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STUDY OF VARIOUS PHYSICO-CHEMICAL PARAMETERS ON GROWTH BEHAVIOR ON DIFFERENT ALGAL ISOLATES FROM SHASTRA DISTRICTS

Simmi Kumari*[1] and Shikha Choudhary[2]

[1]School of Biological and Chemical Sciences, MATS University, Raipur.

[2]Department of Botany S.N.S.R.S. College, Saharsa.

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***Corresponding Author**

Simmi Kumari

School of Biological and Chemical Sciences, MATS University, Raipur.

ABSTRACT

The study of various physico-chemical parameter gives insight of cyanobacterial growth pattern which was measured in dry weight (mg/l) best growth of *M. Aeruginosa* was observed in a Jaworski's medium flowed by Allen arnon medium, BG-11 medium and CHU10 medium respectively Best growth of *O. limnosa* observed in BG-11 medium flowed by Allen arnon medium and Jaworski's medium. *A. circinalis* showed best growth in CHU10 medium flowed by BG-11 medium and Allen arnon medium. Incandescent light supported the best growth of all three algae. The poorest growth was observed in Far red light. Blue light algae grew initially up to 9^{th} day and then ceased growing. The growth and biomass production studied at varying temperature. Maximum growth was observed at 30^0C followed by 25, 35, 40 and 20^0C. While maximum growth of *O. limnosa* at pH 8.5 and that of *A. circinalis* at 8. The growth was recorded in the pH range of 7 to 11. Growth of cyanobacteria in different inorganic sources was concentration dependent which showed the impact of various concentrations of nitrogen sources on *M. aeruginosa*, *O. limnosa* and *A. circinalis*.

KEYWORDS: *Physico-Chemical Parameters, Growth Behavior, Cyanobacteria.*

INTRODUCTION

During warm weather water bodies turn blue-green due to bloom formation. Blue-green algae are the most common attainders in bloom formation because of high nutrient levels, particularly nitrogen and phosphorus concentration chiefly promote reproduction and formation of dense water bloom. Majority of toxin producing Blue-green algae grow well in mineral solutions devoid of organic substance, a slight alkaline reaction proving most suitable environment. A number of workers have been making attempts to grow the new isolate in a variety of medium. At the moment different synthetic media such as JM[1], BG- 11[2], CB[3], MJM[4] are known in which reproducible growth of *M. aeruginosa* has been reported. The cyanobacteria show diversity in the habitat. All the ponds/lakes/rivers inhabiting cyanobacteria have different level of nutrients. The nutrient levels in the natural environment are often considerably lower than in synthetic culture media. The presence of relatively high concentration of essential nutrients and trace metals in synthetic media may be inhibitory to cells whose physiological and metabolic activities are adapted to much lower nutrient concentration.[2]

Monochromatic light of the visible spectrum is involved in many functions such as photosynthesis, growth, morphogenesis, chromatic adaptation and heterocyst differentiation of the cyanobacteria.[5] The pigment content of photosynthetic organism is greatly affected by light qualities.[6] Attempts have been made by a number of workers to optimize growth of *M. aeruginsa* by altering concentration of nutrients. Type and ratio of important nutrients present in media.[4,7] Similarly effect of temperature on growth and toxin production has been reported.[8]

In the present study we have made an attempt to culture the local isolate of *M. aeruginosa*, *Nostoc linkia*, and *Anabaena macrospora*. The growth response of these strains has been studied in various synthetic media under varying physiological factors in order to optimize the growth condition.

MATERIALS AND METHODS

Saharsa district include two subdivision Saharsasadar and Simribakthiyarpur. District is located at 25.88^0N-86.6^0E. It has an average elevation of 41 meters (134 feet). Saharsa and its surrounding areas are a flat alluvial plain forming part of the Kosi river basin. River Kosi passes through different parts of these districts. A large number of water bodies like Chours, Ponds and Ditches are present in this area in which water remains logged throughout the year. These water bodies are contaminated by animals and human activities. Organic waste is being added regularly. So luxuriant growth of blue-green algae takes place in these water bodies.

Ten spots were selected from where algal samples were collected at regular intervals. Water samples were collected in 1 lit. plastic bottles and preserved for laboratory analysis as per standard procedures recommended in APHA.[9] Collected algal samples were brought to laboratory and identified microscopically using standard monographs. The time interval between collection, storage and analysis was intentionally shortened to avoid any major change in chemical characteristics of the sample. The analysis was done for the following parameters as per the methods briefly mentioned against each one.

Growth behavior in different culture media

For optimizing the growth in vitro, growth response was tested in BG-11 medium, Allen and Arnon's medium, Modified Jaworsk's medium, Chu-10 medium, E27 medium, ASN-III medium, DGN medium and Gorhams medium. Initially the growth behaviors were tested 25±2^{0}C and 2200±200 Lux light intensity. Growth was measured from 3rd day to 15th day. In term of dry wt. (mg/l).

Growth behavior in different monochromatic light

The six different Incandescent, Fluorescent, Green, Red, Blue, Far Red lights tested with respect to promotion of growth.

Response of Growth to Temperature

The growth and biomass production studied at varying temperature. The range of temperature was taken from 20to 40^{0}C. the dry weight (mg/l) was measured from 3rd day to 15th day.

Effect of pH on Growth

Effect of pH was on growth of selected strains was tested. For this pH range of medium was taken from pH 7 to pH 11. The growth was measured on 15th day.

Growth response with Different Inorganic Nitrogen source

Nitrogen status of the pond water regulates the bloom formation. So, the impact of nitrogen status on growth was examined. To assess the effect of different inorganic nitrogen sources three salts – Amm. Nitrate, Amm. Chloride and Sodium nitrate were selected. Each salt was mixed in culture medium at different conc. 0.125 mM, 0.25 mM, 0.5 mM, 2.30 mM and 4.30 mM were added in culture medium and growth was measured on 15th day. The growth in different nitrogen sources at different conc.

Growth response with Different Organic Nitrogen Source

Effect of urea and amino acid was tested on the growth of the organism. Seven different Amino acid-Alanine, Lysine, Methionine, Glutamine, Aspartic acid, Glutamic acid, Trytophan were selected. The concentration of urea was 0.25 mM were as amino acids were added at a concentration of 0.50 mM. The growth was measured on 15th days.

Impact of varying phosphate concentration on the Growth

Much information is available regarding the role of phosphorus in bloom formation and species diversity.[10,11] N:P ratio plays vital role in determining the growth of phytoplankton in any water body. To assess the effect of phosphorus 5 different concentration of KH_2PO_4 was added in culture media. The concentration of $KH_2PO_4$was taken as 0.5 mg/l, 2.5 mg/l, 4.5 mg/l and 6.5 mg/l. Growth was measured from 3rd day to 15th day.

RESULT AND **DISCUSSION**

Growth was measured in dry weight (mg/l) best growth of M. Aeruginosa was observed in a Jaworski's medium flowed by Allen Arnon Medium, BG-11 medium and CHU10 medium respectively (Fig- 1.1). Best growth of Oscillatoria limnosa observed in BG-11 medium flowed by Allen arnon medium and Jaworski's medium (Fig-1.2). *Anabanena circinalis* showed best growth in CHU10 medium flowed by BG-11 medium and Allen Arnon Medium (Fig.-1.3).

Growth Behavior in different monochromatic light

The six different light Incandescent, Fluorescent, Green, Red, Blue and Far red tested with respect to promotion of growth. Growth was measured from 0 to 15th day. Incandescent light supported the best growth of all three algae. The poorest growth was observed in Far red light. Blue light algae grew initially up to 9th day and then ceased growing. Result shows in Fig. -1.4 to 1.6.

Response of Growth to Temperature

The growth and biomass production studied at varying temperature. Maximum growth was observed at 300C followed by 25, 35, 40 and 200C. Growth was observed between 15 days in the culture incubated in between 20 to 40^{0}C (Fig.-1.7-1.9).

Effect of pH on Growth

Effect of pH on growth was measured on 15th day. Fig.-1.10 shows the effect of pH on the growth. Maximum growth of M. aeruginosa was observed at pH 9.0. While maximum growth of *Oscillatoria limnosa* at pH 8.5 and that of *Anabaena circinalis* at 8. The growth was recorded in the pH range of 7 to 11. The lower and higher pH of the medium inhibited the growth (Fig. No.-1.10).

Growth response with Different Inorganic Nitrogen source

Best growth of the culture of Oscillatoria limnosa and Anabaena circinalis was seen in the medium supplemented with NaNo3. *M. aeruginosa* also showed best growth in NaNo3 followed by NH4Cl and NH4NO3 while growth of *Oscillatoria limnosa* and *Anabaena circinalis* was good in Ammonium nitrate and poor in Ammonium chloride. Growth was concentration dependent. (Fig.-1.11- 1.13) shows the impact of various concentrations of nitrogen sources on *M. aeruginosa, Oscillatoria limnosa* and *Anabaena circinalis*. Linear increase in growth yield with increasing concentration of nitrogen source was observed.

Growth response with Different Organic Nitrogen Source

Effect of Urea and 7 amino acids was observed on the growth of selected strains of Blue green algae. Growth was measured on 15th day. M. aeruginosa showed best growth in Glutamine flowed by Urea. Aspartic acid and Glutamic acid also supported growth but other amino acid did not support the growth of M. aeruginosa. Growth of *Oscillatoria limnosa* showed best growth in Glutamine flowed by Aspartic acid. Glutamic acid also supported growth. Urea and other amino acids did not support its growth. Growth of *Anabaena circinalis* was supported by Glutamine, Aspartic acid and Glutamic acid. Best growth was observed in Glutamine flowed by Glutamic acid.

Impact of varying phosphate concentration on the Growth

Effect of phosphate was observed in four different concentrations (0.5, 2.5, 4.5 and 6.5 mg/l) KH2PO4 from 0 to 15th day. Maximum growth *of M. aeruginosa* was seen in the medium supplemented with 4.5 mg/l phosphate followed by 6.5 mg/l. Minimum growth was reported at 0.5 mg/l of phosphate. Maximum growth of *Oscilltoria limnosa* and *Anabaena circinalis* was recorded at 2.5 mg/L phosphate (Fig.-5.14- 5.16).

Monochromatic light of the visible spectrum is involved in many functions such as photosynthesis, growth, morphogenesis, chromatic adaptation and heterocyst differentiation of the cyanobacteria. There are few reports of the effect of light on the growth of cyanobacteria.[12] Many workers have earlier studied the specific requirement of the nutrient and impact of physicochemical parameters on the growth of M. aeruginosa (Gorham *et al.*, 1964; Carmichael, 1992; Thakur, 1996; Kumar *et al.*, 2000).[13,14,15]

Fig. No. 5.1 Growth response of M. aeruginosa in different culture media (growth from 0-15th Day)

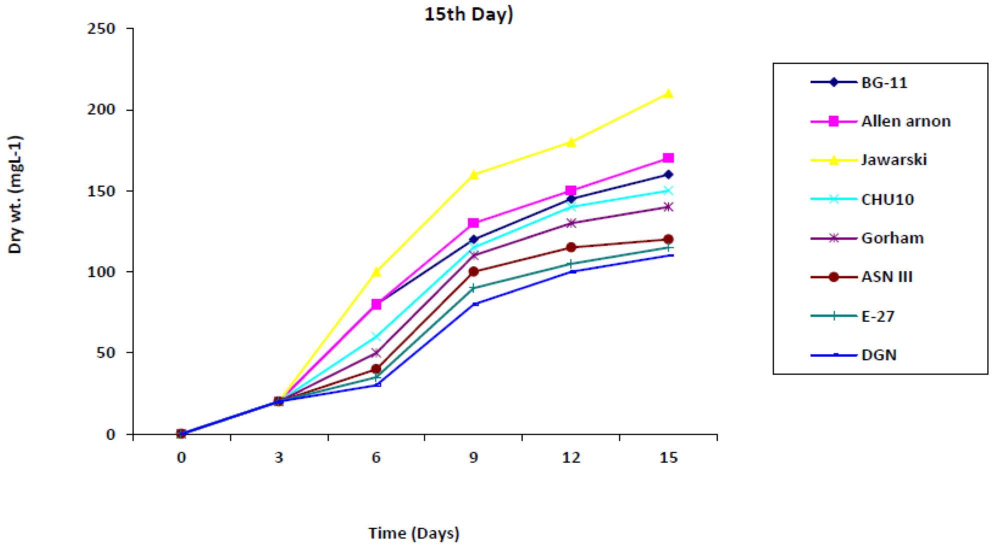
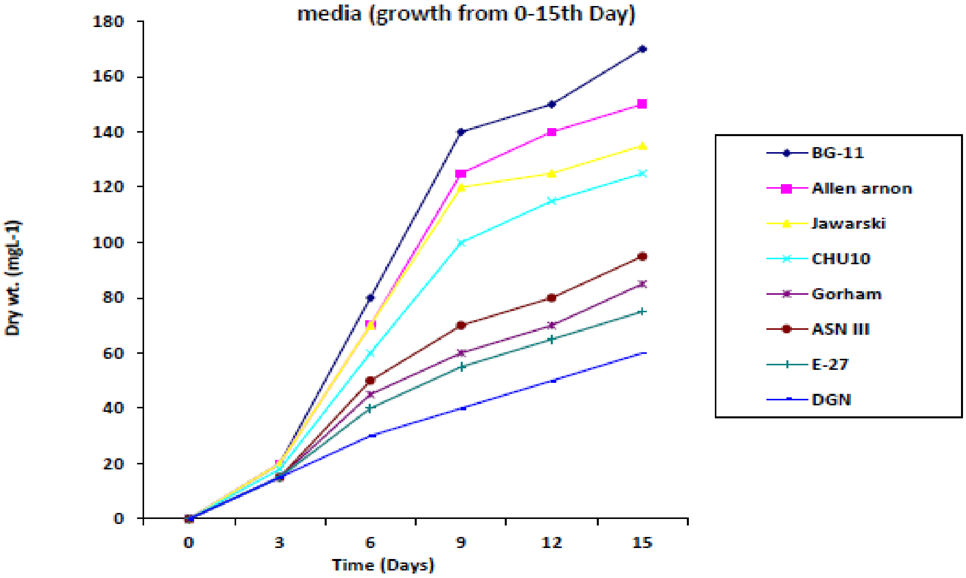


Fig. No. 5.2 Growth response of Oscillatoria limnosa in different culture media (growth from 0-15th Day)



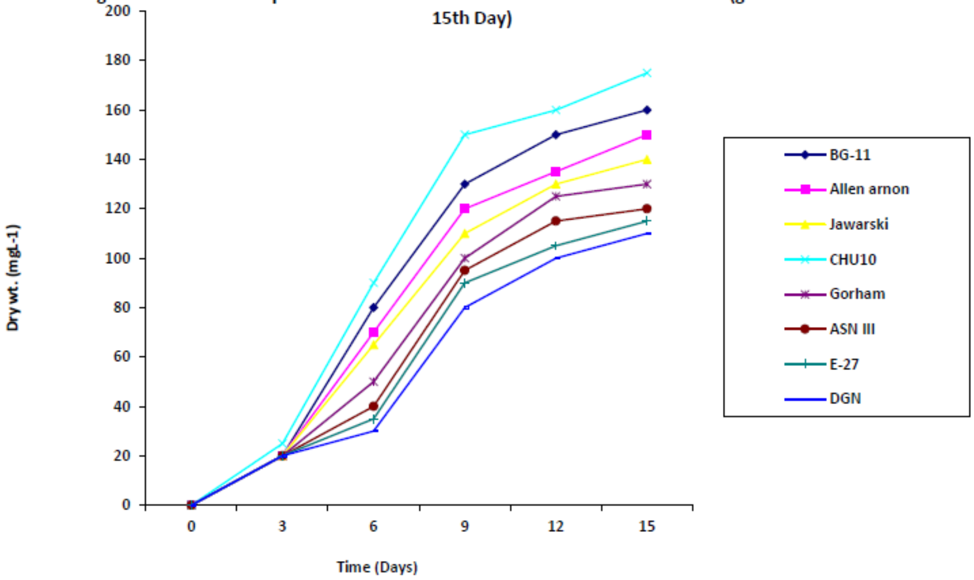


Fig. No. 5.3 Growth response of Anabaena circinalis in different culture media (growth from 0-15th Day)

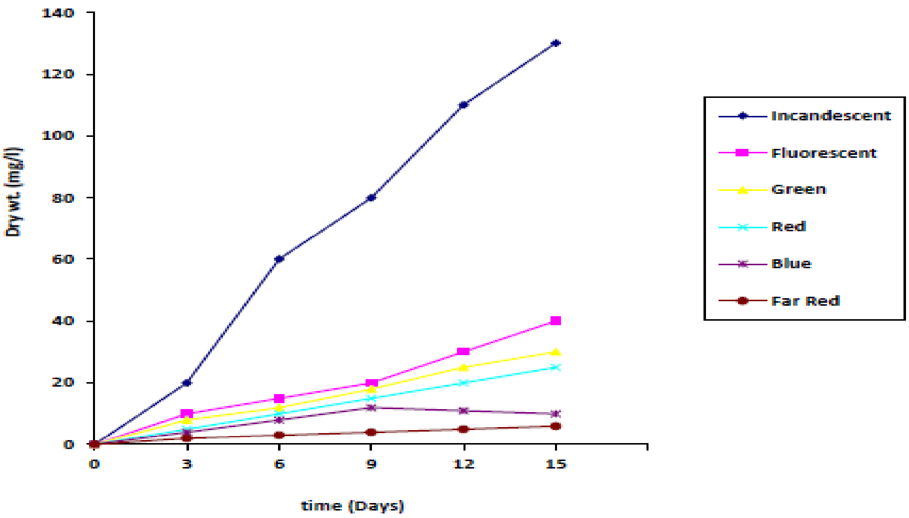


Fig. No. 5.4 Growth behavior of *M. aeruginosa* in different monochromatic light (growth from 0-15th Day)

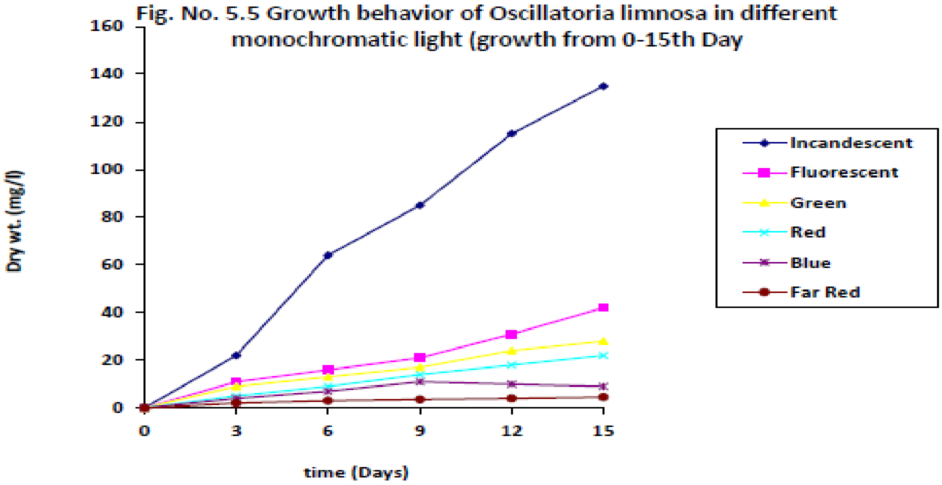


Fig. No. 5.5 Growth behavior of Oscillatoria limnosa in different monochromatic light (growth from 0-15th Day

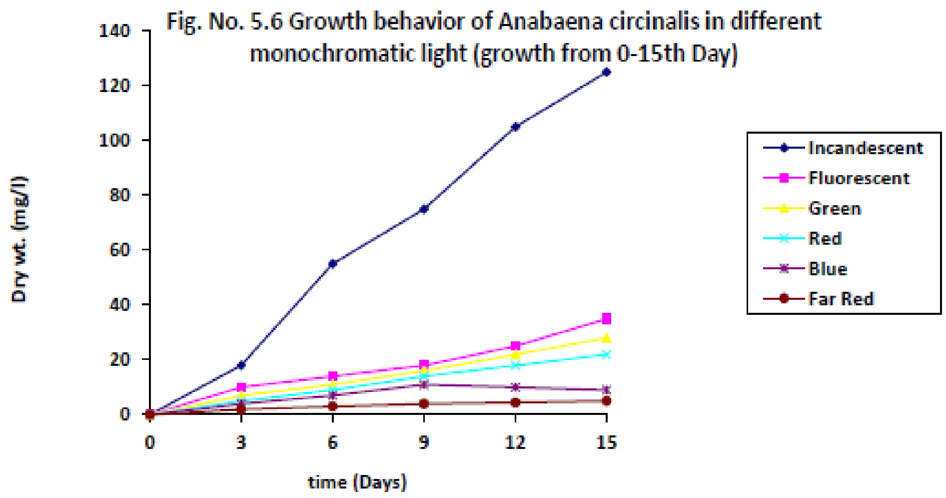


Fig. No. 5.6 Growth behavior of Anabaena circinalis in different monochromatic light (growth from 0-15th Day)
Dry wt. (mg/l)
time (Days)
Incandescent
Fluorescent
Green
Red
Blue
Far Red

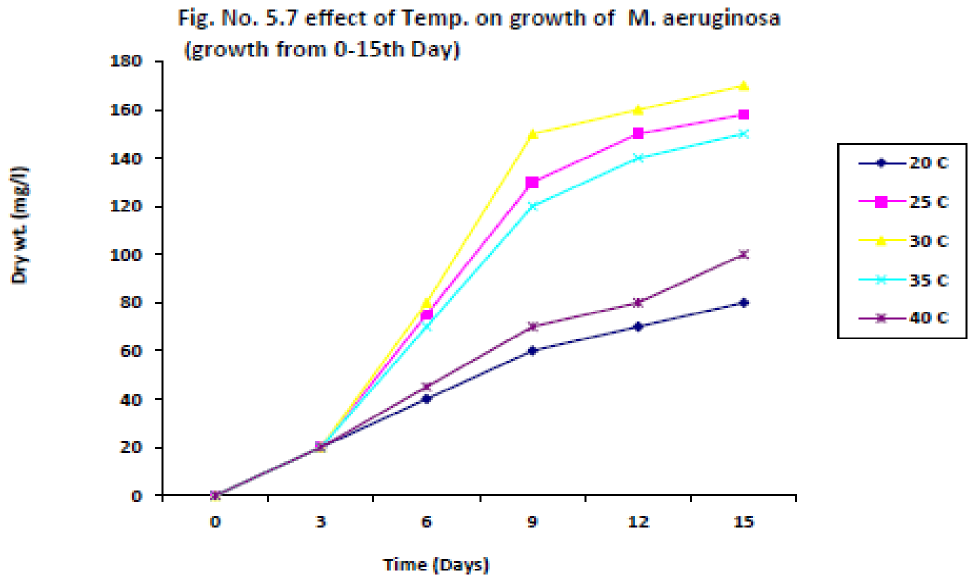


Fig. No. 5.7 effect of Temp. on growth of M. aeruginosa (growth from 0-15th Day)
Dry wt. (mg/l)
Time (Days)
20 C
25 C
30 C
35 C
40 C

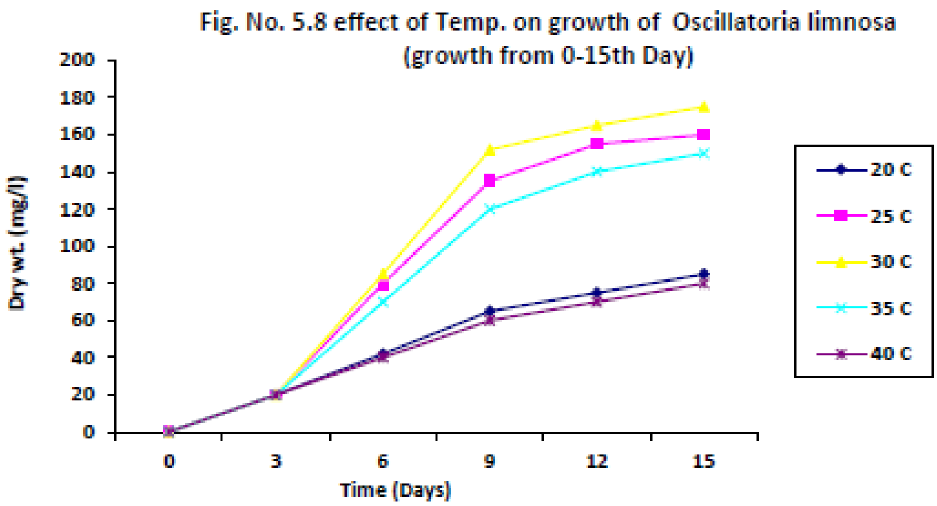


Fig. No. 5.8 effect of Temp. on growth of Oscillatoria limnosa (growth from 0-15th Day)
Dry wt. (mg/l)
Time (Days)
20 C
25 C
30 C
35 C
40 C

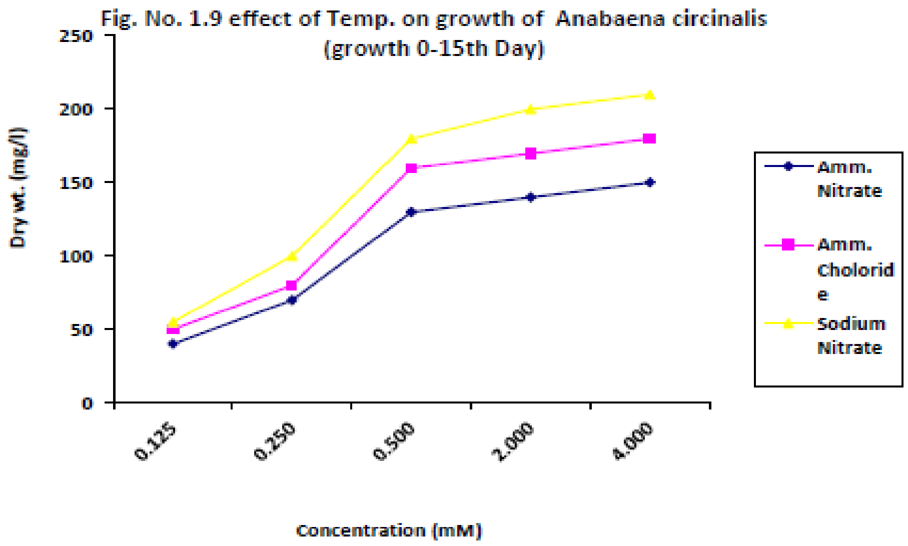


Fig. No. 1.9 effect of Temp. on growth of Anabaena circinalis (growth 0-15th Day)
Dry wt. (mg/l)
250
200
150
100
50
0
0.125
0.250
0.500
2.000
4.000
Concentration (mM)
Amm. Nitrate
Amm. Cholorid e
Sodium Nitrate

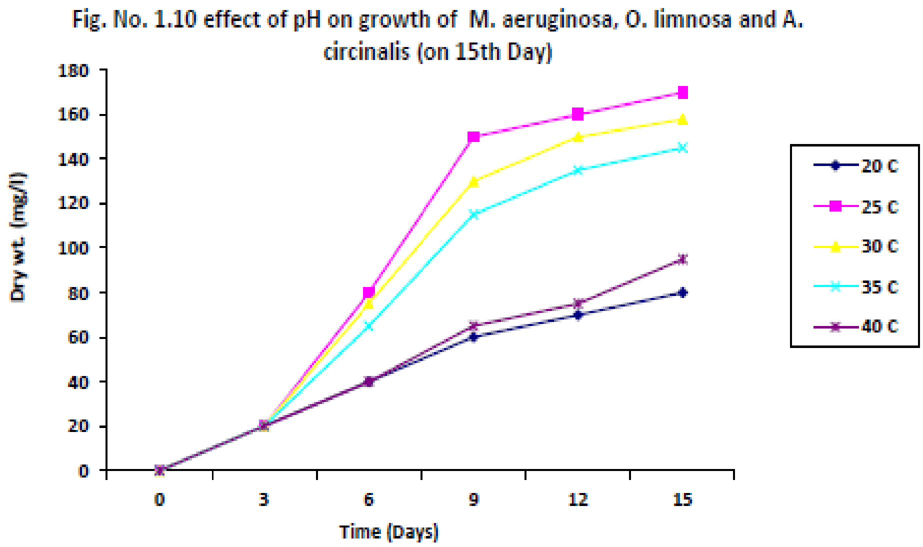


Fig. No. 1.10 effect of pH on growth of M. aeruginosa, O. limnosa and A. circinalis (on 15th Day)
Dry wt. (mg/l)
180
160
140
120
100
80
60
40
20
0
0
3
6
9
12
15
Time (Days)
20 C
25 C
30 C
35 C
40 C

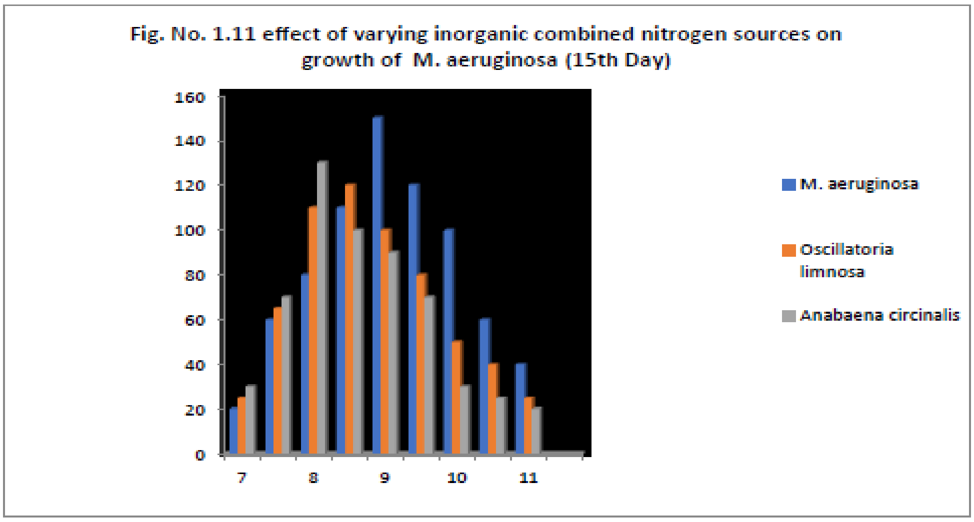


Fig. No. 1.11 effect of varying inorganic combined nitrogen sources on growth of M. aeruginosa (15th Day)
160
140
120
100
80
60
40
20
0
7
8
9
10
11
M. aeruginosa
Oscillatoria limnosa
Anabaena circinalis

Fig. No. 1.12 effect of varying inorganic combined nitrogen sources on growth of O. limnosa (15th Day)

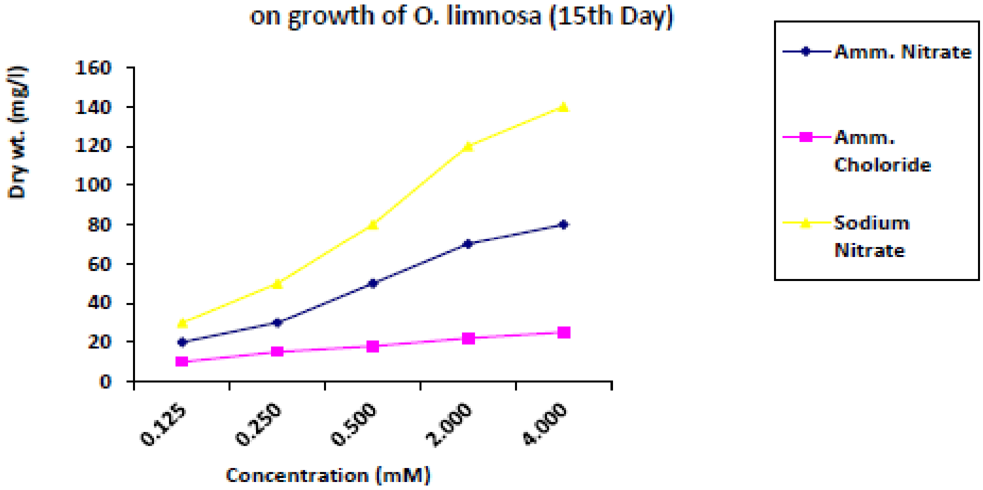
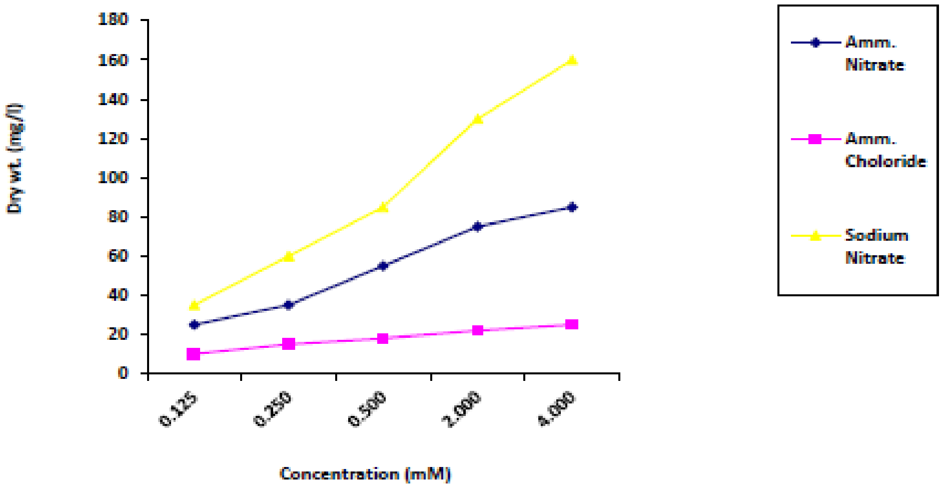


Fig. No. 1.13 effect of varying inorganic combined nitrogen sources on growth of Anabaena circinalis (15th Day)



CONCLUSION

In investigating the nutrition of these algae, and especially relation to their organic nutrient, it is necessary to eliminate the bacteria that occupy the surface of the trichomes and often live in abundance within the gelatinous sheaths. Pure cultures of the diverse *Oscillatoria*, as well as of *Nostoc cuticulare*, where first achieved by Pringsheim and have since been obtained with other species of *Nostoc* and *Anabaena*.

Diverse earlier workers established that blue-green algae often grow well in nitrogen free solutions, with or without organic matter, and this has usually been ascribed to the cooperation of nitrogen fixing Bacteria, which or almost certainly occur within the enveloping mucilage.

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