Oscillatoria Winter Bloom in the Nile River

Mohamed N. Gomaa[1], Abeer S. Amin[1], Diaa A. Gaballah[2], and Aziz M. Higazy[3]

[1]Biology Department, Faculty of Science and Arts-Khulais, King Abdulaziz University
[2]Marine Toxin Lab., Food Toxicology and Contaminants Dept. National Research Center, Cairo, Egypt
[3]Microbiology Department, Faculty of Agriculture, Cairo University, Egypt
Corresponding auther: Mohamed N. Gomaa Email: mngomaa@gmail.com. Cell phone: 00966567755906

Abstract
Cyanobacterial winter bloom occurred in Port-Said at the northern part of Egypt causing an unpleasant taste and odor of drinking water and the frequent clogging of filters in the drinking water treatment plant were the primary problems that trigger this study. The objectives were to explore the changes in cyanobacterial community structure at different sites and to explore the primary factors affecting such structure. Samples of water and water column algae were collected from the Port-Said freshwater canal and sites along the Rosetta branch of the Nile River in Kafr El-Zayat, Edvena and Rosetta from September 2007 to March 2009. Variations in floristic composition, phytoplankton communities and water characteristics were determined. Different ecological parameters exhibited seasonal variations with small changes among different sites. Among the identified species of Cyanophyta, *Microcystis aeruginosa*, *Oscillatoria brevis* and *Oscillatoria princeps* recorded the highest cell counts. For *Microcystis aeruginosa*, the maximum cell counts were recorded in November 2008 and January 2009 at the Edvena site; maximum values of *Oscillatoria brevis* were recorded in January 2009 at the Kafr El-Zayat site, and maximum values of *Oscillatoria princeps* were recorded in January 2009 at the Rosetta site. A canonical correspondence analysis (CCA) of physicochemical parameters and cyanobacterial counts indicate that the maximum counts were positively correlated with TDS, nitrate, ammonium and phosphate.

Key words: Winter Cyanobacterial Bloom, Nile River, *Microcystis aeruginosa*, *Oscillatoria brevis* , *Oscillatoria princeps*.

Introduction

The growth of cyanobacteria and the formation of blooms are influenced by physical, chemical and biological factors (Messineo *et al*. 2008). As a result of the interplay of these factors, there may be large yearly fluctuations in the levels of cyanobacteria and a seasonal variation in dominant species. Cyanobacterial blooms persist in water supplies that contain adequate levels of essential inorganic nutrients, water temperatures generally between 15 and 30˚C, and pH levels between 6 and 9. This means that blooms are most common in eutrophic or hypereutrophic bodies of water and usually occur in late summer or early fall in the temperate zone (WHO, 1999); however, winter blooms are more likely to occur in the subtropical zone (Jacoby *et al*., 1984; Gomaa *et al*., 2000).

Thermal stratification causes reversed nutrient stratification with nutrient layers in deeper layers being unavailable to phytoplankton. Turbulence is considered to reduce cyanobacterial development (WHO, 1999 and Van Ginkel, 2004); however, Harding (1997) found that regular mixing of the system results in higher productivity.

Cyanobacteria are frequently exposed to light due to the presence of specialized gas-filled vesicles, and this may account for their faster growth compared to other microbes found in the same ecosystem (Walsby *et al*., 2006). High light intensities increase cellular iron intake, as Fe^{3+} appears to be converted to Fe^{2+} by light before it is transported into algal cells, which may ultimately be responsible for faster growth (Utkilen and Gjølme, 1995).

Nutrients such as nitrogen and phosphorus are essential for cyanobacterial growth (Villareal and Carpenter, 2003). Several studies have shown that cyanobacteria have a higher affinity for nitrogen and phosphorus than many other photosynthetic organisms (Kaebernick *et al*., 2001 Lawrence *et al*., 2002). The ability of cyanobacteria to store substantial amounts of phosphorus (Metting and Pyne, 1986; Kaebernick *et al*., 2001) allows them to perform two to four cell divisions, which correspond to a 4–32-fold increase in biomass. Low nitrogen to phosphorus ratio has also been observed to favour cyanobacterial blooms. The N:P ratio is not, however, a controlling factor in the development of cyanobacteria as dominant phytoplankton group, but should be linked to nutrient availability and the prediction of which element is likely to become limiting during the algal growth phase (Metting and Pyne, 1986; Kaebernick *et al*., 2001; Van Ginkel *et al*., 2001; Villareal and Carpenter, 2003).

The increasing nutrient load resulting from fertilizer runoff and the discharge of sewage containing nitrogen and phosphorus to the Nile River may be the cause of a cyanobacterial bloom in winter 1995 in the freshwater canal of Port Said, Egypt. An unpleasant taste and odor of drinking water and the frequent clogging of filters in the drinking water treatment plant were the primary problems during this episode (Gomaa *et al*., 2000). This winter bloom has reoccurred each winter since then and has expanded from Port-Said to the cities of Ismailia and Suez along the Ismailia Canal, one of the branches of the Nile River (Gomaa, *et al*., 2000; Amin, 2001; El-Manawy and Amin, 2005; Mohammed, 2010).

A possible expansion of such cyanobacterial winter blooms to other branches of the Nile River is likely to occur. Therefore, the primary objective of this study was to explore the changes in cyanobacterial community structure at different sites along the Port-Said freshwater canal approximately 200 km northeast of Cairo, which represents the area where the problem began, and the end of the Nile River's Rosetta Branch approximately 200 km northwest of Cairo, where the bloom is expected to expand during future seasons. In addition, the correlations between cyanobacterial community structure and measured physicochemical parameters were assessed to explore the primary factors affecting such structure.

Material and Methods

Area of study

Monthly samples of algae and water were collected monthly from 4 sites along the Port Said freshwater canal extending a distance of 35 km away from the water treatment plant and from 3 sites at the end of the Rosetta Branch approximately 100–200 km away from Cairo (Table 1 and Figure 1) to explore the cyanobacterial profile in this particular River Nile ecosystem during the period from September 2007 to March 2009.

Table 1. Location names and detailed information on the locations of the sampling sites.

Location	Site number	Site local name	Sampling location
Port-Said freshwater canal	(I)	"El-Kap"	Approximately 34 km away from the Port-Said city water treatment plant along the freshwater canal.
	(II)	"El-Teina"	24 km from the Port-Said city water treatment plant.
	(III)	"Ras El-Eish"	14 km from the Port-Said city water treatment plant.
	(IV)	"El-Rasua"	Water inlet of the Port-Said city water treatment plant with a width of approximately 10 m and a depth ranging from 1-5 m.
Rosetta Branch	(V)	"Kafr El-Zayat"	Located approximately 123 km northwest of Cairo.
	(VI)	"Edvena"	Located approximately 210 km northwest of Cairo.
	(VII)	"Rosetta"	Located approximately 224 km northwest of Cairo.

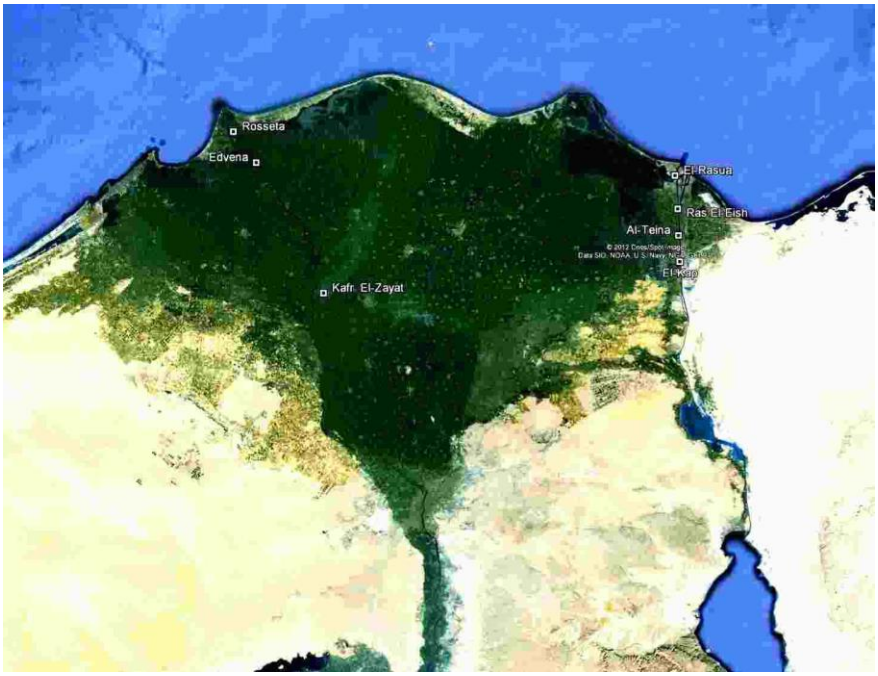

Figure 1. Locations of sites sampled throughout the study period

Physicochemical parameters

All sample collection and field measurements were performed around mid-day.

Portable field meters were used to record the physical parameters of surface water. Water temperature in was measured using a digital thermometer. A turbidity meter (Type 12 FSc Fatramo systems Inc. Chemtrix Inc., Hillsboro, OR, USA) was used for the determination of the turbidity of water samples. The turbidity was expressed in nephlometric turbidity units (NTU). Alkalinity was estimated and expressed as mg l^{-1} equivalent calcium carbonate according to APHA (1985). Water conductivity was directly measured using a conductivity meter (Chemtrix Type 700) and expressed in µmhoscm^{-1}. The oxygen content of water was measured using an oxygen meter (Cole Parmer model 5946-70). A known volume of well

mixed sample of each sampling site was filtered through a standard fiberglass filter. The filtrate was then evaporated in a pre-weighed dish to constant weight at 180˚C. The difference between the dish weight and the final weight represents the total dissolved solids according to APHA (1985) methodology. The pH was measured using a digital pH meter (Cole-Parmer model 5938-50). Nitrate, nitrite, ammonium, and phosphorus were determined according to Parsons *et al*., (1984), and the chemical oxygen demand (COD) was determined according to Strickland and Parsons (1968).

Cyanobacterial productivity and species abundance

The collected water samples were divided into three portions to three sub samples; the first was used for fixation and counting of algae, the second to provide fixed materials for making immediate identifications of algal taxa.

Algal counts and identification of algal species

Counting of phytoplankton species was carried out using the Sedgwick-rafter counting cell, as recommended by APHA (1985). Species identifications were carried out using a phase contrast microscope (Carl Zeiss, Jena) according to Prescott (1978), Humm and Wickes (1980) and Hindak (1984, 1988 and 1990).

Statistical Analysis

A two -way analysis of variance (2-way ANOVA) was used to detect the significance of the differences among the data between seasons at each selected site in one direction and among the different sites in each season in the second direction using GraphPad Prism (version 5), and the mean separation was detected using Bonferroni post-tests (Motulsky, 2007). Canonical Correspondence Analysis (CCA) was used to assess the relationships between phytoplankton species distribution and the environmental parameters. The number of individuals of each algal species and the measured environmental parameters were used in the ordination of this technique (Ter Braak and Prentice 1988). This multivariate direct gradient analysis produces a biplot graphical presentation of the interrelationships between species distribution and environmental variables in each season. CCA was performed using CANOCO for Windows version 4.5.2 (Ter Braak and Smilauer, 2002).

Results

Physicochemical parameters

Variations in water temperature exhibited a general seasonal trend across the different studied sites. A significant difference ($P<0.01$) was detected among different seasons, forming obvious seasonal variations. The water temperature ranged from 26–31˚C all sampling sites during summer and 16–18˚C during winter (Table 2).

Table 2. Temperature, Turbidity, and Conductivity of the water samples collected in different seasons from Port-Said freshwater canal and Rosetta Branch sites indicated as mean of samples and sites ± SE.

Season	Port-Said freshwater canal		
	Temperature	**Turbidity**	**Conductivity**
Autumn 2007	25.9^{B} ± 0.97	19.1^{C} ± 2.38	387^{E} ± 3.3
Winter 2007/2008	19.5^{D} ± 0.48	11.4^{E} ± 0.81	480^{B} ± 4.7
Spring 2008	23.3^{C} ± 0.47	13.1^{D} ± 1.02	398^{D} ± 6.7
Summer 2008	29.3^{A} ± 0.22	21.0^{B} ± 1.36	390^{DE} ± 4.3
Autumn 2008	24.3^{BC}± 0.68	24.9^{A}± 2.12	417^{C}± 6.5
Winter 2008/09	17.6^{E} ± 0.09	9.9^{E} ± 0.54	492^{A} ± 3.2
Rosetta Branch			
	Temperature	**Turbidity**	**Conductivity**
Autumn 2007	25.8^{B} ± 0.97	14.0^{C} ± 0.93	1060^{B} ± 42.0
Winter 2007/2008	18.1^{D} ± 0.27	7.1^{E} ± 1.30	787^{E} ± 29.0
Spring 2008	22.8^{C} ± 0.56	19.0^{B} ± 0.88	561^{F} ± 21.6
Summer 2008	28.3^{A} ± 0.43	21.5^{A} ± 2.39	963^{C} ± 29.4
Autumn 2008	22.2^{C} ± 0.68	10.0^{D} ± 1.31	1144^{A} ± 49.5
Winter 2008/09	16.9^{D} ± 0.09	10.1^{D} ± 0.98	903^{D} ± 21.2

Same letters in each column is not significantly different $P>0.05$

The determination of turbidity levels throughout the period of study indicated seasonal variations ranging between 1.5 and 87 NTU. No significant differences were detected among the different sampling sites in Port-Said, whereas significantly higher values were observed in the sampling sites on the Rosetta Branch ($P<0.05$). Additionally, significant seasonal variations ($P<0.01$) were detected where the highest turbidity (24.9 NTU) was recorded in autumn 2008 in Port-Said followed by summer 2008 in Rosetta and Port-Said (Table 2).

Electrical conductivity values (Table 2) varied widely between the sites along the Rosetta Branch throughout the period of study. Their values ranged between 362 µmhoscm^{-1} and 7670 µmhoscm^{-1} at site I in June 2008 and site V in May

2008, respectively. Significant differences were detected only between Rosetta Branch sites; however, significant differences among seasons ($P<0.01$) were also observed.

No significant differences were detected among the pH values of the different Port Said sampling sites or among seasons. The lowest pH was recorded as 7.33 at site number V "Kafr El-Zayat" on the Rosetta Branch during December 2007. The highest pH of 7.95 was recorded in winter 2009 followed by autumn 2007 (Table 3). The water alkalinity at different sampling sites showed a general seasonal trend. It ranged between 110 and 348 mg l^{-1} except at site V, where the highest alkalinity was recorded, reaching 660 mg l^{-1} in June 2008 (Table 3). Significant differences were detected among the different sampling sites ($P<0.05$) and seasons ($P<0.01$).TDS varied widely between the Port Said canal and Rosetta Branch sampling sites along the study period. The significant highest value, 4260 mg l^{-1}, was recorded at site VII in April 2008. Additionally, significant differences were detected among seasons (Table 3).

The nitrate content of the water samples ranged between 2.5 and 33.3 µg l^{-1} throughout the period of study (Figure 2). Statistical analysis showed significant differences among different sampling sites and seasons. The highest levels were recorded during September 2008 at the Edvena site (VI). The highest levels were recorded during autumn and winter, whereas the lowest values were recorded in summer and spring (Table 4). The trend observed for nitrate was present in the ammonium concentrations of the water samples, where significant differences were detected among different sampling sites and seasons (Table 4). The highest levels were recorded during autumn and winter, whereas the lowest values were recorded in summer and spring. Ammonium levels ranged between a minimum of 188 µg l^{-1} at site V in August 2008 and a maximum of 542 µg l^{-1} at site V in March 2008 (Figure 3).

The nitrite content varied significantly among sampling sites ($P<0.01$) and among seasons ($P<0.05$), with values ranging between 0.2 and 5.5 µg l^{-1} (Figure 4). However, a different trend was observed compared to the patterns of other nutrients; for nitrite, the highest levels were recorded during summer and spring, whereas the lowest values were recorded in winter and autumn (Table 4). No significant trends were observed in the phosphorus level during the different seasons or among the different sites (Table 4), and PO_4 concentrations ranged from 23-44 µg l^{-1} throughout the period of study.

The dissolved oxygen had significant differences among seasons, where the highest content was during autumn and winter. The values ranged between a minimum of 6.3 mg l^{-1} at site V in July 2008 and a maximum value of 9.6 mg l^{-1} at site II in November 2007. No significant differences were recorded among the different sampling sites in Port-Said; however, significant differences were observed among the different sampling sites in the Rosetta Branch (Table 5). COD levels ranged between 1.2 and 37.4 mg l^{-1}; the highest value was observed at site II in September 2007. Statistical analysis indicated significant variations among different sampling sites and seasons (Table 5).

Cyanophyta composition

The cyanobacterial density was monitored monthly from September 2007 to March 2009 at four and three selected sites in the Port-Said freshwater canal and the Rosetta Branch, respectively. The identification of Cyanophyta revealed the presence of two genera, *Oscillatoria* sp and *Microcystis* sp , that represented 70% of the cyanophyte count.

Microcystis aeruginosa Kuetz.

The results from the counts of *Microcystis aeruginosa* Kutzing in the water samples (Figure 5) showed general patterns of seasonal and spatial variability; the highest cell counts were recorded during the winter seasons, and the winter cell count of 2009 was higher than that of 2008. However, low numbers were recorded during spring and summer 2008. Density of *M. aeruginosa* Kuetz. was uniform (20-450 cells $ml^{-1)}$ throughout the period of collection. A sudden increase in the cell count was observed in January and February 2008, and a sudden decrease was recorded in June 2008. Gradual increases in cell counts were observed in autumn 2008, reaching a maximum value of 450 cells ml^{-1} in January 2009, especially at site V (in the Rosetta Branch) and 320 cells ml^{-1} at site IV (at the end of Port-Said freshwater canal), followed by a sharp decrease in cell counts in spring and summer.

Oscillatoria brevis Kuetz.

A similar trend was observed in *Oscillatoria brevis* Kuetz. (Fig. 6) where high cell counts were also observed during winter 2009; however, the density of *O. brevis* was a magnitude of order higher than of *M. aeruginosa;* the cell count for *O. brevis* ranged from 30-4300 cells ml^{-1} throughout the period of sampling collection. A gradual increase in cell counts was also observed in October 2009 for *O. brevis*, reaching a maximum value of 4300 cells ml^{-1} in January 2009 in site V (Rosetta Branch) and 3200 cells ml^{-1} in site IV (at the end of the Port Said canal). This was followed by a sharp decrease similar to that observed in the *M. aeruginosa* 2009 profile.

Oscillatoria princeps Vaucher

Although the cell counts of *Oscillatoria princeps* Vaucher (Figure 7) were not as high as those of *O. brevis*, the same trend was recorded for the seasonal profile in 2008 and 2009. Counts of *O. princeps* Vaucher ranged from 20 to 340 cells ml^{-1} throughout the study period, reaching a maximum in January 2009 with the highest densities being 340 cells ml^{-1} in site VII (Rosetta Branch) and 140 cells ml^{-1} in site II (at the end of the Port-Said canal) followed by a sharp decrease as observed in the *O. brevis* Kuetz., 2009 profile.

Discussion

The winter cyanobacterial bloom in parts of the Nile River was primarily due to *Oscillatoria brevis*. *Microcystis aeruginosa* and *Oscillatoria princeps* were also present during the bloom. Among the seven sites under study, site IV (at the end of the Port-Said canal) and site V (in the Rosetta Branch) seasonal and spatial variability showed the highest cell counts. Therefore, a CCA analysis was performed for these 2 sites to explore the possible causes of such a bloom at these 2 particular sites. Figure 8 represents the output of the CCA analysis in the form of a triplot illustrating the interrelationships among the six seasons of the current study (autumn 2007 to winter 2009), the three studied phytoplankton species and the nine estimated physicochemical parameters of water.

The results showed that the variations in phytoplankton density were primarily affected by the NO_3-N and NH_4-N concentrations followed by PO_4 and NO_2-N. Lower turbidity, alkalinity and temperatures as well as higher electric conductivity also affected the occurrence of higher cell counts of cyanobacteria. Figure 8 demonstrates the seasonal impact on the appearance of the bloom where the highest bloom occurred in winter 2008/2009 followed by autumn 2008 and winter 2007/2008. Summer 2008, spring 2008 and autumn 2008 showed no effect on the appearance of the bloom during these seasons.

In the present study, a winter bloom of *O. brevis* along with *M. aeruginosa* and *O. princeps* was observed where the temperature at sampling sites ranged between 16.9 and 17.6˚C in winter, whereas it ranged between 28.3 and 29.3˚C in summer. This result was previously explained by Robarts and Zohary (1987), who reported that in temperate water bodies, most cyanobacteria blooms are likely to occur during the summer, whereas in tropical and subtropical zones, cyanobacteria bloom in winter.

The water was slightly alkaline as reported earlier (Gomaa *et al*., 2000). Conditions of high pH or low CO_2 availability commonly favour cyanobacterial growth (Shapiro, 1990). On a daily basis, dense aggregations of cyanobacteria result in early morning minima in pH and dissolved oxygen with late afternoon maxima in both variables.

Alkalinity and pH may affect the dominance of cyanobacteria in aquatic ecosystems by influencing the availability of inorganic carbon. Reynolds and Walsby (1975) noted that cyanobacteria generally grow more abundantly in hard-water lakes, most likely because the CO_2 concentration is greater. Because the availability of CO_2 from the bicarbonate-carbonate alkalinity system in water decreases with increasing pH, the cyanobacteria should successfully out-compete other types of algae in low-alkalinity waters, especially during the summer when high photosynthesis rates extract CO_2 from the alkalinity system, thus increasing water pH. The results of this study showed that alkalinity levels ranged between 154 and 223 mg l^{-1} in winter and between 167 and 356 mg l^{-1} in summer. This means that low alkalinity during the winter months may be one of the factors influencing the winter cyanobacterial bloom.

Codd (1999) attributed the dominance of bloom-forming *Aphanizomenon flos-aquae*, *Anabaena circinalis* and *Anabaena oscillarioides* to their ability to rise to the surface of lakes when CO_2 concentrations in the water column were low to take advantage of atmospheric CO_2 for photosynthesis. Both high pH, which algal photosynthesis itself creates, and low alkalinity, which reduces the concentration of CO_2 in the alkalinity system, were conditions associated with bloom formation. The lower turbidity levels reported in the winter periods of this study (2.7 and 26.7 mg l^{-1}) compared to the higher levels in summer (9.7 and 87.7 mg l^{-1}) confirmed that low turbidity levels during winter may be one cause of the winter cyanobacterial bloom. This is primarily because increased turbidity resulting from runoff or phytoplankton growth reduces the light availability below the surface, which in turn affects the intensity of photosynthesis and the distribution of phytoplankton (Communis, 1973 and Chalar, 2009).

In this study, the levels of nitrate, ammonium and phosphorus were higher in winter than in other seasons. The amount of nutrient discharge to the Nile River is approximately the same during all seasons; however, the higher nutrient concentrations in winter are primarily due to the lower water level during winter. These higher levels of nutrients in winter may also help explain the occurrence of cyanobacterial blooms in winter. Cyanobacteria blooms occur when ammonium concentrations are very high (Chellappa *et al.*, 2000) and sufficient phosphate is available (Gabriela and Alessandra, 2004). Thus, both nitrogen and phosphorus have been found to influence phytoplankton abundance (Gabriela and Alessandra, 2004, Addico and Frempong, 2004).

The maximum cell counts recorded in this study for *O. brevis* Kuetz., reaching 4300 cells ml^{-1} in January 2009 in site V (Rosetta Branch) and 3200 cells ml^{-1} in site IV (at the end of the Port Said canal) was much lower than those recorded by Bowling (1994), who reported that cell numbers of a cyanophyte bloom in the Lachlan River valley upstream of Lake Cargelligo during the winter of 1990 exceeded 100,000 cells ml^{-1}. However, our recorded cell counts are not far from the two guidance levels for cyanobacterial growth in sources of drinking water supplies that were established by the World Health Organization (WHO, 1998) and have also been suggested in Australia. The WHO's threshold for alert level 1 is 2000 cyanobacterial cells ml^{-1}, and the threshold for alert level 2 is 100,000 cyanobacterial cells ml^{-1} (Bartram *et al*., 1999). In Korea, an alert system for algal blooms was established in 1997 by the Ministry of Environment (Ahn *et al*., 2003), in which the caution, warning and outbreak levels were defined as cyanobacterial cell densities of 500, 5000 and 1000 000 cells ml^{-1}, respectively.

The *Oscillatoria brevis* winter bloom incident that occurred in the freshwater canal of Port Said, Egypt in 1995 and reoccurred for 4 consecutive years were reported by Gomaa *et al.*, (2000). During these episodes, the frequent clogging of

filters in the drinking water treatment plant along with an unpleasant taste and odor of drinking water were the primary problems. The winter bloom of *O. brevis* then expanded against the current from Port-Said to the cities of Ismailia and Suez along the Ismailia Canal, one of the branches of the Nile River, with the appearance of the new species *Oscillatoria princeps* and *Microcystis aeruginosa* in the bloom profile; however, *O. brevis* remained the dominant bloom component with the highest cell count numbers (El-Manawy and Amin, 2005; Mohammed, 2010). The expected expansion of this winter cyanobacterial bloom to other branches of the Nile River, hypothesized in the objectives of this study, was confirmed by the detection of a cyanobacterial winter bloom with the same species profile in the west branch of the Nile Delta (Kafr El-Zayat site VII of the Rosetta Branch).

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