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**Research Article**

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**DIVERSITY OF BLUE-GREEN ALGAE AND STUDY ON RELATED PHYSICO-CHEMICAL PARAMETERS OF PADDY FIELDS OF CHHATARPUR DISTRICT OF MADHYA PRADESH**

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**ABSTRACT**

Occurrence of Blue-green algae in local paddy fields of Chhatarpur district M.P. has been under taken for the first time in this area. A regular monthly collection has been made during cropping season May to October in year 2013. In the present investigation, rich diversity of Blue-green algae was recorded. Total 66 Blue-green algal species with wide range of thallus structure were found, belonging to various orders i.e., Chroococcales, Oscillatoriales, Nostocales, and Stigonematales etc. Total nine physico-chemical parameters (pH, EC, Temperature, soil N, P, K and Calcium content) were chosen for study. Chlorophyll-a content were also estimated during study period as an index of growth. This study reveals that comparatively lesser number of Blue-green algae were growing in summers in comparison to rainy season. The unicellular forms were abundant during summer while number of filamentous (heterocystous and non-heterocystous forms) increased during rainy season.

**Keywords:** Blue-green algae, Chhatarpur district, Chlorophyll-a, Diversity, Paddy fields, Physico-chemical, thallus.

**INTRODUCTION**

Blue-green algae or cyanobacteria a group of gram negative photoautotrophic bacteria are the one of the most ancient component of earth present in the Archaean and Proterozoic Eras (2.7 billion years ago) that were responsible creating our oxygenic atmosphere through their photosynthetic activities<sup>1</sup>. The paddy fields are the suitable ecosystem for growth and reproduction of blue-green algae<sup>2</sup>. The Blue Green Algae are ecologically beneficial in Paddy fields for sustaining soil fertility, reclaiming of alkaline soil and can contribute 25-30 kg N<sub>2</sub> per season. They are ubiquitous in nature and dominantly present in paddy fields. Various studies were reported on identification of Blue-green algae from various Indian paddy fields 3, 4, 5, 6, 7, 8, 9, 10 and only few from Madhya pradesh 11, 12. The District Chhatarpur occupies a central position in the plateau of Bundelkhand and known after the name of the great warrior

of the region Maharaja Chhatrasal. The District is situated at North East border of Madhya Pradesh. This District is spread over an area of 8687 Square Kms. with longitudes and latitudes of 24.06 - 25.20 on North 78.59 - 80.26 on East respectively. The study of identification of blue green algae has been taken for the first time in this area.

**MATERIALS AND METHODS**

**Collection of samples**

Soil samples were collected from four local paddy fields of Chhatarpur district of Madhya Pradesh, namely, Ishanagar (24°51'51"N, 79°23'19"E), Barat (25°0'39"N, 79°52'38"E), Sahaniya (25°0'36"N, 79°28'57"E) and Alipura (25°10'31"N, 79°20'08"E), stations-1, 2, 3 and 4 respectively. Soil samples were collected at one month interval during morning to noon from the pre-sowing treatment stage of the crop to throughout cropping and cutting season from May 2013 to October 2013.

Representative, randomized and composite soil surface samples collected from 8-10 spots of upper 0.5 cm soil crust from study areas. The sites were selected on the basis of different texture of soils and different water resources by which they irrigated, so that diverse and maximum number of Blue-green algal species can be observed. Station-1 is situated at the bank of Tadper River; station-2 is irrigated by water of Jagatsagar pond, station-3 by Seelap River and station-4 by Dhasan River.

#### Isolation and Identification of samples

Soil samples were mixed well, dried, sieved and 100g representative samples from each field were stored in sterilized polythene bags. BG 1113 and modified Chu No.10 14 were utilized as enrichment medium for isolation of strains. 1 g soil sample was inoculated in 50 mL sterilized BG-11 medium and then flasks were incubated for 30 days at  $28 \pm 20$  C with cool white fluorescent light tubes under a 16/8 h light. The isolation of Blue-green strains was carried out by dilution and pour plate method. The identification of taxa was done following the keys given by Desikachary (1959)<sup>15</sup> and classical manuals of Baker & Bold (1970)<sup>16</sup>. Table 1.

#### Microscopic analysis

Isolated Blue-green algal strains were observed under light microscope, for morphometric analysis. Camera-Lucida sketches were drawn and data related to trichome shape, filament color, akinete and heterocyst shape, size, position, number recorded. Some microphotographs of dominant forms were also taken by a digital camera (Figure.1).

#### Physico-chemical properties of Soil

Total nine different parameters pH, EC, Soil temperature, N, P, K, Organic carbon and Calcium content were taken into consideration for the measurement of fertility levels in soils. The soil temperature, pH and conductivity of the soil samples were determined by using soil thermometer, digital pH meter and conductivity meter respectively. The available soil phosphate, sodium, potassium, total soil nitrogen, organic carbon and calcium content were estimated in the laboratory as per procedures described by Trivedi and Goel (1986)<sup>17</sup>. The data obtained of the soil analysis during the study period are given in Table 2.

**Table 1. Blue-green algae identified in four studied local paddy fields of Chhatarpur district (M.P.)**

S.N.	Name of isolates	Station 1	Station 2	Station 3	Station 4
1	<i>Microcystis robusta</i>	+	-	-	+
2	<i>Microcystis aeruginosa</i>	-	-	+	-
3	<i>Aphanocapsa montana</i>	+	+	-	-
4	<i>Aphanocapsa littoralis</i>	-	+	+	+
5	<i>Aphanocapsa bififormis</i>	+	+	+	+
6	<i>Aphanocapsa virescens</i>	-	+	-	-
7	<i>Aphanocapsa banarensensis</i>	+	+	+	+
8	<i>Aphanothece saxicola</i>	+	+	+	+
9	<i>Gloeothece samoensis</i>	+	-	-	+
10	<i>Chroococcus minor</i>	+	+	+	+
11	<i>Chroococcus turgidus</i>	+	+	+	+
12	<i>Chroococcus varius</i>	+	-	-	-
13	<i>Chroococcus minimus</i>	-	-	+	+
14	<i>Chroococcus minutus</i>	+	-	-	-
15	<i>Chroococcus cohaerens</i>	-	+	-	-
16	<i>Chroococcus limneticus</i>	-	-	+	-
17	<i>Chroococcus indicus</i>	-	-	-	+
18	<i>Gloeocapsa calcarea</i>	-	+	-	+
19	<i>Gloeocapsa compacta</i>	+	-	+	-
20	<i>Gloeocapsa nigrescens</i>	+	+	+	+

21	<i>Gloeocapsa luteofusca</i>	-	-	-	+
22	<i>Gloeocapsa punctata</i>	-	+	-	-
23	<i>Stichosiphon sansibaricus</i>	+	-	-	-
24	<i>Arthrospira spirulinoides</i>	-	-	+	-
25	<i>Arthrospira platensis</i>	+	-	-	-
26	<i>Oscillatoria ornata</i>	-	-	-	+
27	<i>Oscillatoria boryana</i>	-	+	-	-
28	<i>Oscillatoria pseudogeminata</i>	+	-	-	-
29	<i>Oscillatoria limosa</i>	-	-	+	-
30	<i>Oscillatoria angusta</i>	+	-	+	-
31	<i>Oscillatoria miniata</i>	+	-	-	-
32	<i>Oscillatoria proteus</i>	+	-	-	+
33	<i>Trichodesmium thiebautii</i>	-	-	+	-
34	<i>Phormidium ambiguum</i>	-	+	-	-
35	<i>Phormidium corium</i>	-	+	-	-
36	<i>Lyngbya spiralis</i>	+	-	-	-
37	<i>Lyngbya contorta</i>	-	+	-	-
38	<i>Lyngbya mesotricha</i>	-	-	-	+
39	<i>Symploca cartilaginea</i>	-	-	-	+
40	<i>Cylindrospermum indicum</i>	+	-	-	-
41	<i>Cylindrospermum muscicola</i>	+	-	-	+
42	<i>Nostoc entophyllum</i>	-	+	-	-
43	<i>Nostoc paludosum</i>	+	-	-	-
44	<i>Nostoc calcicola</i>	+	-	+	+
45	<i>Nostoc commune</i>	-	+	-	-
46	<i>Nostoc spongiaeforme</i>	-	+	-	-
47	<i>Nostoc ellipsoforum</i>	-	-	+	-
48	<i>Nostoc muscorum</i>	+	-	-	-
49	<i>Anabaena constricta</i>	+	-	-	-
50	<i>Anabaena torulosa</i>	+	-	-	-
51	<i>Anabaena oryzae</i>	-	+	-	-
52	<i>Anabaena naviculoides</i>	--	-	--	+
53	<i>Anabaena orientalis</i>	+	-	+	-
54	<i>Anabaena circinalis</i>	+	-	-	-
55	<i>Aphazimemon flos-aquae</i>	-	-	+	-
56	<i>Scytonema bohneri</i>	-	+	-	-
57	<i>Scytonema subtile</i>	-	-	-	+
58	<i>Scytonema millei</i>	+	-	-	-
59	<i>Microchaete tenera</i>	-	+	-	-
60	<i>Calothrix fusca</i>	-	+	-	-
61	<i>Stigonema aerugineum</i>	+	+	-	-
62	<i>Haplosiphon flagelliformis</i>	+	-	+	-
63	<i>Haplosiphon welwitschii</i>	+	-	-	-
64	<i>Westiellopsis prolifica</i>	+	-	-	+
65	<i>Fischerella muscicola</i>	-	-	-	+
66	<i>Stigonema hormoides</i>	+	-	-	-
<b>Total species</b>		<b>33</b>	<b>24</b>	<b>21</b>	<b>23</b>

\* Present (+), Absent (-)

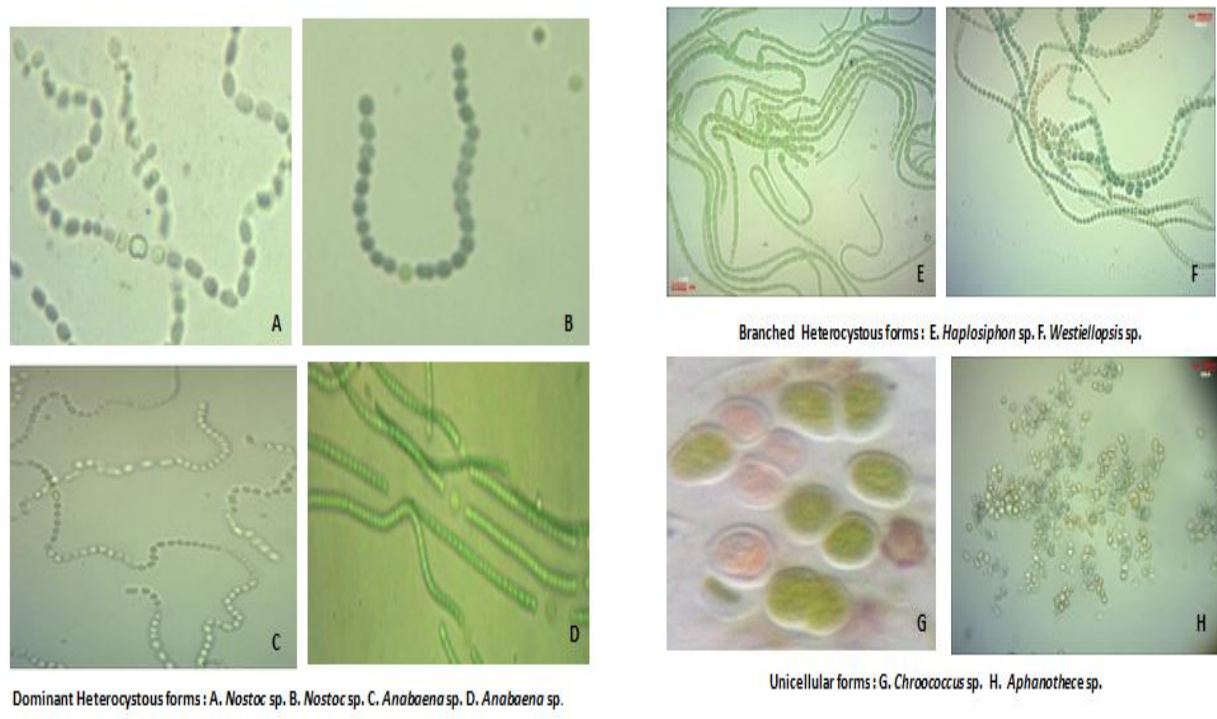


Figure 2. Some micro-photographs of identified dominant forms

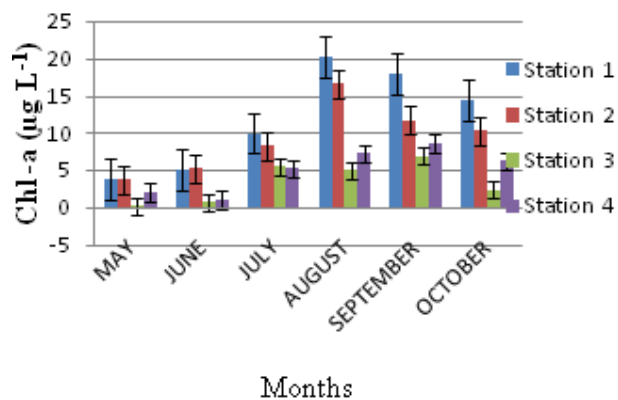


Figure 2. The graph showing variation in Chlorophyll-a in four local paddy fields of Chhatarpur district (M.P.) during May 2013 to October 2013

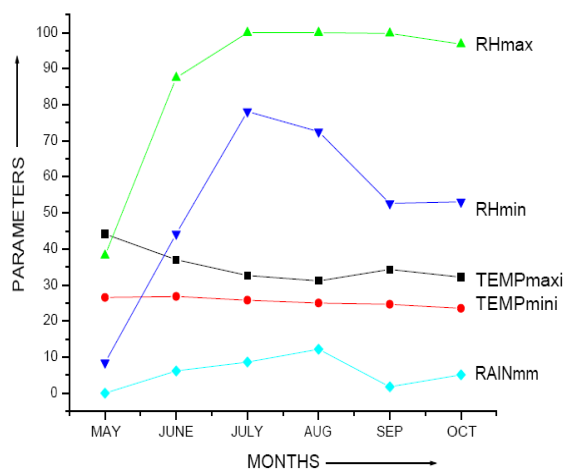


Figure 1. The graph showing monthly variations in different meteorological parameters of Chhatarpur district during study period (during May 2013 to October 2013)

**Table 2. Physico-chemical characteristics of the four local paddy fields of Chhatarpur district of Madhya Pradesh during (May 2013 to October 2013)**

Parameters	Stations	May	June	July	August	September	October
Surface temperature (°C)	1	45±0.200	43.2±0.158	32.5±0.343	32.7±0.186	35.6 ±0.423	30.9±0.310
	2	44±0.520	44±0.210	33±0.450	28 ±0.090	34.6±0.066	29.5±0.005
	3	44±0.040	42±0.364	30.5±0.026	33.7±0.057	32±0.0830	28.5 ±0.165
	4	43±0.030	44±0.095	30.2±0.062	32.5±0.860	34.5 ±0.004	27.9 ±0.019
pH	1	6.5±0.045	6.9±0.024	8.6±0.054	8.97±0.071	9.64 ±0.014	9.42±0.045
	2	5.91±0.02	6.2±0.033	7.8±0.068	7.6±0.051	6.98±0.039	6.87±0.019
	3	3.9±0.007	4.2 ±0.010	4.6 ±0.003	5.0 ±0.012	5.7 ±0.014	5.3±0.020
	4	4.3±0.026	5.2 ±0.008	5.6 ±0.041	5.8 ±0.094	6.8 ±0.057	6.1 ±0.004
Conductivity (µS)	1	3.8±0.004	4.2±0.001	4.65±0.023	4.97±0.002	5.7±0.006	3.98±0.003
	2	2.1±0.046	3.30±0.018	3.45±0.060	4.36±0.120	4.34±0.153	4.29±0.035
	3	0.1±0.082	0.3±0.0091	0.3±0.048	0.4±0.006	0.34±0.016	0.3±0.072
	4	1.2±0.160	2.3±0.185	2.6±0.101	2.8±0.002	2.5±0.080	2.2±0.025
P (Kg/hect)	1	9.78±0.019	8.96±0.082	9.6±0.078	10.5±0.079	10.37±0.134	8.34±0.063
	2	3.2±0.014	2.9±0.005	4.0±0.017	5.4±0.023	5.61±0.097	4.97±0.028
	3	0.89±0.170	2.78±0.001	2.8±0.95	2.6±0.043	2.4±0.001	0.96±0.031
	4	5.6±0.003	9.7±0.165	8.6±0.072	9.8±0.018	3.4±0.039	4.5±0.674
K (Kg/hect)	1	450.90±2.927	458±1.390	456.96±1.803	398±3.459	490±2.684	365.45±2.742
	2	365.45±1.005	456.78±1.249	403.2±1.841	400.9±0.984	406.45±0.662	394.42±0.474
	3	330.4±0.032	345.1±0.056	348.80±0.018	374.20±0.062	321±0.071	298±0.820
	4	276±0.018	289±0.260	323.8±0.045	340±0.045	340±0.038	328±0.009
Soil N (kg/hect)	1	167.30±0.063	123.80±0.048	282.10±0.094	289 ±1.080	245±1.325	280±1.462
	2	190.52±1.260	198.15±1.652	198.27±1.420	200±0.989	212±0.971	202±0.052
	3	210±0.620	256±0.505	200.8 ± 0.925	212.86±1.051	243.30±1.076	195.36±2.929
	4	180.4±0.602	196.4±0.692	188.42±0.764	162±0.831	178±0.527	184±0.810
Organic carbon %	1	0.60±0.002	0.85±0.028	0.75±0.172	0.56±0.118	0.58±0.091	0.54±0.035
	2	0.45± 0.018	0.57±0.282	0.46±0.062	0.48±0.038	0.42±0.073	0.38±0.064
	3	0.32±0.052	0.56±0.163	0.49±0.058	0.43±0.019	0.34±0.030	0.21±0.095
	4	1.05±0.068	1.20±0.003	1.20±0.103	1.23±0.062	1.12±0.041	0.98±0.071
Ca <sup>2+</sup> (ppm)	1	0.49±0.025	0.79±0.084	0.23±0.017	0.36±0.018	0.38±0.056	0.35±0.110
	2	0.26±0.005	0.32±0.650	0.65±0.012	0.53±0.080	0.75±0.032	0.36±0.085
	3	0.63±0.026	0.99±0.016	0.86±0.100	0.65±0.073	1.58±0.041	0.74±0.014
	4	0.07±0.720	0.10±0.029	0.13±0.013	0.12±0.011	0.98±0.025	0.25±0.062

\*Values are expressed as the mean ±S.D; n=3

### Measurement of chlorophyll accumulation and Enumeration of algal cells

Chlorophyll content was estimated using hot methanol extraction method<sup>18</sup>. Enumeration of populations was carried out by MPN (Most Probable Number) technique. The enrichment flasks and MPN tubes were regularly monitored for growth and observed microscopically. The data obtained of monthly chlorophyll estimation and algal counts are shown by Figure 2.

### Meteorological Study

Meteorological parameters notably influence the diversity of Blue-green algae. All the data of meteorological parameters

(Rainfall, Relative Humidity and Temperature) were collected from Agricultural Institute and Department of Bhu-Abhilekh, Chhatarpur. Average meteorological parameters during study period are showing in Figure 3.

### Statistical Analysis

The triplicate sets of data for the various parameters were analyzed by ANOVA (completely randomized design) using Graph pad prism 6.04 (Graph pad Software, Inc., San Diego, CA, USA) statistical software and critical differences values were calculated at P level of 0.05.

## DISCUSSION

### Diversity of Blue-green algae

In studied paddy fields, station-1 showed maximum number of species while least number of species recorded from station 3. The unicellular forms are abundant during summer while heterocystous and filamentous forms found exclusively during rainy season. Total 27 heterocystous, 17 filamentous and 22 unicellular forms were obtained from studied area. Maximum number of species belongs to Nostoc genera, and order Chroococcales.

### Effect of physico-chemical parameters

The physico-chemical properties of collected soils showed much variation, the maximum soil surface temperature ( $44\pm 0.520$ ) °C found in May while minimum ( $27.9 \pm 0.019$ ) °C in October. The pH of soils of all studied paddy fields differ greatly ranged from  $3.9\pm 0.007$  to  $9.42\pm 0.045$ . The soil of site-1 showed alkalinity throughout season. The maximum diversity found in alkaline soil, which favors growth of Blue-green algae. Collected soils showed low concentration of N<sub>2</sub> content, ranged from  $123.80\pm 0.048$  to  $282.10\pm 0.094$  Kg/hect but rich in potassium (K) ranged from  $276\pm 0.018$  to  $456.78\pm 1.249$  Kg/hect. The site-3 had lowest concentration of potassium  $0.89\pm 0.170$  and site-1 showed high concentration upto  $10.37\pm 0.134$  Kg/hect. The phosphorus ranged from  $0.89\pm 0.170$  to  $10.37\pm 0.134$ , showed moderate availability in soil. The calcium content of collected soils ranged from  $0.07\pm 0.720$  to  $1.58\pm 0.041$  %, showed medium concentration level of calcium in soils. The site-4 had maximum concentration of organic carbon (upto  $1.23\pm 0.062$  %) and site-3 had lowest concentration ( $0.21\pm 0.095$ ).

### Meteorological parameters and Chlorophyll-a (Chl-a)

The average monthly maximum temperature (T<sub>max</sub>) was recorded in May (44.16.C) while average minimum temperature (T<sub>min</sub>), found in August (23.52.C). The maximum Relative humidity (100%) found in July and August in study period. The maximum Chl-a value found in September ( $18.0183 \mu\text{g L}^{-1}$ ) and minimum value ( $0.2553 \mu\text{g L}^{-1}$ ) analysed in May. Site-1 showed maximum Chl-a value among four sites throughout season while site-3 showed lowest value.

## CONCLUSION

In this investigation, it is found that among meteorological parameters, relative humidity plays an important role in growth of Blue-green algae as well as available nutrients in soil also increases their number. Since maximum diversity found in alkaline soil, it implies that alkalinity favors diversity and growth of BGA. The soils of all studied paddy fields have nitrogen deficiency and due to water-logged condition, the efficiency of nitrogen fertilizers becomes low in flooded soil. Application of Blue-green algae in paddy fields as biofertilizer increases the fertility level of soil and biological nitrogen fixation will also be improved.

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