Global Journal of Science Frontier Research: C
Biological Science
Volume 17 Issue 4 Version 1.0 Year 2017
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

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*GJSFR-C Classification:* *FOR Code: 069999*


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# Studies on Kinetics of Nitrogen Uptake in Combination with 2, 4-D in Blue-Green Algae of Rice Fields

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## I. Introduction

In tropical countries such as India, the blue-green algae inhabitated mainly in the paddy fields (Singh,1961;Venkataraman,1972) and play a considerable leading role in the ecosystem of rice agriculture (Watanabe and Brotonegoro,1981). The most important functional characteristics of blue-green algae growing in the soils of paddy-fields are the dinitrogen fixation and reclamination of the soil by enriching with nitrogenous substances and metabolites to obtain more crop yield and reduce the application of nitrogenous chemical fertilizers. To obtain a high yield of grains, farmers employ synthetic chemical fertilizers in the rice-fields, which enrich the nitrogen content and phosphate content. With a view to obtain more crop yield, the farmers have been following the modern agricultural practices such as controlling growth of weeds and pests by employing the pesticides and herbicides irregularly and indiscriminately. As a result, pesticides and herbicides not only pile up and cause environmental hazard in paddy fields but also effect the non targeted beneficial organisms such as blue-green algae and fungi inhabitated in the paddy fields. The coincidence of employing the herbicides to eradicate weeds in the rice-fields while the farmers are simultaneously irrigating the fields with eutrophicated water.

At this juncture the eutrophication promotes the growth and survival of blue-green algae while the pesticides and the herbicides normally suppress the growth of weeds and pests simultaneously affecting the non-targeted beneficial blue-green algae of rice-fields. In fact the blue-green algae should not grow in the rice-fields because of the application of the pesticides and herbicides, but after their application where the irrigated eutrophic water used to stagnate, one could observe the abundance of growth of cyanobacteria which could have been influenced by the environmental factors such as nutrients (nitrogen and phosphate) and physical factors (pH, temperature and light). The intrinsic information acquire from rice-field farmers necessitated to pursue the research on nutrients influence on the herbicidal toxicity in cyanobacteria.

The percentage of nitrogen in cyanobacteria would be 8-10% of the total dry weight and nitrogen becomes an important factor in controlling the luxuriant growth of blue-green algae. The cyanobacterial growth and uptake of nitrogenous substances in rice-fields possibly depended on many physical factors (light, rainfall and temperature) and chemical factors (organic and inorganic nutrients such as nitrogen, carbon, phosphate and pH of the soil).

Various species of blue-green algae exhibited different levels of efficacy in assimilation of high or low levels of nutrients, which becomes a great advantage in nutrient poor waters (Sivasubramanian and Rao, 1988). Normally, cyanobacteria prefers to utilize lower concentrations of nitrogenous fertilizers for optimum growth and nitrogen fixation (Anand, 1990). Ammonium-nitrogen often led to poorer growth of blue-green algae *Anabaena doliolum* than nitrate supplied at comparable levels and the concentrations above 0.4 M were toxic (Singh and Srivastava, 1968). Nitrate nitrogen was evidenced as the most suitable nitrogen source for the growth of *Nostoc muscorum* (Kratz and Myers, 1955), *Anabaena doliolum* (Srivastava and Singh, 1968).

In the present investigation *Nostoc spongiaeforme* was selected for studying the kinetics of

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nitrogen and 2, 4-D uptake. The effect of nitrogen and 2, 4-D on the growth has also been investigated.

## II. Materials and Methods

*a) Blue-green algae, culture medium and growth and uptake measurement*

The filamentous, heterocystous and nitrogen fixing *Nostoc spongiaeforme* isolated from local rice-fields of Andhra Pradesh, India were made unialgal and grown in Chu No.10 medium as modified by Gerloff et al.,(1960). The pH was adjusted to 8.5 and stocks and experimental cultures were maintained in culture chambers at 28±2$^0$C and illuminated with fluorescent light emitting 1600 lux and shaken twice a day. Growth was measured by the estimation of chlorophyll-a and protein. Based on the results of short-term experiments (72 hours incubation period), long-term experiments were conducted to study the effect of various types of nitrogen sources $\left(NO_3^-, NO_2^-, NH_4^+\right)$ alone and in combination with fixed dose of 2,4-D (600 $\mu$g per ml) on the growth, uptake and their kinetics in *Nostoc spongiaeforme* up to 25 days of life cycle period with an interval of five days.

The estimation method of nitrate $\left(NO_3^-\right)$ (Brucine sulphanilic method mentioned by Brown et al., 1974), nitrite $\left(NO_2^-,\right)$ (Sulphanilamide and N-(1-naphthyl)- ehtylenediamine - dihydrochloride method, APHA, 1980) and ammonia $\left(NH_4^+\right)$ (Nessler's reagent method, APHA, 1980) were employed in these experiments. 2, 4-D was estimated by hydroxylamine hydrochloride, N, N'-Dicyclohexyl carbodiimide and ethanolic ferric chloride as mentioned by Grzegzolea Klazbita(1979). For chlorophyll estimation, 10 ml algal culture were centrifuged at 5000g for 5 min and O.D. measured to calculate chlorophyll as per the formulae of MacLachlan and Zalik (1963). Proteins were determined by the method of Lowry et al., (1951).

Different concentrations (0.1, 1.0, 1.5 and 2.0 mg per ml) of nitrogen sources i.e. sodium nitrite ($NaNO_2$), potassium nitrate ($KNO_3$) and ammonium chloride ( $NH_4Cl$) alone and in combination with 2,4-D (600 $\mu$g per ml) were supplied in growth medium. Control cultures which do not contain nitrogen source (BM-$NO_3$; BM-$NO_3$ +2,4-D) and pH of the growth media were adjusted to 8.5 with 0.1 N NaOH and 0.1 N HCl, were sterilized and inoculated with small vegetative filaments of 2-4 cells (125 x $10^4$ per ml which was equivalent to 100 mg fresh weight) of *Nostoc spongiaeforme* grown in $NO_3$ free basal medium. (For obtaining starved inoculum, the growing cultures were transferred to the required nutrient deficient media and were allowed to grow for one week in that medium. The starvation was observed by the reduction of pigment in the algal cultures). After every five days of inoculation of algae, growth was measured in terms of chlorophyll-a and protein as mentioned earlier. The uptake of nitrogenous substances was measured as the difference between nitrogen content before and after inoculation with algal cells (5 days). The S/V values indicated the rate of uptake of nitrogen/2,4-D herbicide per hour by the algae and it was calculated by dividing the supplemented nitrogen/2,4-D substrate concentration (S) with uptake of nitrogen or the velocity of the nitrogen (V). The Km (Michaelis constant) values of various nitrogen sources were calculated by plotting the velocity of uptake of nitrogen source (S/V) against substrate (S) concentration to deduce the Km values (Plummer,1977).

## III. Results

*a) Long-term effects of nitrogen sources alone and in combination with 2,4-D*

The recorded results of short-term experiments conducted on the influence of nitrogen nutrients on growth and kinetics in *Nostoc spongiaeforme* were extremely encouraging which lead to design the following long-term experiments to examine the effects of nitrogen sources i.e. potassium nitrate ($KNO_3$), sodium nitrite ($NaN0_2$) and ammonium chloride ($NH_4Cl$) in different doses (0.1, 1.0, 1.5 and 2.0 mg per ml) solely and in combination with a fixed dose of 2, 4-D (600 $\mu$g per ml ) on the growth, uptake and their kinetics in *Nostoc spongiaeforme* in which recording of results was carried out up to 25 days of life duration with a gap of five days.

Prior to the long term experimental studies on the effects of nitrogen sources alone and in combination with 2, 4-D on growth in *Nostoc sponglaeforme*, the effect of different concentrations (500, 1000, 1500, 2000 and 2200) of the herbicide i.e. 2, 4-D on the growth of *Nostoc sponglaeforme* in terms of chlorophyll-a was examined (Fig. 1a). In these experiments, starved inoculums grown in basal medium which do not contain nitrogen (BM-$NO_3$) was inoculated in basal medium (BM-$NO_3$) as control and in basal medium supplemented with different concentrations of 2, 4-D. Among the employed doses, the concentration at 500 $\mu$g 2, 4-D per ml was proved as growth promoter since it enhanced the quantity of chlorophyll-a as compared to control and the growth was gradually inhibited with increasing concentrations and completely retarded at 2200 $\mu$g per ml dose. The data suggested that the alga *Nostoc spongiaeforme* was intrinsically tolerant to the high doses of 2, 4-D when the growth inhibition of algae was measured in terms of chlorophyll-a, it is evident that the 2, 4-D retarded the biosynthesis of chlorophyll-a in this alga.

In long-term experiments, based on the experience of previous experiments on survival, the sub-lethal dose 600 $\mu$g 2, 4-D per ml was selected to examine the toxic effects of 2, 4-D alone and in the

presence of three nitrogenous sources on the growth of *Nostoc spongiaeforme* besides kinetics and uptake of nitrogen sources and 2, 4-D. Nitrogen starved inoculums of *Nostoc spongiaeforme* was inoculated in the basal medium which is normally deficient of nitrogen (BM-$NO_3$) and in the basal medium supplemented with a fixed dose of 600 $\mu$g 2, 4-D per ml (BM-$NO_3$ + 2, 4-D) as control and nitrogen deficient basal medium supplemented with nitrogen sources individually and in presence of 2, 4-D (BM-$NO_3$ + nitrogen sources + 600 $\mu$g 2, 4-D per ml). Studies on growth, kinetics of nitrogen sources alone and in combination with 2, 4-D in *Nostoc spongiaeforme* were measured upto 25 days of the life cycle duration with an interval of 5 days period (Tables 1 and 2). Table 1 illustrates the effect of nitrogen sources alone on the growth in terms of chlorophyll-a of *Nostoc spongiaeforme*. Among the three nitrogen sources, potassium nitrate ($KNO_3$) cultures showed better growth than sodium nitrite ($NaN0_2$) and ammonium chloride ($NH_4Cl$) cultures and control cultures. Normally, chlorophyll-a content was increased with increasing concentrations (0.1, 1.0, 1.5 and 2.0 mg per ml) of potassium nitrate ($KNO_3$) containing cultures. Sodium nitrite ($NaN0_2$) cultures of *Nostoc spongiaeforme* also showed similar type of growth. The chlorophyll-a quantity was reduced when compared with potassium nitrate ($KNO_3$) cultures, whereas higher concentrations (1.5 and 2.0 mg per ml) of ammonium chloride ($NH_4Cl$) showed growth reduction than control cultures (Table 1). Similarly, Table 2 shows the effects of different doses (0.1, 1.0 1.5 and 2.0 mg per ml) of potassium nitrate ($KNO_3$), sodium nitrite ($NaNO_2$) and ammonium chloride ($NH_4Cl$) on the growth of *Nostoc spongiaeforme* in relation to quantities of chlorophyll-a and proteins and on the lethality of 600 $\mu$g 2, 4-D per ml in *Nostoc sponglaeforme*. Among the three nitrogen-supplemented cultures, potassium nitrate ($KNO_3$) cultures increased the growth in terms of chlorophyll-a and proteins than the other two nitrogen sources [sodium nitrite ($NaNO_2$) and ammonium chloride ($NH_4Cl$)]. Potassium nitrate ($KNO_3$) expressed itself as an efficient protector against the lethality of 2, 4-D on the growth of *Nostoc spongiaeforme* as evidenced in the quantitative enhancement of chlorophyll-a or protein reflects the reduction of toxicity of 2, 4-D. As shown in the Table 2, except at 1.5 mg per ml concentration of sodium nitrite ($NaNO_2$) and ammonium chloride ($NH_4Cl$) concentrations exhibited higher levels of chlorophyll than the control cultures (BM-$NO_3$ + 600 $\mu$g 2, 4-D per ml) on $5^{th}$, $10^{th}$, $15^{th}$, $20^{th}$ and $25^{th}$ days. Likewise, protein content of *Nostoc spongiaeforme* cultures supplemented with nitrogen sources was higher than control cultures except at 1.0, 0.1 and 1.0 mg per ml cultures of ammonium chloride ($NH_4Cl$) on $5^{th}$, $15^{th}$, $20^{th}$ day; at 1.5 and 2.0 mg per ml of sodium nitrite ($NaNO_2$) on $5^{th}$ and $20^{th}$ day and at 1.0 mg per ml potassium nitrate ($KNO_3$) on $20^{th}$ day respectively. Thus, the experimental results indicated that the alga *Nostoc spongiaeforme* grown in nitrogen supplemented cultures were deemed to be much more protected against the toxicity of 2, 4-D than their respective control cultures (BM-$NO_3$ + 2, 4-D) as evidenced by the enhancement of chlorophyll-a and proteins. Comparatively, 2, 4-D plus nitrogen supplemented algal cultures showed better growth than nitrogen sources alone containing cultures.

The uptake of nitrogen sources (V) in *Nostoc sponglaeforme* was shown in figs.1 to 15 indicates that the uptake of ammonium chloride ($NH_4Cl$) was higher at all concentrations during the life cycle intervals ($5^{th}$, $10^{th}$, $15^{th}$, $20^{th}$ and $25^{th}$ day) than the other two nitrogen sources. When the cultures of *Nostoc spongiaeforme* were supplemented with different concentrations of nitrogen sources alone (0.1,1.0,1.5 & 2.0) and with a fixed dose of 2, 4-D (600 $\mu$g per ml), the uptake of nitrogen was progressively decreased in all the observed intervals. Comparatively uptake of nitrogen by *Nostoc spongiaeforme* was higher in nitrogen solely containing cultures than in nitrogen plus 2,4-D containing cultures. The S/V values denote the rate of uptake of nitrogen sources by *Nostoc spongiaeforme* at different concentrations solely and in association with 600 $\mu$g per ml 2,4-D. The Km values signify the affinity of the nitrogen substrate (S) and its rate of nitrogen uptake velocity S/V in nitrogen solely cultures and in combination with 2,4-D (600 $\mu$g per ml) containing cultures. At the beginning of the life cycle, *Nostoc spongiaeforme* exhibited low Km values which indicated the high uptake of nitrogen. Later, increasing Km values at every successive period of the growing stages of algae indicate the reduction in uptake of nitrogen from the medium suggesting that the algal cells might have absorbed sufficient nitrogen.

*Table 1:* Kinetics and effect of Nitrogen sources alone on the growth of *Nostoc spongiaeforme*

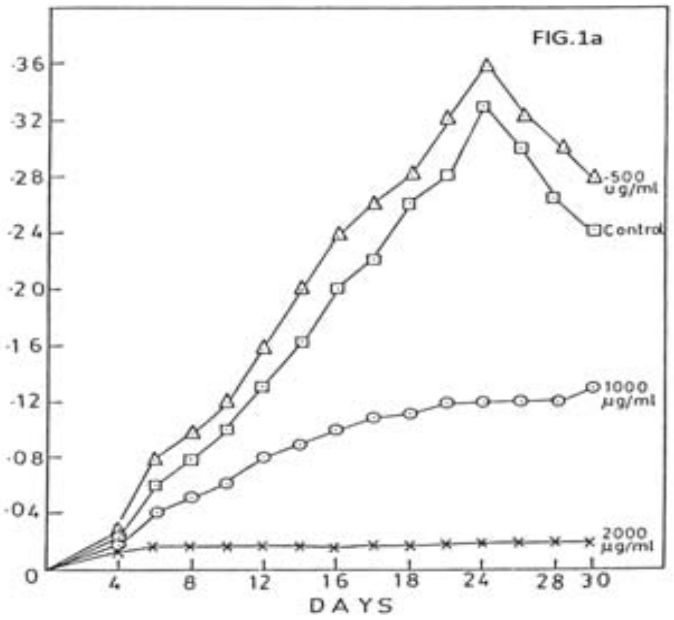
| | 5th day | 10th day | 15th day | 20th day | 25th day |
|---|---|---|---|---|---|
| Concentration (mg per ml) | Chlorophyll-a | Chlorophyll-a | Chlorophyll-a | Chlorophyll-a | Chlorophyll-a |
| BM – $NO_3$ | 0.0905 | 0.0235 | 0.0035 | 0.0048 | 0.0040 |
| BM-$NO_3$+ the following nitrogen sources | | | | | |
| $NH_4Cl$ | | | | | |
| 0.1 | 0.0185 | 0.1132 | 0.0112 | 0.2068 | 0.0243 |
| 1.0 | 0.0329 | 0.0847 | 0.0216 | 0.0038 | 0.0421 |
| 1.5 | 0.0553 | 0.0010 | 0.0034 | 0.0254 | 0.0356 |
| 2.0 | 0.0208 | 0.1054 | 0.0028 | 0.0153 | 0.0258 |
| $NaNO_2$ | | | | | |
| 0.1 | 0.0300 | 0.0936 | 0.0016 | 0.0085 | 0.0285 |
| 1.0 | 0.0650 | 0.0872 | 0.0020 | 0.0046 | 0.0264 |
| 1.5 | 0.0748 | 0.0128 | 0.0028 | 0.0048 | 0.0238 |
| 2.0 | 0.0862 | 0.0735 | 0.0042 | 0.0092 | 0.1041 |
| $KNO_3$ | | | | | |
| 0.1 | 0.0785 | 0.3068 | 0.0325 | 0.0246 | 0.0398 |
| 1.0 | 0.0852 | 0.3217 | 0.0127 | 0.0165 | 0.0146 |
| 1.5 | 0.0898 | 0.1815 | 0.0156 | 0.0258 | 0.3015 |
| 2.0 | 0.0943 | 0.4816 | 0.0242 | 0.0324 | 0.1228 |

*$NH_4$ Cl = Ammonium Chloride; $NaNO_2$ = Sodium nitrite; $KNO_3$ = Potassium nitrate;*

*Table 2:* Kinetics and effect of Nitrogen sources and 2, 4-D on the growth of *Nostoc spongiaeforme*

| Concen-tration (mg/ml) | 5th day | | 10th day | | 15th day | | 20th day | | 25th day | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Chlorophyll -a mg/g | Protein (µg/ 100mg fw) | Chlor-ophyll-a mg/g | Protein (µg/ 100mg fw) | Chlorophyll-a mg/g | Protein (µg/ 100mg fw) | Chloro-phyll-a mg/g | Protein (µg/ 100mg fw) | Chlorophyll-a mg/g | Protein (µg/ 100mg fw) |
| BM – $NO_3$ | 0.0920 | 72.50 | 0.0256 | 40.16 | 0.0042 | 45.66 | 0.0055 | 21.68 | 0.038 | |
| Bm-$NO_3$+2,3-D (600 µg/ml) | 0.0105 | 20.50 | 0.0158 | 25.83 | 0.0015 | 30.50 | 0.0030 | 14.18 | 0.020 | 20.32 |
| BM – Nitrogen + 600 µg 2, 4-D per ml + the following nitrogen: | | | | | | | | | | 10.15 |
| $NH_4Cl$ | | | | | | | | | | |
| 0.1 | 0.0198 | 16.66 | 0.1282 | 61.66 | 0.0135 | 1.66 | 0.234 | 71.06 | 0.0340 | 100.00 |
| 1.0 | 0.0421 | 93.33 | 0.1049 | 74.16 | 0.0228 | 42.5 | 0.0040 | 12.5 | 0.0499 | 23.33 |
| 1.5 | 0.0605 | 73.33 | 0.0013 | 40.83 | 0.0049 | - | 0.0271 | 26.58 | - | - |
| 2.0 | 0.0292 | 44.16 | 0.1162 | 88.33 | 0.0049 | - | 0.0178 | 25.10 | - | - |
| $NaNO_2$ | | | | | | | | | | |
| 0.1 | 0.0358 | 35.52 | 0.1025 | 28.33 | 0.0018 | 57.50 | 0.0098 | 15.83 | 0.0320 | 130.83 |
| 1.0 | 0.0985 | 40.21 | 0.0924 | 40.83 | 0.0025 | 56.66 | 0.0052 | 16.68 | 0.0264 | 80.00 |
| 1.5 | 0.1032 | 40.18 | 0.0248 | 45.83 | 0.0032 | 27.50 | 0.0056 | 28.33 | 0.0246 | 52.50 |
| 2.0 | 0.1548 | 42.12 | 0.0875 | 75.00 | 0.0043 | 41.50 | 0.0105 | 8.33 | 0.1153 | 55.00 |
| $KNO_3$ | | | | | | | | | | |
| 0.1 | 0.0948 | 37.50 | 0.3234 | 175.00 | 0.0379 | 150.00 | 0.0284 | 24.18 | 0.0469 | 166.66 |
| 1.0 | 0.1129 | 45.83 | 0.3970 | 130.83 | 0.0435 | 225.00 | 0.0191 | 13.33 | 0.0161 | 216.25 |
| 1.5 | 0.2499 | 65.00 | 0.2092 | 146.66 | 0.0178 | 123.33 | 0.0254 | 15.83 | 0.3239 | 166.78 |
| 2.0 | 0.3562 | 46.66 | 0.5077 | 168.33 | 0.0357 | 298.33 | 0.0391 | 21.68 | 0.1454 | 250.00 |

*$NH_4$ Cl = Ammonium Chloride; $NaNO_2$ = Sodium nitrite; $KNO_3$ = Potassium nitrate; 2, 4-D = Dichlorophenoxyacetic acid; mM = Milli Molar; = f.w., = fresh weight*



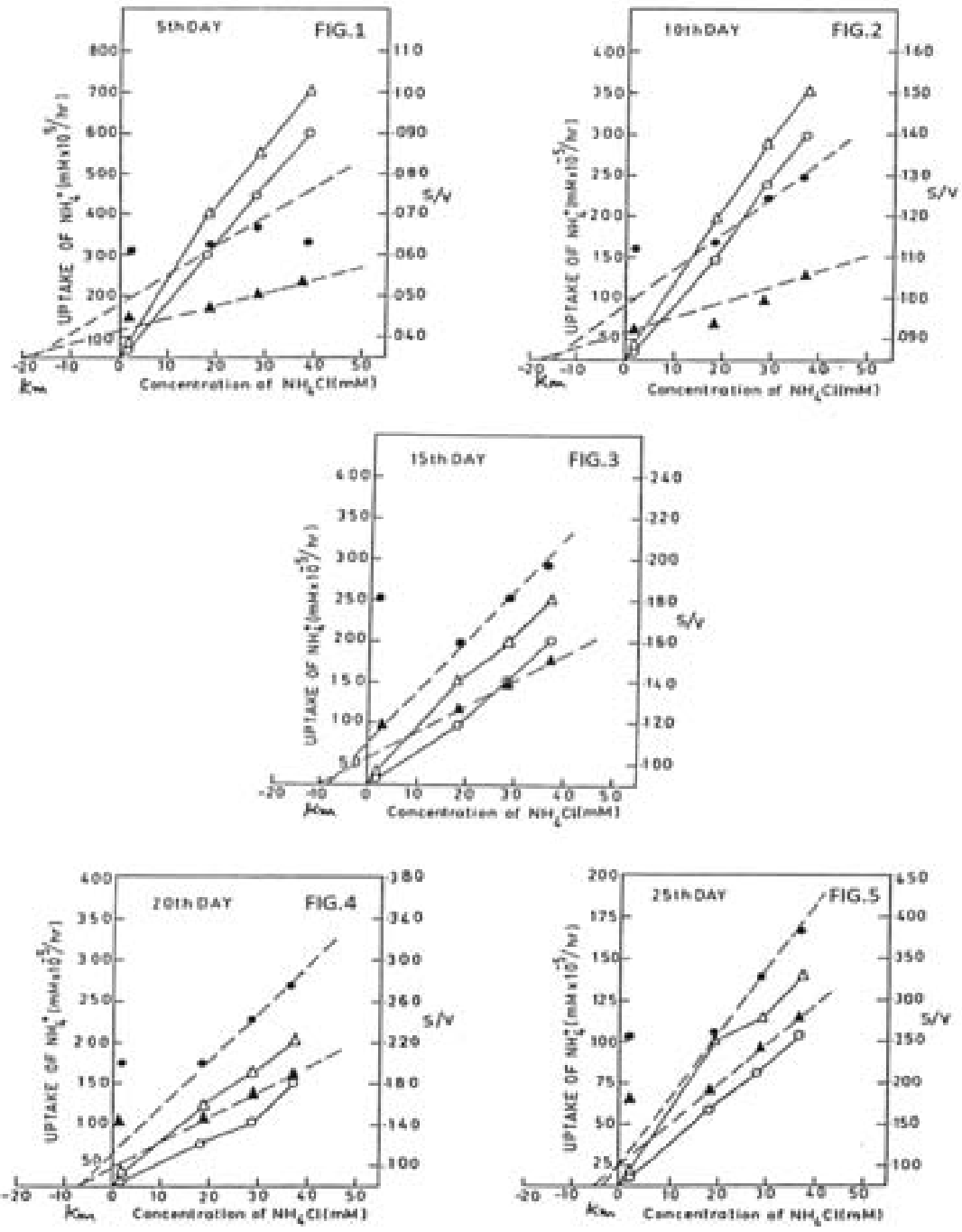

□ - □ : Control

△ - △ : 500 $\mu$g per ml

○ - ○ : 1000 $\mu$g per ml

x - x : 2000 $\mu$g per ml

*Figure 1:* Growth of *Nostoc spongiaeforme* in nitrogen depleted basal media supplemented with graded concentrations of 2, 4-D with normal inoculums by optical density



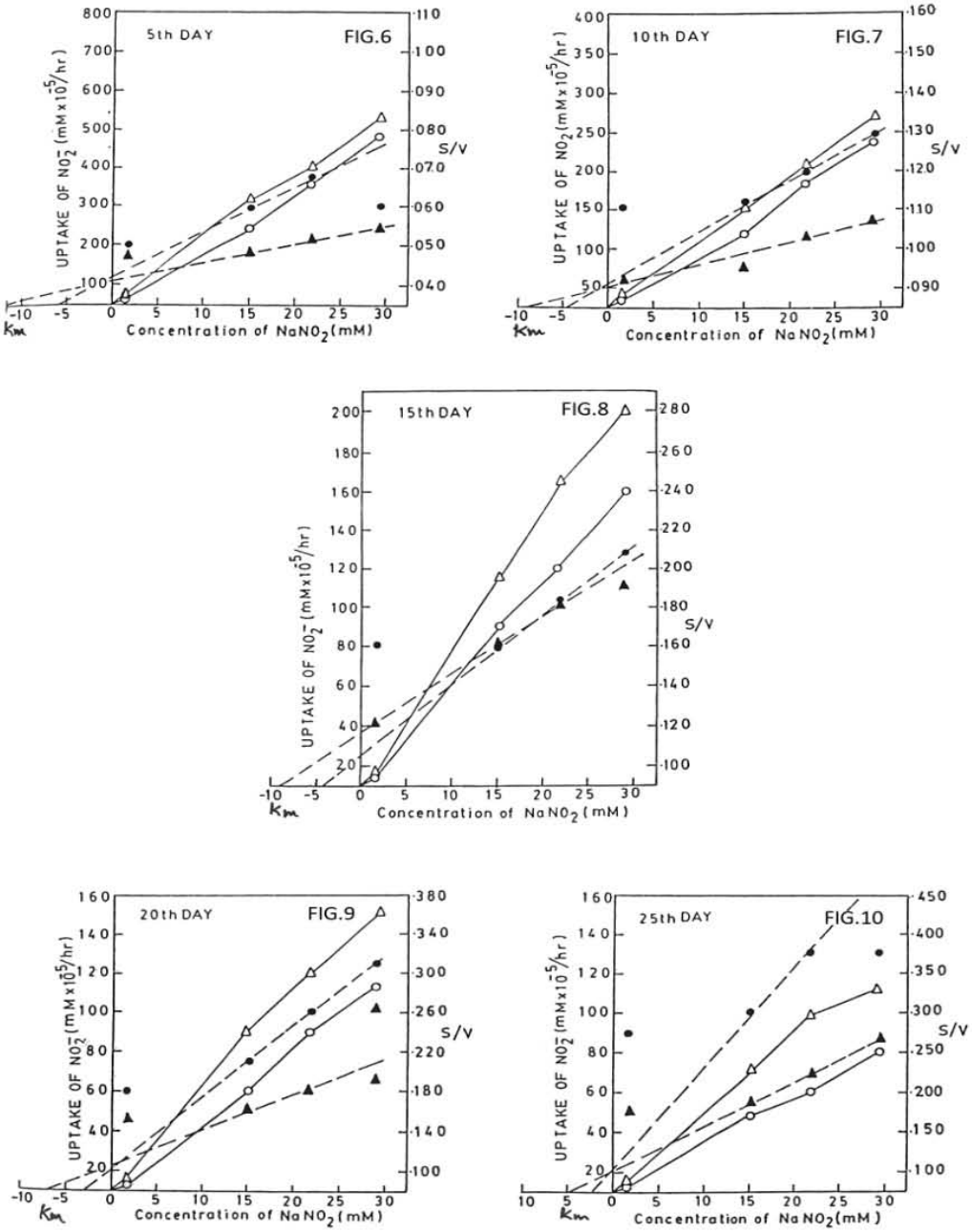

○-○ : Uptake of $NH_4^+$ (m M x $10^{-5}$ / hr.) in $NH_4CL$ cultures.

△-△ : Uptake of $NH_4^+$ (m M x $10^{-5}$ / hr.) in $NH_4CL$ + 2, 4-D cultures.

●-● : S/V (rate of uptake) of $NH_4^+$ in $NH_4CL$ cultures.

▲-▲ : S/V (rate of uptake) of $NH_4^+$ in $NH_4CL$ + 2, 4-D cultures.

*Figure 2:* The uptake of $NH_4^+$ (mM x $10^{-5}$ / hr.) in different concentrations (0.1, 1.0, 1.5, and 2.0 mg per ml) of $NH_4CL$ alone and in association with fixed dose of 2, 4-D (600 $\mu$g per ml) containing cultures of *Nostoc spongiaeforme* on $5^{th}$, $10^{th}$, $15^{th}$, $20^{th}$ and $25^{th}$ day



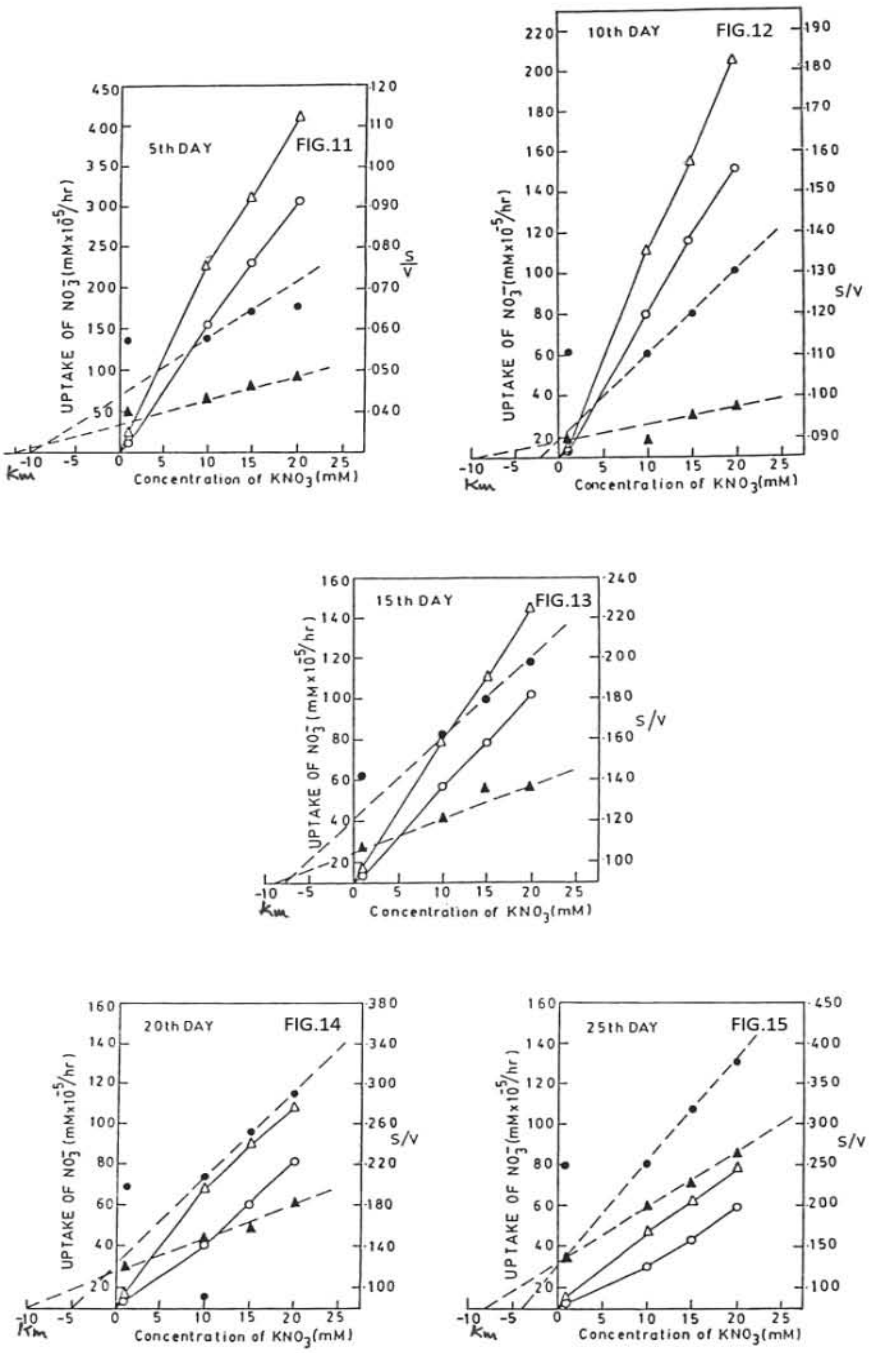

○-○ : Uptake of $NO_2^-$ (mM x $10^{-5}$ / hr.) in $NaNO_2$ cultures.

△-△ : Uptake of $NO_2^-$ (mM x $10^{-5}$ / hr.) in $NaNO_2$ + 2, 4-D cultures.

● - ● : S/V (rate of uptake) of $NO_2^-$ in $NaNO_2$ cultures.

▲ - ▲ : S/V (rate of uptake) of $NO_2^-$ in $NaNO_2$ + 2, 4-D cultures.

*Figure 3:* The uptake of $NO_2^-$ (mM x $10^{-5}$ / hr.) in different concentrations (0.1, 1.0, 1.5, and 2.0 mg per ml) of $NaNO_2$ alone and in association with fixed dose of 2, 4-D (600 $\mu$g per ml) containing cultures of *Nostoc spongiaeforme* on $5^{th}$, $10^{th}$, $15^{th}$, $20^{th}$ and $25^{th}$ day




○ - ○ : Uptake of $NO_3^-$ (mM x $10^{-5}$ / hr.) in $KNO_3$ cultures.

△ - △ : Uptake of $NO_3^-$ (mM x $10^{-5}$ / hr.) in $KNO_3$ + 2, 4-D cultures.

● - ● : S/V (rate of uptake) of $NO_3^-$ in $KNO_3$ cultures.

▲ - ▲ : S/V (rate of uptake) of $NO_3^-$ in $KNO_3$ + 2, 4-D cultures.

*Figure 4:* The uptake of $NO_3^-$ (mM x $10^{-5}$ / hr.) in different concentrations (0.1, 1.0, 1.5, and 2.0 mg per ml) of $KNO_3^-$ alone and in association with fixed dose of 2, 4-D (600 $\mu$g per ml) containing cultures of *Nostoc spongiaeforme* on $5^{th}$, $10^{th}$, $15^{th}$, $20^{th}$ and $25^{th}$ day.

## IV. Discussion

Farmers have been employing nitrogen fertilizers and pesticides simultaneously to eradicate the weeds in the rice-fields and pests on the rice plants with a view to get more crop yield. 2, 4-Dichlorophenoxyacetic acid (2, 4-D) is a harmone type herbicide used for control of many seasonally annual broad leafed weeds in rice-fields where the Cyanobacteria have been reported to be distributed and became resistant to pesticides doses than the doses of pesticides recommended for pest and weed control in rice-field. Tiwari and Pandey (1981) reported the herbicide resistant mutants of *Anacystis nidulans* in the form of filaments at 2 mg per ml concentration of 2, 4-D cultures. Depending upon the nitrogen, phosphorus and carbon sources, the toxicity of 2, 4-D was modified in *Nostoc calcicola*, *Synechococcus aeruginosus* and *Scenedesmus incrassatulus* and the mechanism of antagonistic action involved in these studies was not understood by mere laboratory experiments. Sivasubramanian and Rao (1988) studied the kinetics of nitrogen uptake in the presence of metabolic inhibitors (KCN, PCMB, DCMU) in diatoms and concluded that uptake of $NO_3^-$ and $NH_4^+$ was evidenced by the active absorption by utilizing energy produced in photosynthesis and respiration and partially by a passive diffusion whereas $NO_2^-$ is taken up only through photosynthesis mediated active uptake. Similarly, Hii et. al., (2011) observed the interactive affect of ammonia and nitrate on the nitrogen uptake by *Nannochloropsis* sp. as well as by conducting the short-term experiments Jyothi and Reddy (2017) studied the nitrogen uptake and kinetics in blue-green algae *Nostoc spongiaeforme*.

As evidenced by the above studies, currently it is of great interest to understand the relationship between kinetics of uptake of nutrients by Cyanobacteria and nutrient levels. The assimilation of nitrate by blue-green algae involving nitrate uptake and reduction of intracellular nitrate to ammonium occurred through a transport system having a high affinity for nitrate (Flores et al., 1980) and even low concentrations of nitrate found in their natural aquatic environments induced the nitrate reductase to function. The inorganic nitrogenous substances were considered as significant metabolites for cellular growth and cell constituents. Magee and Burris (1954) studied the nitrogen metabolism in diazotrophic cyanobacteria by incubating with $^{15}N_2$, $^{15}NH_4$, and $^{15}NO_3^-$, and concluded that the amino acid composition of the proteins were the same irrespective of the nitrogen source and incorporated into cellular proteins and cell wall material. Probably the nitrogen substances absorbed by active uptake through a carrier system as mentioned in *Anacystis nidulans* by Flores et al. (1983) and Meeks et al. (1983) and Tischner and Schmidt (1984) or simple diffusion process into cytoplasm where they preceded the nitrogen assimilation pathway, and incorporated into amino acids, proteins and cell materials and thereby increased the biomass and protein content of the algae as reported by Thomas et al. (1977), Wohlhueter et al. (1973) and Ingraham et al. (1983).

The present experimental studies suggested that, comparatively growth of *Nostoc spongiaeforme* was augmented in nitrogen plus 2, 4-D containing cultures than nitrogen alone supplemented cultures. It indicated that these three nitrogen sources significantly increased the growth of algae by antagonizing the 2, 4-D lethality and enhanced the biomass which was evident from the increased levels of chlorophyll-a, and proteins of *Nostoc spongiaeforme* (Tables 1&2). However, among the three nitrogen sources, potassium nitrate ($KNO_3$) was found to be a better protector against 2, 4-D toxicity than sodium nitrite ($NaNO_2$) and ammonium chloride ($NH_4Cl$) in *Nostoc spongiaeforme* cultures as evidenced by augmentation of biomass, chlorophyll-a and proteins.

## V. Conclusion

The present study reveals that the nitrogen uptake capacity of *Nostoc spongiaeforme* was greater in ammonium chloride ($NH_4Cl$) among the employed nitrogen sources. Nitrogen uptake was very high in $NH_4Cl$ supplemented cultures. So that *Nostoc spongiaeforme* is well suited as a phycoremediation organism for $NH_4$ removal from waste waters and effluents.

## Acknowledgement

The authors would like to thank the Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India for providing necessary laboratory facilities.

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