PHYTOPLANKTON VARIABILITY WITH RELATIONSHIPS TO ENVIRONMENTAL FACTORS IN A TEMPORARY SALT LAKE

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Résumé.— *Variabilité du phytoplancton en relation avec les facteurs environnementaux dans un lac salé temporaire*.— Dans ce travail, afin de déterminer l'impact des différentes variables environnementales sur le phytoplancton dans un milieu hypersalé, un suivi des paramètres abiotiques et biotiques a été réalisé au niveau de Sabkhet El Adhibet (sud-est Tunisien), durant les périodes allant de novembre 2005 à avril 2006 et de novembre 2006 à avril 2007 (le site étant sec entre le début du mois de mai et la fin du mois d'octobre). Les résultats obtenus ont permis l'identification de 16 espèces de phytoplancton, appartenant aux groupes des diatomées, chlorophycées, dinophycées, euglénophycées, cryptophycées et cyanobactéries. La plus grande densité microalgale a été observée en décembre 2005 et janvier 2006 avec respectivement 8,6 et 14,6 10^6 cellules.L^{-1}, alors que la plus petite densité a été enregistrée en novembre 2006 avec 0,19 10^6 cellules.L^{-1}. L'analyse de la composition en phytoplancton révèle que ce sont les diatomées et les chlorophycées qui présentent la fraction la plus importante du phytoplancton, avec respectivement un maximum de 97,8 % (novembre 2006) et 95,7 % (janvier 2007). Le pourcentage moyen décroissant, relatif à chaque groupe de phytoplancton, de toute la période d'investigation est comme suit : diatomées (52,1 %), chlorophycées (39,8 %), dinophycées (3,2 %), euglénophycées (3 %), cryptophycées (1,1 %) et cyanobactéries (0,8 %). Toutefois, nous remarquons une grande variation mensuelle de la composition phytoplanctonique avec les diatomées et les chlorophycées présentes au niveau de tous les échantillons, tandis que les dinophycées, les euglenophycées et les cryptophycées sont absentes au niveau de nos échantillons durant certaines périodes de l'année. L'étude de la corrélation, entre la composition et la densité des phytoplanctons avec les différents paramètres abiotiques, montre que c'est l'orthophosphate qui affecte le plus la dynamique et la variation phytoplanctonique dans le milieu. Par ailleurs, aucune corrélation n'a été observée entre le phytoplancton et les autres paramètres environnementaux.

Summary.— To establish a relationship between environmental variables and phytoplankton dynamics, physicochemical characteristics and phytoplankton sampling were performed in Sabkhet El Adhibet (Southeastern Tunisia) monthly from November 2005 to April 2006 and from November 2006 to April 2007 (the site dries annually between May and late October). All measured water quality variables showed considerable seasonal variation, and quantitative and qualitative differences in phytoplankton communities were recorded. The maximum phytoplankton density was recorded in January 2006, whereas lowest values occurred in November 2006. Sixteen species belonging to 6 orders were recorded, among which diatoms (52.1 %) and chlorophytes (39.8 %) were the most abundant. The correlation analysis between phytoplankton (density and composition) and abiotic variables revealed that orthophosphate is the major factor affecting the phytoplankton dynamics, and there were no correlation between phytoplankton and all the other environmental parameters. The PCA analysis of the phytoplankton composition and density revealed two main PCA axis explaining 82.99 % of the variance. The first axis was associated with diatoms, cryptophytes, euglenophytes, dinophytes and total phytoplankton, with a contribution of 21.8, 21.6, 21.3, 17.4 and 17.4 %, respectively. The second axis was correlated with chlorophytes (55.3 %) and cyanobacterias (26.3 %).

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Temporary inland water ecosystems are characterized by fluctuations in water-level and physicochemical characteristics, and sequential response in the biological communities especially plankton species. Hypersaline water spans large areas worldwide, not only in salt production areas (solar salt works, salterns or salinas) but also in natural lakes and lagoons, and in tidal ponds (Javor, 1989). These ecosystems can be used as models for studying adaptation of aquatic organisms to extreme environments. These habitats are shelters for migratory birds and home of unique native flora and fauna well adapted to the extremely harsh conditions (Amat *et al*., 1991; Lenz & Browne, 1991; Gajardo *et al*., 1992; Cohen *et al*., 1999). Phytoplankton and invertebrate populations are two important components of the biological system of salt lakes. Several studies gave information on the composition and ecology of phytoplankton in natural salt lakes and solar salt works located in various countries (e.g. Davis, 1990; Pedròs-Alió *et al*., 2000; Ayadi *et al*., 2004; Dolapsakis *et al*., 2005; Segal *et al*., 2006). Ashton & Schoeman (1983) and Davis (2000) reported that, in salt lakes, distribution and productivity levels of organisms are largely determined by physicochemical factors. In fact, in these ecosystems a large number of factors have been implicated in relation to dynamics of algal populations under natural conditions. These include physical factors (light, temperature, weather, water movements, and flotation), inorganic chemical nutrients (nitrogen, phosphorus, calcium, magnesium, potassium, sulphate, chloride and other trace elements), organic matter, and biological factors (perennation (resting stages), predation, parasitism) (Lund, 1965). Therefore, the functioning of this kind of system is generally driven by factors related to seasonality and hydrology (Comín *et al*., 1999).

Natural salt lakes such as sebkha are impacted by agriculture and urban pollution and highly variable water flow regime, whereas the organic and inorganic substances often rapidly undergo massive fluctuations. There are a number of studies that have dealt with the phytoplankton communities of solar salt works, usually examining a limited number of ponds along the complete salinity range (e.g. Pedròs-Alió *et al*., 2000; Ayadi *et al*., 2004; Segal *et al*., 2006). The main objective of the present study was to determine the relationship between phytoplankton dynamics and the water physicochemical characteristics in the Sabkhet El Adhibet temporary salt lake.

MATERIALS AND METHODS

STUDY AREA

Sabkhet El Adhibet (33°07'7.58"N – 11°24'8.69"E) is an inland water body site located in Southeastern Tunisia. Its total surface is 12 500 hectares, including 500 hectares occupied by industrial salt works. In the salt work water is pumped from the underground brine reservoir with salinity about 280 psu and directly administered in the crystallizer. Artificial trenches delimiting salt works were dug in order to unite the dividing dykes of the basins with an average depth of 0.75 m. These artificial canals in the border of salt work are filled by rain water accumulated during rainy season until May or June and their biotic and abiotic characteristics change depending on precipitation and evaporation rate. However, the saltern is generally filled by rain-water from December to February. For more information about Sabkhet El Adhibet see Ben Naceur *et al*. (2009, 2011).

PHYSICAL PARAMETERS AND BIOLOGICAL ANALYSIS

Samples were collected monthly from November 2005 to April 2006 and from November 2006 to April 2007 in three stations located in the artificial canals in the border of salt work, although these stations were filled with water for only half the year (the sites were dried up annually from May to late October). Samples were taken in the morning between 07 :00 and 11 :00 am. Water temperature, salinity and pH were measured *in situ* using portable multiprob (WTW. Multi/340i/SET). Dissolved oxygen concentration analysis was carried out following the chemical method of Winkler (Aminot & Kérouel, 2004). One litre of water sample was sieved over a 120 µm mesh filter to remove zooplankton, stored in plastic bottles and then refrigerated in the dark and transported to the laboratory (in a time not exceeding 24 hours) in order to analyse orthophosphate, nitrites, nitrates and ammonium (colorimetric method), according to Aminot & Kérouel (2004). Phytoplankton samples were collected by immersing one-litre plastic bottles 5-20 cm beneath the surface. Samples were fixed *in situ* with 2 ml Lugol and 10 ml neutralised formaldehyde solution. Phytoplankton species were identified and their abundance estimated in sedimentation chambers using an inverted microscope (Leitz/Diavert with magnification : 400x and 1000x) according to Utermöhl (1958). Taxonomic identification was based on Geitler (1932), Bourrelly (1966, 1968, 1970) and Germain (1981).

Statistical analysis

Phytoplankton characteristics were correlated with environmental factors using Pearson's correlation coefficient ($P = 0.05$) to find the degree of association between them. Principal Component Analysis (PCA), using Pearson's matrix and principal components (PC) axis scores was carried out by means of the computer program XLSTAT-Pro 7.5. PCA is used to identify patterns in data (monthly variability of phytoplankton) and to express the data in such a way as to highlight their similarities and differences (density and composition of different phytoplankton groups) (see Derry *et al*., 2003; Ariyadej *et al*., 2004; Flanagan *et al*., 2009; Sun *et al*., 2011).

RESULTS

Abiotic parameters

The mean values of water temperature, salinity, pH and dissolved oxygen as well as nutrients concentrations measured in all stations have been reported earlier by Ben Naceur *et al*. (2009). Figure 1 presents the main information about physicochemical parameters in this site as reported by Ben Naceur *et al*. (2009).

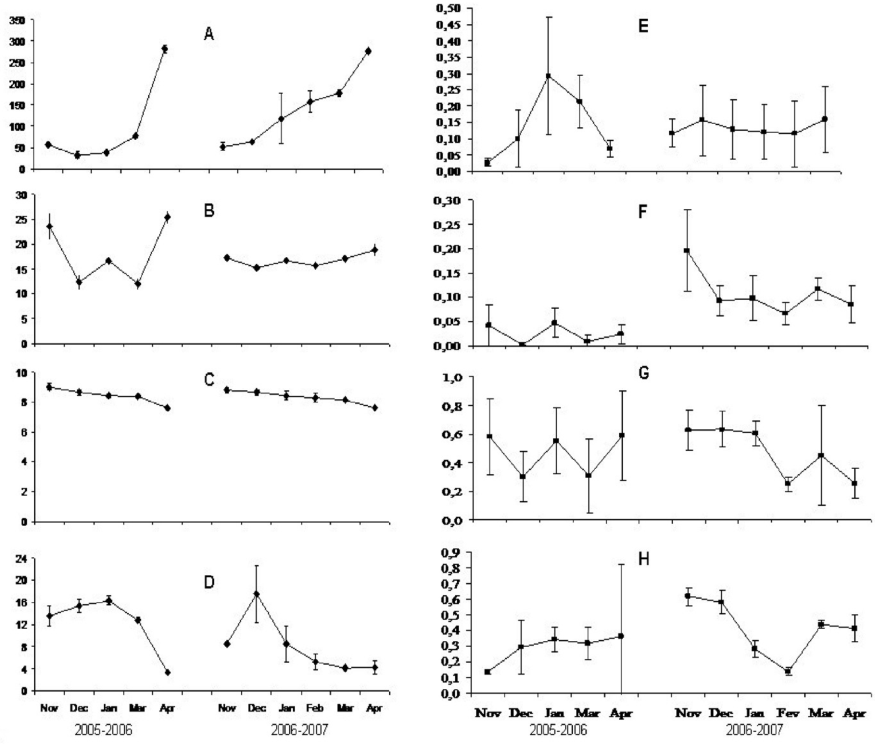

Figure 1.— Monthly abiotic parameters fluctuation during investigation period in Sabkhet El Adhibet. A : salinity (g.L^{-1}), B : temperature (°C), C : pH (pH unit), D : dissolved oxygen (mg.L^{-1}), E : orthophosphate (mg.L^{-1}), F : nitrite (mg.L^{-1}), G : nitrate (mg.L^{-1}), and H : ammonium (mg.L^{-1}) (Ben Naceur *et al*., 2009).

The mean values of minimum and maximum surface water temperatures obtained were 12.1 °C (March 2006) and 25.4 °C (April 2006). Salinity ranged between 32.2 and 281.7 g.L^{-1}. Average values of dissolved oxygen vary from 3.4 to 17.5 mg.L^{-1}. Monthly average pH values ranged from 7.6 to 9. The nutrients analysis showed that the mean values of orthophosphate ranged between 0.027 ± 0.01 mg.L^{-1} (November 2005) and 0.293 ± 0.18 mg.L^{-1} (January 2006). The nitrite minimum concentration was recorded in February 2007 (0.001 ± 0.001

mg.L^{-1}) and the maximum in December 2006 (0.195 ± 0.04 mg.L^{-1}). The mean values of nitrate ranged between 0.25 ± 0.05 mg.L^{-1} and 0.633 ± 0.12 mg.L^{-1} for December 2005 and November 2006, respectively. The ammonium concentrations ranged between 0.137 ± 0.02 mg.L^{-1} (November 2005) and 0.617 ± 0.05 mg.L^{-1} (November 2006).

PHYTOPLANKTON STUDY

The phytoplankton of Sabkhet El Adhibet consisted of 16 taxa belonging to diatoms, chlorophytes, dinophytes, euglenophytes, cryptophytes and cyanobacteria divisions. A list of recorded taxa and divisions is presented in Table I. The relative percentages of phytoplankton taxa in decreasing order were : diatoms (52.1 %), chlorophytes (39.8 %), dinophytes (3.2 %), euglenophytes (3 %), cryptophytes (1.1 %) and cyanobacteria (0.8 %). However, in terms of temporal distribution and abundance, diatoms, chlorophytes and cyanobacteria were present throughout all the investigation period. Cryptophytes and euglenophytes were present only during some months (Fig. 2). During the sampling periods, the phytoplankton density showed a maximum of 14 10^3 cell.L^{-1} in January 2006, when salinity was in its minimum level (Fig. 2). Seasonal successions were varied with a distinct seasonal periodicity occurring between different periods.

TABLE I

Phytoplankton species composition (+ present, - absent)

	2005-2006					2006-2007					
	Nov	Dec	Jan	Mar	Apr	Nov	Dec	Jan	Feb	Mar	Apr
Chlorophytes (10^3 cells.L^{-1})	292	7972	316	2518	1732	3	388	456	239	369	488
Ankystrodesmus falcatus var *acicularis*	-	-	-	+	-	-	-	-	-	-	-
Chlamydomonas sp.	++	+	+	-	++	-	++	+	-	-	+
Dunaliella salina	-	+++	-	+	++	+	+	-	++	++	+++
Oocystis sub marina	-	-	-	+++	-	-	-	-	-	-	-
Tetraedron minimum	-	+	-	-	-	-	-	-	-	-	-
Diatoms (10^3 cells.L^{-1})	229	629	12152	449	187	184	1163	18	2359	1629	344
Cyclotella ocellata	+	+	+	+	+	-	++	-	+	-	++
Cymbella sp.	+	+	+	-	-	-	+	+	+	-	+
Navicula sp.	+	-	++	+	+	+	++	-	+	+	+
Nitzschia sp.	-	++	+++	++	+	++	++	+	+++	+++	+
Amphiprora sp.	-	-	+	++	+	-	+	-	+	+	-
Dinophytes (10^3 cells.L^{-1})	0	23	639	173	12	0	344	1	295	176	0
Gymnodinium sp.	-	+	+++	++	+	-	++	+	++	++	-
Cryptophytes (10^3 cells.L^{-1})	0	5	365	9	0	0	35	1	0	0	0
Cryptomonas ovata	-	+	++	+	-	-	+	+	-	-	-
Euglenophytes (10^3 cells.L^{-1})	0	0	1104	0	5	0	0	0	0	0	0
Euglena sp.	-	-	+++	-	+	-	-	-	-	-	-
Cyanobacteria (10^3 cells.L^{-1})	19	22	14	9	5	1	33	1	168	14	20
Oscillatoria sp.	+	+	-	-	+	+	+	+	++	-	+
Pseudanabaena catenata	+	+	-	-	-	-	+	-	-	-	+
Pseudanabaena constricta	+	-	+	+	-	-	-	-	-	+	+

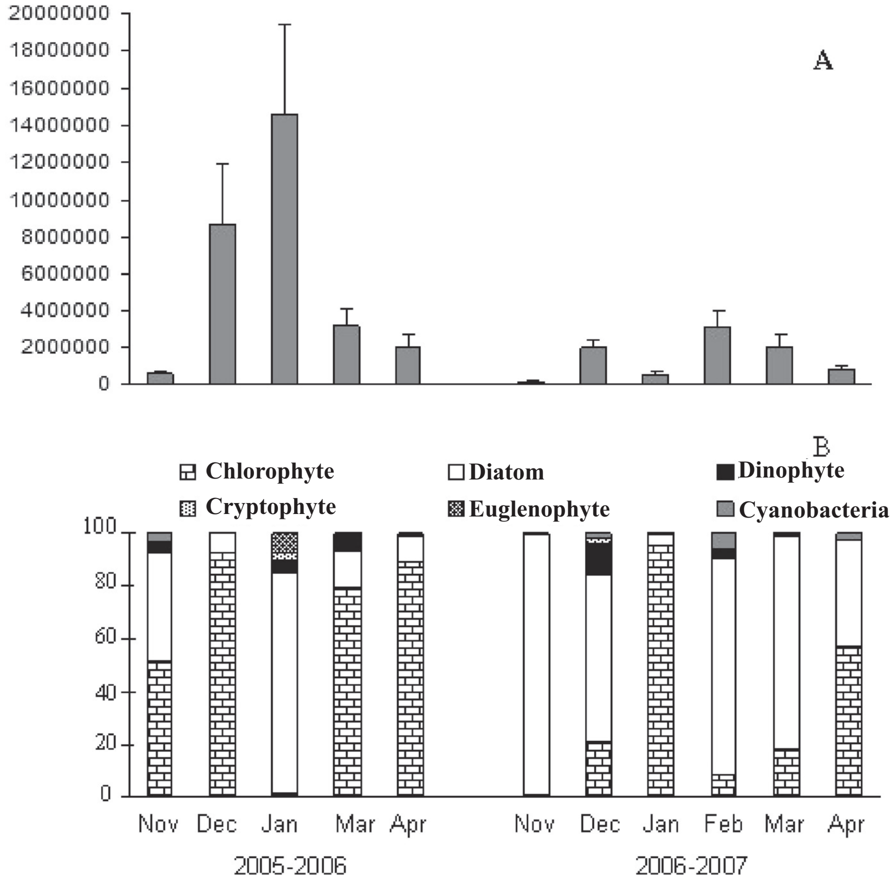

Figure 2.— Phytoplankton variability. (A) phytoplankton density (cells.L^{-1}) ; (B) percentage phytoplankton division composition (%).

Correlation analysis of the phytoplankton (density and composition) and water physico-chemical parameters indicates that total phytoplankton abundance had highly significant correlations with orthophosphate (Pearson's r = 0.640, *P* = 0.05). The phytoplankton community structure shows that chlorophytes and cyanobacteria were not correlated with any environmental variable, and that diatoms, dinophytes, cryptophytes and euglenophytes present highly significant correlations with orthophosphate (r = 0.736, 0.756, 0.757 and 0.733, respectively). However, despite the absence of significant correlations between environmental parameters and phytoplankton density and composition, it is obvious that a weak negative relationship between salinity and all phytoplankton groups (except for cyanobacteria), temperature and nitrite, and all phytoplankton groups, and ammonium and all phytoplankton structure (except for cryptophytes) existed. On the other hand a weak positive relationship was also observed between pH and dissolved oxygen, and all phytoplankton groups except for cyanobacteria (Tab. II).

TABLE II

Coefficients of correlation between phytoplankton and environmental characteristics

	Total phytoplankton	Chlorophytes	Diatoms	Dinophytes	Cryptophytes	Euglenophytes	Cyanobacterias
Salinity	-0.401	-0.238	-0.284	-0.356	-0.331	-0.297	0.102
Temperature	-0.334	-0.405	-0.121	-0.272	-0.092	-0.055	-0.169
pH	0.091	0.124	0.019	0.134	0.055	0.024	-0.006
Dissolved O_2	0.516	0.311	0.359	0.506	0.453	0.394	-0.206
Orthophosphate	**0.640**	-0.115	**0.736**	**0.756**	**0.757**	**0.733**	-0.095
Nitrite	-0.439	-0.584	-0.132	-0.170	-0.145	-0.144	-0.072
Nitrate	-0.126	-0.405	0.090	0.066	0.199	0.178	-0.494
Ammonia	-0.146	-0.174	-0.058	-0.017	0.016	-0.026	-0.448

Significant ratios at P = 0.05 are marked boldface

The PCA analysis of the phytoplankton composition and density revealed two main PCA axis explaining 82.99 % of the variance. Variables were most correlated with the horizontal axis (axis 1 : component 1) than with vertical axis (axis 2 : component 2), with axis 1 explaining 64.14 % and axis 2 explaining an additional 18.85 % of the total variance. The first axis was associated with diatoms, cryptophtes, euglenophytes, dinophytes and total phytoplankton, with a contribution of 21.8, 21.6, 21.3, 17.4 and 17.4 %, respectively, while the second axis was correlated with chlorophytes (55.3 %) and cyanobacterias (26.3 %) (Tab. III ; Fig. 3).

TABLE III

Contribution and portion of each trait in the two first components of phytoplankton variability

	Phytoplankton				Environmental parameters		
	Variables contribution (%)		Component			Variables contribution (%)	
	F1	F2	F1	F2		F1	F2
Total phytoplankton	17.480	11.109	0.886	0.383	Salinity	30.756	0.117
Chlorophytes	0.205	55.395	-0.096	0.855	Temperature	11.898	0.615
Diatoms	21.825	0.337	0.990	-0.067	pH	24.457	0.107
Dinophytes	17.425	6.629	0.884	-0.296	Dissolved O_2	26.437	0.003
Cryptophytes	21.696	0.070	0.987	0.030	Orthophosphate	3.900	0.223
Euglenophytes	21.369	0.104	0.979	0.037	Nitrite	0.132	24.195
Cyanobacterias	0.001	26.354	-0.007	-0.590	Nitrate	0.821	21.461
					Ammonium	0.020	35.602
					Artemia density	1.578	17.677

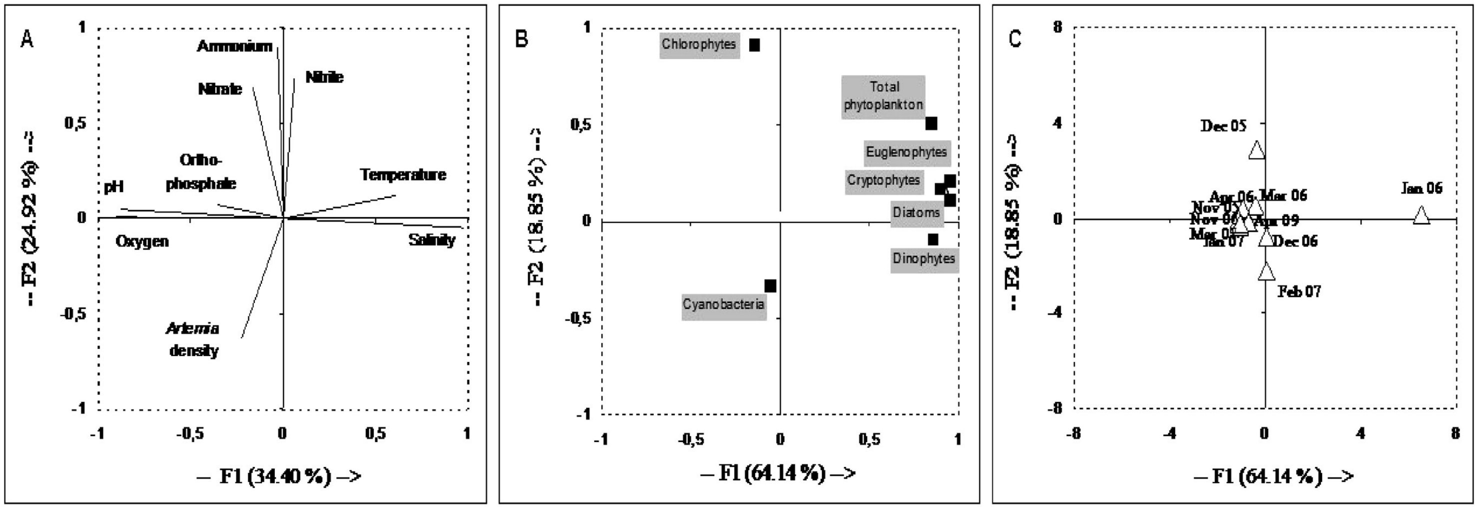

Figure 3.— Principal Component Analysis (PCA) of phytoplankton variability in Sabkhet El Adhibet : (A) environmental parameters, (B) phytoplankton groups and (C) phytoplankton monthly variability.

DISCUSSION

Phytoplankton of coastal wetlands is generally composed of cosmopolitan species able to cope with wide variations in salinity, turbidity, and eutrophy. In areas dominated by a Mediterranean climate, this variability is often arranged in flooding and drying periods according to water availability (Reyes *et al*., 2007). Davis (1990, 2000, 2006), Javor (1983) and Britton & Johnson (1987) reported that in many solar salt works, the low salinity ponds are characterized by clear waters, as microalgae are present in low quantities, a fact that has been attributed to nutrient limitation (Javor, 1983). In our case, phytoplankton reached its maximum density in December 2005 (8.629 10^6 cell.L^{-1}) and January 2006 (14.583 10^6 cell.L^{-1}), when salinity shows its minimum value (32.2 and 38.7 psu, respectively), confirming the results of Segal *et al*. (2006) who reported that water column photosynthesis and biomass decrease markedly with increasing salinity. Moreover, PCA revealed that salinity is the most important environmental factor which contribute (30.75 % according to axis 1) to the temporal variability between the different phytoplanktons.

In previous studies, the phytoplankton community has been reported to be composed mainly of benthic microalgae, i.e. benthic and epiphytic diatoms, dinoflagellates, cyanobacteria and euglenophytes (Javor, 1983; Davis, 1990; Segal *et al*., 2006), although planktonic species such as various nanoflagellates (Chlorophyceae, Prasinophyceae, Chrysophyceae and Cryptophyceae) have also been reported (Dolapsakis *et al*., 2005; Segal *et al*., 2006). In Sabkhet El Adhibet planktonic diatoms and chlorophytes species were found to be the most abundant microalgae. On the other hand, dinophytes, euglenophytes, cryptophytes and cyanobacteria were often observed in the water column samples but were not abundant. Moreover, our results (Fig. 2) show that the abundant species belong mainly to diatoms with mean percentage of 52.1 % of total phytoplankton during the whole investigation period, in consistence with results from other solar salt works (e.g. Britton & Johnson, 1987; Ayadi *et al*., 2004).

The impact of abiotic parameters in phytoplankton density and composition during the two investigation periods was less pronounced. Variations in environmental conditions between different sampling dates did not appear to influence phytoplankton community. However, the results of this study could be considered to support previous observations that phytoplankton responds positively to orthophosphate concentration. Our results confirm the findings of other authors (Wang *et al*., 2007; Lewis & Wurtsbaugh, 2008). Mainstone & Parr (2002) reported that, in aquatic ecosystems, phosphorus is highly active both chemically and biologically, undergoing numerous transformations and continuously exchanging between the particulate and dissolved phases, between the sediment and water column, and between the biota and the abiotic environment. Van Wazer (1973) revealed that, in an aquatic system, the dissolved fractions of phosphorus consist mainly of orthophosphate, and that it is commonly accepted that algae and other aquatic organisms assimilate phosphorus mainly in the form of orthophosphate (Huang & Hong, 1999; Gardolinski *et al*., 2004). Pilkaitytė & Razinkovas (2007) confirm that both centric and pennate diatoms and green algae reacted positively to the phosphate enrichment. Huang & Hong (1999) and Kuang *et al*. (2004) reported that phosphorus is frequently the most limiting nutrient for algal growth both in fresh water, and marine environments (Hu *et al*., 1989; Barak *et al*., 2003; Neill, 2005).

In Sabkhet El Adhibet, a non-significant negative correlation between phytoplankton population and salinity, temperature, nitrite, nitrate and ammonium was observed. Numerous studies revealed the role of nitrogen as limiting nutrient (Pilkaitytė & Razinkovas, 2007; Marcarelli *et al*., 2006; Segal *et al*., 2009). Segal *et al*. (2009) reported that in low salinity phytoplankton was nitrogen-limited but phosphorus-limited in high salinity. Moreover, Camargo *et al*. (2004) reported that in salt works numerous organisms are trapped and slowly die as salinity increases progressively in the evaporating basin; thus, organic matter accumulates and decomposes, both of animal (i.e. crustaceans, fishes, insects) and plant origin (i.e. leaves, phytoplankton). Consequently, the concentration of the nitrogenous compounds, first nitrite and later nitrate (through the process of nitrification), increases through time as salinity increases. In the same way, Nyonje *et al*. (1995) showed that in the salt works at Gongoni (Kenya), chlorophyll *a* increases with increasing salinity and the co-occurring elevated levels of nitrates in the evaporation ponds. Marcarelli *et al*. (2006) mentioned that at salinities greater than 70 $g.L^{-1}$, or with additions of combined nitrogen, N_2 fixation ceased. When N_2-fixing cyanobacteria were absent, the plankton community was routinely nitrogen-limited regardless of salinity, and suggests that nutrient limitation of phytoplankton communities may change depending on salinity levels, because salinity controls whether N_2-fixing cyanobacteria will be present in the phytoplankton community. In Sabkhet El Adhibet, no correlations were found between nitrates and phytoplankton density and composition. This result can be explained by the fact that during the first sampling period (November 2005 to April 2006) salinity was less than 76 psu during the five first month and then peaked from 76.7 psu (in March 2006) and exceeded 250 psu (in April 2006) causing a massive mortality of aquatic organisms. During May, the site dried up and the process of nitrification was stopped.

Evagelopoulos & Koutsoubas (2008) revealed that where salinity is not very high, biodiversity is significant. But in the extreme hyperhaline conditions of the high salinity ponds and the crystallizers, the environment is too harsh and biodiversity is consequently limited, many taxonomic groups are absent and halophilic and halotolerant taxa persist and thrive (Rodriguez-Valera, 1988). In our case, weak negative relationships between the species richness and abundance of phytoplankton and the salinity indicate an agreement with the results of Golubkov *et al*. (2007) who showed that there are weak negative relationships between the species richness of phytoplankton and the salinity.

The PCA analysis revealed that the monthly phytoplankton distribution in Sabkhet El Adhibet showed a clear separation into 4 groups. January 2006 data were plotted almost exclusively on the upper right side of the ordination plot, indicating that this month was characterized by the presence of all phytoplankton divisions, with diatoms representing the most abundant group, and a high phytoplankton density. February 2007 data were plotted on the lower right side of the abscissa, and were associated with high cyanobacteria density. December 2005 data were plotted on the upper part of axis 2, reflecting a high chlorophytes and phytoplankton density.

CONCLUSION

Statistical analysis did not show a clear interaction between the majority of physicochemical parameters (especially salinity and temperature) and phytoplankton composition and density, as has been reported in previous studies (see literature cited above). However, the number of phytoplankton species was much higher in December 2005 and January 2006, when water salinity reached its minimum values. The correlation analysis of the phytoplankton parameters (density and composition) and abiotic variable reveal that orthophosphate is the major factor affecting the phytoplankton dynamics in Sabkhet El Adhibet.

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