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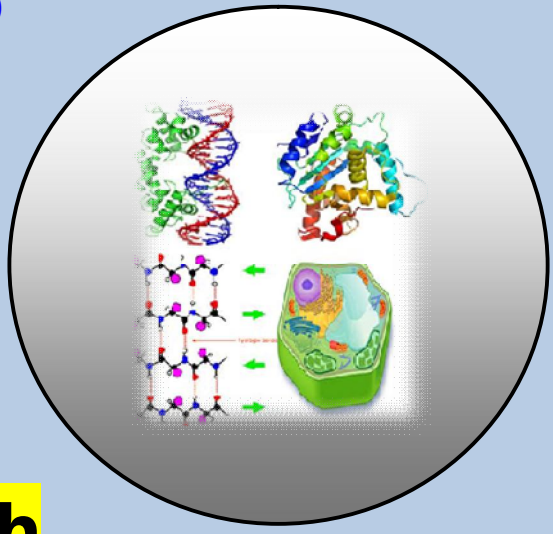

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RESEARCH PAPER

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A Study on Eco-Physiology of *Spirulina* in Relation to Some Environmental Parameters

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ABSTRACT

Physico-chemical characteristics of Surajkund water body in Varanasi (UP) were monitored. High levels of nitrogen (N), phosphorus (P), calcium (Ca), potassium (K) and sodium (Na) indicated that pond was eutrophic. However, contrast to nitrate; ammonia-nitrogen was high in summer and low in winter. The high concentrations of sodium in summer stimulated uptake and transport of CO2 and HCO3 at high PH. Highly significant correlation was observed among studied traits. NO3 exhibited significant negative relation with all traits, though the magnitude varied from NO2 to NH4. More over positive correlation observed among traits only exception was NO3. Calcium, Potassium, Sodium etc showed significant positive correlation with other traits except NO3. Positive correlation maintained by NO2 with other traits where negative value with NO3. Surajkund Microcystisaeruginosa was dominant plankton in the month of December, January and February. Spirulina sp. was found along with the population of Oscillatoria and Chlamydomonas in April, May and June. After 15 days of growth, protein, dry weight, carbohydrate, chlorophyll and carotenoids were estimated for Spirulina platensis.

Key words: Water body, Physico-chemical traits, Spirulina and Association analysis.

INTRODUCTION

Algae comprise one of the most diverse plant groups and contribute to approximately 50% of global photosynthetic activity and over 70% of the world's biomass (**Andersen, 1996**). They are considered to be the first group of organisms to appear and colonize on earth 2.5Gaλ (= Years X 109λ) ago and have broad habit range from polar region to tropical coral reef [**South, G.R and A. Whittick. 1987**].

Algae are very sensitive to the environment. Any alteration in the environment leads to the change in algal communities and reflect the average ecological condition and therefore, they may be used as tools for assessment and evaluation of habitats [**Saikia, M. K; Kalita, S, Sarma,G.C. 2011**]. Among alga, the blue green alga *Spirulina* has attracted worldwide interest as photosynthetic planktonic organism suitable for mass cultivation [**Fox, R.D. 1985; Li, D.M. 1995; Muthukumar, C Muralitharan, G., Vijayakumar, R., Panneerselvam, A. and Thajuddin, N. 2007**]. Efforts are underway to evolve a simple rural technology for producing *Spirulina* biomass and to utilize it as a feed supplement for cattle and poultry. *Spirulina* has a higher protein content, higher growth rate and easy digestibility [**Belay, A., Yoshimichi, O., Miyakawa, K. and Shimamatsu, H. 1993 Vonshak, A, (ed). 1997; Anaga A and Abu GO 1996**]. Malnutrition in developing countries has catalyzed several efforts to intensify the production of protein both from conventional agriculture and from unconventional sources. *Spirulina* because of its many favourable properties has considerable future potential in improving the protein supply to mankind **[Anuradha, V. and Vidhya, D. 2001; Bhattacharya, S. Shivaprakash, M.K. 2005;] Laliberte, G., Olguin, E.J. and de la Noue, J. 1997**]. In India millions of hectares of available land annually rendered unfit for farming because of increasing salinity or alkalinity. The saline alkaline lands occupy an area of about 65 lakh hectares in the country. These lands are characterized by impermeability, silty texture and loose aggregation of soil particles. These saline-alkaline lands are locally known as Usar Land. Due to canal irrigation, the area of user land increasing year after year. User lands show high pH from 8.3 – 10 or even 11. These lands also contain high levels of sodium. Due to high alkalinity and salinity usar land unfit for growth for most of the crops. The problem of soil alkalinity and salinity is due to the salt formation of Na^+, K^+, $Ca^{++}$and Mg^{++} ions with Cl^- and SO_4 mainly, sometimes with NO_3 and CO_3 and to a small extent with HCO_3 which may be nutrients for *Spirulina*. *Spirulina* can grow at high alkalinity up to pH 8 to 11 and prefers high salt content for growth **[Vonshak, A, (ed). 1997; Abdulqader G, Barsanti L and Tredici MR 2000; Lodi A, Binagli L, Solisio C, Converti A and Del Borghi M. 2003; Zeng WL, Cai ZL and Ouyang F. 2001; South, G.R and A . Whittick. 1987**]

MATERIAL AND METHODS

Collection, isolation and purification

Water and soil samples were collected from certain alkaline sites and were analysed as per standard procedure of ***APHA (1989)***. **Growth Measurement-** The growth experiments for isolated strains were conducted in 100 ml flasks. Exponential grown cultures were centrifuged washed with sterile double distilled water and re-centrifuged. The inoculum of filamentous strains was prepared by cyclo-mixing. The following methods were used for growth estimation. **Protein estimation**: Protein content of algal samples was determined by following the method of *Lowery et al* as modified by Herbert at al. **Pigment extraction**: Estimation of Chlorophyll a- Chlorophyll a and caroteniods were extracted in methanol and estimated as per the methodology of *Mackinney*.

Chlorophyll a and carotenoids were recorded for absorbance of 663 and 480 nm. **Carbohydrate estimation**: Total sugar was estimated by *Phenol-sulfuric acid method* using the absorbance of 480 nm, using glucose as standard. **Dry weight**: For the measurement of dry weight, algal cultures of known volume were filtered on Whatman NoI filters and dried in a hot air vacuum even at 45 C for 24 hours. Ammonia was determined as per the method of Solarzano. Dissolved Oxygen in the water sample was estimated by *Winkler's method*. Nitrate in water sample was estimated *by Brucinesulphuric acid method*

RESULTS AND DISCUSSION

Different water bodies in Varanasi such as *Durgakund, Surajkund, Laxmikund, and Laatbhairo* were extensively surveyed for high alkalinity(***Table-3***). User soil and water samples (***Table-2, Figure-1***) [Varanasi] were also collected for isolation of *Cyanobacteria*. All strains were made axenic using standard microbiological techniques and identified with the help of ***Desikachary (1959)***. All these strains were maintained in air conditioned culture room at 26+3 in presence of 75µ E light m^{-2} Sec^{-1} with 18 hr and 6hr light dark periods. Cultures were transferred to fresh agar slants at an interval of 16 days. Following strains were isolated from saline-alkaline habitats and their pH limits were recorded (***Table 2***). In Suraj Kund, a dense surface bloom of *Microsytisaeruginosa* was observed during the month of December, January and February. In the months of April, May and June *Oscillatoria amphibia* dominated in Suraj Kund. *Spirulinasp* and *Chlamydomonassp* were found along with the population of *Oscillatoria* in the months of May, June and July. The physico-chemical characteristics of the pond water recorded in different months are given in ***Table 3***. The pH values varied hourly and monthly also, pH was lowest in December and January and highest during May and June. Figure indicate that pH of water varied greatly from morning to evening; it was highest at 2:30 PM, followed by 6:30 PM, 10:30 PM and 6:30AM. The temperature varied greatly from 19 to 42 C. Similar to pH, temperature was also highest at 2:30 PM followed by evening and morning on the same day. It was highest in May and June and lowest in December and January. Marked increase in dissolved oxygen content of water occurred during day time at 2:30 PM in all the months and followed the same trend like pH and temperature. Hardness, alkalinity and total alkalinity increases considerably from December to June being highest in May, June and lowest in December. The ammoniacal nitrogen was highest in May, June and lowest in December, January. In contrast to this, nitrate nitrogen was minimum in May, June and maximum in December and January. Sodium, Potassium and calcium concentrations in water samples followed similar trends, being highest in May, June and lowest in December and January. After 15 days growth, protein, dry weight, carbohydrate, chlorophyll and carotenoids were 400, 780(mg/l), 125, 6.5 and 4.92 (µg/ml) respectively for *Spirulina platensis*. The specific growth rate and generation times were 0.026 and 38.7 (h) (***Table-1***). Correlation analysis measures the closeness of the linear relationship between chosen variables (Table-***4***).

Table 1. Growth, biomass, chemical composition of *S.platensis* attained at 15 days of growth.

Parameters	*S platensis*
Final growth 0.0 (665m)	1.52
Specific growth rate (generation h_	0.026
Generation time (h)	38.70
Dry weight (mg/l)	780.00
Carbohydrate (µg/ml)	125.00
Protein (µg/ml)	400.00
Chlorophyll(µg/ml)	6.50
Cartenoids(µg/ml)	4.92

Table 2. Collection site, characteristics of Cynobacteria isolated from different water bodies.

Cyanobacterial	*Medium*	*Collection site*	*Characteristics*	*pH for growth*
*Microcystis*aeruginosa	Jaworski medium	Suraj Kund, Laxmi Kund, Laat Bhairo	Unicellular, bloom forming	9-11
Oscillatoriaamphibia	CHU-10	Suraj Kund	Non-heterocystous filamentous	8-9.5
Nostoccalcicola	Allen Arnon	Usar Soil, Bhadooi, Varanasi	He terocystous filamentous	8
Nostoc sp.	Allen Arnon	-do-	-do-	8
Anabaena sp.	Allen Arnon	-do-	-do-	8.2

Table 3. Physico-chemical characteristics of Suraj Kund water in different months.

	December	*January*	*February*	*March*	*April*	*May*	*June*
Hardness (mg/l)	18.6	22.4	21	23.8	24.6	26	28
Alkalinity(mg/l)	20	21	21	38	39	42	42
Total Alkalinity(mg/l)	169	165	164	189	190	199	225
NO_3(mg/l)	9.4	9.3	9.2	8.4	7.8	5.25	3.75
NH_4(mg/l)	0.14	0.21	0.4	0.9	1.7	3.92	4.4
NO_2(mg/l)	0.46	1.6	2.9	3	3.2	3.6	3.7
Na (ppm)	110	117	119	130	135	139	140
K(ppm)	78	89	90	110	124	125	130
Ca(ppm)	23	23.4	24.3	26	28.5	29	29.2

Moreover the value of correlation coefficient nearer to +1 or –1,shows the probability of linear relationship between the variables x and y. Highly Significant correlation observed among studied traits.NO_3exhibited significant negative relation with all traits, though the magnitude varies from NO_2 to NH_4. More over positive correlation observed among traits only exception is NO_3.Calcium, Potassium, sodium etc showed significant positive correlation with other traits except NO_3.Positive correlation maintained by NO_2 with other traits where negative value with NO_3 (***Table-4***).

Table 4. Correlation matrix for physio-chemical traits of Suraj Kund.

	Hardness (mg/l)	Alkalinity (mg/l)	Total Alkalinity (mg/l)	NO_3 (mg/l)	NH_4 (mg/l)	NO_2 (mg/l)	Na (ppm)	K (ppm)	Ca (ppm)
Hardness (mg/l)	1.0000	0.8921	0.8993	-0.8920	0.8891	0.8552	0.9531	0.9481	0.9116
Alkalinity(mg/l)		1.0000	0.8867	-0.8054	0.8264	0.8133	0.9680	0.9682	0.9503
Total Alkalinity(mg/l)			1.0000	-0.9458	0.9200	0.7037	0.8799	0.8881	0.8845
NO_3(mg/l)				1.0000	- 0.9914	- 0.7102	- 0.8528	- 0.8452	-0.8695
NH_4(mg/l)					1.0000	0.7342	0.8763	0.8665	0.8999
NO_2(mg/l)						1.0000	0.9088	0.8780	0.8590
Na (ppm)							1.0000	0.9943	0.9801
K(ppm)								1.0000	0.9866
Ca(ppm)									1.0000

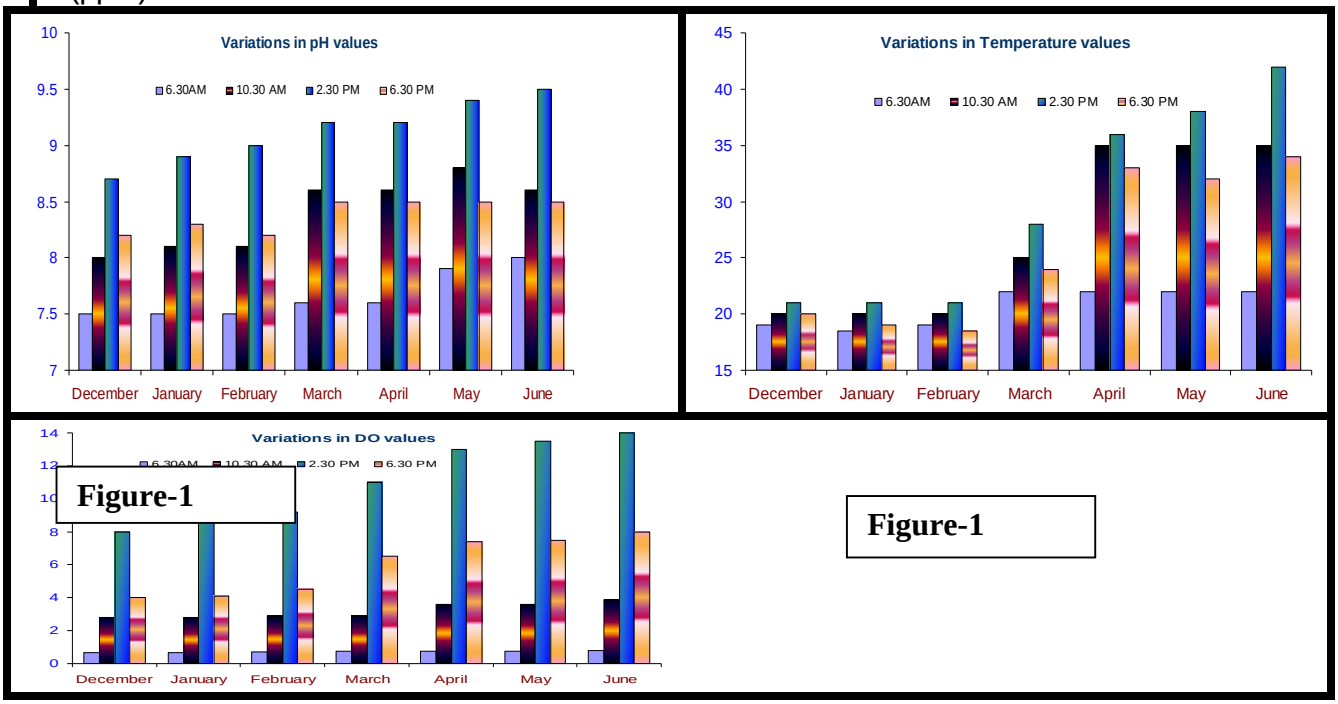

Figure1. Monthly variations in pH, temperature and DO values.

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