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PHYTOPLANKTONIC DIVERSITY AND PHYSICOCHEMICAL CHARACTERISTICS OF KISHORE SAGAR, KOTA, RAJASTHAN

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Wetlands cover the major area of the earth. Aquatic systems are greatly affected by the environmental conditions and the organisms residing within. Kishore Sagar is a artificial lake located in the centre of the city and its water is mainly used for agricultural purposes through a connecting canal system to Rajasthan and Madhya Pradesh. The lake is at a risk by many anthropogenic activities which affect the water quality. It harbours many species of phytoplantons which are mainly responsible for the productivity of any wetland. The present study is an attempt to investigate the water quality of Kishore Sagar in reference of its phytoplankton diversity. The present paper reveals that the phyoplanktonic diversity comprises of 33 species. Total 8 species belonging to 8 genera of Class Chlorophyceae, 17 species belonging to 8 genera of Class Cyanophyceae and 8 species belonging to 6 genera of Class Bacillariophyceae were identified during the study period.

Keywords: Carbon cycle; Diversity; Phytoplankton; Pollution; Water quality.

Introduction

Water forms the basis of life and forms the essential part in urbanization, industrialization and agricultural activities. Sources of pollution may be natural and run-off, agricultural, industrial and domestic wastes. Wetlands cover the major part of the earth. On the basis of current and stratification, fresh waters can be divided as standing waters and running waters[1]. Freshwater aquatic systems sequester a good amount of global carbon through carbon cycle[2,3]. Greatest values of ecosystem goods and services per unit area of all habitats have been assigned to freshwater aquatic ecosystems[4]. Health of an aquatic ecosystem depends on the water quality and the living content residing within including both plants and animals. Phytoplanktons are the important components of the aquatic ecosystems which are free floating, unattached photosynthetic micro-organisms. They are the primary producers which occupy the first trophic level in a food chain. Their abundance is affected by the water quality of an aquatic system. Estimation of phytoplankton diversity can be used as a water pollution indicator[5]. Phytoplanktonic diversity and the physicochemical parameters have been studied at various places of different lakes by many workers[6,7,8,9,10,11,12]. Work done by Kumar *et al.* on Kishore Sagar deals with diversity of diatoms only[13]. This study is an attempt to

investigate the phytoplankton diversity of Kishore Sagar Lake in relation to its water quality.

Study Area

The small lake in Kota better known as Kishore Sagar Talab is an artificial lake which was constructed in 1946 by Prince Dher, Deh of Bundi. It is located in the centre of the city with location 24°25.675" N latitude & 76°37.348" E longitude[13]. It is walled all over with a beautiful seven wonders park at one side and talab ki paal at the opposite side (Fig. 1).

Fig. 1. View of Kishore Sagar Lake showing Jagmandir in the centre

Fig. 2. Dam constructed on lake

Fig. 3. Inlet point with showing human interference

A round of the lake can be taken by road all over. Jagmandir is situated in the middle of the lake which was constructed in 1745 by then queen of Kota for entertainment. It is accessible by boat. A dam is constructed on the lake which is 1500 m long and 5.6 m high (Fig. 2) and is used for distribution of water for crops during Kharif and Rabi seasons through a canal to Rajasthan and Madhya Pradesh. The lake has inlet and outlet of Chambal river water on regular basis[13] (Fig. 3). The talab is almost irregular rectangular in shape. Water is mainly used for agricultural purposes and recreational activities. The lake went under renovation after 2013 to keep the water level up to the mark in such a way that it neither makes lake occupied by green herbs nor it becomes an open land for cattle grazing as was the condition twelve years back in 2005[13]. Deity idols are also immersed every year after ganesh chaturthi and navratri which adds pollution through organic matters and chemicals. Anthropogenic activities are one of the sources of its pollution.

Materials and Methods

Three sampling sites were selected for the present study in Kishore Sagar Talab in April 2017. Surface water samples were collected in one litre capacity plastic bottles between 9 to 11 am in the morning for water quality analysis. Water temperature and pH were determined on the spot. The collected samples were brought to laboratory for the analysis of other parameters like alkalinity, dissolved oxygen, nitrate, sulphate etc. Physicochemical parameters of water were analyzed within 24 hrs of collection using standard methods[5,14,15,16].

The sampling for phytoplankton was done from the study site on the same day of water sampling. The collected samples were fixed and preserved in Lugol's Iodine solution until further analysis in laboratory. For taxonomic identification, a research microscope (Metzer) and a imported camera MD500 was used. The identification of algal samples was done with the help of standard

references[17,18,19,20,21,22,23].

Result and Discussion

Physicochemical characteristics:

Results of physicochemical parameters are summarized in Table 1.

Temperature

Water temperature varies with the environmental conditions. The surface water temperature was observed 28.3°C which is almost same as was observed by Kumar *et al.*[13] They observed temperature of 29°C in the same month during 2005.

Table 1. Mean values of physicochemical parameters of Kishore Sagar for the month April 2017

S.No.	Parameter (Unit)	Month- April 2017
1.	Temperature (℃)	28.30
2.	pH (Units)	8.67
3.	EC (µS/cm)	444.33
4.	Turbidity (NTU)	10.91
5.	Total Alakalinity (mg/L)	126.67
6.	Total Hardness (as CaCO3) (mg/L)	173.33
7.	Calcium Hardness (as CaCO3) (mg/L)	100.00
8.	Magnessium Hardness (as CacO3) (mg/L)	73.33
9.	Chloride (as Cl) (mg/L)	60.00
10.	Nitrate (as NO3) (mg/L)	4.33
11.	Sulphate (as SO4) (mg/L)	18.00
12.	Fluoride (mg/L)	0.67
13.	Total Dissolved Solids (mg/L)	311.00
14.	Total Suspended Solids (mg/L)	34.33
15.	DO (mg/L)	6.93
16.	COD (mg/L)	130.00
17.	BOD (mg/L)	4.13

Turbidity

Along with suspended and colloidal matter, silt and clay, phytoplanktons also increase turbidity of water[15]. It can be used as an index of productivity. Turbidity was measured 10.91 NTU during the study period which is almost a unit higher beyond permissible limit of 10.

pH

The pH value was measured 8.67 units. The reason for higher range of pH can be attributed to presence of bicarbonates and carbonates of alkali and alkaline earth metals, sewage addition and through human interference. The lake water can be said to be 'slightly alkaline' as was investigated by Kumar *et al.* in 2005[13].

Electrical Conductivity (EC)

Electrical conductivity is directly proportional to dissolved ions present in

water[4]. This parameter is affected by the total dissolved salts. It was measured 444.33 µS/cm which is contradictory to what was observed by Kumar *et al*[13]. They measured EC of 293 µS/cm. High conductivity also indicates high productivity. With this high value, the lake can be classified as mesotrophic[10].

Total Alkalinity

In the study period, mean value of 126.67 mg/L was measured for total alkalinity which is on lower side from that was found in 2005 by Kumar *et al* [13]. Mostly total alkalinity is caused due to dissolved bicarbonates. High alkalinity of water indicates high productivity of water. Richness of phytoplanktons increases the productivity of water[15.]

Total Hardness

Property of hardness of water is due to a complex of mixture of ions. The hardness is due to mainly calcium and magnesium ions. Total hardness is due to both calcium hardness and magnesium hardness. In wetlands, soil characteristics influence total hardness of water[12]. Kumar *et al.*[13] has categorized the Kishore Sagar Lake as 'nutrient - rich' as total hardness of more than 60 was observed by them with the result of 94 mg/L. In the present study, total hardness of 173.33 mg/L was observed. Hence with this reading, it can still be categorized as 'nutrient – rich' [13].

Kumar *et al.* observed calcium hardness of 60 mg/L in 2005 while the calcium hardness of 100 mg/L was observed in the present study. The data reveals a jump in the calcium hardness of water in this span of time from 2005 to 2017.

The magnesium hardness was recorded as 73.33 mg/L during the study period which is almost the double of that observed by Kumar *et al* [13]. In this leap of twelve years, the parameter has raised from 34 mg/L (in 2005) to 73.33 mg/L (in 2017).

The total hardness of water comprising of both calcium & magnesium hardness depicts that the water is hard in terms of hardness[16].

Chloride and Fluoride

High chlorine concentration is a pollution indicator. Chloride was estimated as 60 mg/L and fluoride as 0.67 mg/L in the present water body which are both below the permissible limits. As the water is supplied for crops, the water is safe for agricultural purposes being below 250 mg/L.

Nitrate and Sulphate

Nitrate is the highly oxidized form of nitrogen. Nitrate – Nitrogen was found to be 4.33 mg/L and sulphate as 18 mg/L. Values for nitrate content higher than 10mg/L are toxic for human consumption. The nitrate and sulphate concentrations have been increased from 2005 to 2017 when compared to the work of Kumar *et al.*[13]. They observed 0.106 mg /L of nitrate – nitrogen and 3.2 mg/L of sulphate. Higher values of nitrogen as nitrate tend to increase net productivity of aquatic ecosystems. The reason attributed to this increased concentration of nitrate is increased disposal of domestic effluents, sewage disposal, decayed vegetables, animal matter and anthropogenic activities which has increased from 2005 to 2017 with increasing population and hence indicate polluted status of the lake. Sulphates add hardness to water. Sulphates may be added from sedimentary rocks through leaching from their weathering, being converted to sulphate compounds adding in water[16].

Total Dissolved Solids (TDS) and Total Suspended Solids (TSS)

TDS is a parameter that is due to various salts like chlorides, sulphates, nitrates, phosphates, carbonates and bicarbonates of elements like sodium, magnesium, iron etc.

dissolved in water. It is an estimate of dissolved organic and inorganic waste[5]. TDS value of 311 was observed during the study period. Higher TDS value may be the reason for higher EC. Mean TSS value found was 34.33. TSS is amount of suspended salts of metals.

Dissolved Oxygen (DO)

DO is one of the most important parameters for water quality. Chemical, physical and biological processes of a water body have an influential effect on the DO level. DO levels are important for the maintaining aquatic life. The mean value of dissolved oxygen was estimated 6.93 mg/L in Kishore Sagar in the present study which is suitable for the fishes. In the past years, the DO levels have raised from 3.6 mg/L (2005) to 6.93mg/L (2017). As there is regular periodic inlet and outlet of water through dam, there remains a fluctuation in the organic matter addition to the lake water. This may be the reason for higher DO level during the study period. Due to higher temperatures in summer months, DO levels remain on lower side due to increased biological oxidation[16].

Biological Oxygen Demand (BOD)

BOD value of 4.13 mg/L was estimated during the study period. The BOD is directly proportional to the amount of oxidizable organic matter present in water and is an important parameter for pollution detection[15]. The BOD values lie somewhere between reasonable and tolerable category[16] of water. BOD values suggest the capacity of a water body for self purification.

Chemical Oxygen Demand (COD)

COD is a major parameter for pollution measurement. Like BOD, it is also a measure of oxygen requirement. COD values are always higher than BOD values as found in this period of study also. COD value of 130 mg/L was measured during the study period.

Algal diversity

The phytoplankton diversity of Kishore Sagar Lake comprises of various types of single-celled, colonial and filamentous forms of algae belonging to different classes *viz.* Chlorophyceae, bacillariophyceae (both belonging to centrales and pennales) and cyanophyceae as listed in table 2.

Systematic Enumeration and Description of some of the studied species

Chlorophyceae

Coelastrum microporum Nagaeli

Cells spherical or polygonal arranged to form hollow spherical or many sided coenobium with 2-64 cells, with the narrow end outwardly directed, cells 3.75-15.00 µm long, cells adjoined laterally by very short interconnecting protuberances of the mucilaginous sheath[17,20,23].

Eudorina elegans Ehrenberg

Cells spherical and evenly spaced out within the colonial mucilaginous matrix to the edge leaving a clear zone at the centre, cells arranged in transverse bands or tiers[17,18,22].

Pandorina morum Bory.

Colony spherical or oval, cells pear shaped, 8-32 densely/ compactly packed cells embedded in mucilage that extends beyond the cells at the colony edge, two equal length flagella present, cells 8-20 µm long[17,22].

Pediastrum duplex Meyen var. duplex

Colony 16-32 celled, walls smooth with lens shaped space between the inner cells, cells quadrate, the inner side of marginal cells distinctly concave, peripheral cells quadrate, the outer margin extended into two tapering truncate , blunt-tipped processes, colony upto 50-55 µm in diameter, cells 8-10 µm in diameter[17,22.23].

Scenedesmus quadricauda (Turp.) de Breb

Cells ovoid or ellipsoid, adjoined by their lateral wall to form a row of 4 in a single series, colony linear, two outer cells of the series bear a long curved spine at their poles [17,18,20].

Table 2. Phytoplankton diversity observed in Kishore Sagar during study period of April 2017

Chlorophyceae	Cyanophyceae	Bacillariophyceae
Coelastrum microporum	*Aphanocapsa roeseana*	*Cyclotella bodanica*
Eudorina elegans	*Arthrospira platensis*	*Fragilaria crotonensis*
Pandorina morum	*Chroococcus dispesus*	*Melosira varians*
Pediastrum duplex	*C. tenax*	*Navicula radiosa*
Scendesmus quadricauda	*C. turgidus*	*Nitzschiza acicularis*
Staurastrum rotula	*Merismopedia elegans*	*N. dissipata*
Tetraedon trilobulatum	*M. glauca*	*Synedra acus*
Trebauria crassipinia	*M. tenuissima*	*S.ulna*
	Microcystis aeruginosa	
	M. flos- aquae	
	M. robusta	
	Oscillatoria princeps	
	O. tenuis	
	O. proboscidea	
	Phormidium tenue	
	Spirulina major	
	Spirulina subtilissima	

Staurastrum rotula Nordst

Cell not compressed, apex of the cell extended, poles bearing 3 or more arms or lobes so that the cell appears radiating when seen from the top[17,18,20].

Tetraedron trilobulatum (Reinsch) Hansgirg

Cells triangular, 19.5-21.4 µm in diameter, angles of cells broadly rounded[23].

Treubaria crassipina G.M. Smith

Cell tetrahedral, each apex with a long stout spine, spines broader at the base and stout, decidedly tapering, processes are not toothed at the tip[17,20].

Cyanophyceae

Aphanocapsa roeseana de Barry

Colony irregular and embedded with mucilage, cells spherical to sub-spherical, light green to yellowish and cells 5.26 µ broad and 7.89 µ long[19].

Arthrospira platensis (Nordst.) Gomont

Trichomes slightly constricted at the cross walls, not attenuated at the ends or only a little attenuated, more or less regularly spirally coiled, end cells broadly rounded, distance between the spirals 43-57 µ, spirals 2-6µ long and 26-36µ broad[19].

Chroococcus dispersus (v. Keissler) Lemm.

Cells 4-8, 16 or more in a tabular mucilaginous free swimming colony, with round margins, without sheath, 3-4 µ broad, with sheath 5-6 µ diam., individual envelopes often totally gelatinized, not lamellated, colourless[19].

Chroococcus tenax (Kirchn.) Hieron

Cells mostly in groups of 2-16, blue green , sheath colourless to brown, distinctly lamellated[19].

Chroococcus turgidus (Kutz.) Nag

Cells spherical or ellipsoidal single or in groups of 2-4, sheath colourless and distinctly lamellated[19].

Merismopedia elegans A. Br.

Colonies small or big, 16-4000 celled, light blue, cells spherical or oblong, more or less closely arranged, light blue,cell 3.5-7 µ broad and 4.8-6µ long[19].

Merismopedia glauca (Ehrenb.) Nag.

Colonies mostly small with 16-64 cells , pale blue green, rarely more, 45-150 µ diameter, cells oval to spherical, closely arranged, pale blue-green , cell 3-6 µ broad and 4.5 µ long[19].

Merismopedia tenuissima Lemm.

Cells closely packed in colonies of 16-100 cells, subspherical, 1.3-2 µ broad, sometimes individual cells with distinct mucilaginous envelopes[19].

Microcystis aeruginosa Kutz.

Colonies when young round or slightly longer than broad, solid, when old becoming clathrate with distinct hyaline colonial mucilage, cells spherical, generally with gas vacuoles, cell 3-7 µ diameter[19].

Microcystis flos-aquae (Wittr.) Kirchner

Colonies roughly spherical, ellipsoidal or somewhat elongate often squarish in optical section, not clathrate with indistinct colonial mucilage, cells spherical , with gas vacuoles, cell 3-7 µ diameter[19].

Microcystis robusta (Clark) Nygaard

Colonies round at first but become irregularly elongate later, distinct sheath present, cells 6-9 µ diameter, spherical, without gas vacuoles[19].

Oscillatoria princeps Vaucher ex Gomont

Thallus light green to brownish , trichome more or less straight ,cap slightly present , rounded calyptras present, end cell thick walled, non contructed septa, cells 21.04 µ broad and 3.5- 7 µ long[19].

Oscillatoria proboscidea Gomont

Thallus light to dark blue green, trichome almost straight without constrictions at cross walls, slightly curved or spirally coiled sometimes end cells flatly rounded, capitates, cells 2-4 µ long[19].

Oscillatoria tenuis Ag. ex Gomont

Thallus thin , blue green , slimy, trichome straight ,4-10 µ broad, not capitate, not attenuated at the apices, end cell more or less hemispherical[19]

Phormidium tenue (Menegh) Gomont

Thallus thin, membranous, pale blue green, expanded, trichome straight or slightly bent, attenuated at the ends, calyptra absent, end cell acute-conical, septa not granulated, cell 2.5-5 µ long and 1-2 µ broad[19].

Spirulina major Kutz. Ex Gomont

Trichome 1.2 – 1.9 µ broad, regularly spirally coiled, blue green, spirals 2.5-4 µ broad and 2.7-5 µ distant, apex not attenuated, terminal cell rounded without calyptras, cell 1.2-1.9 µ broad, spiral 2.7-5 µ long[19].

Spirulina subtilissima Kutz. Ex Gomont

Trichome 0.6-0.9 µ broad, regularly spirally coiled, bright blue green or yellowish, distance between the spirals 1.25-2 µ, spirals 1.5-2.8 µ broad[19].

Bacillariophyceae

Cyclotella bodanica Eulenstein ex Grunow

Intermediate zone covered with irregular rows of puncta, marginal zone with 11 striae in 10 µm, frustules 23 µm in diameter[25].

Fragilaria crotonensis Kitton.

Broadly elliptical valve, bilaterally symmetrical frustules, Striae 14-15 in 10 µm and rib like in appearance, frustule 13-120 µm long and 4-16 µm wide[22,25].

Melosira varians Agardh

Cylindrical cells forming chains, convex valve face covered with small spines, frustules 6-30 µm in diameter[22].

Navicula radiosa Kutz

Valves lanceolate, apices acutely rounded, proximal striae radiate and distal covergent.,

central area expanded, with polar raphe fissures on one side, frustule 45-70 µm long and 10-12 µm broad, striae 10 -12 in 10 µm [24].

Nitzschia acicularis W. Smith

Small frustules, pointed extremeties,linear and long valve with parallel margins, fine striae, slightly constricted rounded apices,

frustules 4.5 µm wide and 65-70 µm long[25].

Nitzschia dissipata (Kutz.) Rabenhorst.

Valves linearly lanceolate , raphe sub central and fibulate, series of dots visible along one margin, frustule 40-95 µm long and 2-4 µm broad, striae 20-30 in 10 µm [22,24].

Synedra acus Kutzing

Capitate valve end, Valve not inflated at middle, long, central area linear and clear, frustule 90-100 µm and 4-7 µm wide, striae 15 in 10 µm[25].

Synedra ulna (Nitz.) Ehr.

Valves linear with constricted, poles broad, pseudoraphe narrow, central area small, frustule 100-250 µm long and 4-6 µm wide, striae 8-10 in 10 µm[24,25].

Conclusion

The physicochemical parameters studied in the present study show that they fall in the permissible limits. The standards being in permissible limits suggest that the water is safe for irrigation and agricultural purposes. Occurrence of some pollution indicator genera like *Microcystis*, *Oscillatoria*, *Nitzschia*, *Scenedesmus* suggest the polluted status of the Kishore Sagar Lake. Hence being nutrient – rich, the water of lake can be said to be polluted. The results obtained for various physicochemical parameters and phytoplanktons studied during the month of April, the lake can be categorized as of mesotrophic nature. The lake needs more attention, protection and management as further study on phytoplankton composition and distribution can be used in biomonitoring of this wetland.

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References

1. Maitland PS 1990, Biology of Freshwaters. 2nd edition. Chapman & Hall, New York.
2. Valk AG 2012, The Biology of Freshwater Wetlands. 2nd edition. Oxford University Press.
3. Whitton BA 2012, Ecology of cyanobacteria II: their diversity in space and time. Springer Science & Business Media.
4. Dodds WK and Whiles MR 2010, Freshwater ecology: concepts and environmental applications of limnology. 2nd edition. Academic press.
5. Gupta PK 2007, Methods in environmental analysis: water, soil and air. *Agrobios*.
6. Gaur KS, Sharma V, Sharma MS, Modi R and Verma BK 2014, Water quality assessment in relation to trophic status of the Rana Pratap Sagar Dam and the Chambal river (Rajasthan) India. *World Journal of Environmental Biosciences* **3**(1) 19-33.
7. Gopal B, Goel PK, Sharma KP and Trivedy RK 1981, Limnological study of a freshwater reservoir, Jamwa Ramgarh (Jaipur). *Hydrobiologia* **83**(2) 283-294.
8. Mishra V, Sharma SK, Sharma BK, Upadhyay B and Choudhary S 2012, Phytoplankton, Primary Productivity and Certain Physico-Chemical Parameters of Goverdhan Sagar Lake of Udaipur, Rajasthan. *Universal*

Journal of Environmental Research and Technology **2**(6) 569-574.
9. Srivastava N, Harit G and Srivastava R 2009, A study of physico-chemical characteristics of lakes around Jaipur, India. *Journal of environmental*

biology **30**(5) 889-894.
10. Sharma R, Sharma MS, Sharma V and Malara H 2008, Study of Limnology & Microbiology of Udaipur Lakes. Proceedings of Taal 2007: The 12th World Lake Conference. 1504-1508.
11. Balai VK, Sharma LL and Ujjania NC 2015, Phytoplankton diversity in Lake Jaisamand, Rajasthan (India). *Journal of Applied & Natural Science* **7** (2) 592-597.
12. Murulidhar VN and Murthy VNY 2015, Dynamics of Phytoplankton & their Correlation with Physicochemical Characteristics in Gulur Wetland, Tumakuru District, Karnataka, India. *Asian Journal of Natural & Applied Sciences* **4**(1) 37-47.
13. Kumar A, Sharma LL and Aery NC 2008, Physicochemical Characteristics and Diatom as Indicators of Trophic Status of Kishore Sagar, Rajasthan. Proceedings of Taal 2007: The 12th World Lake Conference, 1804-1809.
14. APHA- AWWA-WPCF 1999, Standard methods for the Examination of Water & Wastewater. 20th edition. Washington DC.
15. Aery NC 2010, Manual of environmental analysis. Ane Books Pvt Ltd.
16. Maiti SK 2004, Handbook of Methods in Environmental Studies Vol. 1: Water & Wastewater Analysis. ABD Publishers.
17. Edmondson WT 1959, Freshwater Biology. 2nd edition. John Wiley & Sons Inc.
18. Prescott GW 1954, How to know the Freshwater Algae. Brown Company Publishers, Dubuque, Iowa.
19. Desikachary TV 1959, Cyanophyta, Monograph on blue green algae. ICAR. New Delhi.
20. Smith GM 1994, Manual of Phycology: An Introduction to the Algae and their Biology. Scientific Publishers.
21. Palmer CM 1980, Algae & Water Pollution. Castle House Publisher Ltd., England.
22. Bellinger EG and Sigee DC 2015, Freshwater Algae- Identification, Enumeration and Use as Bioindicators. 2nd edition. Willey Blackwell.
23. Philipose MT 1967, Chlorococcales, ICAR, New Delhi.
24. Meeravali SN, Prabhakara RC and Venkata RRR 2015, Floristic Studies on Diatom Flora Selected Water Bodies of Ananthapuram District Andhra Pradesh India. *International Journal of Recent Scientific Research* **6**(10) 6923-6930.
25. Narayan P and Barupal GK 2015, A Systematic Account of the Fresh Water Diatom from Kaylana Lake of Jodhpur District, Rajasthan (India). *International Journal of Recent Scientific Research* **6**(4) 3435-3439.