



Microalgal diversity in response to differential heavy metals-contaminated wastewater levels at North Nile Delta, Egypt

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Background: The most hazardous wastewater sources in the northern part of the Middle Nile Delta, Egypt; receiving a massive amount of agricultural, industrial, and sewage drainage are Kitchener drain which is one of the tallest drainage systems, and Burullus Lake which represents the 2nd largest Egyptian coastal lake.

Results: The current work is to determine the abundance and frequency of cyanophytes, chlorophytes, and bacillariophytes and the correlation between them and environmental abiotic components. Among sixty nine microalgal species, 19 species are belong Cyanophyta, 26 belong Chlorophyta and 24 belong Bacillariophyta. Genus *Scenedesmus* (Chlorophyta) was the most abundant in the study area (13 species), followed by Genus *Oscillatoria* (9 species) and Genus *Navicula* (7 species). *Nostoc muscorum* and *Chlorella vulgaris* were the most common and recorded in all sites (100% of the locations) under study. The application of the two-way indicator species analysis (TWINSPAN) and detrended correspondence analysis revealed agglomerating of 4 groups (communities) at 4th level of classification and reasonable segregation between these groups. Zinc, cadmium and lead were showed the highest levels (0.26 ± 0.03 , 0.26 ± 0.06 , and 0.17 ± 0.01 ppm, respectively).

Conclusions: The correlation analysis between water and community variables indicated a high negative correlation of total algae richness with nickel ($r = -0.936$, $p < 0.01$). Cyanophyta and Bacillariophyta were correlated negatively ($r = -0.842$, $p < 0.01$). However, Chlorophyta showed a negative richness with each of Ni and Pb ($r = -0.965$, -0.873 , respectively) on one hand and a high positive correlation was revealed ($r = 0.964$) with all environmental variables on the other hand.

Keywords: Burullus Lake, heavy metals, Kitchener drain, microalgae, sewage drainage

Introduction

Microalgae are microscopic, photosynthetic, fast growing aquatic organisms (Suthers et al. 2019) that inhabit wide range of diverse habitats; freshwater, brackish, saline water and wastewater environmental conditions (Neto and Pinto 2018). So they can degrade and/or absorb nutrients, heavy metals, organic and inorganic components as well as other pollutants from wastewater that might be required for their growth (Supeng et al. 2012). In this respect, Srimongkol et al. (2022) stated that quaternary and tertiary wastewater remediation can be sustained by using microalgae cultures as they have the ability to degrade complex pollutants.

The dominant species that are recorded in the mild polluted zones belong to genera *Nitzschia palea* and *Gomphonema parvulum*, *Cocconeis*, and *Chamaesiphon* were previ-

ously recorded in unpolluted zone (Venkatachalapathy et al. 2013). A good indicator of organic pollution was *Navicula accomda* whereas *Gamophema* was reported in severe polluted water (Archibald 1972). In narrow scale, wastewater algal-treatment is conventional and beneficial for growth in pH, salt etc., it fixes carbon dioxide and produces biomass with greater feasibility.

Any metal or related metalloid that has high-density and is not biodegradable producing toxicity at low strength and environmental pollution would be referred as heavy metal (Herrera-Estrella et al. 2001). Some heavy metals are essential for plant growth (e.g., Fe, Zn, Ni, Cu, Mn, and Co) whereas, they have a vital biochemical function or physiological role in plants. Such essential heavy metals cause toxic effects at high levels. Other elements are not essential for plant since they have not any biochemical function or



physiological role and produce toxic effects at low strengths (e.g., Pb, Hg, and Cd) (Gaur and Adholeya 2004). Cr, Pb, Hg, Cd, and the other heavy metals are highly toxic to living organisms could be removed or mitigated by microalgae and cyanobacteria that is known as phycoremediation. Different mechanisms depending on the identity of treated heavy metal, algal species and the treatment conditions could be mediated the phycoremediation process (González-Dávila 1995; John 2003; Suresh Kumar et al. 2015).

Singh et al. (2021) recorded a variable algal effective removable of N (90%–98.4%), P (66%–98%), Pb (75%–100%), Zn (15.6%–99.7%), Cr (52.54%–96%), Hg (77%–97%), Cu (45%–98%), and Cd (2%–93.06%) from heavy metal-contaminated aquatic systems. So, microalgae are considered good bio-indicators for pollution in the environment.

Previous studies of heavy metals biosorption revealed that microalgae have the greatest potential compared to other biosorbents. Algae were more efficient in heavy metals biosorption by about 15.3%–84.6% greater than other microorganisms; bacteria and fungi (Brinza et al. 2007; Mustapha and Halimoon 2015; Sweetly 2014). Recently, Priya et al. (2022) stated that using microalgal-based bioremediation strategy in wastewater treatment instead of traditional methods has grown in importance due to their ability to produce a large biomass with short life span and assimilate great amounts of CO₂.

Early, Shehata and Bader (1985) found that the most algal communities in river Nile were green algae, blue-green algae, and diatoms. Also, Talling et al. (2009) examined the phytoplankton community's composition and abundance in regions of Nile system and reported that diatoms are the majority in seasonal sequences which ended by high turbidity of nutrients-rich floodwater. Under current water defiance, figuring a map for distribution, abundance and

frequency of algal community in response to heavy metal toxicity-tolerance in current study is very important to design further phycoremediation strategy. Statistical analysis namely, the two-way indicator species analysis (TWIN-SPAN) and detrended correspondence analysis (DCA) techniques were used to reveal the distribution and classification of algal communities, the effects of environmental constituents namely heavy metals levels in wastewater of each of Kitchener drain and Burullus Lake on algal communities composition and to detect the correlation between different water-heavy metals constituents and algal communities distribution.

Materials and Methods

Study area

The study area was a part of the Nile Delta, Egypt which comprises about 63% of Egypt's productive agricultural area (Fig. 1). It is bounded by the Mediterranean Sea at the north with an area of about 22,000 km² (Shaltout and Al-Sodany 2008). The northern part of Nile Delta is characterized by three Northern shallow brackish lakes, namely from east to west: Manzalla, Burullus, and Idku, which are joined to the Mediterranean by narrow gaps in the sandy bars. Lake Burullus is a shallow brackish lake extending for 47 km along the deltaic Mediterranean coast of Egypt with an area of about 57,426 ha (Abu Al-Izz 1971). El-Gharbia Main Drain (about 68.3 km length) discharges, partly into Lake Burullus and Mediterranean Sea. This drain catches its drainage water from south Nile Delta at east El-Gharbia Governorate to Lake Burullus at the North Nile Delta passing through Kafr El-Sheikh Governorate (El-Shinnawy 2002). It receives domestic, industrial and

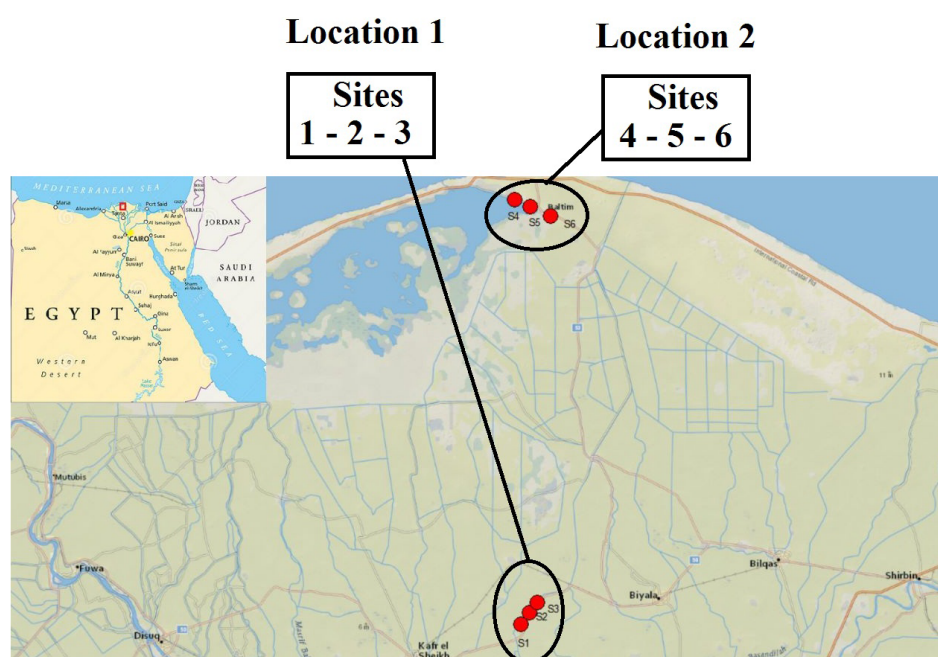


Fig. 1 Map of Kafr El-Sheikh Governorate showing the locations of collected samples, namely the Kitchener drain and Burullus Lake.

agricultural wastes from different pollution sources, and its severe stress on biological community is due to the interaction of all these pollutants. The assessment of its water quality has become a critical issue and an important demand due to the concern that it affects many important ecosystems and the fresh water will be a scarce resource in the future.

Collection of water samples and locations

Two highly polluted sites in north Delta, Egypt were chosen to carry out this study namely; Kitchener drain (31°02'39.6"E and 31°08'51.4"N) and Burullus Lake (31°04'30.4"E and 31°33'29.9"N) (Fig. 1). Water samples (from about 20 cm depth) were collected in triplicates into 2 liter sterilized capacity randomly from the different sites at the two studied locations during winter, January 2021. Abiotic measurements were applied either in field or in lab.

Analysis of macro and micronutrient

Water samples were digested before analysis by concentrated HNO_3 and concentrated HCl . Four elements, sodium (Na), calcium (Ca), potassium (K), magnesium (Mg), and six heavy metals, iron (Fe), zinc (Zn), lead (Pb), nickel (Ni), chromium (Cr), and cadmium (Cd) were measured using Atomic Absorption Spectrometry type (GBC Avanta E, Victoria, Australia) according to instrument manual.

Physicochemical analysis of wastewater samples

Physical analysis

Temperature, pH, electrical conductivity, and total dissolved salts values of different wastewater samples were recorded directly in sampling locations using portable field meters; thermometer (SH-104; YuYao Shuanghe Electron Instrument Co., Ltd., Zhejiang, China), pH meter (model; Hanna Instruments, Nusfalau, Romania), digital conductivity meter (model; Hanna Instruments) and total dissolved solids (TDS) meter (Milwaukee, made in Europe).

Chemical analysis

As described by Allen (1974); soluble carbonates and bicarbonates were estimated by titration against 0.01 N HCl using phenolphthalein and methyl orange as indicators, respectively. For determination of sulphate content in wastewater samples, gravimetrically method by using 5% solution of BaCl_2 was followed as described by United Nations Environment Programme (2004) and American Public Health Association (1976), while chlorides were estimated by titration against $\text{N}/35.5 \text{ AgNO}_3$ (Patnaik 1997).

Identification of collected samples microalgae (biological measurement)

Using light microscopic characterization, the microalgae species were morphologically identified (Hegewald and Schmidt 1992; Khaybullina et al. 2010; Komárek et al.

2013). Names of the isolated algal morphospecies were taxonomically validated following the Algae Base international database (Guiry and Guiry 2013).

Refresh of algal strains

For refreshing algal strains, 1 mL aliquot of each sample was transferred to sterile 25 mL flasks containing 10 mL of BG11 and Bold basal media. After one incubation week at $80 \mu\text{E}/\text{m}^2$, 24 hours white fluorescent light and 30°C , green, yellowish green and/or blue green color were recorded (El-Naggar et al. 1999).

Data and statistical analysis

Multivariate analyses were applied to evaluate the recognized microalga communities in the study area using classification and ordination techniques from presence percentage of 69 microalgae species. Applied classification technique was the TWINSpan, while DCA was used for ordination (Gauch and Whittaker 1981; Hill 1979a, 1979b; Hill and Gauch 1980). Species turnover (beta-diversity) is calculated as a ratio between the total number of species recorded in a certain site and its alpha diversity (Whittaker 1972). Relative evenness or equitability (Shannon–Weaver index) of the importance value of species was expressed as $\hat{H} = -\sum^s P_i (\log P_i)$, where S is the total number of species and P_i is the relative importance value (relative cover) of the species. The relative concentration of dominance is the second group of heterogeneity indices and expressed by Simpson's index: $C = \sum^s (P_i)^2$, where S is the total number of species and P_i is the relative importance value (relative cover) of species. About these indices, available details could be obtained from Pielou (1975) and Magurran (1988). In order to detect correlations between Cyanobacteria parameters and environmental data, canonical correspondence analysis (CCA) according to Ter Braak and Smilauer (2002) was conducted with species recorded in different sites of Kitchener drain and Burullus Lake variables using the second matrix (Al-Sodany et al. 2018). Means, standard deviations and one-way analysis of variance (ANOVA) were calculated for the means of the water variables in relation to sites (altitudes) to assess the heterogeneity of samples around their means. These techniques were according to IBM SPSS statistics 20 (IBM Co., Armonk, NY, USA).

Results

Sixty nine microalgal species (19 Cyanophyta, 26 Chlorophyta, and 24 Bacillariophyta) were detected along the different locations of the study area belong to 36 genera (Table 1). *Scenedesmus* had the highest contribution in the study area (11 species), followed by *Oscillatoria* (9 species) and *Navicula* (7 species), while *Nostoc muscorum* and *Chlorella vulgaris* had the highest contribution (100% of

Table 1 Algal species composition and their percentage abundance in different sites of Kitchener drain and Burullus Lake at North Nile Delta, Egypt

Species	Code	Locations/sites						P (%)	Total No. of species/location	
		Kitchener			Burullus				Kitchener	Burullus
		1	2	3	4	5	6			
Cyanophyta										
<i>Anabaena affinis</i>	<i>Anab aff</i>						12.9	16.7		1
<i>Aphanothece microscopica</i>	<i>Apha mic</i>		2.4					16.7	1	
<i>Chroococcus minutus</i>	<i>Chr minu</i>		2.4					16.7	1	
<i>Chroococcus minor</i>	<i>Chr mino</i>		2.4					16.7	1	
<i>Cyanarcus hamiformis</i>	<i>Cyan ham</i>		2.4					16.7	1	
<i>Lyngbya hieronymusii</i>	<i>Lyng hier</i>		2.4					16.7	1	
<i>Merismopedia elegans</i>	<i>Meri ele</i>						6.5	16.7		1
<i>Nostoc commune</i>	<i>Nos commi</i>						3.2	16.7		1
<i>Nostoc muscorum</i>	<i>Nos musc</i>	7.4	7.3	1.8	4.4	11.1	3.2	100.0	3	3
<i>Nostoc comminutum</i>	<i>Nos commu</i>						3.2	16.7		1
<i>Oscillatoria prolifica</i>	<i>Osci pro</i>	22.2						16.7	1	
<i>Oscillatoria anguina</i>	<i>Osci ang</i>		2.4					16.7	1	
<i>Oscillatoria sancta</i>	<i>Osci san</i>					11.1		16.7		1
<i>Oscillatoria bornetii</i>	<i>Osci bor</i>		2.4					16.7	1	
<i>Oscillatoria minima</i>	<i>Osci min</i>							16.7		1
<i>Oscillatoria limosa</i>	<i>Osci lim</i>		2.4					16.7	1	
<i>Oscillatoria princeps</i>	<i>Osci pri</i>		2.4					16.7	1	
<i>Oscillatoria curviceps</i>	<i>Osci cur</i>		7.3					16.7	1	
<i>Oscillatoria tenuis</i>	<i>Osci ten</i>	29.6						16.7	1	
Chlorophyta										
<i>Chlorella vulgaris</i>	<i>Chlo vul</i>	22.2	9.8	5.5	17.4	11.1	16.1	100.0	3	3
<i>Chlorella ellipsoidea</i>	<i>Chlo ell</i>		4.9					16.7	1	
<i>Characium limneticum</i>	<i>Char lim</i>						3.2	16.7		1
<i>Chlorococcum humicola</i>	<i>Chlo hum</i>		2.4					16.7	1	
<i>Cosmarium</i> sp.	<i>Cosmarium</i>			1.8		5.6	3.2	50.0	1	2
<i>Chlamydomonas globosa</i>	<i>Chla glo</i>					11.1		16.7		1
<i>Crucigenia tetrapedia</i>	<i>Cruc tet</i>				8.7			16.7		1
<i>Crucigenia quadrata</i>	<i>Cruc qua</i>			1.8				16.7	1	
<i>Kirchneriella lunaris</i>	<i>Kirc lun</i>					5.6		16.7		1
<i>Pandorina morum</i>	<i>Pand mor</i>			3.6			12.9	33.3	1	1
<i>Palmodictyon viride</i>	<i>Palm vir</i>					5.6		16.7		1
<i>Quadrigula chodatii</i>	<i>Quad cho</i>			1.8				16.7	1	
<i>Scenedesmus abundans</i>	<i>Scen abu</i>		7.3				3.2	33.3	1	1
<i>Scenedesmus dimorphus</i>	<i>Scen dim</i>		4.9	14.6				33.3	2	
<i>Scenedesmus opoliensis</i>	<i>Scen opo</i>		2.4	1.8				33.3	1	
<i>Scenedesmus bernardii</i>	<i>Scen bar</i>			3.6				16.7	1	
<i>Scenedesmus bijuga</i>	<i>Scen bij</i>			12.7		5.6		33.3	1	1
<i>Scenedesmus acutiformis</i>	<i>Sce acum</i>			1.8				16.7	1	
<i>Scenedesmus acuminatus</i>	<i>Sce acut</i>			1.8				16.7	1	
<i>Scenedesmus quadricauda</i>	<i>Scen qua</i>			5.5				16.7	1	
<i>Scenedesmus incrassatulus</i>	<i>Scen inc</i>		14.6	14.6	4.4			50.0	2	1
<i>Scenedesmus hystrix</i>	<i>Scen hys</i>		4.9					16.7	1	
<i>Scenedesmus arcuatus</i>	<i>Scen arc</i>		2.4	9.1		5.6		50.0	2	1
<i>Selenastrum minutum</i>	<i>Sele min</i>			1.8				16.7	1	
<i>Westella linearis</i>	<i>West lin</i>		2.4	1.8			3.2	50.0	2	1
<i>Elakatothrix viridis</i>	<i>Elak vir</i>					5.6		16.7		1
Bacillariophyta										
<i>Amphora ovalis</i>	<i>Amph ova</i>						3.2	16.7		1
<i>Campylodiscus bicostatus</i>	<i>Camp bic</i>			1.8	17.4			33.3	1	1
<i>Campylodiscus noricus</i>	<i>Camp nor</i>				13.0			16.7		1
<i>Caloneis amphisbaena</i>	<i>Cal amph</i>			1.8				16.7	1	
<i>Craticula cuspidate</i>	<i>Crat cus</i>			1.8				16.7	1	
<i>Cymbella tumidula</i>	<i>Cymb tum</i>					5.6		16.7		1
<i>Cymbella aequalis</i>	<i>Cymb aeq</i>						6.5	16.7		1

Table 1 Continued

Species	Code	Locations/sites						P (%)	Total No. of species/location	
		Kitchener			Burullus				Kitchener	Burullus
		1	2	3	4	5	6			
<i>Diatoma mesodon</i>	<i>Diat mes</i>				8.7			16.7		1
<i>Diatoma vulgaris</i>	<i>Diat vul</i>				4.4			16.7		1
<i>Martyana martyi</i>	<i>Mart mar</i>				4.4			16.7		1
<i>Navicula lanceolata</i>	<i>Navi lan</i>			1.8				16.7	1	
<i>Navicula oblonga</i>	<i>Navi obl</i>	7.4						16.7	1	
<i>Navicula ignota</i>	<i>Navi ign</i>					5.6		16.7		1
<i>Navicula radiosa</i>	<i>Navi rad</i>			1.8			3.2	33.3	1	1
<i>Navicula cryptocephala</i>	<i>Navi cry</i>			1.8				16.7	1	
<i>Navicula rhynchocephala</i>	<i>Navi rhy</i>					5.6		16.7		1
<i>Navicula angusta</i>	<i>Navi ang</i>			1.8			3.2	33.3	1	1
<i>Nitzschia solita</i>	<i>Nitz sol</i>	7.4						16.7	1	
<i>Pinnularia viridis</i>	<i>Pinn vir</i>	3.7						16.7	1	
<i>Stauroneis phoerocenteron</i>	<i>Stau pho</i>			1.8			3.2	33.3	1	1
<i>Stepanodiscus neoastraea</i>	<i>Step neo</i>				4.4			16.7		1
<i>Synedra rumpens</i>	<i>Syne rum</i>					5.6		16.7		1
<i>Synedra ulna</i>	<i>Syne uln</i>		7.3					16.7	1	
<i>Tabellaria flocculosa</i>	<i>Tabe flo</i>				13.0			16.7		1

P is the presence percentage of algal species in the six locations.

Table 2 The population abundance of the different algal groups, throughout the different sites of the two studies locations

Divisions	Sites						Locations	
	1	2	3	4	5	6	Kitchener	Burullus
Cyanophyta								
Total groups	16	15	1	1	4	12	32	17
No. of genera	2	6	1	1	2	4	6	4
No. of species	3	11	1	1	2	6	13	7
Chlorophyta								
Total groups	6	23	46	7	10	13	75	30
No. of genera	1	4	8	3	7	6	9	11
No. of species	1	10	16	3	8	6	20	14
Bacillariophyta								
Total groups	5	3	8	15	4	6	16	25
No. of genera	3	1	5	5	3	4	8	10
No. of species	3	1	8	7	4	5	12	16
Total								
Total groups	27	41	55	23	18	31	123	72
No. of genera	6	11	14	9	12	14	23	25
No. of species	7	22	25	11	14	17	45	37

the locations). Regarding sites, 45 algal species (32 species of them as a unique species) were recorded in Kitchener drain, and 37 species (24 species of them as a unique species) in Burullus Lake. As indicated in Table 1, the species: *Nostoc muscorum* (Cyanophyta), *Chlorella vulgaris*, *Cosmarium* sp., *Pandorina morum*, *Scenedesmus abundans*, *S. arcuatus*, *S. bijuga*, *S. incrassatus*, *Westella linearis* (Chlorophyta), and *Campylodiscus bicostatus*, *Navicula radiosa*, *N. angusta*, *Stauroneis phoerocenteron* (Bacillariophyta) are recorded in both Kitchener and Burullus sites. However, *Nostoc muscorum* (Cyanophyta), *Chlorella vulgaris*, *Scenedesmus dimorphus*, *S. incrassatus*, *S. arcuatus*, and *Westella linearis* (Chlorophyta) are the most dom-

inant in Kitchener drain, while Lake Burullus was dominated by *Nostoc muscorum* (Cyanophyta), *Chlorella vulgaris*, and *Cosmarium* sp. (Chlorophyta).

On the other hand, location 1 at Kitchener drain was characterized by high diversity in all microalgal species as compared with Burullus Lake except the number of genera in both Chlorophyta and Bacillariophyta and the number of Bacillariophyta species (Table 2). It had the highest value of total number of cyanobacteria (13 species = 93.6% of the total species), Chlorophyta (20 species) and total algal species (45 species = 64.3% of the total species).

The application of the TWINSpan to the data set of the presence percentage of 69 microalgal species in 6 locations,

resulted in agglomerating of 4 groups (communities) at 4th level of classification (Fig. 2). The application of the DCA indicates reasonable segregation between these groups. The first group (community I) are dominated by 8 algal species (*Crucigenia tetrapedia*, *Campylodiscus bicostatus*, *C. noricus*, *Diatoma mesodon*, *D. vulgaris*, *Martyana martyi*, *Stepanodiscus neoastrea*, *Tabellaria flocculosa*), community II are dominated by 3 algal species (*Nostoc muscorum*, *Chlorella vulgaris*, *Scenedesmus incrassatulus*); community III are dominated by 5 algal species (*Oscillatoria prolifica*, *O. tenuis*, *Navicula oblonga*, *Nitzschia solita*, *Pinnularia viridis*) and community IV are dominated by 54 species.

The application of CCA on the cyanobacteria communities and water variables indicated that some cyanobacteria species are correlated with cadmium and zinc such as *Nostoc muscorum* and *Oscillatoria sancta*, while others are correlated with carbonates and nickel such as *Oscillatoria prolifica* and *O. princeps* (Fig. 3). Regarding to Chlorophyta, some microalgae such as *Westella linearis* and *Scenedesmus abundans* are correlated with Zn and Cd; *Scenedesmus bijuga*, *S. arcuatus*, *S. dimorphus*, and *S. incrassatulus* are correlated with carbonates and Fe; *Chlorella vulgaris*, *Pandorina morum*, *Characium limneticum*, and *Crucigenia tetrapedia* are correlated with most of water variables and *Cosmarium* sp. is correlated with pH (Fig. 3). On the other hand, some Bacillariophyta such as *Synedra ulna*, *Nitzschia solita*, and *Pinnularia viridis* are correlated with carbonates, while *Campylodiscus bicostatus*, *Tabellaria flocculosa*, and *Navicula lanceolata* are correlated with the most water variables (Fig. 3).

Generally, TDS, EC, sulphates, chlorides, sodium, magnesium, and potassium are differed significantly between water of different sites (Table 3). The Kitchner sites had the highest levels of zinc, cadmium, and lead (0.26 ± 0.03 , $0.26 \pm$

0.06 , and 0.17 ± 0.01 ppm, respectively), while the lowest of almost water variables. The Lake Burullus had the highest values of the most water variables. On the other hand, the correlation analysis between water and community variables (Table 4) indicated a highly negative correlation of total algae richness with nickel ($r = -0.936$, $p < 0.01$) and Bacillariophyta richness with Cyanophyta richness ($r = -0.842$, $p < 0.01$). The Chlorophyta richness had a negative correlation with nickel and lead ($r = -0.965$, -0.873 , $p < 0.01$, respectively) on one hand, but displayed a highly positive correlation ($r = 0.964$, $p < 0.01$) with all environmental variables on the other hand.

Discussion

Water properties are considered the key driving factors for abundant microalgae in lakes and water canals. Temperature and salinity are the main factors in controlling species distribution and abundance (Zakaria and El-Naggar 2019). Tavsanoğlu et al. (2015) showed that the changes in salinity will have a substantial impact on aquatic ecosystems, where it can result in osmotic stress on cells, uptake or loss of ions, and effects on the cellular ionic ratio. Dissolved oxygen is considered one of the maximum important factors controlling the biota in the aquatic habitat. Relatively high oxygen was observed during summer and autumn in Naubaria site which could be an indication of high phytoplankton counts. Increased human activity and discharged sewage containing high levels of nutritional salts as well as other toxic compounds had a significant impact on the biotic factors of several sites along the Mediterranean Sea coast (Farrag et al. 2019; Shaban et al. 2020). The obtained results confirm that the water properties and



Fig. 2 Dendrogram of the 4 groups or communities (I-IV) derived after the application of two-way indicator species analysis (TWINSpan) classification technique.

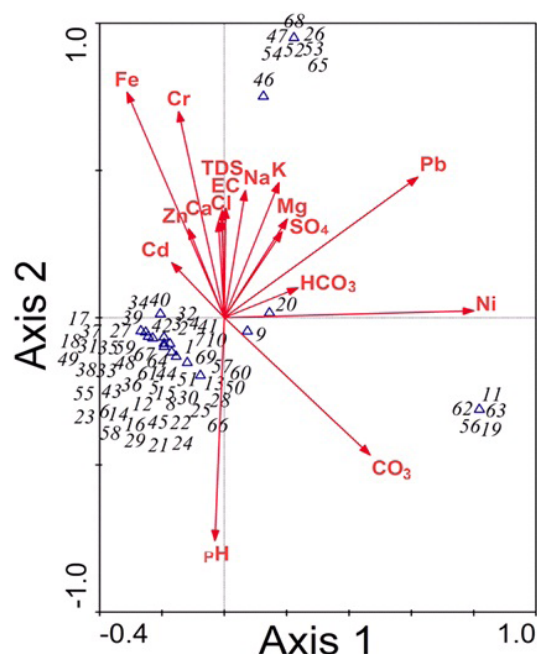


Fig. 3 Canonical correspondence analysis biplot with water characteristics (→) and species (Δ) of algae in Lake Burullus (Egypt): The algae species are organized as the following: 1: *Anabaena affinis*; 2: *Aphanothece microscopica*; 3: *Chroococcus minutus*; 4: *Chroococcus minor*; 5: *Cyanarcus hamiformis*; 6: *Lyngbya hieronymusii*; 7: *Merismopedia elegans*; 8: *Nostoc commune*; 9: *Nostoc muscorum*; 10: *Nostoc comminutum*; 11: *Oscillatoria prolifica*; 12: *Oscillatoria anguina*; 13: *Oscillatoria sancta*; 14: *Oscillatoria bornetii*; 15: *Oscillatoria minima*; 16: *Oscillatoria limosa*; 17: *Oscillatoria princeps*; 18: *Oscillatoria curviceps*; 19: *Oscillatoria tenuis*; 20: *Chlorella vulgaris*; 21: *Chlorella ellipsoidea*; 22: *Characium limneticum*; 23: *Chlorococcum humicola*; 24: *Cosmarium*; 25: *Chlamydomonas globosa*; 26: *Crucigenia tetrapedia*; 27: *Crucigenia quadrata*; 28: *Kirchneriella lunaris*; 29: *Pandorina morum*; 30: *Pinnularia viride*; 31: *Quadrigula chodatii*; 32: *Scenedesmus abundans*; 33: *Scenedesmus dimorphus*; 34: *Scenedesmus opoliensis*; 35: *Scenedesmus bernardii*; 36: *Scenedesmus bijuga*; 37: *Scenedesmus acutiformis*; 38: *Scenedesmus acuminatus*; 39: *Scenedesmus quadricauda*; 40: *Scenedesmus incrassatulus*; 41: *Scenedesmus hystris*; 42: *Scenedesmus arcuatus*; 43: *Selenastrum minutum*; 44: *Westella linearis*; 45: *Amphora ovalis*; 46: *Campylodiscus bicostatus*; 47: *Campylodiscus noricus*; 48: *Caloneis amphibaena*; 49: *Craticula cuspidate*; 50: *Cymbella tumidula*; 51: *Cymbella aequalis*; 52: *Diatoma mesodon*; 53: *Diatoma vulgaris*; 54: *Martyana martyi*; 55: *Navicula lanceolata*; 56: *Navicula oblonga*; 57: *Navicula ignota*; 58: *Navicula radiosa*; 59: *Navicula cryptocephala*; 60: *Navicula rhynchocephala*; 61: *Navicula angusta*; 62: *Nitzschia solita*; 63: *Pinnularia viridis*; 64: *Stauroneis phoerocenteron*; 65: *Stepanodiscus neoastreae*; 66: *Synedra rumpens*; 67: *Synedra ulna*; 68: *Tabellaria flocculosa*; 69: *Elakatothrix viridis*.

quality between the studied sites/sites on the one hand and between wastewater and brackish water on the other side, strongly influence the abundance and distribution of microalgae examined. The strong spatiotemporal variation in the correlations between algae and water quality indices suggested that the limiting factor for the dominant algae growth depends on seasonality and locations (Li et al. 2014; Wang et al. 2021). Our results mostly aligned with Heneash et al. (2022), where the species variation patterns

were significantly related to the environmental heterogeneity patterns. The quality of water sampling sites ranges towards contaminated environments and saline water. This may be owing to municipal pollutants which are pumped directly into the sampling sites by agricultural, industrial drains and sewage, leading to the seawater around the sampling sites becoming unsuitable for maritime life as a result of continuous pollution (Alprol et al. 2021).

As Kitchner drain is a freshwater canal that reached by several nutrients and pollutants (Badawy et al. 2022), Cyanophyta were recorded as the highly abundant organisms even at low and high pollutants, which explain their potential role in bioremediation of environment (Singh et al. 2016). After establishment the Egyptian High Dam, Kitchner drain and Lake Burullus receive almost regular freshwater drainage input due to regular passage of fresh water as well as regular and constant irrigation process (Dumont and El-Shabrawy 2007). This system leads to raising the water level to about 25–60 cm above sea level in the lake, preventing the seawater from entering the lake through the Burullus inlet (Dumont and El-Shabrawy 2007). The increase in freshwater budget combined with a reduction in salinity has resulted in the dominance of freshwater organisms which caused shifting in the biodiversity of the lake with hypereutrophy condition throughout the whole year (Younis 2019). These reports strongly explain abundant of several fresh water species in both locations (Kitchner drain and Lake Burullus) like *Nostoc muscorum* and *Chlorella vulgaris*. Here, the link between abiotic component and biotic microalgae one side and the brackish water and drain canal in the other side based on changes in the water quality and phytoplankton community are investigated. Chlorophyta and Cyanophyta are highly recorded in Kitchner drain, while Bacillariophyta are highly abundant in Burullus Lake. Kitchner drain is one of the biggest drain in Egypt that contains a lot of pollutants (e.g., Pb, Zn, and Cd), so it may explain high abundant and diversity of Cyanophyta and Chlorophyta. Obtained results came in agree with Søndergaard et al. (2011), Seif et al. (2018), and Fernandez-Figueroa et al. (2021). Bacillariophyta are almost abundant equally in both investigated environments.

High diversity of Bacillariophyta in Burullus Lake locations may reflect quite high salt concentration and high pollutants. Recorded TDS values of Burullus Lake was about 2,953.33 that higher than that recorded for Kitchner drain (781.33); and due to very narrow golfs to sea and high level of lake compared to sea, the establish environment becomes ideal for the growth of Bacillariophyta. Heneash et al. (2015) observed that emergence of Chlorophyta from dominant groups is abnormal recorded in the Mediterranean Sea water, which is accustomed to record Bacillariophyta and Dinophyta as dominant groups.

The correlation analysis between water and community variables indicated a high negative correlation of total algae

Table 3 Physicochemical variables of collected water samples from Kitchener drain and Burullus Lake (n = 3)

Parameters	Kitchener	Burullus	F-value
TDS (ppm)	781.33 ± 12.50	2,953.33 ± 144.34	674.27***
pH	7.66 ± 0.14	7.68 ± 0.34	0.013
EC (μS)	1.87 ± 0.04	6.07 ± 0.15	2,291.24***
CO ₃ (mequ/L)	0.004 ± 0.001	0.0024 ± 0.00	16.00
HCO ₃ (mequ/L)	0.01 ± 0.00	0.01 ± 0.00	2.57
SO ₄ (mequ/L)	0.04 ± 0.00	0.06 ± 0.00	60.63***
Cl (mequ/L)	0.03 ± 0.00	0.18 ± 0.02	152.65***
Na (mg/L)	325.27 ± 37.14	1,117.57 ± 39.20	645.74***
Ca (mg/L)	106.93 ± 1.50	118.27 ± 14.62	1.78
K (mg/L)	26.94 ± 4.43	63.08 ± 6.99	57.17**
Mg (mg/L)	86.53 ± 5.60	115.87 ± 0.64	81.20***
Zn (mg/L)	0.26 ± 0.03	0.20 ± 0.07	2.09
Fe (mg/L)	13.47 ± 5.62	20.11 ± 7.00	1.64
Ni (mg/L)	0.42 ± 0.27	0.54 ± 0.07	0.50
Cd (mg/L)	0.26 ± 0.06	0.17 ± 0.06	3.12
Cr (mg/L)	0.32 ± 0.06	0.43 ± 0.12	2.22
Pb (mg/L)	0.17 ± 0.01	0.18 ± 0.01	1.50
Total algae richness	18.33 ± 10.02	13.67 ± 2.52	0.61
Cyanophyta richness	5.00 ± 5.29	2.67 ± 2.08	0.51
Chlorophyta richness	9.33 ± 8.02	5.33 ± 2.08	0.70
Bacillariophyta richness	4.00 ± 3.61	5.67 ± 1.15	0.58

Data are the mean ± standard deviation.

F-values followed with ** and *** indicated significant and highly significant variance ($p \leq 0.01$). F-value is the ratio of variance resulted from application of one way ANOVA.

Table 4 Correlation between water variables and the algal groups

Variable	Total algae	Cyanophyta	Chlorophyta	Bacillariophyta
TDS	-0.353	-0.304	-0.384	0.338
pH	0.026	-0.056	0.089	-0.042
EC	-0.367	-0.331	-0.385	0.342
CO ₃	-0.070	0.171	-0.014	-0.414
HCO ₃	-0.505	0.399	-0.677	-0.475
SO ₄	-0.524	-0.398	-0.508	0.284
Cl	-0.346	-0.294	-0.382	0.340
Na	-0.424	-0.350	-0.443	0.343
Ca	-0.158	0.052	-0.310	0.177
K	-0.515	-0.417	-0.503	0.327
Mg	-0.546	-0.321	-0.564	0.233
Zn	0.310	0.408	0.186	-0.172
Fe	0.095	-0.466	0.083	0.770
Ni	-0.936**	-0.084	-0.965**	-0.296
Cd	0.379	0.489	0.293	-0.340
Cr	-0.082	0.059	-0.245	0.230
Pb	-0.788	-0.125	-0.873*	-0.037
All	-	0.299	0.964**	0.156
Cyano	-	-	0.077	-0.842*

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

richness as reported by Szymańska-Walkiewicz et al. (2022) who focused on measuring the effects of abiotic factors— heavy metal concentrations (Ba, Bi, Cr, Cu, Mn, Fe, Ni, Pb, Zn) on phytoplankton composition, biomass and photosynthetic activity. The lakes differed in salinity: freshwater versus brackish versus transitional lakes. Regardless of the lake type, the predominant group was cyanobacteria (~80%), but their percentage was lower in the brackish lake.

Infiltration of the Baltic Sea waters resulted in lower concentrations of heavy metals in the lake waters of iron, zinc, lead, and positive effects of some heavy metals on the biomass of Chlorophyta and Bacillariophyta.

Chlorophyta showed a negative richness with each of Ni and Pb as reported by Cytotoxicity of lead and cobalt on cyanobacteria was reported by El-Naggar et al. (1999). Toxic effect of nickel on microalgae was attributed to inducing

the reactive oxygen species (ROS) that directly effect on a variety of differentially expressed genes. These genes are involved in redox homeostasis, nitrogen metabolisms, fatty acids, and DNA metabolism (Guo et al. 2022).

Conclusions

The abundance and frequency of investigated microalgae have significant correlation with the environmental abiotic components. Species belong Chlorophyta are the most abundant species (26) followed by Bacillariophyta (24) and Cyanophyta (19). Genus *Scenedesmus* (Chlorophyta) was the most abundant in the study area (11 species), followed by Genus *Oscillatoria* (9 species). *Nostoc muscorum* and *Chlorella vulgaris* were the most common and recorded in all sites (100% of the locations) under study. The correlation analysis between water and community variables indicated a high negative correlation of total algae richness with nickel ($r = -0.936$, $p < 0.01$). Cyanophyta and Bacillariophyta were correlated negatively ($r = -0.842$, $p < 0.01$). Chlorophyta showed a negative richness with each of Ni and Pb ($r = -0.965$, -0.873 , respectively) in one side and a high positive correlation was revealed ($r = 0.964$) with all environmental variables on the other hand.

Abbreviations

TWINSpan: Two-way indicator species analysis

DCA: Detrended correspondence analysis

CCA: Canonical correspondence analysis

ANOVA: One-way analysis of variance

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Authors' contributions

All authors contributed to the study conception and design. Material preparation, sample assembly, data collection and analysis were performed by MYKE, ASA, EEM, and EGE. All authors contributed in writing the first draft of the manuscript. Statistical analysis was performed by YMAS, ASA, and MYKE. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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