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Investigation of the microalgae diversity and physico-chemistry of Ozi Lake as preliminary steps in bioprospecting for bioactive molecules against SARS-COV-2

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Abstract

Benefits from the ecosystems have elicited various research interests, traversing different disciplines, including biomedical and medical. This study investigated Ozi lake microalgae component whose metabolites have been reported to act against SARS-CoV-2. Water samples were collected two times per month for twelve months, at fortnightly intervals. The microalgae in the water samples were studied using a light microscope, and identified with microalgae photographs contained in standard textbooks, journal articles and microalgae database websites; the physico-chemical parameters were analyzed after the methods of American Public Health Association. One hundred and eighty-three microalgae species were recorded, of which thirty-one species belonging to the three genera of Chlorella, Euglena, and Spirulina have been reported to be effective against COVID-19. The microalgae species and abundance data showed temporal and spatial variations; Chlorella had one species, with recorded populations mean of 82 ± 14 cells/mL; Euglena had 29 species, with recorded populations mean of 189 ± 28 cells/mL; and Spirulina had one species with recorded populations mean 1 ± 0 cell/mL. Descriptive statistics, ANOVA, Pearson correlations and t-test analyses were carried out against the microalgae and physico-chemical parameters. Chlorella, and Euglena showed wider populations variation between months of investigation than across locations. Seasonal variations in the abundance of the microalgae showed that Chlorella, and Euglena were higher during dry season months than rainy season. Euglena had significant correlations with pH, TDS, COD, nitrate, Fe, DO, and rainfall. Ozi lake is veritable harbour and breeding grounds for auspicious microalgae species; including those reported to have shown promising use against COVID-19 pandemic.

Key words: Microalgae, Chlorella, Euglena, Spirulina, Ozi lake, Bioprospecting, SARS-CoV-2

Introduction

The metabolites of algae – microalgae and macroalgae – make them important raw materials in the food, pharmaceutical, energy, cosmetics and drugs industries. Mayer et al. (2021) reported that compounds extracted from

algae have demonstrated antiviral activities against different human viruses. Compounds such as lectin, carrageenan, ulvans, and fucoidans from red, green and brown algae act as prophylactic and bio-therapeutic agents against COVID-19. Alam et al., (2021) provided useful data on broad range

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of molecules and compounds of algae origin, acting in many ways against various human viruses (e.g. SARS-CoV-2; HIV, etc). Halitunal derived from the marine microalga *Halimeda tuna* had showed antiviral activities against murine coronavirus A59 in vitro (KoeHN et al., 1991). Ulvans from green algae are also considered potential biotherapeutic agents against SARS-CoV-2 (Pereira and Critchley, 2020). Astaxanthin from *Haematococcus pluvialis*, when used in conjunction with primary antiviral drugs has been reported to benefit patients with COVID-19 by improving their health and reducing their recovery time (Talukdar et al., 2020).

Siedenburg and Cauchi (2022) opined that *Spirulina* plays antiviral activities which have both prophylactic and therapeutic effects against SARS-CoV-2; Garcia-Ruiz et al. (2022) reported anti-SARS-CoV-2 activities by biomolecules extracted from *Spirulina* and *Chlorella*; Sani et al. (2021) opined that abundant supply of selenium and zinc from *Chlorella* play antiviral activities against SARS-CoV-2. Nakashima et al. (2021) also reported an inhibition of influenza by *Euglena* extracts – including the inhibition of infection by flu strains that are already resistant to common anti-flu drugs such as oseltamivir and amantadine. The beta glucans from *Euglena* have been strongly recommended as adjuvant in COVID-19 vaccine administration (Cordova-Martinez et al., 2021). Microalgae-derived nutrients had also supported COVID-19 patients by improving their immunity. A diet containing vitamins and minerals as immune nutrients has been considered good strategy to fight against COVID-19 (Jovic et al., 2020); and some microalgae-based foods are rich in vitamins. For instance, the microalgae *Tetraselmis suecica* and *Isochrysis galbana* are rich sources of vitamins C Ferraces-Casais et al. (2012), while *Dunaliella tertiolecta* and *Chlorella stigmatophora* are rich in vitamins B1, B2, B12 and E (McDermid and Stuercke, 2003; Ferraces-Casais et al., 2012). *Euglena* produces vitamins A, C, E, essential amino acids, and a good source of polyunsaturated fatty acids (O'Neill et al., 2015). As result of improved nutrition, Tang and Suter (2011) reported weight gain in HIV patients who were supplemented with microalgae food.

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The reports about the uses of some phytometabolites to treat COVID-19 the latter which still menace the global human populations sparked our interests in carrying out this survey on the microalgae biodiversity of Ozi Lake ecosystem as a potential supply point of these auspicious microalgae. Our objectives include: 1) to survey Ozi Lake water for microalgae which have been reported to ameliorate COVID-19; 2) to investigate the spatial and temporal diversity and abundance of these microalgae; to investigate the composition of some ecologically important physico-chemical parameters that influence the microalgae species compositions and abundance; and 4) to investigate the interactions between the physico-chemical parameters and these microalgae of this lake water.

Materials and methods

Study Area

Ozi Lake located at 6° 42'10.7"N and 7°32'52.9"E in an elevation of 191 m a.s.l. (Ozoko, 2015) is situated in Opi Agu, southeastern Nigeria. Opi Agu has derived savanna vegetation (Nweze, 2006); hence there are patches of remnant tropical rainforest dotting the areas. The soils of the area are related to the vegetation; having the rainforest soil with more humus and the lateritic soil on the savanna vegetated zone (Uzoije et al., 2014). The area experiences two seasons annually – the wet season (March – October) and dry season (November – February), with the cold, dry hamattan period dominated by the North-East Trade wind in December to January (Nweze, 2006). More than 98% of the total annual rainfall was reported to fall during the wettest 8 months of the year; leaving less than 2% to the driest 4 months of the year. Ozi Lake is suggested to be of tectonic origin by Ofomata (1975) and Ozoko (2015). Ozoko (2015) reported that the lake has maximum length and width of 160 m and 70 m respectively; with maximum depth and areal extent of 7.5 m and 11200 m² respectively. Ozoko (2015) described the water of Ozi Lake as oligotrophic and of very good water quality (having total phosphorus of 6.52 µg/l). Ozi Lake receives and discharges water by seepage interaction with groundwater with no visible input from surface waters such as stream, river or other lake. The lake water has mild acidity (pH 5.6) with low alkalinity level (12.50 mg/l) (Ozoko, 2015).

Water Samples Collection for Phytoplankton and Physico-chemical Studies

Water samples for phytoplankton and physico-chemical parameters studies were collected from the lake at fortnightly interval at five different locations (designated

L1, L2, L3, L4, and L5) for 12 months using plastic containers of different volumes (300 ml for phytoplankton study and 5 L for physico-chemical studies). The 5 sampling locations were navigated with canoe rowed with paddle; and the locations were strategically chosen – one location each at the extremes of the transects traversing the maximum length (designated L1 and L5), and maximum width (designated L2 and L4), and L3 location at the point where the two transect lines (L1L5 and L2L4) intercepted. At L1, L2, L3, L4, and L5 water samples were collected from subsurface; and collection was carried out between the hours of 10 am to 2 pm. The water samples for phytoplankton investigation were preserved with Lugol's iodine solution in the ratio of 1: 100 of the sample (Bellinger and Sigee, 2010); taken to the laboratory for microscopy. In the laboratory the phytoplankton species identification and counting were done using a wet mount preparation under binocular light microscope (Olympus model). The wet mounts were viewed at different magnifications (especially X40 and 100X). The species as seen under the light microscope were matched with microalgae photographs obtained from reliable microalgae databases (e.g. AlgaeBase), and from algae drawings in some standard textbooks. The microalgae populations count was done by counting the number of microalgae cells in 1 drop of the sample (ca. 0.03 ml) and extrapolating to its equivalent value in 1 ml; recording the values in cells/ml. The water samples for chemical studies were analyzed for pH, electrical conductivity, total dissolved solids, total suspended solids, bicarbonates, sulphates, chlorides, potassium, sodium, calcium, magnesium, silicates, cadmium, nitrates, COD, BOD, iron, phosphates, zinc, copper, lead, arsenic, manganese, aluminum, chromium, total hardness, dissolved oxygen, total alkalinity, and carbonates following the procedures in the American Public Health Association (APHA, 1985) except where otherwise stated. The depth of the water, water temperature and pH were measured in situ using wooden pole, mercury-in-glass thermometer and pH meter (Hanna model) respectively; from the marks made on the wooden pole, respective depths for each location were measured and recorded in cm; the temperature values obtained were recorded in degree Celsius (°C)

Meteorological (Climatic Characteristics) Data

The meteorological data on precipitation (mm) were collected from a nearest meteorological station located in the University of Nigeria, Nsukka, about 28 km from the lake.

Statistical Analyses

The IBM SPSS (Version 20, USA) was used to analyze the means, standard errors, t-test, ANOVA, and Pearson correlation of the data collected from the study

Results

Meteorological Data

The monthly rainfall varied between the twelve months of the study (October, 2021 to September, 2022) (Figure 1)



Figure 1: Monthly rainfall (mm) in Ozi Lake during the period of study

Microalgae Diversity Data

One hundred and eighty-three phytoplankton species belonging to seven phyla of Bacillariophyta, Charophyta, Chlorophyta, Chrysophyta, Cryptophyta, Cyanophyta and Euglenophyta were recorded during this study; out of which only the Chlorophyta, Cyanophyta and Euglenophyta contained the algae genera so far reported to be used in treating and/or ameliorating COVID-19. These genera are the *Chlorella* (Chlorophyta), *Euglena* (Euglenophyta) and *Spirulina* (Cyanophyta). The Euglenophyta (*Euglena spp.*) were more abundant with total recorded populations mean of 189 ± 28 cells/mL and 29 species, with these species recorded across the five locations investigated and the twelve months of the study; the Chlorophyta (*Chlorella spp.*) were next in abundance, and had total recorded populations mean of 82 ± 14 cells/mL with only one species, which was recorded in all the locations and months of study except in May; the Cyanophyta (*Spirulina spp.*) was the least in abundance, having populations mean of 1 ± 0 cells/mL and one species, recorded only in L5 and in July (Figure 2). The spatial investigations revealed variations in the populations mean of *Chlorella spp.*, *Euglena spp.*, and *Spirulina spp.* The population means of *Chlorella spp.* across the five locations showed that L3 and L1 had the

lowest and highest populations means of 54 ± 19 and 111 ± 40 cells/mL, respectively. The populations means of *Euglena spp.* showed lowest and highest populations means of 108 ± 19 and 311 ± 103 cells/mL in L4 and L3 respectively; the result revealed significant mean differences across the locations (Figure 2B). *Spirulina sp.* occurred only in L5; ANOVA result showed significant mean difference between L5 and the other locations (Figure 2C). The temporal data obtained also revealed variations in the abundance of the recorded species. *Chlorella spp.* had the lowest and highest populations means of 0 ± 0 and 190 ± 68 cells/mL in the months of May and August respectively; the means of some months were significantly different (Figure 2D). *Euglena spp.* had the lowest populations mean of 40 ± 15 cells/mL in November and April, and highest populations mean of 654 ± 231 cells/mL in December; some monthly means differed significantly

(Figure 2E); *Spirulina spp.* was recorded only in July; with populations mean of 7 ± 4 cells/mL which differed significantly with means of the other months (Figure 2F). The microalgae populations across the dry and wet seasons also revealed means variation; the t-test analysis however showed that it was only *Euglena* that had significant mean-difference between the wet and dry seasons populations (Figure 3). The Pearson correlations analysis ($p \leq 0.05$ & 0.01) (Table 1) revealed that *Chlorella* had no significant correlations with any of the physico-chemical parameters investigated; *Euglena* showed significant positive correlations with TDS, COD, As, Cr, DO, and significant negative correlations with pH, total hardness, Ca, dissolved nitrates, Fe, and rainfall; *Spirulina* showed significant positive correlations with pH, and rainfall, and significant negative correlation with DO.

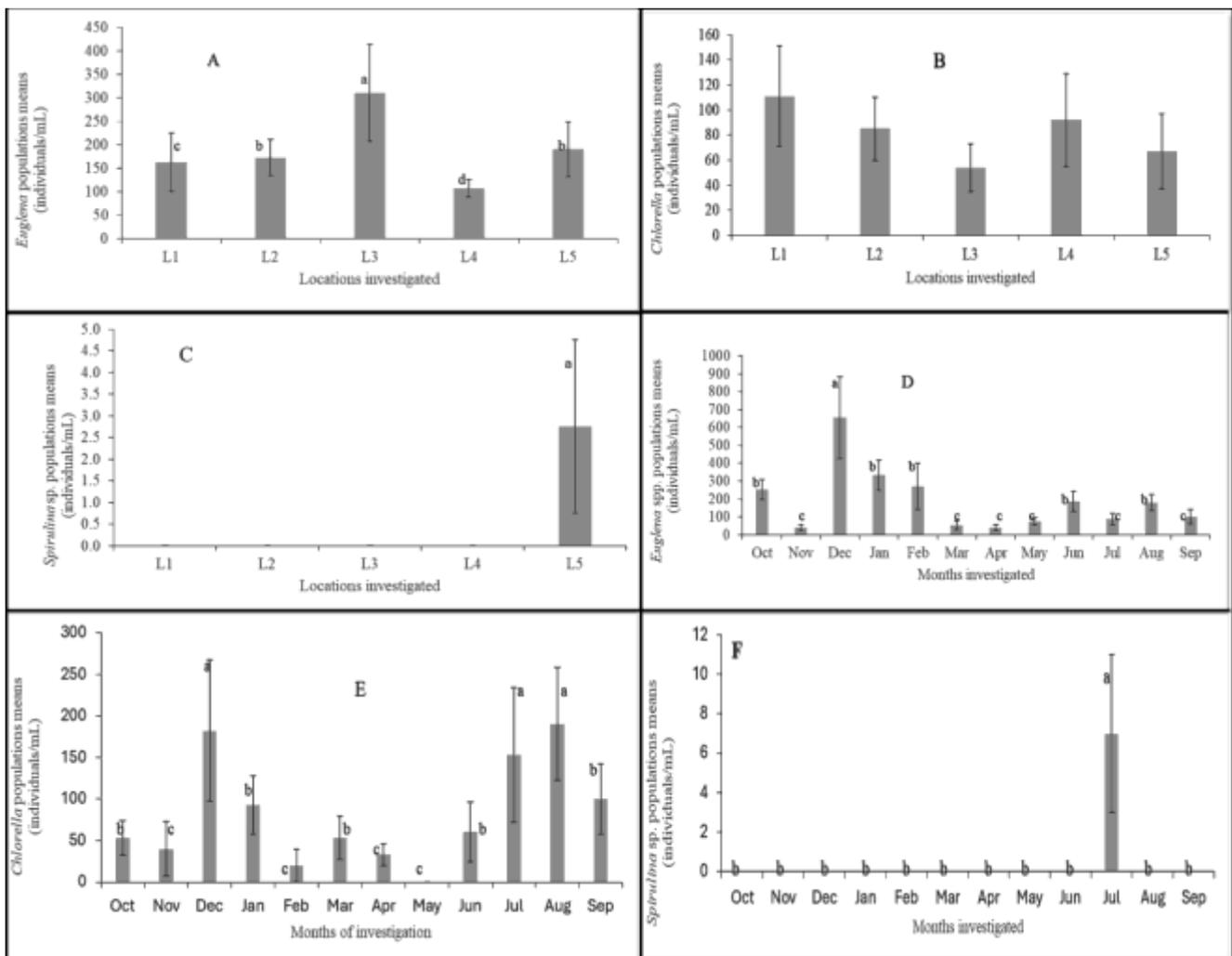


Figure 2: Bar charts for the microalgae populations means across the locations and months during the period of the study

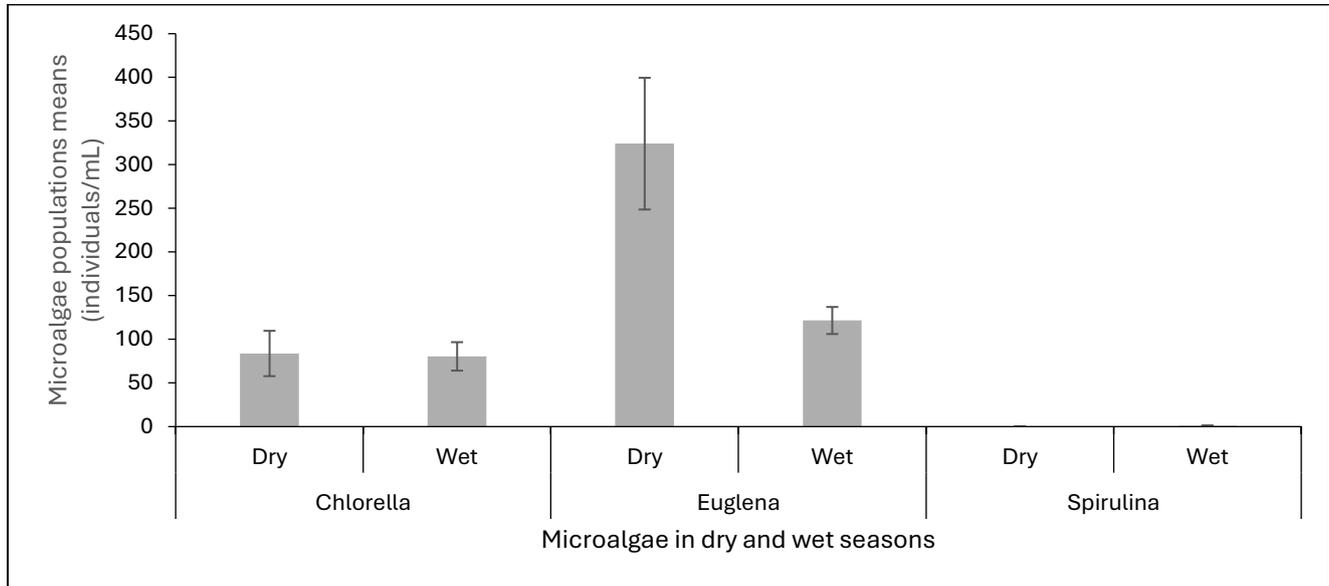


Figure 3: Bar charts for the microalgae populations means across the wet and dry season

Table 1. Pearson correlations analysis for the physico-chemistry and microalgae in Ozi Lake during the period of the study.

	Chlorocella sp0. (Cells/mL)	Euglena spp0. (Cells/mL)	Spirulina sp0. (Cells/mL)
pH	0.084	-0.324**	0.199*
EC (mg/L)	-0.142	-0.150	-0.068
TDS (mg/L)	-0.102	0.283**	-0.070
TSS (mg/L)	0.121	-0.108	-0.040
Bicarbonates (mg/L)	-0.007	-0.039	0.081
K (mg/L)	-0.072	0.033	-0.091
BOD (mg/L)	0.078	-0.136	0.164
COD (mg/L)	-0.091	0.253**	-0.118
Total hardness (mg/L)	0.106	-0.209*	0.159
Total alkalinity (mg/L)	-0.021	-0.037	-0.002
Carbonates (mg/L)	0.054	-0.100	0.070
Na (mg/L)	0.020	-0.029	0.009
Ca (mg/L)	-0.086	-0.182*	0.028
Cd (mg/L)	-0.042	0.078	-0.062
Nitrate (mg/L)	-0.062	-0.213*	0.001
Mg (mg/L)	0.104	-0.085	0.060
Mn (mg/L)	-0.147	-0.115	-0.052
Fe (mg/L)	-0.010	-0.205*	-0.049
Zn (mg/L)	-0.021	-0.026	-0.080
Cu (mg/L)	-0.069	0.084	-0.054
Pb (mg/L)	-0.062	-0.096	-0.046
As (mg/L)	0.053	0.207*	-0.051
Al (mg/L)	-0.092	0.074	-0.080
Cl (mg/L)	0.017	0.067	-0.068
Phosphates (mg/L)	-0.058	-0.041	-0.049
Cr (mg/L)	0.117	0.404**	-0.065
Sulfate (mg/L)	-0.060	0.002	-0.053
DO (mg/L)	-0.096	0.200*	-0.185**
Depth (mg/L)	0.137	0.066	0.166
Water Temp (°C)	-0.161	-0.137	-0.118
Monthly rainfall (mm)	-0.006	-0.259**	0.211*
Chlorocella sp0. (cells/mL)	1	0.015	-0.068
Euglena spp0. (cells/mL)		1	-0.052
Spirulina sp0. (cells/mL)			1

P significant at 0.05(*) and 0.01()**

Discussion

The recorded microalgae species of Ozi Lake water conform typically with microalgae of tropical water ecosystems (Ude and Nweze, 2021); which included the genera that had been reported to be used in the treatments of COVID-19; and these genera were *Chlorella*, *Euglena*, and *Spirulina*. The high number of microalgae species (183 species) recorded during this study showed that Ozi Lake water is oligotrophic (Nweze, 2009); supporting earlier report (e.g. Ozoko, 2015) that Ozi Lake water is oligotrophic. *Euglena* were richer in species diversity (29) and abundance (189 ± 28 cells/mL) than *Chlorella spp.* and *Spirulina spp.* which had one (1) species each with populations means of 82 ± 14 and 1 ± 0 cells/mL respectively. The richer species and greater abundance of the Euglenophyta (*Euglena spp.*) than the Chlorophyta observed during this study is a deviation from the desmids-dominant phytoplankton compositions characteristic of lakes in Opi area (e.g. Nweze, 2006); this may relate to changes in the physico-chemical and grazing factors that may currently prevail in this water body (Ndebele-Murisa et al. 2010). Some lakes in the Opi area, including Ozi Lake, have been ventilating between oligotrophic and mesotrophic (Ozoko and Ifediegwu, 2013; Ozoko, 2015). Desmids characterize oligotrophic lakes (Nweze, 2006; Bellinger and Sigee, 2010), while *Euglena* are more in organic polluted lakes (Ndebele-Murisa et al., 2010). Ozi Lake's catchment basin has grasses making up vast component of the vegetation which attract nomadic cattle grazing; dung from the cattle which go to this water to drink could possibly elevate pollution of the water. Again, the regular animal sacrifices made in and around this water body could also result in its organic pollutions.

The microalgae populations across the investigated locations and months showed variations. *Chlorella sp.*, and *Euglena spp.* showed similar temporal trends in their abundances; having their maximum populations in the months with relatively high insolation (December, January, and August); this trend became clearer when the wet and dry seasons populations of these microalgae were compared, with significantly higher abundances of *Euglena spp.* in the dry than wet season months. The spatial distributions of the microalgae revealed that the locations with less human activities (L3 and L5) had greater abundance than the locations with greater human activities (L1 and L4). The microalgae populations means had wider variations across the months than across locations; the narrower population means variations across the locations suggested how close these locations are to one another; Ozi Lake water has small surface area.

Total dissolved solids and chemical oxygen demand showed significant positive correlations with the populations of *Euglena* suggesting that the nutrients available for these organisms are soluble inorganic salts. pH showed a significant negative correlation with *Euglena spp.* suggesting that an increase in the acidity of the water might be removing the nutrients available for the microalgae. Electrical conductivity (EC), Ca, water temperature, and monthly mean rainfall showed significant negative correlations with *Euglena*, suggesting that the ions that contribute to EC, and Ca may form the nutrients of the microalgae and, are hence reduced by feeding as the populations of the microalgae increased. Temperature rise may decrease the nutrients available for algae by increasing the reactions that alter the forms of the nutrients; moreover, rises in temperature often lead to increase in metabolisms of the Lake zooplankton and their consequent food consumptions (grazing on the microalgae). Increased rainfall can impact negatively on microalgae abundance by diluting their biomasses (Ude and Nweze, 2021), and again by passively reducing the nutrients available to microalgae via dilution (Okogwu and Ugwumbah, 2012); furthermore, rise in the volume of lake water from rainfall will yield additional habitat for primary consumers/zooplankton, consequently leading to the population rise of the latter, and their grazing pressure on the primary producers – *Chlorella* and *Euglena* are good choice food for the primary consumers.

Conclusion

Ozi lake is a good harbour for varieties of microalgae and, hence could be a sourcing point for these important microalgae; the latter have found industrial application in the cosmetic, food, and drug industries. The three genera of *Chlorella*, *Euglena* and *Spirulina* recorded in this study have been reported to treat or ameliorate SAR-COV-2 (COVID-19).

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Data availability

This manuscript has no associated data.

Consent for publication

Not applicable.

Competing interests:

The authors declare no conflict of interest

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