International Journal of Applied Environmental Sciences
ISSN 0973-6077 Volume 11, Number 1 (2016), pp. 211-228

http://www.ripublication.com

Phytoplankton structure, biochemical, stoichiometry and elemental composition in Lake Nasser, Egypt.

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Abstract

The relationship between environmental variables and elemental, biochemical composition of phytoplankton species in Lake Nasser were studied. In the lake 130 phytoplankton species were recorded, the lake is dominated mainly by *Cyclotella glomerata* (Bacillariophyceae), *Planktolyngbya limnetica*, *Eucapsis minuta* (Cyanophyceae). Phytoplankton organic carbon content at the lake was 32%. The elevation of NO_3 (365 µg/l) and CO_3 (21 mg/l) at the east of the lake was concurrently with the highest proportion of inorganic C, N and algal protein content. C/N ratio decreased thus Redfield ratio indicating a high growth rate of phytoplankton with increasing protein content. C/H and O/C ratio for natural phytoplankton samples were less than one, which means unsaturated aliphatic compounds at metabolites categories of phytoplankton. RDA analysis cleared that, *Cyclotella glomerata* (dominant at the north area) was sensitive to flushing, tolerant to nutrient deficiency and had higher protein content. *Planktolyngbya limnetica* (dominant at the middle) was more sensitive to pH, phosphorus is non-limiting factor and characterized by elevation in lipid content. *Eucapsis minuta* (dominant at the south) was tolerant to mild light deficiency and contains a maximum value of carbohydrates and Chlorophyll *a*.

Keywords: Algae, FGs codons, Stoichiometry,

Introduction

Lake Nasser represents the main national freshwater reservoir of Egypt and the main strategic importance for sustaining Egypt's water policy [1]. Phytoplankton has been used as indicators of environmental [67] and climatic changes [44]. It constitutes the basis of food chain in aquatic environment [49]. Phytoplankton growth is regulated by essential resources such as light and nutrients [23]. Phytoplankton communities in Lake Nasser are rich with density and biomass [41] and increases to southwards [10]. The taxonomic group of bacillariophyceae was the most important group and key players in the global carbon cycle in Lake

Nasser [70], followed by cyanophyceae [29]. Phytoplankton classes varied significantly in their content of protein, carbohydrate and lipid. Chlorophyceae and Prasinophyceae were normally richer in carbohydrate than species from other classes [13]. Carbon is the principal structural component of phytoplankton (24±80% of dry weight) and used as standard element in quantifying plant biomass production and bioenergetics [6]. The other important macroelements in algae are nitrogen and phosphorous, which are the main components for the growth rate process [66]. There are few studies concerning the qualitative and quantitative information of the elements in algae and what their role in the different species [32]. The elemental composition of microalgae is a fusion of the evolutionary history of plastid, resulting in modifications in genetic constraints and selection pressures associated with environmental variables [51]. Ecological stoichiometry shows that not only the carbon content, but also the carbon-to-nutrient ratio is important in producing high plant biomass [61]. Algal food quality and its biochemical content may also vary with algal stoichiometry [24].

The objective of this study was to determine how the elemental composition of phytoplankton and deviations from the Redfield ratio at Lake Nasser changed due to phytoplankton structure**.** Investigate the effect of physico-chemical variables on dominant phytoplankton species and its biochemical content in the littoral area of the Lake.

Material and Methods

Lake Nasser is a vast reservoir in southern Egypt and one of the largest man-made lakes in the world [1]. The Lake extends about 350 km are within the Egyptian territory between 22^o 00\ to 23^o 58\ N and 31^{o}19 \ to 33^{o}15\ E. The total surface area of the Lake is 5237 km^2 at 180 m over mean Sea level [4] and [35]. The length of eastern shoreline is almost double that of the western shoreline [40] and [42] (Fig.1.)

Seasonally, water samples were collected using 1.5 L Ruttner sampler from 9 sites at both east (E) and west (W) area of Lake Nasser during 2013 (Table 1,Fig.1). Chemical analysis and phytoplankton differentiation were determined at each sample subsurface (S) and near bottom (B). Biochemical contents of phytoplankton and stoichiometry were subsurface.

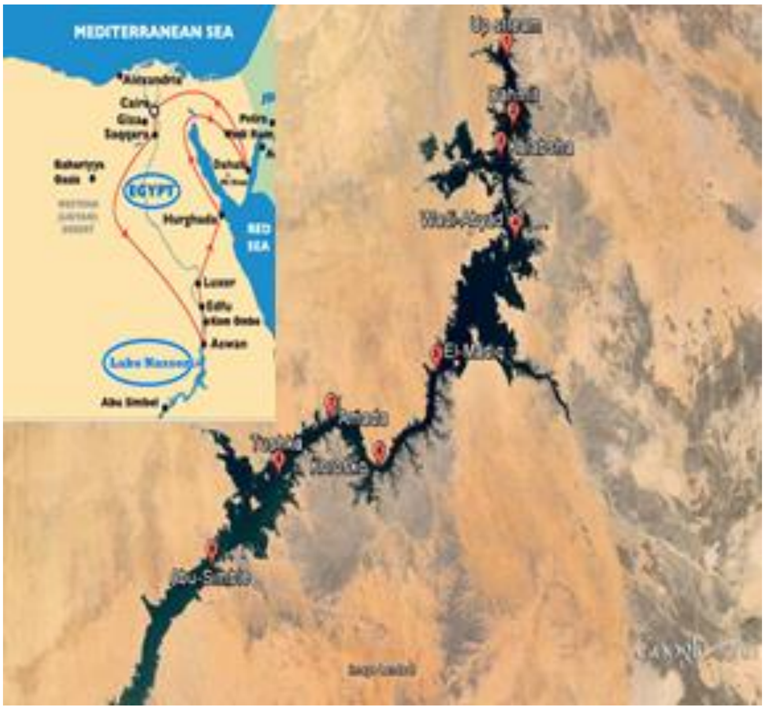

Figure 1: The location of the selected littoral sites in Lake Nasser at the north (N), middle (M) and south (S) part of the lake.

Table 1: The sampling locations in Lake Nasser.

Code	Sites	location	Latitude		Longitude		Distance from High Dam (Km)
1	High Dam	North	23°	56.24'	32°	51.89'	2.9
2	Dahmit		23°	51.1'	32°	53.78'	21
3	Kalabsha		23°	33.41'	32°	52.04'	47.7
4	Wadi-Abyad	Middle	23°	20.08'	32°	56.12'	74.3
5	El-Madiq		22°	56.47'	32°	37.68'	130.1
6	Korosko		22°	37.65'	32°	24.14'	167.2
7	Amada	South	22°	43.97'	32°	5.45'	199
8	Tushka		22°	36.32'	31°	55.21'	240
9	Abu-Simble		22°	19.71'	31°	37.14'	268.8

Electrical conductivity (mS cm^{-1}) was measured by using a conductivity meter (S.C.T.33 YSI), Transparency (cm) by Secchi disc and water temperature by an ordinary thermometer. pH by using Orion Research Ion Analyzer 399A pH meter.

CO_3^{2-} and HCO_3^- were measured titrimetrically against standard H_2SO_4 (0.02 N) using phenolphthalein and methyl orange indicators. Dissolved oxygen (DO) was determined by azide modification method. Concentrations of nitrite (NO_2), nitrate (NO_3), ammonium (NH_4) and orthophosphate (PO_4) in water samples were determined using the colorimetric techniques [8].
For phytoplankton analysis water samples collected from the Ruttner samler (500 mL) was preserved in 4% neutral formalin [43]. Uttermohl method was applied for counting (until 400 counted individual) and identification of phytoplankton species [9] using inverted microscope (ZEISS 1M4738). The main references used in identification were: [33, 22, 12, 60, 50, 62 and 19].

For biochemical analysis water sample was sieved and filtered through zooplankton net (100 µm mesh size) to separate macrozooplankton. Defined volume of water sample was refiltered on glass microfiber filter GF/F. Biuret method [20] was used for protein analysis which involves the use of a Biuret reagent, subsequent absorbance measurements at 550 nm and comparison to a bovine standard absorbance at the same wavelength. Total carbohydrate content was determined by the sulphuric acid hydrolysis method, glucose as a standard [25]. Lipid extraction was performed using Sulphophosphovanillin procedure [16]. Chlorophyll *a* (Chl. *a*) was extracted in acetone (90%) overnight, prepared and measured spectrophotometry and calculated applying trichromatic equation [8]. Trophic state index was calculated according to [14]. The elemental composition was based on the calulations of [28].

Total caloric content was calculated on the proximate biochemical composition according to [68], by the relative caloric coefficients (5.56 for proteins, 4.1 for carbohydrates and 9.45 for lipids).

Statistical analysis: Redundancy analysis (RDA) was used as method to extract and summaries the variation in between biotic factors as dominant species, biochemical content and elemental composition of phytoplankton and abiotic physico-chemical parameters in the different sites. RDA is a direct gradient analysis technique which summarises linear relationships between components of response variables. Data were analyzed using CAnoco 5.0 [64] and Statistica 8.0.

Results and Discussion

The aquatic environment as physical and chemical characteristics of Lake Nasser has been depending on flood events. During the last few decades, there has been much interest in the routes influencing the development of phytoplankton populations, primarily in relation to physico-chemical factors [45]. The chemistry of water describes the general hydrobiological relationship [59]. Water in the east side of the Lake was more transparent than the west one and transparency was gradually decreased southward in both sides (Table 2). The southern sites were generally affected by the turbidity or abundant suspended organic materials than the northern sites. Water temperature fluctuated between 20.5 °C to 25.5 °C at east side of the lake and from 18.7 °C to 26.3 °C at the west side (Table 2). Temperature has a significant positive correlation with Chl. *a* (R= 0.42). Light transparency and temperature are the most important physical variables controlling on the aquatic environment [3]. pH is normally alkaline with a slight local variations at the lake and it increased in the bottom layers than the surface. pH was correlated with Chl. *a* and Cyanophyceae abundance (r =0.23 and 0.56, P < 0.001). Gharib and [29] also showed that there was a close linear correlation between pH, DO and Chl. *a*. The highest conductivity value of 226 µS/cm was recorded in the north at the west area. This result coincided with [65]. DO fluctuate from 4.4 to 6.8 mg l^{-1} at both sides during the study period. Alkalinity of the lake was within the allowable limits of Bureau of Indian Standards BIS (200 mg/lit) [48]. N:P ratios give valuable information on nutrient limitation. N: P < 7 was most frequently noted in the Lake water, indicating N is the limiting phytoplankton growth [69].

Table 2: Annual average of physico-chemical variables studied in the littoral area of the Lake.

	Parameters	Littoral area	Sites								
			North			Middle			South		
			St_1	St_2	St_3	St_4	St_5	St_6	St_7	St_8	St_9
Physical parameters	Temp.	East	23.6	22.5	24.3	24.7	20.5	23.9	25.5	25.4	25.0
		West	23.4	23.5	23.4	24.5	21.9	25.6	26.3	24.7	18.7
	pH	East	7.9	8.0	8.2	8.3	8.3	8.4	8.6	8.4	8.3
		West	8.1	8.1	8.3	8.1	8.4	8.5	8.5	8.3	8.5
	EC	East	218	215	210	205	206	205	212	210	200
		West	226	208	208	205	201	210	212	209	
Chemical parameters	Trans. Cm	East	313	385	345	348	295	215	208	250	180
		West	363	357	353	320	245	223	167	230	260
	PO_4 (µgL^{-1})	East	78	84	79	71	23	24	39	39	115
		West	74	73	67	71	20	34	35	95	35
	NO_2 (µgL^{-1}	East	14	38	9	5	7	9	16	16	31
		West	12	10	12	4	5	9	21	26	60
	NH_4 (µgL^{-1})	East	73	105	65	48	67	77	58	73	42
		West	151	94	162	38	83	162	47	93	41
	NO_3 (µgL^{-1}	East	213	151	211	365	323	238	295	286	323
		West	358	198	155	198	334	402	453	350	518
	CO_2 (mg L^{-1})	East	21	12	19	11	19	20	16	14	14
		West	11	17	16	12	10	14	9	10	13
	HCO_3 (mg L^{-1})	East	120	115	110	113	99	95	111	110	107
		West	132	115	127	109	102	112	112	110	108
	DO (mg L^{-1})	East	5.0	4.4	4.7	5.3	5.3	5.0	5.5	5.9	6.1
		West	5.3	4.9	4.7	4.6	5.2	5.1	5.8	5.5	6.8

Phytoplankton was chosen as the most sensitive indicator of changing nutrient conditions because of the potential rapidity of population responses [15] and plays a significant role in maintaining the biological balance in the aquatic environment [63]. Microscopic examination of phytoplankton during study period revealed that a total of 130 species were obtained in the littoral area of the Lake and belonging to 7 classes. Phytoplankton community structure is mainly composed of Bacillariophyceae (42 sp.) representing about 51.7% and Cyanophyceae (37 sp.) constituting 31.7% then Chlorophyceae (36 sp.) forming 13.4%, this result coincided with [2]. Cyanophyceae was observed during the study as first predominant class in summer [30]. While, Dinophyceae (6 sp.), Chrysophyceae (5 sp.), Creptophyceae (3 sp.) and Euglenophyceae (1 sp.) persisted as frequent or rare forms. Bacillariophyceae density was higher in the east than the west side, in reversely with Cyanophyceae and Chlorophyceae. Bacillariophyceae was positively associated with NO_3 (R= 0.17). [5] stated that Bacillariophyceae are favoured in nutrients rich environment mainly nitrates. Growing of diatoms are declining with water temperature and PO_4 where R= -0.43 and -0.24, respectively [55]. Phytoplankton assemblage structure are considered as important indicators and widely used for ecological status assessment [38]. The highest phytoplankton densities at the east area was 1800 x 10^4 cell/L in winter and reduced to 35 x 10^4 cell/L in spring, while the maximum in the west was 1475 x10^4 cell/L in winter and minimized to 25 x10^4 cell/L in spring. Seasonal phytoplankton crop reached its maxima (751 x 10^4 cell/L) in the west area during winter. The highest annual value of 530 x10^4 cell/L was noted at middle area of the east. Annual trend line of phytoplankton crop increase southward in the surface water layer, but it reverse in the bottom layer as shown in Fig (2). Regional variation of phytoplankton reflects its response to changing environmental conditions and grazing [17]. The highest species diversity (87 spp) was recorded during spring while the lowest (45 spp) was obtained during autumn. Phytoplankton species number decreased to half and responds rapidly to changes in the aquatic environment [34]. Phytoplankton was more abundant in the littoral area than in the middle of the open area [37] in press.

Most dominant phytoplankton species were identified in to functional groups (FGs), named alphanumerical codes, according to their sensitivities and tolerances as described by [4, 54, 56 and 46], with its percentage abundance in the two littoral area of the Lake (Table 3).

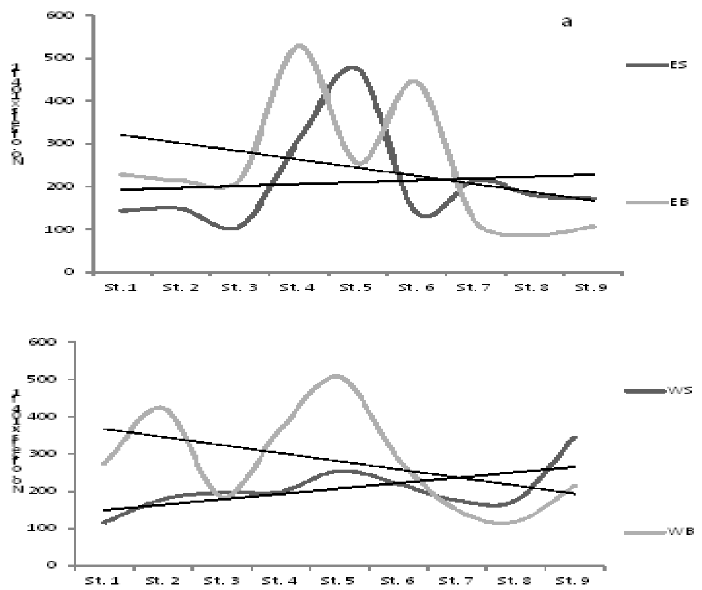

Figure 2ab: Temporal, spatial distributions of phytoplankton crop at the littoral area of Lake Nasser. ES: East surface, EB:east bottom WS:west surface, WB:west bottom.

Table 3: Trait of the dominant phytoplankton functional groups according to their sensitivities and tolerances (Reynolds et al., 2002; Reynolds 2006 and Padisák et al. 2009) and their percentage in the different zones of the Lake

Code	Dominant phytoplankton species in Lake Nasser	Annual percentage in the two littoral area		Tolerances	Sensitivities
		Eastern Lakeside	Western Lakeside		
A	*Cyclotella glomerata*	25.6%	25.2%	Nutrient deficiency	pH rise
D	*Cyclotella ocellata*	6.7%	6.9%	Flushing	Nutrient depletion
	Aulacoseira granulate	13.9%	10.6%	Mild light and C deficiency	Stratification Si depletion
P	*Staurastrum paradoxium*	7.8%	15.1%	Mild light and C deficiency	Stratification Si depletion
XI	*Ankistrodesmus fusiformis*	16.4%	17%	Stratification	Nutrient depletion
S1	*Planktolyngbya limnetica*	38.3%	39.4%	Highly light deficient	Flushing
L0	*Merismopedia punctate*	-	12.5%	Segregated nutrients	Prolonged or deep Mixing
N	*Cosmarium sp.*	5%	-	Nutrient deficiency	Stratification, pH rise
	Coelastrum microborum	7%	-	-	Settling into low light
	Scenedesmus quadricuda,	-	12.7%	-	Settling into low light
J	*Pediastrum simplex,*	3.3%	-	-	Settling into low light
	Oocystis solatoria,	7.2%	-	Low nutrients High turbidity	CO2 deficiency
F	*Dictyosphaerium pulchellum*	4.1%	6.7%	Low nutrients High turbidity	CO2 deficiency

Cellular composition is used as an important biomarker tool in clarifying the taxonomy of algae [36]. Protein peak was found at the north of the two littoral areas where *Cyclotella glomerata* (Bacillariophyceae) represent the dominant species 48.6 %. Annual trend line of protein refers to its contents were decreased southward in the studied region (Fig. 3). *Cyclotella cryptica* contain 260 mg/g protein used as an alternative feed for aquaculture [47].

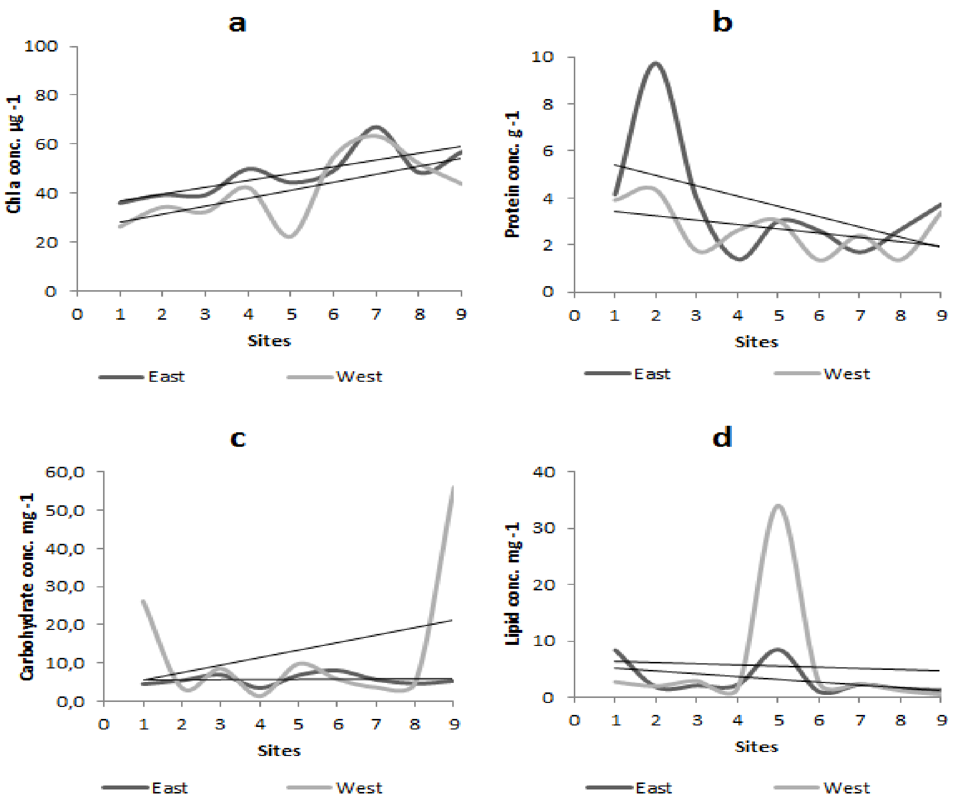

Figure 3a, 3b, 3c,3d : Fluctuations in biochemical contents and chlorophyll *a* of phytoplankton at the two littoral area of Lake Nasser

Phytoplankton at the north of the lake attained the highest calorific value, especially at the east side 34 Kcal/l. currently with the elevation of the mean value of protein contents (4.6 g/l) and Chl. *a* (51 µg/l) at the same sector. Seasonal variations in calorific value of the studied phytoplankton indicated that winter as well as spring had the highest energy value of 21 Kcal/l in the algal biochemical structure which attributed to the maximum protein compositions during both seasons (4 g/L) and increasing in the carbohydrates (17.25 mg/L), lipids (7 mg/L) and phytoplankton standing crop of 393 x10^4 cell/L during winter. The environmental variables interacted with the observed variations in energy content of the phytoplankton in fresh water lake, the quality of phytoplankton varied with energy content in the engulfing phytoplankton [18]. Microalgae varied significantly in their content of protein, carbohydrate and lipid which are important indicators for ecological status assessment [38]. In our study, Chl. *a* concentrations was located in the range of 5.0-140 µg/1 for both littoral sides. The Lake can be described as eutrophic according to TSI where TSI was fluctuated between 59.1 (W) and 60.4 (E) in the lake [14] and [26]. Protein is one of the most abundant macromolecules in the studied algal community (3.7 g/L at E and 2.7g/L at W) as shown in Table 4. Protein constituting a major part of the cell mass under nutrient enrichment conditions [71]. The elevation of the mean value CO_3 (16 mg/L) at the east of the lake, indicated that the largest proportion of inorganic C in water body were incorporated into algal protein. Carbon fixed in photosynthesis incorporated rapidly into amino acids [27]. In contrast, the proportion of storage compounds as carbohydrates (5.6 and 13.3 mg/L) and lipids (3.2 and 5.6 mg/L) for east and west area decreases in determined algal community. Protein ratio decreases when the phytoplankton community is in stationary phase or cells become destroyed [57].

Table 4: Mean elemental formulae for the primary metabolites and stoichiometric ratio of phytoplankton community at the littoral area of Lake Nasser.

Formula		East	West
Chlorophyll *a* (µg/l)	$[C_{46}H_{52}O_5N_4Mg]$	51.03	41.36
Proteins (g/l)	$[C_{130}H_{217}O_{45}N_{39}S]$	3.7	2.7
Carbohydrates (mg/l)	$[C_{17}H_{28}O_{14}]$	5.6	13.3
Lipids (mg/l)	$[C_{53}H_{89}O_6]$	3.2	5.6
C/N		3.6	3.8
C/H		0.6	0.33
O/C		0.33	0.31

The elemental phytoplankton compositions C, N, H and O were higher at the east side of the lake than the west one, this may related to phytoplankton community was dominated by Bacillariophyceae which represent 53.1% (E) and thus would be expected to have a relatively large amount of allocation to elemental compositions than the other classes. Organic carbon content of phytoplankton in investigated area was 32% of the elemental compositions. Phytoplankton contributes most of the organic carbon available to pelagic food webs [54]. The primary importance of H and O content are estimate their relationship with the amount of oxygen consumed during formation of phytoplankton organic material [7]. The usual average C/N ratio according to Redfield et al. (1963) is 6.6. However, microalgae at the study area had a high progress rate where the C/N ratio decreased to 3.6 (E) and 3.8 (W) at the lake

(Table 4), as a result of increasing the proportion of proteins at the same area thus support the idea that these algae were not nitrogen-limited.

Table 5: RDA Analysis 'Var-part-3groups-Simple-effects-tested', results. Total variation is 1558.143, explanatory variables account for 71.0% (adjusted explained variation is 51.7%)

Summary Table:

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.4798	0.1417	0.0478	0.0289
Explained variation (cumulative)	47.98	62.15	66.93	69.82
Pseudo-canonical correlation	0.9432	0.8194	0.6108	0.6265
Explained fitted variation (cumulative)	67.55	87.50	94.24	98.31
Permutation Test Results:				
On All Axes	pseudo-F=3.7, P=0.002			

The result of the RDA clearly show the seasonal differences, in winter the plankton assemblages clearly separate from other season and plankton is mainly dominated by *Cyloellata* species, while in summer *Planktolyngbya limnetica* and *Aulacoseira* became dominated.(Fig 4) When variance partitioning was also included in the analysis the temporal, environmental and biochemical parameters allow us the separate analysis of these factors. The 6 environmental variables and 5 dominant phytoplankton species at the different selected sites, as in Figure 5. Analysis indicated that, the most important factors affecting on phytoplankton distribution are NO_3, NO_2, PO_4, pH, Transparency and NH_4. RDA cleared that, *Planktolyngbya limnetica* was dominated at the middle area, more related to pH while phosphorus is non-limiting factor for its growth. Both *Aulacoseira granulate* and *Eucapsis minuta* are tolerant to mild light deficiency and more dominated at the south area. *Cyclotella glomerata* and *C. operculata* are dominated at the north area, sensitive to flushing and tolerant to nutrient deficiency especially (NO_2, NO_3 and PO_4), as shown in table 3. The species compositions are influenced by a change of environmental variables especially nutrient, transparency and temperature [21]. RDA (Fig, 5) showed an elevation in lipid at the middle of the littoral area of the lake which probably due to increasing of annual phytoplankton crop (508 x 10^4 cell/L) where the most leading species was *Planktolyngbya limnetica* Lemmer contains a high level of saturated fatty acids (39.7%) [52]. While the maximum value of carbohydrate and Chl. *a* were obtained at the southern area of the lake (Fig. 5), which accompanied with the maximum ratio of 34.8 and 13.4% for both *Eucapsis minuta* and *Aulacoseira granulata*.

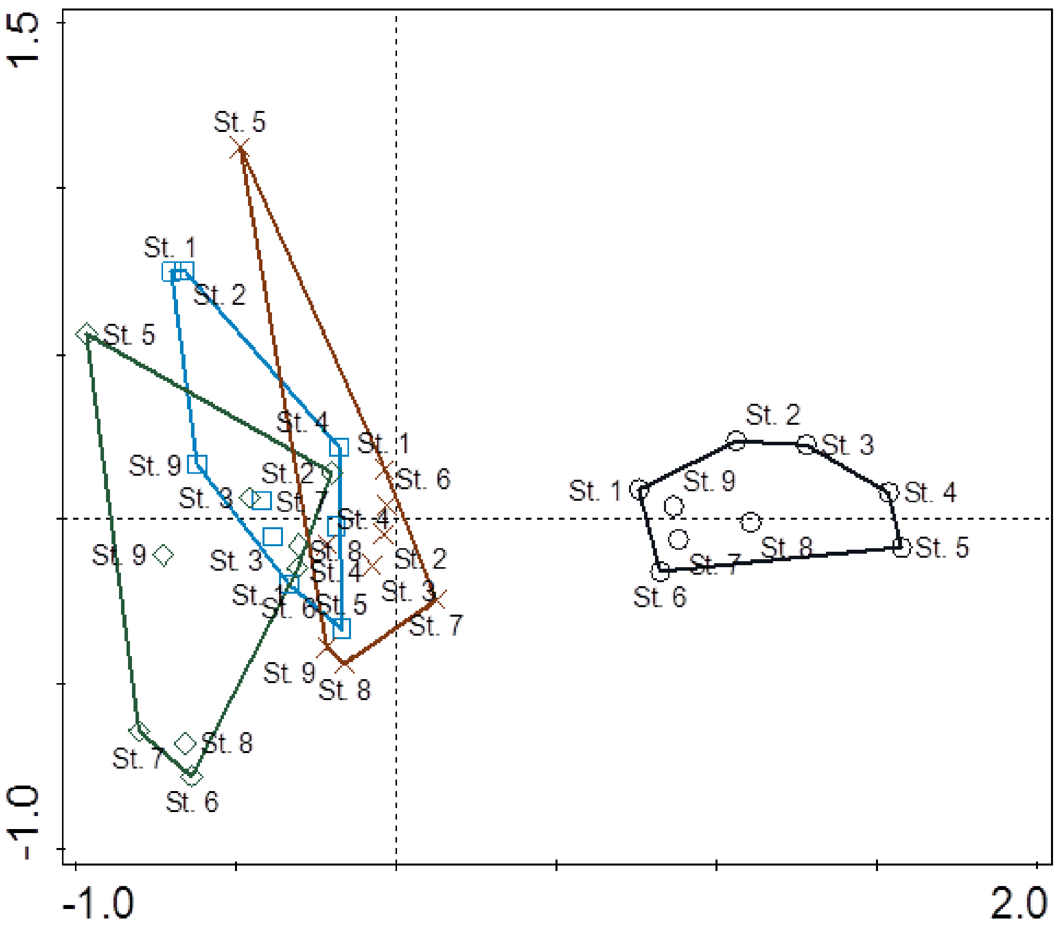

Figure 4: RDA unconstrained ordination of the dominant phytoplankton species (Objects) and selected environmental (Variables) in the different sites of the Lake.

RDA (Fig, 5) cleared that protein is highly coordinate with the elemental compositions of studied phytoplankton. The stoichiometry and diversity of phytoplankton frequently returns the supply of nutrients and light [31]. The stoichiometry of phytoplankton depends on the physiological state of the cell [11].C/H and O/C ratio for natural phytoplankton samples were less than 1. Which pointed to the metabolites categories of studied phytoplankton contain unsaturated aliphatic compounds such as alkenes and aliphatic tail of fatty acids [58].

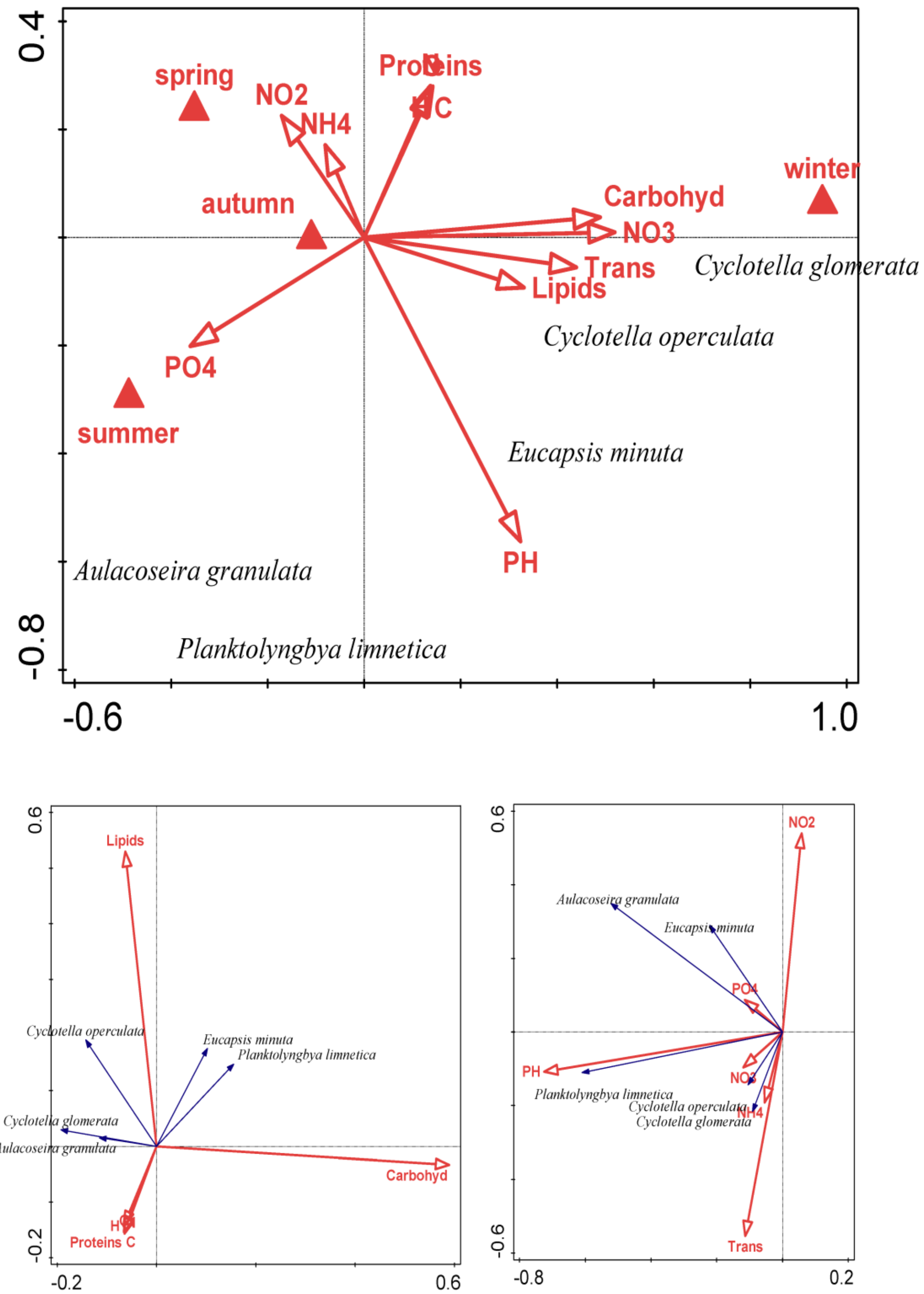

Figure 5: RDA unconstrained ordination of the dominant phytoplankton species (Objects) and selected environmental (Variables) in the different sites of the Lake, for the temporal, environmental and biochemical parameters

Conclusion

Examination of elemental contents and biochemical parameters in natural algal communities can yield important insights into the effects of physico-chemical data and nutrient composition of water body on the energetic value of algae. Bacillariophyceae can be classified as high elemental and biochemical composition group at the lateral area of Lake Nasser. A continuous monitoring should be organized to analyze the elemental composition of species from diverse taxonomic groups under a variety of environmental conditions. Furthermore, results of the study may be an alarm for all stakeholders, who are concerned about the lake changes as a result of the construction of the Renaissance Dam in Ethiopia.

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