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Article

# Effects of Water Quality on Diversity of Freshwater Green Microalgae in Semiurban Areas

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**Abstract:** Recent climate change and water pollution has affected algal diversity resulting in emergence of more tolerant species. They can be the indicators of water quality, pollution and seasonal changes. Present study revealed the green algal diversity and distribution pattern according to water parameters and season in the Lucknow City, India. The water sample were collected from seven sites within Lucknow and outskirts. The occurrence of a total of 36 taxa of green algae belonging to 11 genera are recorded. It has been observed that less polluted water is dominated by *Spirogyra*; where conductivity, TDS and salinity of water were low. Whereas the more polluted water was dominated by desmids. Dominance index was found highest in site 4 (0.61), where only three taxa were identified; whereas the lowest (0.13) was measured in site 1, where nine taxa were identified. This study also recorded several algal taxa for the first time from Lucknow.

**Keywords:** diversity index; distribution pattern; green algae; taxonomic identification; water parameter

#### 1. Introduction

Rapid economic growth, urbanization, pollution and climate changes worldwide pose a serious threat to biodiversity loss and ecosystem dysfunction. As in the aquatic ecosystem, algae are the predominant primary producer they are playing a major role in the ecosystem. Green algae are largely freshwater organism may grow on moist terrestrial substrate. They are grass-green colour due to the presences of pigments chlorophyll a and b,  $\alpha$  and  $\beta$  carotenes and xanthophylls. They are unicellular, multicellular or filamentous. According to the newer classification proposed by Leliaert *et al.* (2011, 2016) green algae are segregated into two phyla, Chlorophyta and Charophyta. The members of phylum Chlorophyta are morphologically diverse, including one to eight flagella and non-motile (coccoid) unicells and include about 4300 species worldwide. Whereas phylum have certain unique enzymes, lateral flagella in flagellate members and mitosis by phragmoplasts.

From India, so far 7,411 species of algae are known, which is 14.98% of the total Indian flora Mao & Dash (2019). Their diversity and distribution are varied according to the habitat, season and water quality. Some of the algae are known to be pollution indicators. Green algae usually grow better in less polluted ponds and streams; however, some species could present in heavily polluted water (Dasgupta *et al.* 2017). The parameters such as pH, conductivity, salinity, total dissolved solids (TDS), dissolved oxygen (DO) are rapid and reliable indicators of water quality of particular water body (Patil *et al.* 2012).

In India, despite several algal diversity studies carried out in different places, but there is an apparent lacuna in the study of algal diversity in relation to pollution and seasonal effects. Lucknow, the capital of Uttar Pradesh and an important historical city has witnessed rapid urbanization in the recent past, which has led to changes in algal flora. However this city has been rarely explored for algal diversity and distribution pattern. Only a few reports are available on green algal flora of Uttar

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Pradesh and especially on Lucknow. Gupta (2012) reviewed and revealed the occurrence of a total of 703 taxa of Chlorophyta of Uttar Pradesh. Some enumeration of fresh water algal flora of Uttar Pradesh has also been carried out by Suseela *et al.* (2015). Kumar and Suseela (2004) reported 85 taxa of Chlorophyta from Uttar Pradesh. Singh (2015) have studied phytoplanktonic diversity of Gomti River and Lucknow ponds in relation to seasonal variation and pollution. Recently the initiative was taken by Dasgupta *et al.* (2017) to determine the relation between water parameters and algal flora in late summer in different sites of Lucknow and revealed occurrence of a total of 40 taxa belong to 32 genera of 4 different phyla namely Cyanophyta, Chlorophyta, Bacillariophyta and Euglenophyta in eleven study sites of urban water bodies near Lucknow city in summer. However, none of the studies were focused on a thorough exploration of green algal species, their diversity indices and distribution pattern relation to water quality and season.

Nowadays, the crisis in taxonomic and biodiversity studies stimulated us to determine the relation between water parameters and green algal diversity in seven different sites of Lucknow. Morphological analysis was carried out to determine the taxonomic composition of green algae in aquatic ecosystems, both qualitative and quantitative. Diversity indices and a topographical map of algal distribution were determined by mathematical analysis. Water parameters were analyzed to reveal the water quality of a particular site.

#### 2. Material and Methods

### 2.1. Collection of Microalgae

Lucknow is spread over an area of 310 km² in the central plain of the Indian subcontinent. Samples have been collected from field research stations at Banthra, CSIR-NBRI, Lucknow which cover the 85-hectare land surface. The moist bricks and surrounding waters, water bodies, agricultural fields, different plantations and forest were assessed in four sites in Banthra Field Research Centre, CSIR-NBRI (N 26° 41′ 34.584″; E 80° 50′ 3.443″), two sites in Indian Institute of Sugarcane Research (ICAR-IISR) (N 26° 50′ 21.408″; E 80° 55′ 23.268″) and Sinik pond (Kanpur road) ((N 26° 49′ 45.48″; E 80° 54′ 34.919″) during winter season (December, 2018) (Figure 1).

Site 1: Banthra (moist bricks and surrounding water)

Site 2: Banthra (soil of Ziziphus jujuba plantation and surrounding water)

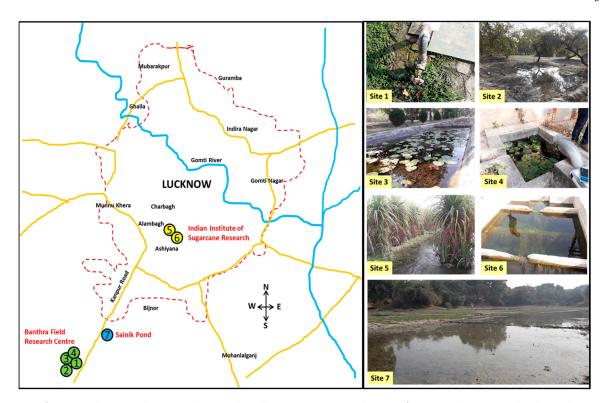
Site 3: Banthra (Lotus pond)

Site 4: Banthra (small water tank)

Site 5: IISR (sugarcane field soil and water)

Site 6: IISR (water pumping station)

Site 7: Sinik pond (Kanpur road)



**Figure 1.** The map showing the sample collection sites in Lucknow. **Site 1:** Banthra (moist bricks and surrounding water); **Site 2:** Banthra (soil of jujube plantation and surrounding water); **Site 3:** Banthra (lotus pond); **Site 4:** Banthra (small water tank); **Site 5:** IISR (sugarcane field soil and water); **Site 6:** IISR (water pumping station); **Site 7:** Kanpur road (Sinik pond).

# 2.2. Analysis of Water Parameters

Water parameters such as temperature (T $^{0}$ C), pH, dissolved oxygen (DO in mg/L), conductivity (Cd. in  $\mu$ s/cm), total dissolved solid (TDS in mg/L), salinity (Sl. in  $^{0}$ %) were measured at sampling site by multi-parameter analyzer (HQ 40d multi, HACH).

# 2.3. Identification

The collected algal samples were observed under the Leica DM 500 light microscope attached with Leica EC3 Camera and computerized image analysis system. Taxa were identified by using the standard publications of Prescott (1951) Scott and Prescott (1961), Tiffany and Britton (1952), Philipose (1967), Prasad and Misra (1992) and nomenclature were updated from Guiry and Guiry (2019; http://www.algaebase.org).

#### 2.3. Mathematical Calculation of Diversity

Diversity indices such as Dominance (D), Simpson's Index (1-D), Shannon and Weaver's Index (H'), Species Evenness (E), Menhinick's Index (MN), Margalef's richness index (ML) and Berger-Parker Dominance (BP) were calculated for each site on the basis of number of taxa (S), the number of individuals of each species in the sample (n), the total number of individuals in the sample (N) or algal density (cells/mL) (Harper, 1999). The analysis was also carried out using the PAST3 statistical software.

Algal density was calculated by the following equation.

Algal density (cells/ml) (N) = 
$$(C \times 1000 \text{ mm}^3)/(A \times D \times F)$$
 (eq. 1)

Where C = number of cells counted, A = area of the field, D = depth of field, F = number of fields counted

Dominance (D) of taxa in a sample is represented by the equation below

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$$D = \sum \left(\frac{n}{N}\right)^2 \tag{eq. 2}$$

Species diversity is represented by Simpson's Index of Diversity (Simpson, 1949)

$$Simpson's index = 1 - D (eq. 3)$$

Shannon and Weaver (1963) index of diversity (H') is represented by the equation below:

$$H' = -\sum(p) \times ln(p) \tag{eq.4}$$

Where, H' is the index of species diversity, p = n/N, the proportion of total sample belonging to particular species.

Buzas and Gibson's evenness = 
$$(e^H)/S$$
 (eq. 5)

Menhinick's richness index = 
$$\frac{s}{\sqrt{n}}$$
 (eq. 6)

Margalef's richness index = 
$$(S-1)/ln(n)$$
 (eq.7)

Berger-Parker Dominance (Berger and Parker, 1970) is one of the best and simplest index was considered by May (1975). It is a simple measure of the numerical importance of the most abundant species.

$$d = Nmax/N (eq.8)$$

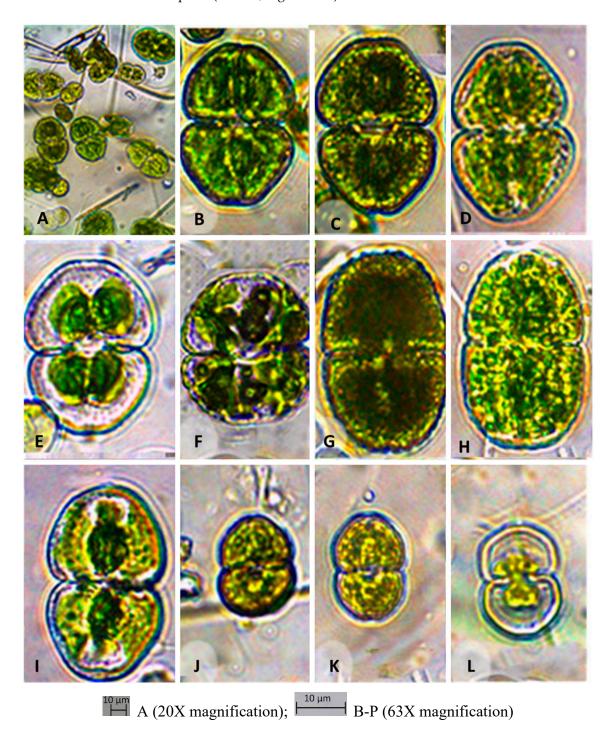
Where Nmax is the number of individuals in the most abundant species.

# 3. Results

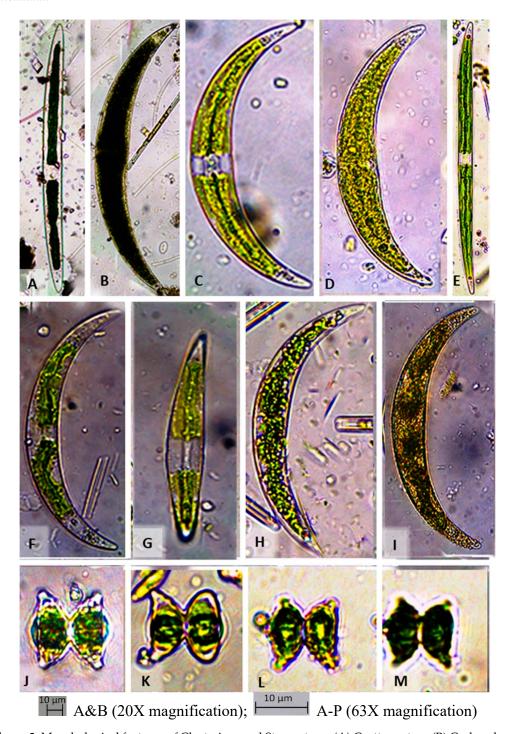
#### 3.1. Water Parameters of Study Sites and Diversity of Green Algal Species

A total of 36 taxa of green algae belonging to 11 genera have been identified from the seven study sites. Water parameters such as temperature, pH, DO, conductivity, TDS and salinity, as well as algal flora (Table 1). As the sampling was carried out in winter, the temperature of surface water at the point of sampling was ranged from 15.8°C to 23.8°C, which stimulated the growth of low temperature loving algae. Variation in pH was found in the range of 7.56 to 8.65. It has been observed that sampling sites having high algal density showed a high concentration of DO such as 19.06 (site 7), 14.14 (Site 2), 11.45 (site 1) and 11.6 (site 3) mg/L. Sainik pond (site 7) having a large surface area exposed to air showed the maximum DO concentration. Conductivity (650 µs/cm), TDS (361 mg/L) and salinity (0.31%) were found higher in Banthra moist bricks and surrounding water (site 1) which was dominated by seven different species of Cosmarium such as C. pyramidatum, C. subspeciosum, C. variolatum var. skujae, C. maculatum, C. pericymatium, C. granatum, C. angulosum var. concinnum and two species of Closterium such as C. moniliferum var. concavum, C. dianae (Table 1, Figure 2). The samples collected from soil of jujube plantation and surrounding water (site 2) were showed higher DO (14.14 mg/L), lower conductivity (572 µs/cm), TDS (277 mg/L) and salinity (0.28 %), which was typified by mixed flora of Closterium praelongum, Scenedesmus bijugatus, Oocystis gigas and 4 different species of Spirogyra such as S. maxima, S. crassa, S. elongate and S. inflata (Table 1, Figures 3–5). There are several lotus ponds in Banthra (site 3) with different green algal flora (conductivity 572 μs/cm, TDS 277 mg/L and salinity 0.28 <sup>0%</sup>). Most of them are dominated with mixed taxa of Closterium, Scenedesmus and thick filaments of Spirogyra such as C. ehrenbergii var. malinvernianum, C. parvulum, C. incurvum, C. leibleinii var. boergesenii, S. bijugatus var. bicellularis, S. decimina, S. neglecta (Table 1, Figures 3–5). Small water tank in Banthra (Site 4) was found to be colder (15.8 °C) than other sites with very low concentration of DO (4.10 mg/L). Conductivity was measured 663 μs/cm, TDS 323 mg/L, salinity 0.32 % and typified by Closterium attenuatum, Closterium rectimarginatum and Oocystis

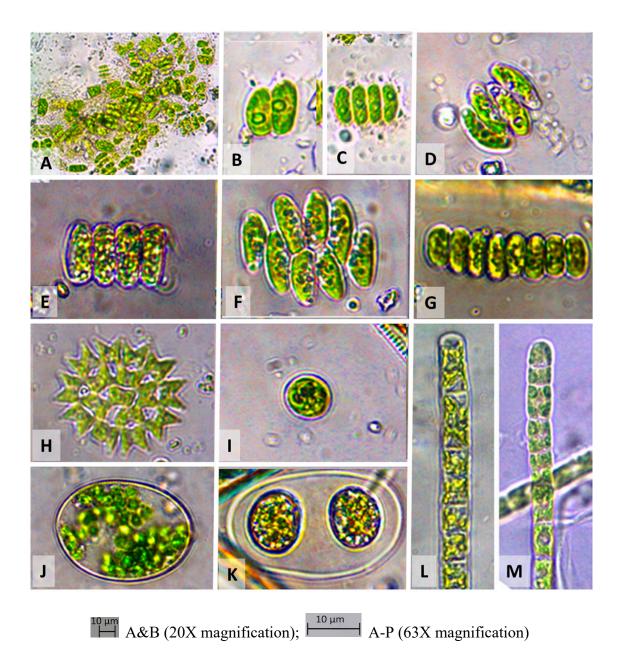
solitaria (Table 1, Figures 3 ad 4). Sugarcane field water was found to be very low in DO (2.27 mg/L) and (site 5) was dominated by a different variety of *Scenedesmus bijugatus* and *Scenedesmus quadricauda*. Some filamentous green algae such as *Zygnema micropunctatum*, *Ulothrix subtilissima*, *Spirogyra novae-angliae* were also found in water of sugarcane field (Table 1, Figures 3 and 4). We have also collected the sample from the tanks of water pumping station of IISR (site 6) (conductivity 579 µs/cm, TDS 281 mg/L and salinity 0.28 %) which was dominated by the thick filaments of *Spirogyra crassa* and *Spirogyra fluviatilis* associated with desmids *Cosmarium granatum* (Table 1, Figures 2, 4 and 5). An open pond (Sinik pond) situated in Kanpur road (Site 7) showed highest DO concentration (19.06 mg/L) as well as lowest conductivity (435µs/cm), TDS (210 mg/L) and salinity (0.21%). The mixed flora of desmids *Cosmarium pyramidatum*, *Cosmarium formosulum*, *Staurastrum avicula* var. *lunatum*, other green algae such as *Scenedesmus bijugatus* var. *alternance* f. *parvus*, *Pediastrum duplex*, *Chlorococcum infusionum*, filaments of *Spirogyra crassa*, *Spirogyra aequinoctialis* and *Spirogyra triplicate* were identified from Sainik pond (Table 1, Figures 2–5).



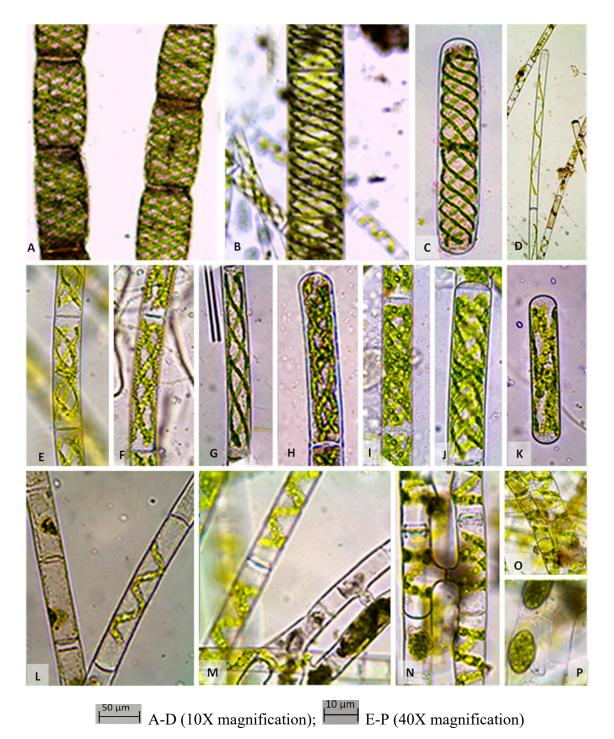
**Figure 2.** Morphological features of *Cosmarium* (**A**) Abundance of *Cosmarium* in a moist brick wall of Banthra garden, CSIR-NBRI; (**B-D**) *C. pyramidatum*; (**E**) *C. subspeciosum*; (**F**) *C. formosulum* (**G**) *C. maculatum* (**H**) *C. variolatum* var skujae; (**I**) *C. paricymatium*; (**J-K**) *C. granatum*; (**L**) *C. angulosum* var. *concinnum*.



**Figure 3.** Morphological features of Closterium and Staurastrum (A) C. attenuatum (B) C. ehrenbergii var. malinvernianum (C) C. parvulum (D) C. moniliferum var. concavum; (E) C. praelongum; (F) C. incurvum (G) C. rectimarginatum; (H) C. dianae (I) C. leibleinii var. boergesenii; (J-M) S. avicula.



**Figure 4.** Morphological features of *Scenedesmus, Pediastrum, Chlorococcum, Oocystis, Zygnema* and *Ullotrhix* **(A)** Abundance of *Scenedesmus* in sugarcane agricultural field of ICAR-IISR, (B) *S. bijugatus* var. *bicellularis*; (C) *S. bijugatus*; (D & F) *S. bijugatus* var. *graevenitzii*; (E) *S. quadricauda*; (G) *S. bijugatus* var. *alternance* f. *parvus*; (H) *P. duplex*; (I) *C. infusionum*; (J) *O. solitaria*; (K) *O. gigas*; (L) *Z. micropunctatum*; (M) *U. subtilissima*.



**Figure 5.** (A) Spirogyra maxima (Hassall) Wittr.; (B) S. crassa; (C & K) Single Spirogyra cell; (D) Spirogyra elongata (Vaucher) Kuetzing; (E) S. aequinoctialis (F) Spirogyra decimina; (G) Spirogyra triplicata; (H)S. neglecta (I) Spirogyra fluviatilis; (J) Spirogyra novae-angliae; (L) Spirogyra inflata, (M-O) conjugation; (P) Zygospore.

**Table 1.** Various parameters measured for water samples collected from seven study sites and their green algal diversity.

Si	ite	Water parameters	Algae
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	Т	pН	DO	Cd	TDS	Sl	Cells/ml	Total	Taxa
	0 <b>С</b>		mg/L	μs/cm	mg/L	0%		taxa	
1	20.6	7.78	11.45	650	361	0.31	51210±120	9	Cosmarium pyramidatum [Figure 2
									B-D]
									Cosmarium subspeciosum [Figure 2
									E]
									Cosmarium variolatum var. skujae.
									[Figure 2 H]
									Cosmarium maculatum [Figure 2 G]
									Cosmarium pericymatium [Figure 2 I]
									Cosmarium granatum [Figure 2 J-K]
									Cosmarium angulosum var.
									concinnum [Figure 2 L].
									Closterium moniliferum var.
									concavum [Figure 3 D]
									Closterium dianae [Figure 3 H]
2	19.0	8.65	14.14	572	277	0.28	60000±80	7	Closterium praelongum [Figure 3 E]
									Scenedesmus bijugatus. [Figure 4 C]
									Oocystis gigas [Figure 4 K]
									Spirogyra maxima [Figure 5 E]
									Spirogyra crassa [Figure 5 B]

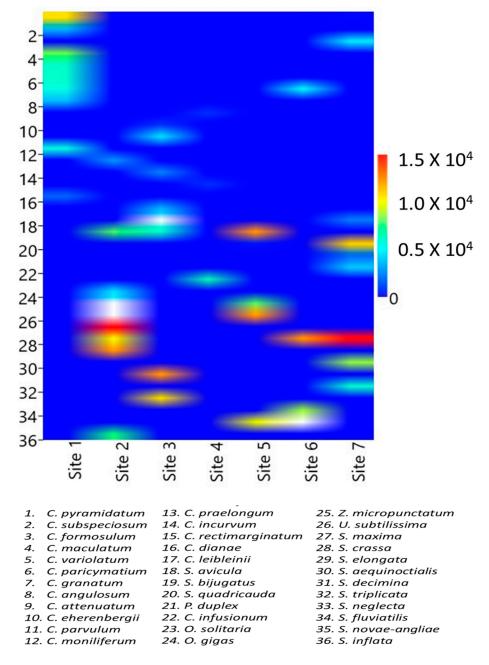
									Spirogyra elongata [Figure 5 D]
									Spirogyra inflata [Figure 5 L]
3	18.0	8.04	11.06	625	304	0.30	40100± 52	7	Closterium ehrenbergii var.
									malinvernianum [Figure 3 B]
									Closterium parvulum [Figure 3 C]
									Closterium incurvum [Figure 3 F]
									Closterium leibleinii var. boergesenii
									[Figure 3 I]
									Scenedesmus bijugatus var.
									bicellularis [Figure 4 B]
									Spirogyra decimina [Figure 5 F]
									Spirogyra neglecta [Figure 5 H]
4	15.8	7.56	4.10	663	323	0.32	8500±30	3	Closterium attenuatum [Figure 3 A]
									Closterium rectimarginatum [Figure 3
									G]
									Oocystis solitaria [Figure 4 J].
5	23.8	7.58	2.27	569	276	0.27	42300±45	5	Scenedesmus bijugatus var.
									graevenitzii [Figure 4 D, F]
									Scenedesmus quadricauda [Figure 4 E]
									Zygnema micropunctatum [Figure 4
									L]

	•								
									Ulothrix subtilissima [Figure 4 M]
									Spirogyra novae-angliae [Figure 5 J]
6	23.2	7.69	7.27	579	281	0.28	25700±58	3	Cosmarium granatum [Figure 2 J-K]
									Spirogyra crassa [Figure 5 B]
									Spirogyra fluviatilis Figure 5 I]
7	22.5	8.10	19.06	435	210	0.21	53840±60	9	Cosmarium pyramidatum [Figure 2 B-
									D]
									Cosmarium formosulum [Figure 2 F)
									Staurastrum avicula var. lunatum
									[Figure 3 J-M]
									Scenedesmus bijugatus var. alternance
									[Figure 4 G].
									Pediastrum duplex [Figure 4 H]
									Chlorococcum infusionum [Figure 4 I]
									Spirogyra crassa [Figure 5 B]
									Spirogyra aequinoctialis [Figure 5 E]
									Spirogyra triplicata [Figure 5 G]

# 3.2. Topographical Map of the Algal Distribution Pattern

The two-dimensional topographical map was drawn with a colour scale to depict the overview of algal species densities in a particular site (Figure 6). In two sites densities of 1.5 X 10<sup>4</sup> cells/ml was found and depicted by deep red colour in the map; *S. maxima* (no. 27) in site 2 and *S. crassa* (no. 28) in site 7. The density of 1.2 X 10<sup>4</sup> cells/ml (orange colour) was contributed by *S. elongata* (no. 29) in site 2, *S. crassa* (no. 28) in site 6, *S. bijugatus* (no. 19) and *U. subtilissima* (no. 26) in site 5. About 1.0 X 10<sup>4</sup> cells/ml density was depicted by yellow colour contributed by *C. pyramidatum* (no. 1) in site 1, *S. crassa* (no. 28) in site 2, *S. neglecta* (no. 33) in site 3, *S. quadricauda* (no. 20) in site 6. Cell density of 0.5 X 10<sup>4</sup> cells/ml was represented by light blue colour in the map and contributed by different species (more than 9 numbers of species). Gradually darker the blue colour lesser densities were represented. Zero

density means the absence of species was represented by dark blue colour and the major portion of the map is blue as many of the total identified species were present in a particular site and absent in others.



**Figure 6.** Two-dimensional topographical map with colour scale on the overview of algal species densities in a particular site. Taxa were represented by numbers in Y axis and sites are plotted in X axis. Deep red colour in map represents species density  $1.5 \times 10^4$  cells/ml,  $1.2 \times 10^4$  cells/ml by orange colour ,  $1.0 \times 10^4$  cells/ml by yellow colour,  $5 \times 10^3$  cells/ml by light blue colour and absence of particular species was represented by dark blue colour.

# 3.3. Diversity Indices

Diversity indices are the mathematical representation of species diversity in a community represented in Figure 7. Diversity indices provided more information about community composition than simply species richness (i.e., the number of species present); they also have taken the relative abundances of different species into account. The Dominance index represented the quantitative measure of species diversity, lower the value lower the species diversity. Dominance index was found highest in site 4 (0.61) where only three taxa were identified, whereas the lowest one was

measured in site 1 (0.13) where 9 taxa were identified. Simpson's and Shannon-Weaver's diversity index were measured on the basis of number of species present, as well as the relative abundance of each species in each sites. We have found the highest value in site 1 (0.87 and 2.105) where the number of species (9 taxa) and abundance were more and the lowest one in site 4 (0.61 and 0.38). Species Evenness refers to the frequencies of the different taxon making up a flora of a particular site, 1 is absolute species evenness of a particular site. Generally when species diversity increased evenness increased simultaneously as observed in site 1 (0.91) which consisted of 9 taxa; but exceptionally the greater evenness (0.94) was also observed in site 4 where only 3 taxa were identified. Berger-Parker Dominance is a simple measure of the numerical importance of the most abundant species. The abundance of species is greater when a number of taxa is low. In the present study the site 4 showed the highest Berger-Parker Dominance (0.76) as it consisted of only 3 taxa. Margalef's and Menhinic's index were used as a simple measure of species richness (Margalef, 1958). The measurements depended on number of species and number of individuals at a particular site. The sites consisted of a higher number of species had higher value of Margalef's and Menhinic's index such as site 1(0.74 and 0.04) and 7 (0.73 and 0.04) respectively.

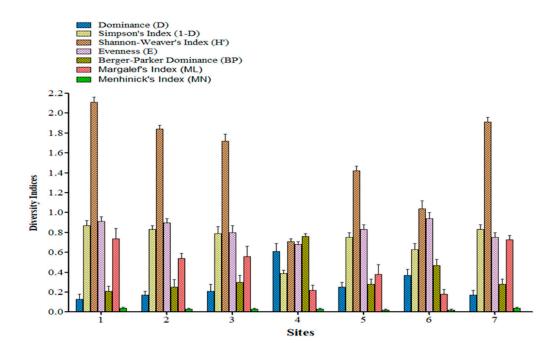


Figure 7. Diversity indices of seven study sites.

#### 4. Discussion

Algal community structure depends on the place, water parameters and seasonal variations. Nowadays, algal flora is very much affected by the changes in the environment, global warming and pollution related problems. Some species may become endangered and many of them could be newly introduced in the particular place. They can be effectively used as an indicator of water quality and pollution. Many of the research papers are presented the taxonomic (morphological) identification of different taxa available in different places of India (Patil *et al.*, 2011; Kalita *et al.*, 2015; Ramesh and Aruna, 2015; Handa and Jadhav, 2015); but limited publication is available related to the taxonomic (morphological) identification, diversity and distribution of algae with respect to water quality or seasonal variation. We have also studied the water parameters of collection sites, plotted the overview of distribution and density of algal species in particular sites and measured the diversity indices. Morphological features were analyzed and compared with several published monographs for correct identification of the taxa and regenerate the taxonomic keys for the particular species. As the sampling was carried out in winter season (15.8°C to 23.8°C) thereby the 36 green algal taxa

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identified can be marked as low temperature loving algae. The pH of the water was found within the optimum ranges of pH (7.0-9.0) for algal growth (Munir et al.. 2015). It has been observed that sampling sites having high algal density showed a high concentration of DO, which is obvious as algae contribute directly to DO concentration during photosynthesis. Large water surface exposed to air also dissolve more oxygen as observed in Sainik pond where the diversity of algae was also in a greater extent. TDS and salinity these are the important parameters to measure water quality. After analyzing the distribution and occurrence of the green algal species we have observed that less polluted water is dominated by Spirogyra; where conductivity, TDS and salinity of water were low (site 2, 5 and 7). Whereas the more polluted water was dominated by desmids (site 1 and 4). Sometimes we have seen that the particular place influences the growth and dominancy of particular species. Perhaps the bricks and rocky surface facilitate the growth of Cosmarium as observed in Banthra moist bricks and surrounding water (site 1). We have also observed the dominancy of Scenedesmus in sugarcane agricultural field soil (site 5). Earlier studies have reported that plant roots release a variety of organic compounds, sugars, amino acids, organic acids etc. which may influence the algal flora in the plant rhizosphere (Neumann and Romheld, 2001). Probably the root exudates of sugarcane influence the growth of Scenedesmus, as it is known to be growing on external carbon sources in heterotrophic mode (Dasgupta et al., 2015). However the overall water quality of these water bodies in winter were within the range of standard norms (DO 5.0-7.0 mgL<sup>-1</sup>, pH 6.5-8.5, conductivity <750 mgL<sup>-1</sup>, TDS <500 mgL<sup>-1</sup>) of World Health Organization (WHO), Bureau of Indian Standard (BIS), Central Public Health and Environmental Engineering Organization (CPHEEO), The Environment Protection Rule, 1986 (EPR) and Central Pollution Control Board (CPCB), Govt. of India. Whereas as reported by Dasgupta et al. (2017) polluted water with higher conductivity, TDS and salinity were mostly dominated by blue-green algae and diatoms. Some of the genus and taxa identified in the present study have also been reported previously from different sites of Lucknow (Singh, 2015). There are few Chlorophycean genera has been reported previously (Kumar and Suseela, 2004; Singh, 2015) but all the species identified in the present study are new to the records of Lucknow except C. angulosum, O. gigas and P. duplex which was identified earlier from Lucknow by Dasgupta et al. (2017).

#### Conclusion

Rapid economic growth, urbanization, industrialization and pollution contributed to worldwide changes in the environment and water quality, which also reflected in the diversity and distribution of microalgal communities. The study revealed that in winter water quality was within the standard norms and *Spirogyra* and desmids can act as indicators of water quality. Mostly less polluted water with low conductivity, TDS and salinity was typified by a greater diversity of *Spirogyra* and highly polluted one dominated by different species of desmids. This is an extensive work on green algal species of Lucknow and most of the species identified are new to the records of Lucknow. In-depth analysis of community composition, simple species richness and relative abundances of different species in a particular site were measured by two-dimensional topographical map and mathematical measurements of diversity indices.

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