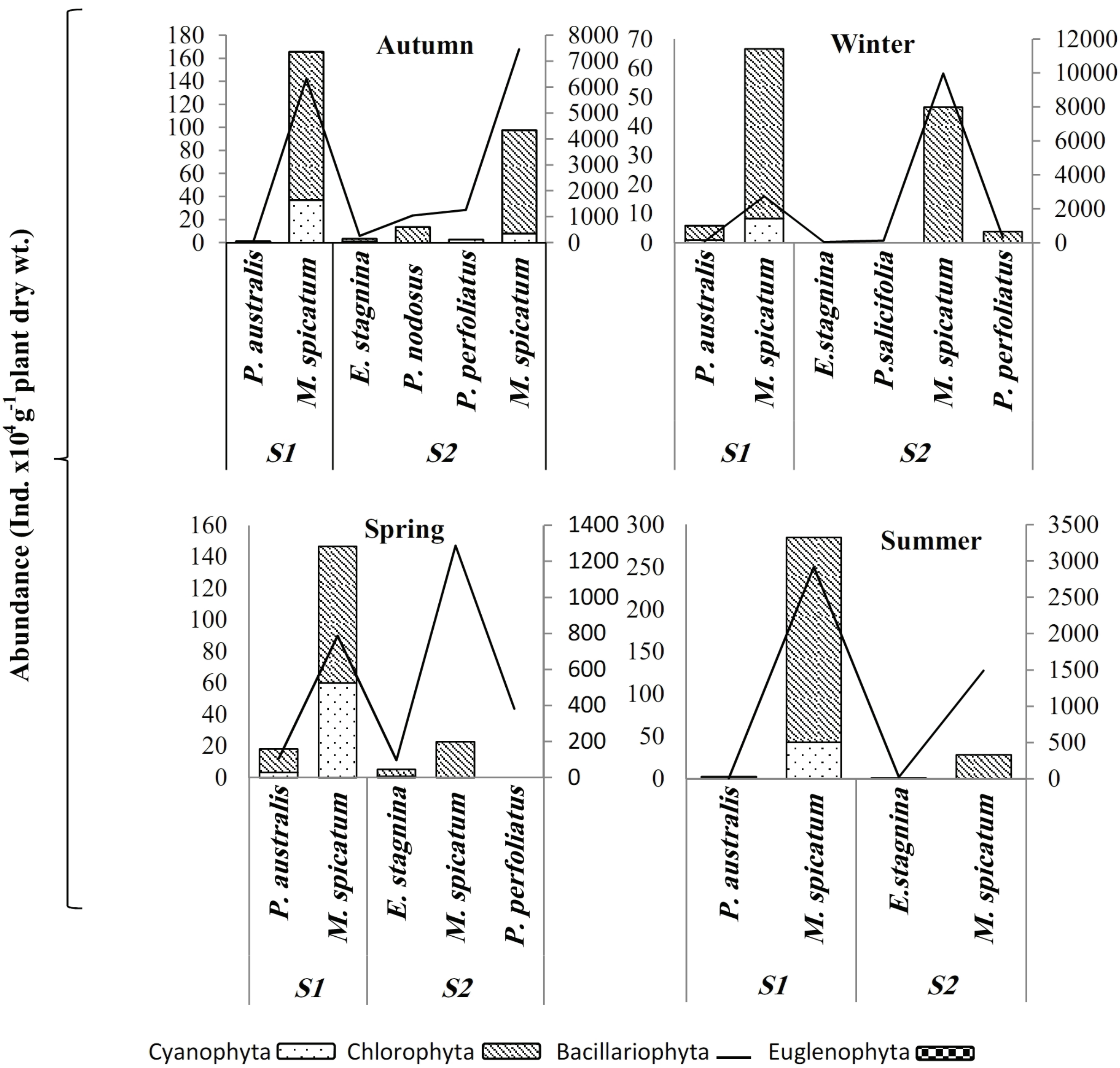

Figure 2. Abundance of epiphytic algal groups recorded on the investigated macrophytes

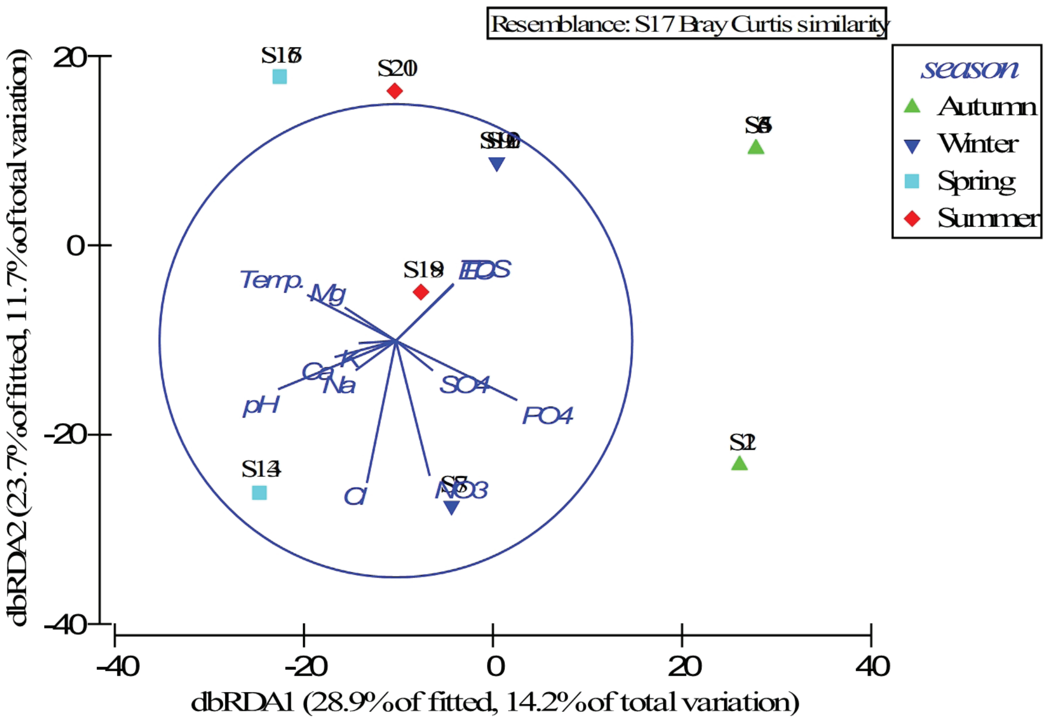

Figure 3. Distance-based redundancy analysis (dbRDA). Relationships between the ordination of the sites and host plant based on epiphytic microalgal species composition and environmental factors.

with culture, promoted the epiphytic algal on *M. spicatum* (Song et al. 2017), whereas, the biomass of epiphytic algae on individual macrophyte may depend on the host plant (Toporowska et al. 2008).

Bacillariophyceae was the most dominant group in all the aquatic macrophytes followed by Chlorophyceae, Cyanophyceae and Euglenophyceae. These results agreed with other studies as Salman et al. (2013) and Fawzy (2016). Diversity index values of epiphytic diatoms were mostly higher than green and blue-green algae (Konsowa 2007). The highest algal species diversity was observed in winter and autumn. This is may be attributed to the highest values of some nutrients such as nitrate, phosphate and sulphate that were recorded in the winter and autumn. Biolo et al. (2015) results suggested that the composition and taxonomic similarity was probably temporally related to the influence of environmental variables (abiotic variables, flood pulses and hydrometric levels). In the eutrophic Lake Verevi (Estonia), the epiphyton algae biomass maximum was estimated in autumn (Laugaste & Reunanen 2005). The total of algal species was varied according to the host plant. The highest number of algal species was found on the stem of Myriophyllum spicatum and Phragmites australis; while, the lowest on the stem of Echinochloa stagnina in summer at site 2. The variation in the total number of epiphytic microalgal species may be due to several factors such as plant growth period, chemical and physical factors (Dere et al. 2002).

The highest algal counts were recorded on the stem of aquatic macrophytes *Myriophyllum spicatum* in winter at unpolluted site 2, while the lowest total algal cell counts were recorded on the stem of *Phragmites australis* at polluted site 1 in summer. This is may be due to the water quality, type of host plant, and variation of nutrients (Kupferberg 2003). This supported previous results of PERMANOVA that presented a significant difference in epiphytic algae among the host plants.

In this study, the most dominant algal species were *Pseudanabaena limnetica* and *Scenedesmus acutus*, *Ulnaria ulna* and *Cyclotella meneghiniana*, while other algal species were *Aphanocapsa thermalis* and *Ulothrix* sp., attached on the stem of *Phragmites australis* also, *Actinastrum hantzschii* and *Encyonema* sp., that grow on *Myriophyllum spicatum* were the rare recorded species. Most epiphytic algae are essentially facultative and are not exactly associated with a host species (Wahl & Mark 1999), some are known as specific and obligate epibionts on certain hosts (Pearson and Evans 1990). Cyanophyta such as *Pseudanabaena limnetica* have a wide range of tolerance to physical disturbance including the fluctuation of water level and large amounts of suspended solids (Albay & Akçaalan 2003).

The other factors that may affect the diversity and abundance of epiphytic algae are the environmental conditions such as nutrients, salinity, light availability and hydrodynamic regime (Frankovich et al. 2006), biological processes such as grazing (Hillebrand et al. 2000), and physiological responses (Ruesink 1998), which may elucidate the succession of other groups on each host plant during the study period. The analysis of dbRDA highlighted the importance of water temperature, pH, phosphate, nitrate, chloride and total dissolved salts that were more evident in changing the structure of microalgae during the different seasons. The maximum of epiphytic algae diversity in this study was estimated in winter and autumn, while the lowest in spring and summer; this may be according to temperature and photoperiods as well as other factors.

The density and distribution of epiphytic microalgae in Nile River were dependent on the variation of pH, nutrient, transparency of water and temperature (Aboellil & Aboellil 2012). Nitrate nitrogen showed the maximum content in the winter at site 1. In fact, the enrichment of P and N_2 in eutrophic water is usually accompanied with an increase of planktonic algae and epiphytic microalgae (Tóth 2013). These variations in water characteristics, therefore, lead to qualitative and quantitative changes in the epiphytic microalgal organisms (Janne & Jani 2005). The chemical disturbance of nutrients may contribute more effects with other factors that can cause a decrease in the assemblage similarity over time. This environmental disturbance induced variation in the abundance and diversity of microalgae, also chemical constituents (Abou-Aisha et al. 1997). Sundbäck and Snoeijs (1991) found that the addition of nutrients led to certain changes in the dominance of diatom species, but the changes were clearer at the macroscopic level than in the microflora. Actually, there is no selective base for epiphytes and there is a great controversy about the cause of choice of one species to a specific host. Many authors reported different results, for example, Patrick et al. (1968) found differences in the composition of species as a function of substrate that represent the presence of selectivity between the host and the species of epiphytic microalgae, but Sullivan (1984) found a resemblance in the communities of epiphytic diatoms, representing non-selectivity between the substrates and epiphytic diatoms.

4. CONCLUSION

The seasonal investigation of epiphytic algae on aquatic macrophytes showed the variations in nutrient content and the pollution in the water affect the distribution, abundance and diversity of the epiphytic microalgal communities that, in turn, would reflect the physico-chemical analysis of water.

References

Abe, S., Uchida, K., Nagumo, T. & Tanaka, J. (2007) Alterations in the biomass-specific productivity of periphyton assemblages mediated by fish grazing. Freshwater Biology, 52, 1486–1493.

Aboellil, A. & Aboellil, A.H. (2012) Colonization Abilities of Microflora to Attach Aquatic Plants. Global Journal of Science Frontier Research, Biological Sciences, 12, 21–27.

Abou-Aisha, K.M., Kobbia, I.A., El Abyad, M.S., Shabana, E.F. & Schanz, F. (1997) Seasonal changes in Cystoseira myrica and phosphorus input at two sites of the Red Sea Egyptian Coast. Water, Air and Soil Pollution, 93, 199–211.

Albay, M. & Akcaalan, R. (2003) Comparative study of periphyton colonisation on common reed (Phragmites australis) and artificial substrate in a shallow lake, Manyas, Turkey. Hydrobiologia, 506(1), 531–540.

Algarte, V.M., Siqueira, N.S., Murakami, E.A. & Rodrigues, L. (2009) Effects of hydrological regime and connectivity on the interannual variation in taxonomic similarity of periphytic algae. Brazilian Journal of Biology, 69(2), 609–616.

Al-Saboonchi, A.A. & Al-Manshad, H.N. (2012) Study of Epiphytic Algae on *Ceratophyllum demersum* L. from Two Stations at Shatt Al-Arab River. Journal of Thi-Qar Science, 3, 57–63.

Anderson, M.J., Gorley R.N. & Clarke. K.R. (2008) PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth.

Biolo, S., Algarte, V.M. & Rodrigues, L. (2015) Composition and taxonomic similarity of the periphytic algal community in different natural substrates in a neotropical floodplain, Brazil. African Journal of Plant Science, 9(1), 17–24.

Borowitzka, M.A., Lethbridge, R.C. & Charlton, L. (1990) Species richness, spatial distribution and colonisation pattern of algal and invertebrate epiphytes on the seagrass *Amphibolis griffithii*. Marine Ecology Progress Series, 281–291.

Bulgariu, L. & Bulgariu, D. (2012) Direct determination of nitrate in small volumes of natural surface waters using a simple spectrophotometric method. Reviews in Analytical Chemistry, 31(3), 201.

Davies, O.A. (2009) Epiphytic diatoms growing on Nypa Fructican of Okpoka Creek, Niger Delta, Nigeria and their relationship to water quality. Research Journal of Applied Sciences, Engineering and Technology, 1, 1–9.

Demir, A.N., Fakioğlu, Ö. & Dural, B. (2014) Phytoplankton functional groups provide a quality assessment method by the Q assemblage index in Lake Mogan (Turkey). Turkish Journal of Botany, 38, 169–179.

Dere, Ş., D. Karacaoğlu, & Dalkiran, N. (2002) A study on the epiphytic algae of the Nilüfer Stream (Bursa). Turkish Journal of Botany, 26, 219–234.

Dewis, J. & Freitas, F. (1970) In physical and chemical methods of soil and water analysis. Soil Bulletin, No. 10. Food and Agriculture Organization of the United Nations, Rome.

El-Karim, M.S.A., Fishar, M. & El-Gawad, S.S.A. (2009) Epiphytic Algae and Macroinverteb rates Communities of *Myriophyllum spicatum Lemm* and Their Cascade in the Littoral Food Web of Lake Nasser, Egypt. Global Veterinaria, 3(3), 165–177.

Fawzy, M.A. (2016) Spatial distribution of epiphytic algae growing on the aquatic macrophytes *Phragmites australis* and *Echinochloa stagnina* at Assuit-Egypt. Minia Science Bulletin, 27(2), 1–26.

Frankovich, T.A., Gaiser, E.E., Zieman, J.C. & Wachnicka, A.H. (2006) Spatial and temporal distributions of epiphytic diatoms growing on *Thalassia testudinum* Banks ex König: Relationships to water quality. Hydrobiologia, 569, 259–271.

Gaiser, E.E., McCormick, P.V., Hagerthey, S.E. & Gottlieb A.D. (2011) Landscape patterns of periphyton in the Florida Everglades. Critical Reviews in Environmental Science and Technology, 41, 92–120.

Ganf, G. (1974) Diurnal mixing and the vertical distribution of phytoplankton in a shallow equatorial lake (Lake George, Uganda). The Journal of Ecology, 611–629.

Hassan, F.M., Salman, J.M., Alkam, F.A. & Jawad, H.J. (2014) Ecological Observations on Epipelic Algae in Euphrates River at Hindiya and Manathira, Iraq. International Journal of Advanced Research, 2(4), 1183–1194.

Hillebrand, H., Worm, B. & Lotze, H.K. (2000) Marine microbenthic community structure regulated by nitrogen loading and grazing pressure. Marine Ecology Progress Series, 204, 27–38

Jackson, M.L. (1958) Soil chemical Analysis constable. Ltd. Co., London 498.

Jackson, M.L. (1960) Soil chemical analysis. Constable and Co., London, pp, 261–262.

Janne, S. & Jani, H. (2005) Relationships between local population persistence, local and regional occupancy of species: distribution patterns of diatoms in boreal streams. Journal of Biogeography, 32, 1971–1978.

Konsowa, A. (2007) Spatial and temporal variation of phytoplankton abundance and composition in the hypersaline Bardawil Lagoon, North Sinai, Egypt. Journal of Applied Sciences Research, 3, 1240–1250.

Kormas, K.A., Nicolaidou, A. & Reizopoulou, S. (2006) Temporal variation of nutrients, chlorophyll a and particulate matter in three coastal lagoons of Amvrakikos gulf Ionian Sea, Greece. Marine Ecology, 22, 201–213.

Kramer, K. & Lange-Bertalot, H. (1991) Süßwasserflora von Mitteleuropa, Band 2/4, Teil 4: Achanataceae, Kritische Ergänzungen zu Navicula (Lineolatae) und Gomphonema Gesmatliteraturzeichnis Teil 1–4. Gustav Fischer Verlag, Stuttgart Jena, 437.

Kupferberg, S. (2003) Facilitation of periphyton production by tadpole grazing: functional differences between species. Freshwater Biology, 37, 427–439.

Laugaste, R. & Reunanen, M. (2005) The composition and density of epiphyton on some macrophyte species in the partly meromitic lake Verevi. Hydrobiologia, 547, 137–150.

Liboriussen, L. & Jeppesen, E. (2003) Temporal dynamics in epipelic, pelagic and epiphytic algal production in a clear and a turbid shallow lake. Freshwater Biology, 48, 418–431

Lund, J.W.G. & Canter-Lund, H. (1995) Freshwater algae: their microscopic world explored. Biopress ltd. The Ochard Clanage Road, Bristol BS3 2JX England, pp. 306.

McArdle, B.H. & Anderson, M.J. (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology, 82, 290–297.

Messyasz B. & Kuczyńska-Kippen N. (2006) Periphytic algal communities: a comparison of *Typha angustifolia* L. and *Chara tomentosa* L. beds in three shallow lakes (West Poland), Polish Journal of Ecology, 54(1), 15–27.

Metzner, H., Rau, H. & Senger, H. (1965) Untersuchungen zur synchronisier-barkeit ein zelner pigment managel. Mutanten von *Chlorella*. Planta, 65, 186-194.

Mormul, R.P., Thomaz, S.M., Da Silveira, M.J. & Rodrigues, L. (2010). Epiphyton or macrophyte: Which primary producer attracts the snail Hebetancylus moricandi? American Malacological Bulletin, 28(1-2), 127–133.

Patrick, R., Cairns, J. & Scheier, A. (1968) The relative sensitivity of diatoms, snails and fish to twenty common constituents of industrial wastes. Progressive Fish-Culturist, 30, 137–140.

Pearson, G.A. & Evans, L.V. (1990) Settlement and survival of Polysiphonia lanosa (Ceramiales) spores on *Ascophyllum nodosum* and *Fucus vesiculosus* (Fucales). Journal of Phycology, 26, 597–603.

Ruesink, J.L. (1998) Diatom epiphytes on *Odonthalia floccosa*: The importance of extent and timing Journal of Phycology, 34, 29–38.

Salman, J.M., Hadi, S.H., & Mutaer, A.A. (2013) Spatial and temporal distribution of phytoplankton and some related physical and chemical properties in al–abasia river (Euphrates), Iraq. International Journal of Geology, Earth & Environmental Sciences, 3(3), 155–169.

Schwarzenbeck, G. & Biederman, W. (1948) Komplexone.X. Erdalkalikomplexe von 0, 6-di-oxyazofarbstoffen. Helvetica Chimica Acta, 31, 678–687.

Sheen, R.T., Kahler, H.L. & Ross, E.M. (1935) Turbidmetric determination of sulfate in water. Industrial & Engineering Chemistry Analytical Edition, 7, 262–265.

Song, Y.Z., Wang, J.Q. & Gao, Y.X. (2017) Effects of epiphytic algae on biomass and physiology of *Myriophyllum spicatum* L. with the increase of nitrogen and phosphorus availability in the water body. Environmental Science and Pollution Research, 24, 9548–9555.

Sullivan, M.J. (1984) Community Structure of Epiphytic Diatoms from the Gulf Coast of Florida, USA. In: Proceedings of the Seventh International Diatom Symposium, Mann, D.G. (Ed.), Otto Koeltz-Science Publishers, Philadelphia, pp. 373–384.

Sundbäck, K. & Snoeijs, P. (1991) Effects of nutrient enrichment on microalgal community composition in a coastal shallow water sediment system: an experimental study. Botanica Marina, 34, 341–358.

Thomaz, S.M., Dibble, E.D., Evangelista, L.R., Higuti, J. & Bini, L.M. (2008). Influence of aquatic macrophyte habitat complexity on invertebrate abundance and richness in tropical lagoons. Freshwater biology, 53(2), 358–367.

Toporowska, M., Pawlik-Skowrońska, B. & Wojtal, A. (2008) Epiphytic algae on *Stratiotes aloides* L., *Potamogeton lucens* L., *Ceratophyllum demersum* L. and *Chara* spp. in a macrophyte-dominated lake. Oceanological and Hydrobiological Studies, 37, 51–63.

Tóth, V.R. (2013) The effect of periphyton on the light environment and production of *Potamogeton perfoliatus*, L. in the mesotrophic basin of Lake Balaton. Aquatic sciences, 75, 523–534.

Verhulst, S. (2013) Response of the Epiphytic Algal Communities to Experimentally Elevated Nutrient Levels in Intertidal Salt Marsh Habitats. UNF Thesis and Dissertations, 435.

Wahl, M. & Mark, O. (1999) The predominantly facultative nature of epibiosis: experimental and observational evidence. Marine Ecology Progress Series, 187, 59–66.

Wetzel, R.G. (1993) Microcommunities and microgradients: linking nutrient regeneration, microbial mutualism, and high sustained aquatic primary production. Aquatic Ecology, 27, 3–9.

Williams, C.H. & Twine, J.R. (1960) Flame photometric method for sodium, potassium and calcium. In: K. Peach & Tracey (Eds.). Modern Methods of Plant Analysis. Springer Verlag, Berlin, Goettingen, Heidelberg, 5, 3–5.