

Effect of temperature on growth of *Mastigocladus laminosus*

Dr.A.C.Mongra

Professor & Head, Department of Biomedical Engineering ,Adesh Institute of Engineering &Technology (Punjab
Technical University) Faridkot-151203, India

Abstract: The *Mastigocladus lamiosus* has ability to fix carbon and nitrogen of atmosphere and due to this dual ability this can be explored further for algal biofertilizer in rice field where temperature remain above or around 45 °C in rainy season. No growth of any of the thermal strains of cyanobacteria isolated from hot water spring Tattapani (HP) was obtained under laboratory conditions using spring water as nutrient medium. The maximum growth of *Mastigocladus laminosus* on the basis of chlorophyll-a concentration (4.2 µg chl-a/ml culture) after 15 days was obtained in Allen and Arnon's medium (pH 7.2) without nitrogen source at 45°C and cool fluorescent light (12 W/m²). Mass culturing of this species under laboratory conditions was done using laboratory fabricated fermentor for continuous use of the culture. The optimum growth with highest frequency of heterocyst (6%) of this strain was found at 45°C with an exponential phase upto 15 days. Significant decrease in the growth rate was observed at suboptimal temperatures of 25° and 35°C. This type of study is useful in exploring the possibility of use of thermal cyanobacteria *Mastigocladus laminosus* as biofertiliser in rice field

Keywords- *Mastigocladus lamiosus*, growth temperature, thermophilic cyanobacteria ,Algal Biofertilizer

I.INTRODUCTION

Thermophilic cyanobacteria are interesting study organisms for basic as well as for applied research. Their ancestors are possibly the oldest primary producer organisms common in the distant past, and they perhaps used thermal springs as refugia (Hindak 2008). Among cyanobacteria *Mastigocladus laminosus* was described from Karlovy Vary (Bohemia) by Cohn (1862), later it was approved by Kastovský (2001) and Kastovsky and Komarek (2001). It is considered in many textbooks as a typical thermal cyanobacteria, growing in temperatures < 60 °C, pH > 7.5 and low salinity. Its taxonomic position, however, is complicated, because of its extreme morphological variability. *Mastigocladus laminosus* ranks in the second position with regards to its widespread occurrence and distribution in hot springs. The optimum growth temperature of the thermophilic strain of *Mastigocladus laminosus* range from of 42°C to 50°C, while the maximum limit was reported in the range of 63°C to 64°C (Brock, 1978; Castenholz, 1969; Holton, 1962).

The *Mastigocladus laminosus* reported in Indian hot springs has optimum tolerance range of about 40-50°C. Castenholz (1970) isolated various polymorphic strains of *M. laminosus* which have higher temperature tolerance range (60- 65°C). It appears that there are large numbers of thermal strains of *M. laminosus* exist in the nature, they basically differ each other from their thermotolerance. The difference among these thermal strains and also the difference from the same non-thermal genus is basically as a result of considerable degree of physiological and biochemical specialization within the cells of thermal cyanobacteria in order to tolerate very high temperature. This thermostability is brought about with the high degree stability of proteins (tertiary structure), enzymes and membrane systems in cells for proper functioning of all the vital processes necessary for survival, especially photosynthesis. Murata et al., 1979).

Compared to other thermal cyanobacteria, *M. laminosus* is highly tolerant to other abiotic stresses in addition to high temperatures. The filaments occasionally become dehydrated under dry weather or salt stress and tends to revive upon favourable conditions. In most of the thermal ecosystem, nitrogen is one of the limiting factor for growth of the photoautotrophs. *M. laminosus* has advantage over others with respect to its ability to fix atmospheric nitrogen for sustaining its own optimal growth and as well as supply the N-nutrients continuously to the remaining non N₂-fixing photoautotrophs. Thus *M. laminosus* plays an important role in balancing the nitrogen status

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 1, January 2014

of thermal spring. Due to this unique property of photosynthesis and nitrogen fixation, the *M. laminosus* often thrives as static single community of cyanobacteria in hot spring while others almost in the process of disappearance under nutrient limiting conditions and again appear when favourable conditions become available (Roger, 1986). This growth and primary productivity of other thermal cyanobacteria are largely influenced by *M. laminosus*. It has been reported that heterocyst bearing filamentous blue-green algae such as *Mastigocladus* make significant contribution to the nitrogen budget by fixation of atmospheric nitrogen in hot stream (Stewart, 1968). *M. laminosus* has been demonstrated as a pioneer species for a laboratory model of cyanobacterial mat as it controlled the nitrogen content budget of spring and hence influence the growth of other cyanobacteria (Bryanskaya et al. 2008)

Keeping in view of the role of thermal *Mastigocladus* strain in regulating the nitrogen balance in the spring water which often supports the growth of other phytoplankton under similar ecosystem, the use of *M. laminosus* as potential nitrogen biofertilizer is realised. This useful strain can be exploited as non-polluting biofertilizer in tropical alkaline submerged rice field where temperature often remains higher in most of the period

II. MATERIALS & METHODS

Algal samples were collected from the different sites of thermal spring "Tattapani" in many replicates. Pre-sterilised screw cap glass vials of 15ml capacity were used for collection purpose. Randomly selected 1cm² blocks of algal patch were scrapped and collected in separate vials. Remaining volume of the vials was filled with natural spring water and these were brought to laboratory for isolation. Streak plate technique was used for clonal isolation of species of *Mastigocladus*.

Table 1: Composition of different media

Chemical	Media (All value are in mg/l medium)				
	AA+N (Allen & Arnon) (Basal Huges medium) (1955)	CHU (Chu No.10) (1942)	BBM (1949)	DM (Bold Basal medium) (1976)	MH (Castenholz's medium) (1958)
NaNO ₃	-	-	250	689	1500
Ca(NO ₃) ₂ .4H ₂ O	-	23	-	-	-
KNO ₃	303	-	-	103	-
NaCO ₃	-	20	-	-	20
NaSiO ₃ .5H ₂ O	-	-	4	-	58
NaCl	117	-	25	8	-
MgSO ₄ .7H ₂ O	124	25	75	100	75
CaCl ₂ .2H ₂ O	15	-	25	-	27
CaSO ₄ .2H ₂ O	-	-	-	60	-
KH ₂ PO ₄	-	-	175	-	-
K ₂ HPO ₄	457	10	75	-	39
Na ₂ HPO ₄	-	-	-	111	-
Nitritotriacetic acid	-	-	-	100	-
Micro-elements: Concentration(ppm) To add 1ml/ litre in all medium					
H ₃ BO ₄	2.86				
MnCl ₂ .4H ₂ O	1.81				
MnCl ₂ .4H ₂ O	222				
ZnSO ₄ .7H ₂ O	0.079				

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 1, January 2014

CuSO ₄ .5H ₂ O	1.26
Na ₂ MoO ₄ .2H ₂ O	0.137
CoCl ₂ .6H ₂ O	0.040
<p>In all medium , iron was provided as Fe –EDTA. The stock solution of Fe-EDTA was prepared according to Jacobson(1951) with modification of Waris (1953) the concentration of iron in this solution was 1 mg/ml. In all the media 10 ml of stock solution of Fe-EDTA was used .pH of the media was always adjusted to 7.5 The culture vessels and media were sterilized in an autoclave at 15 lbs/inch² pressure for 15 minutes</p>	

The cyanobacterium was maintained in sterilized glass jar of volume 5 litres containing mineral medium, Allen and Arnon free from nitrogen in temperature controlled incubator. The 10ml cell suspension fragmented and inoculated into the medium (3 litres) under aseptic condition and was continuously bubbled with air through a sterilized cotton filter from air pump. Two identical sets of this apparatus at 45°C and other at 25°C ±2°C were set. The cultures were constantly illuminated to a light intensity at 2500 lux from 20 Watt fluorescent lamps, kept at a distance of 10cm from glass jar. Fresh algal suspension was harvested for each experiment from the outlet nozzle connected with a sterilized PVC tube. The nutrient media were recycled again in to the glass jar by opening the inlet nozzle, whenever it was required. The inlet nozzle directly connected with the sterilized medium already prepared in a 4 litre flask. The whole set up is shown in the **Fig. 1**. The 45°C grown culture is referred to as HTG (High Temperature Grown) and 25 ±2 °C grown culture is referred to as LTG (Low Temperature Grown) in the text.

Fig 1 Experimental set up for growing of. Mastigocladus laminosus



Filamentous Mastigocladus. laminosus grown at 45°C in incubator as above was centrifuged, washed and pellet obtained was fragmented using glass beads in to possible uniform size. Uniform size fragments were centrifuged and suspended in 15ml of Allen and Arnon medium without nitrogen. 1ml each of suspension was inoculated in fifteen 100ml conical flasks containing 100 ml AA-N media. Five replicates (5 flasks) each were kept at 25°C, 35°C and 45°C in separate incubator over constant rotating shakers. At regular intervals, 2ml of cell suspension was withdrawn, centrifuged and pellet was dried in aluminium foil at 40°C from respective growth temperature. Growth was determined by estimating chlorophyll-a in 80% acetone (5ml) on equal dry weight basis for organism grown at different temperatures.

III.RESULTS &DISCUSSION

III .1 Growth in relation to temperatures, heterocyst frequencies and media

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 1, January 2014

The Mastigocladus laminosus has been described as one of the most widely distributed thermophilic alga (Fig. 2 and Fig 3).

Fig 2 Filaments of thermal Mastigocladus lamionsus with heterocysts

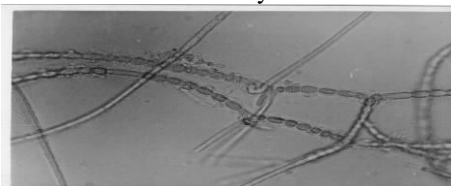
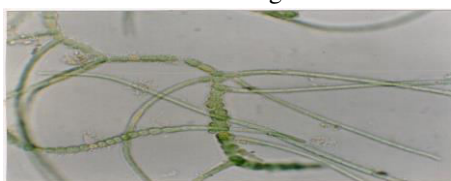


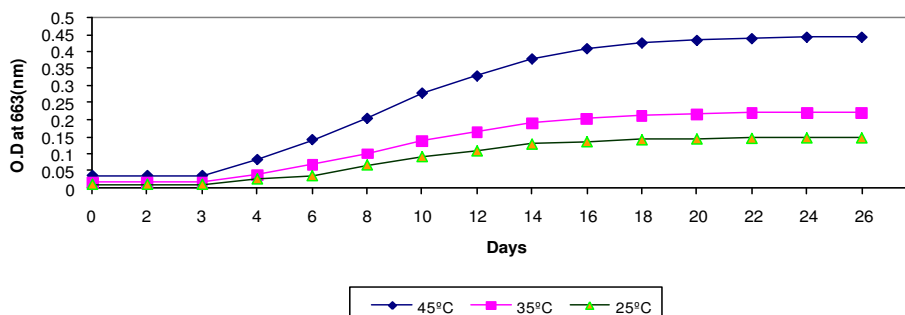
Fig3 Filaments of thermal Mastigocladus lamionsus with dividing cells



It is filamentous and branched with heterocysts (Schwabe 1960). This species is particularly unique in being the most heat tolerant nitrogen fixing species found to-date (Stewart,1970). While it can be found throughout the world, it is the dominant species in Iceland and New-Zealand where *S. lividus* is not found. Mastigocladus laminosus has been reported to be growing at temperatures ranging between 45°C and 60°C depending on the strains (Schwabe 1960; Binder et al., 1972). However, this strain of Mastigocladus laminosus did not appear to be a strict thermophilic form, as it could grow well below 45°C in the laboratory. It is important to point out here that the same organism occasionally can tolerate higher temperatures (more than 45°C) in its native hot spring but eventually loss its tolerance under laboratory conditions. The phenomenon is related to membrane function which is responsible for providing thermostability (Fork et al., 1979; Murata et al., 1979) and which could have undergone a considerable degree of alteration in response to synthetic media and to various other controlled conditions including abiotic factors to which the organism was exposed in the laboratory. Miller et al (2006, 2007) have recently compared 37 strains of Mastigocladus laminosus isolated from sites throughout the world, analyzed 839 nucleotides of the 16S rRNA gene, and reconstructed phylogenies for the nitrogen metabolism genes. They concluded that, although the species is cosmopolitan, its populations are genetically differentiated on local geographic scales and genetically isolated by distance. A common ancestor may have been located in the Yellowstone area (USA). In the present investigation, the isolated strain also showing variability in tolerance of temperature in spring Tattapani (HP) in respect to already reported strains, showing the variability in geographically scales in temperature tolerance due to genetically differentiation. In Thermal springs, new strains possessing attractive biochemical pathways and unusual metabolic products for biotechnological applications (Jaromir Lukavsky et al 2011)

The growth of the cyanobacterium at three temperatures 25, 35 and 45°C as shown in Fig. 4. At 45°C after an initial lag period of three days, the organism attained exponential growth for the next 15 days and thereafter reached to a stationary phase i.e. the organism survived without further multiplication. Similar growth patterns were observed at 35°C and 25°C. However growth drastically declined at these two temperatures (25 and 35°C). The optimum growth temperature of the thermophilic strain of Mastigocladus laminosus has been reported in the temperature range of 42°C to 50°C, while the maximum limit was reported in the range of 63°C to 64°C (Brock 1978, Castenholz 1969, Holtan 1962) with a 1.5 doubling per day. Castenholz(1970) isolated various strains of polymorphic species of Mastigocladus laminosus and successfully grew them in defined medium. These isolates were collected from hot springs with temperatures ranging between 32°C to 62°C. But M. laminosus from

Fig.4 Growth of Mastigocladus laminosus at different temperatures



International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 1, January 2014

Tattapani under investigation did not grow so well in DM medium compared to Allen and Arnon medium which was free from nitrogen source. This showed that this isolate of Mastigocladus laminosus collected from Tattapani is different both physiologically and morphologically from other strains which could grow well in DM medium as reported by Castenholz(1969a),table 2.

Table2.Growth of Mastigocladus laminosus in different media ($\mu\text{g chl-a/ml}$)

(pH adjusted for all the media 7.5 , Light intensity 2,500 lux, Temperature 45⁰C and the age of the culture during the estimation was 15 days)

Growth media	AA (- N) Allen & Arnon (1955)	BBM Bold Basal medium (1949)	DM Castenholz,s medium (1976)	MH Basal Huges medium (1958)	Chu- 10 Chu No. 10 (1942)
Cholorophyll-a	4.2	2.3	1.0	1..0	1.4

The optimum growth with highest frequency of heterocyst (6%) of this strain was found at 45⁰C with an exponential phase upto 15 days, on the growth temperature at 25 &35 ,the heterocyst numbers found to be less in comparison showing the haemophilic character for best growth of this cyanobacterium

Table3 :Heterocyst frequency of Mastigicladus laminosus at different growth temperatures (Heterocyst frequency equals to number of heterocysts present per hundred vegetative cells)

Growth Temperature (⁰ C)	Heterocyst frequency (%)
25	1
35	3
45	6

Medium DM has salinity of about 1002 mg/l (total dissolved solids) which is in the common range of value for the large number of hot springs including present Tattapani hot water spring. However, this medium DM does not resemble natural spring water with respect to minerals content. For example, medium is highly enriched in nitrate and nitrogen: phosphorus ratio of about 5. There is little fluoride and no added silicate or bicarbonate which are the most common three ions of major alkaline hot springs.

III.2 Growth in relation of photosynthetic pigments :

III.2.1Photosynthetic pigments

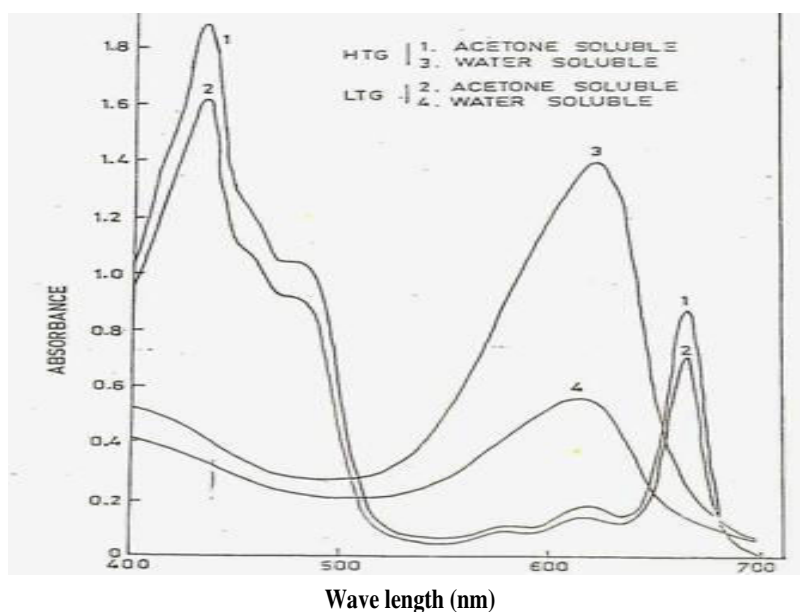
Absorption spectra of chlorophyll-a, carotenoids and phycocyanin are shown in Fig. 5

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 1, January 2014

Fig5 Absorption spectra of chl.a and carotenoids (1,2) and phycocyanin (3,4) of *Mastigocladus laminosus*

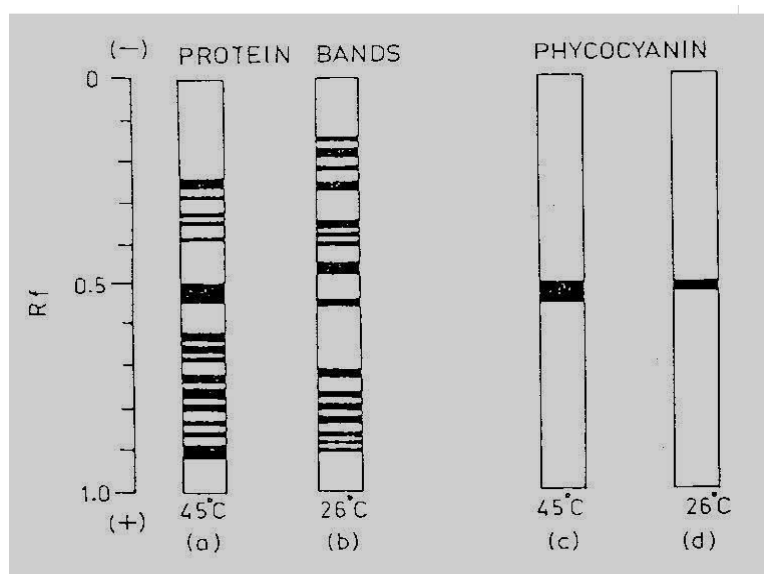


It was observed that on the basis of equal dry matter, phycocyanin content of the cyanobacterium was significantly less in low temperature grown cell (25°C, LTG) compared to their high temperature counterpart (45°C, HTG). The ratio of chlorophyll-a to phycocyanin decreased with increasing growth temperature suggesting that phycocyanin constituted the major accessory photosynthetic pigment at high growth temperature, as shown in **Table 2**. Sheridan and Ulik (1976) reported that phycocyanin increased relative to chlorophyll-a when growth temperature of one of the thermal strains of cyanobacteria *Synechococcus lividus* was increased from 35°C to 55°C. This change in photosynthetic pigment composition resulted in an obvious colour shift from yellow green to blue green as the temperature of culture increased. The concentration of chlorophyll-a was found to be less affected relative to phycocyanin at high temperature.

III.2.2 Protein profile at different temperatures

Total protein profile at the high temperature grown cells (45°C, HTG) and low temperature grown cells (26°C, LTG) in *M. laminosus* showed 15 and 16 bands respectively as shown in **Fig. 6**

Fig 6. Electrophoretogram showing the total protein bands in low and high temperature grown alga



The number of protein bands are consistent with the earlier findings i.e. most of the cyanobacteria e.g. non-thermal *Synechococcus cedrorum* and filamentous forms, *Anabaena* sp., *Tolypothrix* sp. etc. exhibited 16 to 20 protein bands in disc gel electrophoresis (Jacob,1979). A total of 24 protein bands with different electrophoretic mobilities have been reported in *Synechococcus cedrorum* grown at 26°C (Gupta, 1980). Out of these 24 protein bands detected in *S. cedrorum*, thirteen bands were found to be similar to the LTG grown at 26°C with electrophoretic mobilities of (0.16, 0.20, 0.23, 0.27, 0.36, 0.40, 0.42, 0.47, 0.55, 0.73, 0.77, 0.86 and 0.90). Depending upon their relative mobilities, 9 protein bands were found to be common in both LTG and HTG (0.36, 0.40, 0.55, 0.73, 0.77, 0.80, 0.83, 0.86, 0.90). Comparative distribution patterns of the different protein bands in HTG and LTG revealed that LTG possessed a number of protein bands with relatively low electrophoretic mobilities (0.16, 0.20, 0.23) suggesting that all these proteins were of higher molecular weight. Similarly protein bands in the HTG gel demonstrated seven bands of electrophoretic mobilities (0.25, 0.30, 0.35, 0.63, 0.66, 0.70, 0.75) and these bands were not present in LTG. These dissimilarities in protein distribution in LTG and HTG, suggested that shifting of the *M. laminosus* from its optimal growth temperature (45°C) to sub-optimal temperature (26°C) for longer period (20days) possibly caused a change in protein constituents or appearance of new proteins with either low or high molecular weight.

Only one phycocyanin band was observed in LTG and HTG as distinct sharp blue coloured band with relative mobilities of 0.52 and 0.55 respectively as shown in Fig. 6(c,d). Two phycocyanin bands reported by Jacob (1979) in non-thermal cyanobacteria, *S. elongatus* (REM values, 0.31 and 0.30) and *S. cedrorum* (REM values, 0.30 and 0.38) appeared to be different from this thermal alga with respect to their relative electrophoretic mobility.

IV CONCLUSION

The present study reveals that optimum growth with highest frequency of heterocysts (6% of this strain was found at 45°C) with an exponential phase up to 15 days. Significant decrease in growth rate was observed at supraoptimal temperature of 25°C & 35°C. No growth of any of the thermal strains of cyanobacteria was obtained under laboratory conditions using spring water as nutrient medium. The maximum growth of *M. laminosus* on the basis of chlorophyll-a concentration (4.2 µg chl-a/ml culture) after 15 days was obtained in Allen and Arnon's medium (pH 7.2) without

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 1, January 2014

nitrogen source at 45°C and cool fluorescent light (12 W/m²). Mass culturing of this species under laboratory conditions was done using laboratory fabricated fermentor for continuous use of the culture. The optimum growth with highest frequency of heterocyst (6%) of this strain was found at 45°C with an exponential phase upto 15 days. Significant decrease in the growth rate was observed at suboptimal temperatures of 25° and 35°C. All the major photosynthetic pigments e.g. chlorophyll- a, C-Phycocyanin and carotenoids were present in this strain. Phycocyanin increased relative to chlorophyll-a at higher growth temperatures of 45 and 50°C compared to 35°C. Electrophoresis of the protein extract obtained from the organism grown at 26°C and 45°C indicated a change in the protein pattern with respect to their relative electrophoretic mobility. The study reflects that, this useful strain can be exploited as non-polluting biofertilizer in tropical submerged rice fields where temperature often remains higher in most of the period of the year.

REFERENCES

- [1] Allen, M.B and Arnon, D.I. Studies on nitrogen fixing blue-green algae. 1. Growth and nitrogen fixation by *Anabaena cylindrica*. Lemm.Plant.Physiol, Vol 30,pp.366-372,1955
- [2] Binder, A., Locher, P. and Zuber, H. Concerning the large scale cultivation of the thermophilic cosmopolitan *Mastigocladus laminosus* in Icelandic hot springs. Arch. Hydrobiol, Vol. 70, pp.541-555,1972
- [3] Bryanskaya, A. V.,Orleanskii, V. K.,Dagurova, O. P. A laboratory model of the cyanobacterial mat from Kotel'nikovskii hot spring (Baikal region). Microbiology,Vol. 77, pp.490-496, 2008
- [4] Brock, T.D. In: Thermophilic microorganisms and life at high temperatures. Springer-Verlag, New York. 1978
- [5] Castenholz, R.W. The thermophilic cyanophytes of Iceland of the upper temperature limit. J. Phycol., Vol 5,pp. 360-368 ,1969a
- [6] Castenholz, R.W. Thermophilic blue-green algae and the thermal environment. Bact. Rev., Vol 33,pp.476-504,1969b
- [7] Castenholz, R.W. Laboratory culture of thermophilic cyanophytes. Schweiz. Z. Hydrol., Vol 32,pp.538-551,1970
- [8] Cohn, F. Über die Algen des Karlsbader Sprudels, mit Rücksicht auf die Bildung des Sprudelsinters. Abhandlungen der Schlesischen Gesellschaft und Vaterländische Kultur. Vol.5, pp.37-55, 1862
- [9] Fork, D.C., Murata, N. and Sato, N. Effect of growth temperature on the lipid and fatty acid composition and the dependence on temperature of light induced redox reactions of cytochrome-f and light energy redistribution in the thermophilic blue-green alga *Synechococcus lividus*. Plant physiol., Vol 63, pp. 524-530, 1979
- [10] Gupta, R.K. Physiological and biochemical studies on a unicellular blue-green alga *Synechococcus cedrorum*. Ph.D. Thesis, BHU, Varanasi, India. 1980
- [11] Jacob, L. Serological and biochemical studies on certain blue-green algae. Botany Ph.D. Thesis, Banaras Hindu University, Varanasi, India 1979
- [12] Jaromir Lukavsky, Sevdalina Furnadzhieva, Plamen Pilarski Cyanobacteria of the thermal spring at Pancharevo, Sofia, Bulgaria Acta Bot. Croat.,Vol. 70, No. 2, pp.191-208, 2011
- [13] Kastovsky, J. The phototrophic microvegetation of seminatural thermal springs in Karlovy Vary, Czech Republic. PhD Thesis, University of South Bohemia., Budijovice http://botanika.bf.jcu.cz/thesis/pdf/KastovskyJ_Mgr97.pdf, 2001
- [14] Kastovsky, J., Komarek, J. Phototrophic microvegetation of thermal springs in Karlovy Vary, Czech Republic. In: Ester, J., Seckbach, J., Vincent, W. F., Lhotsky, O. (eds.), Algae and extreme environments. Nova Hedwigia, Suppl.,Vol. 123, 107-119, 2001
- [15] Hindak, F., On *Chlorogloeopsis fritschii* (Cyanophyta/Cyanobacteria) from thermal springs in Slovakia and from saline lake in Tunisia. Algological Studies. Vol. 126, pp.47-64,2008.
- [16] Holton, R.W. Isolation, growth and respiration of a thermophilic blue-green alga, Am. J. Bot.,Vol. 49,pp. 1-6, 1962
- [17] Miller, S. R., Castenholz, R. W., Pedersen, D. Phylogeography of the thermophilic cyanobacterium *Mastigocladus laminosus*. Applied and Environmental Microbiology Vol. 73, pp.4751-4759, 2007
- [18] Miller, S. R., Purugganan, M. D., Curtis, S. E., Molecular population genetics and phenotypic diversification of two populations of the thermophilic cyanobacterium *Mastigocladus laminosus*. Applied and Environmental Microbiology. Vol. 72, pp.2793-2800, 2006
- [19] Murata, N., Wada, H., Omata, T. and Ono, T. Low temperature stress and membrane lipid phase in the blue-green algae. Effect of stress on photosynthesis. Adv. Agric. Biotechnol., R. Marcelle, H. Ceijsters and M. Van Poucke (eds.) Martinus Nijhoff "Dr. W. Junk." Pub vol. 99, pp. 193-155, 1979
- [20] Roger, P.A., Tirol, A., Ardales, S. and Watanabe, I. Chemical composition of cultures and natural samples of N₂-fixing blue-green algae from rice fields. Biol. Fertil. Soils, Vol 2, pp.131-146, 1986.
- [21] Schwabe, G.H. Über den thermobionten Kosmopolitan *Mastigocladus laminosus* Cohn. Blau-Algen und Lebenstraum V. Schweiz Z. Hydrol.,Vol. 22, pp.757- 792, 1960
- [22] Sheridan, R.P. and Ulik, T. Adaptive photosynthesis response to temperature extreme by the thermophilic cyanophyta *Synechococcus lividus* J. Phycol., Vol.12, 251-261. 1976.
- [23] Stewart, W.D.P. Nitrogen input in to aquatic ecosystems. In: "Algae, Man and Environment (D.F.) Jackson, ed.) pp. 53-72 University Press, Syracuse, N.Y 1968 .
- [24] Stewart, W.D.P. Nitrogen fixation by blue-green algae in Yellowstone thermal areas. Phycologia, Vol.9, pp. 261-268, 1970 .