STUDIES ON NITROGEN FIXING BLUE-GREEN ALGAE IN RICE SOILS OF WEST BENGAL

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BY

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CERTIFICATE

This is to certify that the work recorded in the thesis entitled "STUDIES ON NITROGEN FIXING BLUE-GREEN ALGAE IN RICE SOILS OF WEST BENGAL" submitted by Sri Tapas Kumar Ghosh for the award of the Degree of Doctor of Philosophy in Agricultural Chemistry and Soil Science of the Bidhan Chandra Krishi Viswa Vidyalaya, is the faithful and bonafide research work carried out under our personal supervision and guidance. The results of the investigation reported in the thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have been duly acknowledged.

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Tapas Kr. Ghosh.
(TAPAS KUMAR GHOSH)

Dated, Kalyani,
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CHAPTER NO. 1

INTRODUCTION

INTRODUCTION

Rice, the most important food grain crop in India, covers an area of about 40.71 million hectares. The fertility levels of wetland rice soils in tropical Asia including India is generally low, and among all the nutrient elements nitrogen has been found to be the most limiting one. Moreover, for an average yield of rice, the amount of nitrogen removed by the crop is about 100 kg ha^{-1} which means removal of 4 million tonnes of nitrogen from about 40 million hectares of gross rice area in India. To compensate this removal an amount of 40 million tonnes of nitrogen are required to be returned to the soil every year to maintain its productivity constant assuming that nitrogen use efficiency for low land rice does not exceed 40% of the applied amount. Although the importance of fertilizers in agriculture is well realised in India, this appears to be a far cry in the near future at the present level of global energy crisis. The development and extensive use of high yielding varieties of rice, which require high dose of nitrogen fertilizers has made the problem much more complex. In this context biological fixation of nitrogen in soil has assumed great importance particularly with reference to rice cultivation in developing countries like India.

The conditions in the wetland rice fields provide an unique environment for luxurious growth of the photoautotrophic blue-green algae, many or which possess the capacity to fix atmospheric nitrogen. Since the pioneering work of De (1939) the importance of these algae in the nitrogen economy of Indian rice soils has been well appreciated. A modest estimate of the quantum of nitrogen fixed by these organisms in the field under favourable condition ranges from 25 to 40 kg ha^{-1}. (De and Mandal, 1956). However, failure of many 'algalization' experiments indicate that information regarding the distribution of different forms of blue-green algae in different soils may be one of the pre-requisites in any attempt of their utilization as a supplementary source of nitrogen in those soils.

It is known that nitrogen fixing blue-green algae grow better in soils of neutral to alkaline reaction. But, in India, the total area under acid soil has been estimated to be 71.23 m. ha (Mandal <u>et al</u>, 1982) and the state of West Bengal is known to have three types of acid soils such as red and laterite acid soils in the western part of the state, alluvial acid soils in the terai zone and acid sulphate soils of Sunderban area. Information regarding the distribution of different forms of blue-green algae in such soils is meagre. Therefore, the distribution of different forms of blue-green algae in some rice soils (generally acidic) from terai zone of West Bengal and their nitrogen fixing ability have been studied.

Pesticides, now-a-days are considered indispensable part of farm practices. The rate of use of pesticides in India, considered one of the lower users, has been estimated to be 300 g ha^{-1} (Anonymous, 1985). A good portion of these pesticides ultimately reaches the soil either by 'fall out' or by 'direct soil application'. Both the abiotic and biotic factors in soils are likely to be influenced directly or by their metabolic products depending on the nature of the pesticide used. Blue-green algae, one of the important constituents of soil biotic factors may come in contact with pesticides either when soil algae become exposed to crop protection chemicals or when pesticides are applied to protect them from grazing by zooplanktons, particularly during the preparation of inoculam. A clear understanding of toxicity level of pesticides to particular strain of blue-green algae is, therefore, necessary for preparation of inoculam from that strain.

Any change in N_2-fixing activities of blue-green algae due to pesticides, will also affect the overall nitrogen economy of the soils. Therefore, in order to judge the possible effects of pesticides on blue-green algae, factors other than growth should also be taken into consideration. These include N_2-fixation by blue-green algae and release of fixed or biomass nitrogen into different forms. Accordingly, the effects of carbofuran (Furadon 3G), a commonly used insecticide in rice culture to control the major rice pest brown plant hopper on the growth of and N_2-fixation by blue-green alga <u>Aulosira fertilissima</u> vis-a-vis pattern of accumulation of fixed and biomass nitrogen was studied in two separate experiments.

Apart from blue-green algae, many other diazotrophos occur in rice fields. More recent addition to the list of diazotrophos active in rice ecological system is the hetero-trophic soil bacteria chiefly the Spirillum sp. associated with roots and is known to fix nitrogen significantly at the expense of organic materials excreted by plant roots. However, the activity of such root associative bacteria may frequently be limited by shortages of energy sources, which they generally receive either from roots of host plant or from soluble and easily decomposable organic matter in the rhizosphere soil. The growth of blue-green algae, besides providing a large amount of O_2, enriches the soil system with organic matter either liberated extra-cellularly by living cells or added in the form of dead cells (Fogg et al, 1973). This may exert an influence on the proliferation, association, and N_2-fixing activity of Azospirillum sp. in the rice N_2-fixing system. Accordingly the effect of inoculation of blue-green algae on the nitrogen fixing activity in soil and root systems during the growth cycle of rice vis-a-vis the yield was studied.

It has been well established that blue-green algae play a vital role in the maintainance and building up of rice soil fertility. Many trials have also been conducted to increase rice yield by inoculating fields with strains of blue-green algae with high N_2-fixing potentiality, either singly or in combination. But this process, also called 'algalization' have been tested on a 'black box' basis where only grain yield was measured. Although grain yield is certainly most important measure for assessment but it will not explain the mode of action of blue-green algae nor allow improvements of the technology of algalization. In paddy fields the death of algal biomass is most frequently associated with soil decication at the end of the cultivation cycle and therefore more attention has been paid on the residual soil fertility than an immediate benefit to the standing crop. But in paddy fields inoculated with blue-green algae, algal bloom occurs early which is infrequent under natural conditions. So, decomposition by lytic micro-organisms and grazing by aquatic fauna which are considered principal means by which nitrogen is made available to the crop, are likely to occur at the begining of the growth cycle. This along with the part of fixed nitrogen exudated by the inoculated blue-green algae may influence the nitrogen status of the soil vis-a-vis the yield and nitrogen uptake of the crop. Since all the processes of N_2-fixation, nitrogen - release, mineralization, immobilization, loss and uptake by plants may be simultaneous and continuous process, experiments were conducted to study the algalization effect in presence of chemical fertilizers and organic sources of nitrogen on the distribution pattern of different forms of nitrogen in soil and rice yield.

CHAPTER NO. 2

REVIEW OF LITERATURE

2.0 REVIEW OF LITERATURE

2.1 Occurrence of nitrogen fixing blue-green algae (Cyanobacteria) in tropical rice soils with special reference to India.

Rice is an exclusive crop plant of aquatic habitat, largely raised in submerged soils. The most important characteristics of such soils is the existence of a layer of standing water, which exercises a profound influence on the physico-chemical and biological properties of the soil below. The principal biological consequences of water-logging are suppression of aerobic soil microflora with a concomitant increase in the population of anaerobic microflora specially in the anoxic zone and development of photosynthetic biomass of algae in the oxic-photic zone. Physiologically algae present in rice fields can be classified into N_2-fixing and non-N_2-fixing forms, the former belonging exclusively to the procaryotic blue-green algae.

Since the importance of nitrogen-fixing blue-green algae in maintaining the nitrogen economy of rice field soils was established by the pioneering work of De (1939), voluminous research has been done showing that these algae are the most promising nitrogen fixing agents and are widely distributed in abundance in the tropical rice fields (Singh, 1961, Sankaram, 1971, Watanabe and Yamamoto, 1971, Stewart, 1973, Yamaguchi, 1976, Singh, 1978, Roger and Kulasooriya, 1980). Because of its photoautotrophic nature and luxurious growth in aquatic environment, nitrogen fixation by this organism in rice soils of different agroclimatic regions has gained importance. Although free living blue-green algae are abundant in submerged soils, no generalization can be made regarding their nitrogen fixing potentiality which depends on many factors, of which soil pH, fertility status of soil, temperature and sunlight are more important. The qualitative and quantitative compositions of blue-green algae and their variation at different periods of the year in a rice soil, may to some extent provide an index of their nitrogen fixing potentiality in that soil. Accordingly, more and more attention are being paid to study the distribution of different forms of nitrogen fixing blue-green algae with a view to evaluate their importance in nitrogen supply in different rice growing soils particularly of the tropical countries including India.

Among the different types of blue-green algae occuring in rice soils, the heterocysts bearing species are considered as the most common forms of nitrogen fixers. About fifty two species belonging to the families Nostocaceae, Rivulariaceae, Scytonemataceae and Stigonemataceae known so far as atmospheric nitrogen fixer are listed below :

Family and genus :

Nostocaceae

Anabaena - A. naviculoides (De, 1939), A. gelatinosa

(De, 1939), *A. variabilis* (De, 1939 and Bortels, 1940), *A. ambigua* (Singh, 1942), *A. fertilissima* (Singh, 1942), *A. cycadeae* (Winter, 1935), *A. cylindrica* (Bortels 1940 and Fogg, 1942), *A. humicola* (Bortels, 1940), *Anabaena* Sp. (Drewes, 1928), *A. azollae* (Venkataraman, 1962), *A. flosaquae* (Davis *et al*, 1966), *A. levenderi* (Cameron and Fuller, 1960)

Anabaenopsis - *A. circularis* (Watanabe, 1951 and 1959 a), *Anabaenopsis* Sp. (Watanabe, 1951 and 1959 a).

Aulosira - *Aulosira fertilissima* (Singh, 1942)

Cylindrospermum - *C. gorakhporense* (Singh, 1942), *C. licheniforme* (Bortels, 1940) *C. majus* (Bortels, 1940), *C. sphaerica* (Venkataraman, 1961 b).

Nostoc - *N. calcicola* (Bortels, 1940), *N. commune* (Herisset, 1952), *N. cycadeae* (Watanabe and Kiyohara, 1963), *N. endophytum* (Stewart, 1962), *N. paludosum* (Bortels, 1940), *N. muscorum* (Burris *et al*, 1943), *N. punctiforme* (Drewes, 1928), *N. sphaericum* (Pankow and Martens, 1964).

Rivulariaceae :

Calothrix - C. brevissima (Watanabe, 1951, 1959a)
C. elenkinii (Taha, 1963, 1964), C. parietina (Williams and Burris, 1952), C. scopulorum (Stewart, 1962).

Scytonemataceae :

Scytonema - S. arcangelii (Cameron and Fuller, 1960)
S. hofmanni (Cameron and Fuller, 1960)

Tolypothrix - T. tenuis (Watanabe 1951, 1959a),

Stigonemataceae :

Fischerella - F. major (Pankow, 1964), F. muscicola (Pankow, 1964, Mitra, 1961).

Hapalosiphon - H. fontinalis (Taha, 1963, 1964),

Mastigocladus- M. laminosus (Fogg, 1951)

Stigonema - S. dendroideum (Venkataraman, 1961 a)

Westiellopsis- W. prolifica (Pattnaik, 1966)

Besides these heterocystous forms there are also evidences of nitrogen fixation by pure cultures of some unicellular and non-heterocystous filamentous blue-green algae anacrobically. These include two strains of genus Aphanothece, five strains of Gloeothece, three strains of Synechococcus and two strains of Dermocarpa, Xenococcus Sp., Myxosarcina Sp. eight strains of Chroococcidiopsis, seven strains of Pleurocapsa, five strains of Oscillatoria, four strains of Pseudanabaena and sixteen strains of Lyngbya-Plectonema- Phormidium group (Singh, 1978, Fay and Fogg, 1962, Cameron and Fuller, 1960, Wyatt and Silvey, 1969, Rippka et al, 1971, Dugdale and Goering, 1967, Watanabe, 1959a, Stewart and Lex, 1970, Rippka and Waterbury, 1977, Stewart et al, 1979).

In India rice is grown mostly in slightly acidic to slightly alkaline soils under waterlogged condition in low-lying areas. These conditions provide a satisfactory environment for growth of nitrogen fixing blue-green algae. Although systematic and detailed surveys as regards their distribution have not been conducted, only a few studies in specific rice growing areas have been reported.

Singh (1961) made survey of rice fields of Uttar Pradesh and several places of Bihar, where the pH of the soils was above 7.5, and reported that there was dominant, widespread and universal occurrence of the community of blue-green algae, comprised mainly of Aulosira fertilissima Ghose intermingled with filaments of Anbaena ambiqua Rao, Anabaena fertilissima Rao and Cylindrospermum gorakhporense Singh. He further observed that in soils of Uttar Pradesh, a general mixture of nitrogen fixing algal species appeared initially, which was soon followed by a 'huge and pure' growth of thick brownish gelatinous mass of Aulosira fertilissima, and as the soil dried, species of Cylindrospermum became dominant. He purified the above four species and found that a considerable amount of nitrogen was fixed by each of them but the greatest fixation, amounting to 8.05 mg per 100 ml of the N-free medium in 45 days, was obtained with Aulosira fertilissima Ghose.

Pandey (1965 a,b) examined rice soils of Ballia and Ghazipur districts of Uttar Pradesh and recorded 107 species of algae, more than two thirds of which belonged to Cyanophyceae.

Mitra (1951) screened sixteen samples of soil from North and South India for the presence of algae and observed that ordinary alluvial soils from rice fields yielded a far larger number of algal species than other types of cultivated soils. Cyanophyceae constituted more than half of the total number of species recorded in the soils, in which nitrogen fixing species of genera Nostoc, Anabaena and Calothrix were common in most of the soils he studied.

Under the aegis of a scheme financed by the ICAR major rice growing areas of the erstwhile Madras state covering 11 districts were surveyed. Seven hundred and ninety eight collections of blue-green algae obtained from a number of places were found to belong to 169 species falling under

31 genera distributed over six families. A very large number of them were found to have nitrogen fixing ability. Members of the Cyanophyceae belonging to the genera Nostoc, Cylindrospermum, Anabaena and Aulosira were the dominant forms in the soils examined, while Scytonema and Stigonema coming under the genera Mastigocladopsis occurred sporadically (Anonymous, 1965).

A detailed comparative study of the algal flora of the acidic rice field soils from seven districts of Kerala State revealed that Cyanophyceae constituted 20 to 76 percent and Chlorophyceae 25 to 100 percent in the rice fields of the surveyed area. Some forms like Aulosira fertilissima and Calothrix brevissima were found to be present in all fields, others were localised. Among the nineteen forms of blue-green algae recorded,seven were known to fix atmospheric nitrogen (Aiyer, 1965). Amma et al, (1966) reported the presence of species of Nostoc and Anabaena even in the peaty soils of Kuttanad in Kerala. Sundara Rao (1964) found in the rice soils of erstwhile Madras and Kerala an ubiquitous occurrence of Aulosira fertilissima, which appeared to have great affinity for rice field conditions.

Khurana and Venkataraman (1968) studied the algal population in six paddy field soils of South India and found that blue-green algae predominated over other groups of algae. The nitrogen fixing blue-green algae harboured were Nostoc *muscorum*, *Nostoc punctiforme*, *Anabaena cylindrica*, *Anabaena fertilissima* and *Westiellopsis prolifica*. *Nostoc muscorum* was found in all soils, while other forms were localized in distribution. They also noticed that *Aulosira fertilissima* commonly encountered in the rice field soils of Bihar and Uttar Pradesh as a dominant form was not present in the rice field soils of some parts of South India.

Pattanaik (1966) examined rice soils of Cuttack (Orissa) and observed an abundance of *Westiellopsis prolifica* Janet. With critical experiments by using ^{15}N technique, he observed that this species fixed 14.09 mg nitrogen after 30 days of growth in 500 ml of combined N-free medium at 35°C, whereas Singh (1971, 1978), surveyed the different blocks of the farm of the Central Rice Research Institute, Cuttack, and reported that the dominating N_2-fixing blue-green algae were species of *Aulosira*, *Cylindrospermum*, *Nostoc* and *Anabaena*.

Saha and Mandal (1979) examined ten alluvial low land rice soils of West Bengal and observed an abundant growth of filamentous heterocysts bearing blue-green algae in all the soils, in which the dominant genera were *Nostoc*, *Anabaena* and *Cylindrospermum* followed by *Scytonema*, *Calothrix* and *Mastigocladus*. The extent of nitrogen fixation carried out by them ranged from 27.66 to 73.33 g soil^{-1} week^{-1} in these soils. Among the different unialgal species isolated, three of *Nostoc* and one of Scytonema were found to be more efficient fixers, Nostoc muscorum being the most both in presence and absence of combined nitrogen.

Venkataraman (1975) found the presence of N_2-fixing species of blue-green algae in 33% of the 2213 soil samples collected from rice fields of different states of India, the dominant species being Aulosira fertilissima and species of Nostoc in some states. Species of Nostoc and Anabaena were, however, ubiquitous in all regions in varying proportions and formed the major constituents of the algal biotypes in 9 out of 15 states.

Watanabe (1959a) and Watanabe and Yamamoto (1971) made a far-reaching search for N_2-fixing blue-green algae in the rice fields of South and South east Asia and found that out of more than 1000 strains collected several were strong N_2-fixers. They purified a good number of the species belonging to the genera Tolypothrix, Cylindrospermum, Calothrix, Anabaena, Nostoc, Anabaenopsis and ascertained their N_2-fixing ability, which varied from 1.37 to 5.20 mg N/100 ml of culture medium. It was found that there was a great variations in N_2-fixing ability of the same species collected from different places. Venkataraman (1972) also observed a similar variation in N_2-fixation by different strains of Nostoc punctiforme, collected from thirteen different agroclimatic locations in India, when examined under identical conditions *in vitro*. Shtina *et al*, (1968) also supported the view that different strains of one species differed in rate of growth and nitrogen accumulation.

The relationship between the occurrence of N_2-fixing blue-green algae and soil characteristics has also been investigated by some workers. Bortels (1940) observed a positive correlation between the fertility of the soil and nitrogen fixing blue-green algae. Gonzalves and Gangala (1949) examined rice growing soils of five centres in Maharashtra state viz. Thana, Goregaon, Dahisar, Khandla, and Lonavla and attempted to correlate the occurrence of algal flora with the physical and chemical properties of soils such as texture, pH, calcium content etc. and concluded that no single factor could alone influence the algal composition of a particular soil which seemed to be determined by a number of factors such as pH, moisture content, soil texture, chemical composition etc.

Okuda and Yamaguchi (1952,1956a) observed no relationship between soil texture or organic matter content and algal growth in paddy soils of Japan. Algal growth was depressed at pH 3-5 and was poor in soils of low P content.

De (1936) measured increases in soil N in ten rice soils of the different districts of undivided Bengal now in Bangladesh. The correlation coefficient (r) between pH and N_2-fixation by blue-green algae in soils was found to be 0.79**. Henriksson et al, (1975) noted a significant positive correlation (r=0.66**) between acetylene reduction and pH in seven Indian and six Swedish rice soils not amended with phosphate. However, relation between acetylene-reduction and extractable K, P or Mg were not statistically significant.

Saha and Mandal (1979), observed positive correlations between nitrogen fixation and soil characters such as pH, organic carbon, total nitrogen, available phosphorus, exchangeable calcium, cation exchange capacity and MPN value of blue-green algae in some alluvial rice soils of West Bengal. Available nitrogen had shown negative correlation with the nitrogen fixation in those soils but none of these correlations was statistically significant.

As in India and Japan, studies on algae from rice fields has also been done in other countries. Egyptian rice oils have been reported to harbour a wide variety of blue-green algae including many N_2-fixing species belonging to the dominant genera Calothrix and Anabaena (El-Nawawy et al, 1962). Taha (1963), isolated Hapalosiphon fontinalis, Anabaena variabilis and Calothrix elenkinii from the rice fields of Egypt in pure cultures. Similarly EL-Ayouty (1966) isolated Anabaena Variabilis, A. torulosa, Cylindrospermum muscicola, Calothrix parientina, Nostoc muscorum, Nostoc paludosum and Nostoc Sp.

from Giessen Soils. Shakeeb (1970) isolated Nostoc muscorum and Phormidium fragile, while El-Borollosy (1972) isolated Nostoc linckia var. arvense, Nostoc piscinale, N. paludosum and Cylindrospermum Sp. from soils of Delta.

Jutono (1973) studied the composition of blue-green algal population in flooded rice soils in the Jogjakarta district of Central Java and found that the members of the families Nostocaceae viz. Cylindrospermum, Anabaenopsis, Aphanizomenon, Anabaena, Nostoc, Nodularia, Aulosira occurred ubiquitously in those soils. Application of nitrogenous fertilizers caused a reduction in numbers of N_2-fixing blue-green algae.

Bunt (1961) recorded species of Nostoc and Anabaena in soils of Northern Australia. He concluded that N_2-fixing algae formed only a minor part of the rice field flora, presumably because of heavy application of ammonium nitrogen to the soil and addition of as much as 5 ppm of coper sulphate to the irrigation water.

Materassi and Balloni (1965) reported that the species of Nostoc and Anabaenopsis were common in Italian soils, where N_2-fixing species constituted about 35 percent of the flora under dry conditions and about 70 percent under waterlogged conditions.

The occurrence of blue-green algae in cultivated and non-cultivated soils in Whitman county, Washington, was studied over a 12 month period by Zimmerman et al, (1980). Nostoc and Anabaena were found in the soils, the former being ubiquitous. However, their infrequent occurrence and low densities in the cultivated soils indicated that nitrogen input by them is not so significant in these soils. Rapid loss of surface soil, moisture, seasonal climatic fluctuation, slightly acidic reaction and agricultural practices were considered to be the probable reasons for smaller numbers and diversity of blue-green algae in the cultivated soils, while the uncultivated soils were found to support more numbers and larger diversity.

Abdullah et al, (1983) studied the composition of algal biomass in one rice field soil sample from Iraqui marshes and obtained 43 taxa by enrichment culture techniques in which Microcoleus chthonoplastes and Nostoc muscorum were found to be the dominant species which were heterocystous and therefore presumed to be N_2-fixers were an important component of the algal crop in the field. They also studied the influence of environmental factors on the growth of these algae and observed a substantial growth at 45°C, but at 48°C they eventually died. 6 taxa showed good growth in medium enriched with 0.5 M NaCl;

3 taxa grew in 1M NaCl, but growth was very slow. They suggested that field research on these algae might be useful to increase the nitrogen status of the soil without the use of nitrogenous fertilizers in that region.

The occurrence of N_2-fixing blue-green algae in 12 flooded rice soils of USA, was reported by Wilson and Alexander (1979). They also studied the effect of various soil factors on the growth of these algae in soils and observed that in P-supplemented flooded soils, these algae generally developed readily at pH 7.3 or greater but poorly at pH 6.5 or less. Iron additions stimulated the activity of inoculated blue-green algae in a phosphate amended flooded soil of pH 7.9 but not in a soil with a pH of 5.5. They further found that N_2-fixation and development of indigenous blue-green algae were correlated with pH and the levels of extractable K and of Ca and Mg in those soils, but not with the organic matter content, inorganic N, or extractable P and Fe.

Although studies so far made regarding the occurrence of N_2-fixing blue-green algae in rice soils of India have shown that they are fairly abundant but the information collected can not be deemed to be adequate and commensurate with the needs of the vast rice growing areas spread out in many states of the country. Information in this regard in respect of the low land rice soils of West Bengal, a major rice growing state in

India, are very meagre. It was, therefore, considered worthwhile to collect information regarding the distribution of different forms of blue-green algae in traditional rice growing soils of West Bengal and to study the relationship between their occurrence, N_2-fixation and the soil characteristics. Such a knowledge would help markedly in successful use of N_2-fixing blue-green algae, as nitrogenous bio-fertilizer in the cultivation of rice in this area.

2.2 Effects of pesticides on the growth of and nitrogen fixation by blue-green algae (Cyanobacteria).

The introduction of chemical pest control measures has brought into use many powerful organo-chemicals most of which either directly or indirectly enter into the soil. Some of them are used as soil application, while the others are used in the form of aerial spray, a good portion of which finally enter into the soil mass as fall out. Although these chemicals are designed to kill definite target groups of organisms they are likely to produce some effects on other organisms also because when added to the soil they are acted upon by a wide group of organisms. As a result of this they either produce a deleterious effect on some of the non-target organisms or may conceivably be metabolized with a modification of their activity. Such influences are, however, determined by the chemical nature of the pesticide, its concentration and persistence in soil, and may have a bearing on the establishment and biological activities of introduced, as well as the native blue-green algae in soil. In fact tolerance to pesticides has been made one of the main criteria for selection of a strain as seeding material for algalization (Stewart, 1980). Although considerable work has been done on the effect of various pesticide chemicals at their different concentrations, on the growth and activity of different species of blue-green algae, most of such work is confined to laboratory liquid culture experiments.

Venkataraman and Rajyalakshmi (1971 and 1972) tested the growth of twenty eight strains of blue-green algae belonging to five different genera viz. *Anabaena*, *Nostoc*, *Aulosira*, *Tolypothrix* and *Anacystis* against varying concentrations of 2 fungicides (viz. cerasan and dithane) and 6 herbicides (viz., 2,4-D, delapron, propazine, cotoron, diuron and linuron) under laboratory cultural condition and observed that most of the strains could tolerate a high level of all the pesticides, such levels being many times higher than those recommended for field application.

Ahmad and Venkataraman (1973) further examined the tolerance of *Aulosira fertilissima* to different concentrations of several herbicides and insecticides like MCPA, MCPB, stam F-34 and lindane, parathion, endrin, diazionone, BHC and sevin in culture medium and found that none of the chemicals had any adverse effect on the growth of this alga at their recommended doses. The alga could tolerate even a concentration of 100 ppm of MCPA, MCPB and Stam F-34. Excepting BHC most of the insecticides upto the concentration of 10 ppm stimulated its growth.

Singh (1973) studied the effect of BHC, lindane, diazionone and endrin on the N_2-fixing blue-green algae *Cylindropermum* sp., *Aulosira fertilissima* and anaerobic N_2-fixer *Plectonema boryanum* 594 and observed that among the four pesticides used, BHC was most toxic. The sensitivity however, varied from species to species. In another study, Singh (1974) reported that the species of *Cylindrospermum* could tolerate upto a concentration of 800 ppm of 2,4-dichlorophenoxy acetic acid under laboratory cultural condition.

Gangawane and Saler (1979) concluded that blue-green algae could tolerate fungicide concentrations of 100 to 1,000 ppm depending on the nature of the pesticide as well as the strain of the algae in culture medium. Difolatan, even at a concentration as high as 300 ppm had produced stimulatory effect on the growth of *Aulosira fertilissima*. Thimet 10 G had no harmful effects on *Westiellopsis*, *Aulosira*, *Tolypothrix* and *Calothrix* at concentrations of 1000, 500, 500 and 300 ppm (active ingredient) respectively, but *Nostoc* was unable to tolerate a concentration even as low as 1 ppm (Gangawane, 1979).

Batterton *et al*, (1971) studied the growth responses of blue-green algae to aldrin, dialdrin, endrin and their metabolites in laboratory culture and concluded that the metabolic products of these pesticides were inhibitory to the growth of algae. Wright *et al*, (1977) reported that 3-4 Dichloroaniline, the primary product of propanil degradation was inhibitory for some blue-green algae, but its effect was much less than the propanil itself at the field doses of application.

All these works mainly dealt with the tolerance limits and growth of different strains of blue-green algae against various concentrations of certain pesticides. However, emphasis has also been given to understand the effect of pesticides in general and their mode of action on the growth and N_2-fixing activities of blue-green algae in particular. Vaishampayan et al, (1978) observed inhibitory effect of stam F-34 on the growth and heterocyst differentiation of Nostoc muscorum was similar to that of DCMU. Some pesticides like butachlor (machete) have been demonstrated to have a mutagenic action on blue-green algae (Singh and Vaishampayan, 1978, Singh et al, 1979).

Alachlor (lasso) like butachlor also showed mutagenic properties when tested in Nostoc muscorum systems. The mutagenicity of the two herbicides appeared to be considerably higher than that of MNNG (N-methyl-N'-nitro-N-nitrosoguanidine). A dose of alachlor considerably higher than that of butachlor was needed to obtain a comparable level of growth inhibition and mutagenesis, thus suggesting that butachlor was a more efficient mutagen than alachlor, (Singh et al, 1979).

The pH of medium, light intensity and size of the algal population influence the toxicity significantly. Kar and

Singh (1977) studied the effect of pH, light intensity and population size on the toxicity of the pesticide furadan (3% carbofuran as the active ingredient) on N_2-fixing blue-green alga Nostoc muscorum and observed that the pesticide was more toxic to the alga in the medium of pH 5-6 as compared to that of pH 7.5-10. The toxicity of the pesticide was found to be gradually decreasing with increase in light intensity and the level of population.

By repeatedly growing and removing blue-green algae Anabaenopsis raciborskii and Anabaena aphanizomenoides from BHC containing medium, Das and Singh (1977a) observed a gradual loss in the toxicity of the pesticide. They (1977b and c) studied the effect of BHC on blue-green algae A. raciborskii, A. aphanizomenoides and Microcystis flos-aquae and observed that the former two were very sensitive and concentration as low as 100 ppm retarded growth and N_2-fixation. However, A. raciborskii and M. flos-aquae tolerated higher concentration of herbicide 2,4-D. Infact addition of lower concentrations, 10-100 ppm, of this herbicide encouraged growth and N_2-fixation whereas 800-1500 ppm were lethal to the cells. Kar and Singh (1977) also reported an increase in growth and N_2-fixation of alga Nostoc muscorum at lower concentration (25 ppm) of furadan whereas at higher concentrations of 500-1000 ppm the growth was inhibited.

Inger (1970) reported that the two herbicide, 2,4-D and MCPA, at concentrations usually recommended for field application, inhibited N_2-fixation by Nostoc muscorum, Nostoc muscorum, Nostoc punctiforme and Cylindrospermum sp., while Da Silva et al, (1975) expressed the opinion that insecticides generally have little effect on algal N_2-fixation; however he observed an inhibitory effect of malathion.

Pandey (1985) observed that propanil inhibited heterocyst differentiation and N_2-fixation together with a decrease in total protein, neuclic acids, carbohydrate contents and a loss of photosynthetic pigments and chlorophyll a synthesis in the culture of cyanobacterium, Nostoc calcicola.

Lal et al, (1987) reported that the blue-green algae Anabaena sp. and Aulosira fertilissima were quite sensitive to DDT, fenitrothion and chloropyrifos; the effects however depended on the type and nature of the insecticide, the organisms and the experimental conditions. Nitrogenase activity was stimulated by DDT in Anabaena but inhibited by it in Aulosira fertilissima, whereas fenitrothion and chloropyrifos inhibited N_2-ase activity in both.

The above cited information regarding the growth and N_2-fixation of several strains of blue-green algae against various concentrations of certain insecticides, herbicides and fungicides are mainly based on the results of the laboratory cultural experiments, which can only give an index of the sensitivity of the strains to the pesticides. The results can hardly be extraploted to actual soil conditions, where many competetive organisms are present, a large percentage of which possesses the capacity to degrade pesticides besides the effect of soil itself.

Raghu and Mac Rae (1967) reported a marked stimulation of growth of the indigenous algae in two submerged tropical rice soils due to addition of BHC to the flood water @ 5,6 and 50 kg/ha as active compound. They attributed the stimulation to the elimination by the insecticide of small animals which feed on the algae. No detrimental effect upon the total algal population was found when gamma BHC was applied at the rate of 50 kg/ha, which was 10 times the rate recommended to control the rice stem-borer. They also detected the qualitative changes in the occurrence of major algal groups and observed that blue-green algae were more abundant in the treated soils whereas green algae and diatoms in the untreated soils. Much larger amounts of algal tissue were produced in the flood water treated with gamma-BHC.

Muralikrishna and Venkateswarlu (1984) observed that parathion, carbaryl and endosulfan, when applied to the soil at the recommended levels of field application (5 to 10 ppm) were not harmful to the algal population. The application of these pesticides to the non-flooded soils at the 5 ppm level slightly enhanced the population of algae while higher concentrations resulted in gradual inhibition, especially under flooded conditions. Parathion caused greater inhibition than the other two. The frequency of the algal forms, however, remained unchanged due to the application of these chemicals except for certain unicellular forms which were inhibited by the addition of parathion to the non-flooded soils.

Megharaj et al, (1988) in a three week's incubation study further examined the effect of carbofuran addition on the quantitative and qualitative occurrence of algal population in soil. They reported that carbofuran at low levels (0.5 and 1 kg ha^{-1}) either did not affect or slightly enhanced the algal population whereas higher levels were toxic to algae under non-flooded condition, while at the level of 2 kg ha^{-1} under flooded conditions it significantly increased the algal population.

They also suggested that carbofuran application in the soil generally favoured the growth of filamentous blue-green algae. The selective favourable actions of certain herbicides like pentachlorophenol, on the growth of N_2-fixing blue-green algae in rice soils were also reported by Ishizawa and Matsuguchi, (1966); Watanabe, A, (1967); Ibrahim, (1972); El-Nawawy and Hamdi, (1975); Roger and Kulasooriya, (1980). But some pesticides like chloropicrin have been reported to affect all algae without discrimination (Ishizawa and Matsuguchi, 1966).

Habte and Alexander (1980) reported that propanil strongly inhibited the Nitrogenase activity of blue-green algae in flooded soil but did not affect the rice plants, protozoa, or N_2-fixing bacteria.

In a comparative study between liquid cultural conditions and soil culture systems, Saha et al, (1984) reported that the fixation of nitrogen and chlorophyll content of blue-green algae, Nostoc muscorum and Nostoc piscinale were markedly increased by the application of the insecticide phorate (10% active ingredient) at lower concentrations (5-10 ppm). Nostoc muscorum was more efficient than Nostoc piscinale. The former performed well upto 50μg/ml in liquid culture and upto 250μg/g in soil culture. The toxicity of the higher concentrations of the insecticide gradually decreased with the increase in the growth period of the algal species. Higher concentrations were less lethal in soil culture than in liquid medium for both the algal species.

It is apparent from the review of literatures that apart from the studies concerning the effects of some important pesticides on the growth of N_2-fixing blue-green algae in culture medium, little information is available regarding their effects on algal N_2-fixation either in culture medium or in soil conditions. However, some general trends on the effects of pesticides on blue-green algae can be derived, which are (i) blue-green algae seem to be more resistant than other algae to pesticides some of which even stimulate their growth upto concentrations above the levels recommended for field application and (ii) insecticides are generally less toxic to blue-green algae than other pesticides. The growth stimulation of N_2-fixing algae due to pesticides application in rice soils generally results in greater N_2-fixation and consequently availability of nitrogen. The hydrolysable and non-hydrolysable -N contents in soil systems may change leading to the benefit of the crop either directly or indirectly.

Concentrated attention, is therefore required to be paid to ascertain the effect of pesticide application on the establishment of the introduced, as well as the native blue-green algae and their nitrogen fixation in submerged soils as well as the changes in the different forms of nitrogen fixed by the algae with a view to understand their implication in nitrogen economy of rice field soils.

2.3 Fixation of nitrogen by blue-green algae (Cyanobacteria) in rice soil and its effect on the root associative nitrogen fixing bacteria.

This part of the review is divided into two sections - the first part deals with the fixation of nitrogen by blue-green algae in rice soils while the second part deals with the effect of the blue-green algae on the nitrogen fixation by root associative bacteria in rice.

i) Fixation of nitrogen by blue-green algae in rice soils.

Since De (1939) first pointed out the role of blue-green algae in maintaining the N-fertility of rice soils voluminous studies have been conducted to evaluate this role. However, amounts of nitrogen fixed by blue-green algae in flooded rice soils have not yet been satisfactorily estimated because of technical difficulties in the assessment (Roger et al, 1987). Most of the information regarding the amount of nitrogen fixed by blue-green algae have come from laboratory incubation experiments and very few attempts have been made to evaluate the contribution of nitrogen by blue-green algae under actual field conditions. De and Mandal (1956) reported algal nitrogen fixation to the extent of 13.8 to 44.4 lb N $acre^{-1}$ in six cropped but unfertilized rice soils of West Bengal, by weekly analyses of gases in soil atmosphere. In the rice fields of Bihar algal nitrogen fixation to the extent of 14 kg N ha^{-1}

was reported by Prasad (1949).

Using ^{15}N, Mac Rae and Castro (1967) demonstrated nitrogen fixation to the extent of 10-15 kg N ha^{-1} $year^{-1}$ at the International Rice Research Institute, Phillippines and using a similar technique Yoshida and Ancajas (1973) obtained values of 40-80 kg N ha^{-1} $year^{-1}$.

The algal nitrogen fixation in three ivory coast rice soils was found to be 4.51 - 8.28 μg N g^{-1} with C_2H_2 - reduction technique and 7.0 - 7.33 μg N g^{-1} day^{-1} with Kjeldhal method (Rinaudo et al, 1971).

Saha and Mandal (1979) reported algal nitrogen fixation rate of 27.66 - 73.33 μg N g^{-1} $week^{-1}$ (from a laboratory incubation experiment) in ten neutral alluvial rice soils of West Bengal, while Reddy and Patrick (1979) reported a value of 57 μg N g^{-1} $year^{-1}$ in Crowley silt loam soil incubated under flooded condition in light using ^{15}N tracer technique. The average of 38 evaluations of amounts of N_2-fixed in flooded rice soils was found to be 27 kg, the highest value being 80 kg N ha^{-1} $crop^{-1}$. An amount of 30 kg N ha^{-1} $crop^{-1}$ is considered to be a reasonable estimate of N_2-fixation by blue-green algae under field condition (Roger et al, 1987). Algal nitrogen fixation in rice soils of acidic nature was observed to be very low. However, inoculation of N_2-fixing blue-green algae following correction of soil acidity with lime, significantly increased the N-content of the soil which was further increased with the addition of P (Okuda and Yamaguchi, 1955; Saha and Mandal, 1980 a). The amount of N_2-fixation by blue-green algae however, depends greatly on the soil and other environmental conditions.

Effect of phosphorus :

The N_2-fixing activity of blue-green algae increases with the addition of phosphorus. Addition of phosphates to rice soils either in soluble form as potassium phosphate or in insoluble form as calcium phosphate stimulated the fixation of nitrogen by blue-green algae (De and Sulaiman, 1950).

Application of superphosphate at the rate of 60 lbs of P_2O_5 per acre was found to stimulate algal N_2-fixation to the extent of 3.00-20.4 lbs N $acre^{-1}$/3-20 kg N ha^{-1} (De and Mandal, 1956).

Effect of nitrogen :

The nature and quantity of fertilizer nitrogen have a considerable influence on the proliferation of blue-green algae and nitrogen fixation by them, the effect of ammonium nitrogen being more pronounced than nitrate nitrogen in this regard. While high nitrate nitrogen showed no effect on nitrogen fixation ammonia nitrogen inhibited the process (Allen, 1956), the latter at higher concentrations was found to inhibit both expression and synthesis of nitrogenase (Rai et al, 1980, Singh et al, 1972) and cause cell lysis (Stewart, 1964). However, Bottomley et al, (1977) reported inability of ammonia and ability of nitrate to completely repress heterocyst differentiation and nitrogenase activity in *Anabaena* sp. CA. Venkataraman (1979) however, did not find any reduction in nitrogen fixation in presence of ammonium nitrogen upto 40 ppm. Kaushik (1987) observed 22-30% N_2-ase activity even in presence of 100 ppm NO_3-N and concluded that NO_3-N per se did not cause complete repression of the nif gene products. Singh (1978) reported that heterocysts differentiation and N_2-fixation by blue-green algae, in the presence of combined nitrogen sources, was depressed but the degree of depression varied from species to species. Saha and Mandal (1980 b) also reported that application of increasing levels of Urea-N suppressed the N_2-fixing activity of different cultures of blue-green algae in some alluvial rice soils,the extent of suppression, however, varied with different species. In pot and field experiments also such suppression was observed due to application of nitrogenous fertilizers. Abou-el-Fadl et al, (1964) in pot experiments using silt loam soil showed that the beneficial effect of inoculation of *Tolypothrix tenius*, was enhanced by addition of organic matter but markedly decreased due to addition of $(NH_4)_2SO_4$. Yoshida et al, (1973) observed that although N-fertilizer increased algal growth, but generally there were more blue-green algae in pots without N-fertilizer. N_2-fixation decreased markedly with the addition of 200 kg N ha^{-1}, either as $(NH_4)_2SO_4$ or as NH_4Cl, the extent of decrease being more with the latter (98%) than with the former (72%). It was completely inhibited by the addition of 400 kg N ha^{-1}.

In field study, Alimagno and Yoshida (1977), using C_2H_2-reduction technique, observed higher algal N_2-fixing activity in unfertilized field (18 to 33 kg N. ha^{-1}) than in the field fertilized with N-fertilizers (2.3 to 5.7 kg N ha^{-1}). Watanabe et al, (1980) also found that in flood water, the N_2-ase activity contributed by blue-green algae was marginally higher (61%) than that of the soil and plant in unfertilized plots (-NPK) and largely lower (16%) in fertilized plots (+NPK).

The foregoing discussion clearly shows that application of nitrogenous fertilizers depress algal N_2-fixation even in field condition but the effect depends on the forms of nitrogen used and the species of algae involved. However, reports are also not uncommon that nitrogen at lower doses or in presence of phosphorus did not much affect the algal N_2-fixation particularly in cropped system.

Singh (1975) and Pattnaik and Singh (1977) observed that use of lower doses of N such as 10-20 ppm as NH_4Cl enhanced the

N_2-fixation by algae in rice fields. They also found that NO_3-fertilizers even in high concentrations did not inhibit rather it stimulated N_2-fixation by some species of blue-green algae (Gloeotrichia sp.) on longer incubation. Shtina et al, (1968) also reported that lower doses of N-application increased the algal growth.

Ibrahim et al, (1971) observed that the inoculation with N_2-fixing blue-green algae in conjunction with N and P significantly increased the contents of soluble N in soil from 8.9 to a maximum of 22.5 ppm in a pot experiment planted with rice. Algalization with Tolypothrix tenuis along with P and Mo increased 50 per cent organic N in soil, and this was not much affected by N fertilizer (Chopra and Dube, 1971).

The accretion of N in soil system by blue-green algae may be enough to support a good crop of rice. The quantity of N left by the inoculation of blue-green algae in rice soil after crop harvest was found to be about 13 to 70 lb per acre (Willis and Green, 1948, Prasad, 1949; Watanabe et al, 1951; Nishigaki et al, 1951; 1953; Singh 1961). However, in long term field trial no appreciable increase in the amount of N in soils after harvest of crop could be detected by Aiyer et al, (1972). Saha and Mandal (1980 c) observed a significant increase of both total and available N-contents in wet soils at crop harvest, due to inoculation of different cultures of blue-green algae, but after air-drying there was marked decrease of the same, suggesting that most of the N added to the soil by blue-green algae did not persist after air drying the soil.

Algal N_2-fixing activity during cultivation cycle of rice :

The growing period of rice is divided into two stages, viz. "vegetative" and "reproductive". The period upto maximum tiller formation is termed as vegetative while that from maximum tiller formation to fertilization of the ovary, as reproductive stage. Each of these stages differs in nutritional requirements. The uptake of nitrogen by the rice plant is highest from four-leaf stage to tube-formation; it is less high in flowering and it still less high in ripening. Therefore, apart from total nitrogen fixation, the fixation at different growth stages of rice may be important so far its contribution to the nitrogen requirement of rice plants is concerned.

The continuous changes of availability of nutrients particularly of phosphorus, in water-logged rice fields, might have an influence on the growth of N_2-fixing blue-green algae during the growth cycle of rice. Moreover, a number of reports indicated that rice crop itself stimulated the algal N_2-fixation by providing sufficient CO_2 through respiring roots and weak shading by the plant canopy (De and Sulaiman, 1950; Watanabe and Cholitkul,1979). A few reports are available concerning the variations of algal N_2-fixation during the cultivation cycle of rice, most of which are from International Rice Research Institute (IRRI) where C_2H_2 reduction assay technique was used. Yoshida and Ancajas (1973) compared algal N_2-fixing activity in non-fertilized planted and unplanted flooded soils during the wet and dry seasons by measuring the C_2H_2 reduction activity of flood water. During the wet season, the activity remained always higher in unplanted than in the planted fields. In the latter; C_2H_2 reduction activity reached its maximal value 3 weeks after transplanting thereafter it decreased upto the ninth week and finally remained very low. This was related to limiting light intensities, which decreased with the increase in the density of the plant cover. The amount of N fixed in flood water was estimated to be 3 kg N ha^{-1} in a planted as compared to 11 kg N ha^{-1} in an unplanted field during wet season. The effect of plant cover was, however, nil during dry season.

Watanabe and Lee (1975) and Watanabe _et al_, (1977) observed that, in unfertilized plots, algal C_2H_2 reduction activity in flood water showed two peaks, a small one at the beginning of the crop season and a larger one near or after harvest both in dry and wet seasons.

Alimagno and Yoshida (1977) compared C_2H_2 reduction activity during a cultivation cycle in a fertilized and unfertilized paddy fields both covered with black cloths. Both of them exhibited no activity, suggesting that blue-green algae were the principal N_2-fixing agents in these fields. In the cropping season, a much higher N_2-fixing activity was observed in the unfertilized than in the fertilized field when they remained uncovered.

In long term fertility plots, Watanabe et al, (1978 a) assayed periodicaly C_2H_2 reduction activity and observed that in plots where NPK fertilizers were applied, algal N_2-fixing activity was depressed. In unfertilized plots, however, N_2-fixing algae bloomed in both wet and dry seasons near or after harvest of rice. Algae biomass and its activity were much higher in the dry than in the wet season. They pointed out that the predominance of algal activity at or after harvest time is, presumably, due to changes in light intensity brought about by the maturing or removal of the rice plant.

In Japan, Wada et al, (1978) measured the N_2-fixing activity in rice field soils at different times of cultivation cycle and observed a very low value before flooding, which increased after flooding and reached peak at maximum tillering stage, declined thereafter, and attained a very low value after drainage.

The above review of literature reveals that a peak of algal N_2-fixing activity may occur any time during the rice cultivation cycle. However, the results are too scarce to allow definite conclusion regarding the variations of algal N_2-fixation during the cultivation cycle. A predominant effect of light intensity in relation to the season and the plant cover is clear to some extent and an inhibitory effect of N fertilization is confirmed.

Availability of algae fixed-N :

It is well established that the growth of N_2-fixing blue-green algae significantly improves the nitrogen fertility of rice soil. Their growth has also been shown to result in increase in soluble or available -N contents of soil (Chopra and Dube, 1971, Ibrahim et al, 1971; Saha and Mandal, 1980 c). However, information on how much, when, and in what ways the algae fixed N become available in the soil system and to rice are still very scarce and mostly hypothetical (Roger and Kulasooriya, 1980).

Nitrogen fixed by blue-green algae in waterlogged rice soil may become available to rice plant mainly by two possible ways (i) through exudation during their early part of growth, and (ii) through microbial decomposition after the death of the cell. According to Fogg et al, (1973) blue-green algae liberate as much as 20 to 40 percent of the nitrogen fixed as various nitrogenous substances into the culture medium. However, no information is available on the exudation of fixed N by blue-green algae under field conditions but it is expected that only a part of it is available to rice, some being either reincorporated by the microflora or lost through volatilization and denitrification (Roger and Kulasooriya, 1980).

Release of algae fixed - N through microbial decomposition after the death of the cells appears to be the principal means by which N is made available in soil and hence to the crop. Watanabe and Kiyohara (1960) reported that a strain of Bacillus subtilis decomposed several N_2-fixing blue-green algae very rapidly; about 40 percent of the N from autolized cells and 50% of the nitrogen from fresh cells were converted to NH_4^+ within 10 days.

If the algae blooms develop early in the cycle, decomposition by lytic microorganisms and grazing by aquatic fauna occurs during the same cycle. This situation may occur either with spontaneously growing blue-green algae or when rice fields are inoculated with it. This is somewhat similar to that in the treatments where dried blue-green algae were surface applied. When algae blooms develop later in the cycle, most of the algal material will dry on the surface of the soil after the harvest of the rice crop and decompose subsequently after it is incorporated into the soil by ploughing at the begining of the next crop season. This is similar to the situation where dried blue-green algae are incorporated. Taking these two situations as representative Wilson et al, (1980) in a green house experiment, recovered in a rice crop 36% of the N from ^{15}N labelled Aulosira sp. when spread on the soil and 50% when incorporated into the soil. Tirol et al, (1982) studied uptake of ^{15}N from Nostoc sp. by rice in pot and field experiments; the quantity of applied algal material was equivalent to that of a dense algal bloom and corresponded to 20 kg N ha^{-1}, 290 kg DW ha^{-1} and 13t FW ha^{-1}. Availability of ^{15}N from incorporated blue-green algae was between 23 and 28% for the first crop of rice and between 27 and 36% for the first and second crop together. Surface application of algae reduced ^{15}N availability 14 to 23% for the first crop and 21 to 27% for the first and second crop together.

The reasons for high availability of algae nitrogen, as reported by Wilson et al, (1980) than that measured under similar experimental conditions by Tirol et al, (1982) were, according to Tirol et al, (1982), related to the nature of the algal material, the method of its preparation, and the nature of the strain. The inconsistencies in recovery of algal nitrogen by rice were also interpreted as an effect of the benthic infauna by Wilson and Segers (1985) who showed that in flooded soil, the uptake of algal N and total N by rice was affected by tubificids (oligochaetes) which reduced recoveries of algal N by rice by making soil N available through mineralization process.

The pot experiment by Tirol et al, (1982) demonstrated that for the first crop, algal ^{15}N was less available than $(NH_4)_2SO_4$- ^{15}N but for two succeeessive crops their availability was very similar, indicating the slow-release nature of algal N. After two crops, 57% of the ^{15}N from blue-green algae and 30-40% of ^{15}N for $(NH_4)_2SO_4$ remained in the soil suggesting the less suceptibility of algal N to losses than mineral N. By periodically estimating the extractable mineral-N contents of soil, Saha et al, (1982) observed that incorporation of fresh blue-green algae dominated by Aulosira sp., released about 12-35 percent of their N into soil within 7-35 days under waterlogged condition. Mian and Stewart (1985) in pot experiment examined the release of nitrogen into available form from ^{15}N labelled dry biomass of Anabaena variabilis and Nostoc muscorum thoroughly mixed with silt loam Bangladesh soil of Sonatolah series. They found that in the absence of rice plant, within 60 days, 43 and 45 percent of applied ^{15}N of Anabaena and Nostoc was released, respectively, of which 93-96 percent was lost as N_2 through denitrification. In presence of rice plants, however, 49 and 53 per cent of the total ^{15}N was released from these two species; about 14 and 13 percent was lost by denitrification and after 60 days, 51 and 47 percent remained in the soils as the undecomposed part of the Anabaena and Nostoc species, respectively. Of the total N assimilated by the rice plant, 61 and 62 percent was supplied by Anabaena and Nostoc respectively and the rest was obtained from the soil used.

Results of these studies indicate that the biomass-N of blue-green algae becomes available to rice plant quite rapidly. In all the works only biomass-N from the supplied algal material was considered; the relative contribution of the supplied algal material to the total biomass in pots/ fields and algal N_2-fixation in the system was not considered. But in practice, N_2-fixation by either indigenous or inoculated algae, release of fixed-N through exudation or decomposition, N-immobilization by soil microflora, loss of N through various means and uptake by rice plant may be a simultaneous and continuous process. Therefore, more direct information on the dynamics of algal fixed-N in wetland rice soils are needed.

ii) Effect of the growth of blue-green algae on root associative bacterial N_2 fixation with rice.

Besides, the algal N_2-fixation in soil-water system, heterotrophic soil bacteria associated with rice root may also fix significant amount (Rinaudo et al, 1971; Yoshida and Ancajas, 1971; Gilmour et al, 1978). Yoshida and Ancajas (1973) found that N_2-ase activity (C_2H_2 reduction) of washed roots of rice plant was much higher than that in the adjacent soil and concluded that the N_2-fixation by organisms close to the root surface might be a major source of nitrogen in unfertilized rice fields.

Watanabe et al, (1978b) studied the seasonal changes of C_2H_2 reduction activity with rice plant (Stems and roots) and calculated a value of 90 m mol C_2H_4/m^2 and 50 m mol C_2H_4/m^2 in the wet and dry season respectively.

Sims and Dunigan (1984) reported that diurnal N_2-ase activity of excised roots varied when the rice was in the early reproductive phase but not during the ripening (grain hardening) phase. Such variation could not be explained by fluctuations in soil temperature alone. They also observed seasonal variation in N_2-ase activity and found maximum activity during the early reproductive phase.

The population and activity of heterotrophic N_2-fixing bacteria associated with rice roots may determine the extent of associative N_2-fixation. Their association is higher in wetland than in dryland rice plants (Watanabe and Cholitkul, 1979, Watanabe, 1986). Watanabe and Cholitkul (1979) examined two rice varieties, IR-26 and IR-36, and detected the most probable number of N_2-fixing rhizoplane bacteria per g fresh weight of root, ranged from 2 to 6 x 10^7 in the glucose yeast-extract medium and 2 to 15 x 10^5 in the malate yeast-extract medium while that in histosphere ranged from 1-18 x 10^7 in the former medium and 3-72 x 10^5 in the latter one. They also found that most of the bacteria in the malate medium resembled Spirillum, but the bacteria in glucose media differ from any of the reported N_2-fixing bacteria. However, they resembled Achromobacter. Till now, little is known about the composition of N_2-fixing micropopulations associated with the rice roots. In the tropics, root associative N_2-fixation with rice is caused chiefly by colonization of Sprillum sp. viz. Azospirillum brasilense (Laksmi Kumari et al, 1976; Nayak and Rao, 1977; Silva and Dobereiner 1978).

Besides, the qualitative and quantitative composition of N_2-fixing bacteria associated with roots, their activity is also frequently limited by shortages of carbon and energy in addition to some other intrinsic and extrinsic factors. In the root zone of rice, energy yielding compounds come mainly from two sources :

i) Roots i.e. root exudates, root lysates and root litter and

ii) Soluble and easily decomposable constituents of root adjacent soil.

Blue-green algae are known to provide a great variety of extracellular substances during their growth period which include carbohydrate, amino acids, peptides and other nitrogenous compounds. These may diffuse to root zone and may influence the root associative N_2-fixation with rice. Besides, they also liberate large amounts of O_2 and CO_2 due to their photosynthetic and respiratory activity and enrich the soil with organic matter contents which may have influence on the root associative nitrogen fixation in rice. Information regarding the influence of the growth of blue-green algae on root associative bacterial N_2-fixation with rice system is meager.

For better nitrogen management in rice cultivation an understanding of the interactions of these two potential sources for N_2-fixation in rice system, is needed.

2.4 Effect of nitrogen-fixing blue-green algae (Cyanobacteria) on growth, yield and nitrogen nutrition of rice plant.

Algal N_2-fixation in paddy soils is of special significance because the products become available to the biological system quite rapidly and thus support a good crop growth. Definite proof of N-transfer from blue-green algae to rice plants (Renaut *et al*, 1975; Venkataraman 1977, Wilson *et al*, 1980 ; Tirol *et al*, 1982);and other higher plants (Mayland and McIntosh, 1966; Stewart, 1967 b) has been obtained using 15_N as a tracer. Higher crop yield due to growth and succession of N_2-fixing blue green algae in rice fields may also provide an indirect evidence of the use of algal fixed nitrogen by rice plants.

De and Sulaiman (1950), in a pot experiment, observed that successive growth of indigenous blue green algae significantly increased the rice yield in the fourth and fifth year and in the last year yields in all cases were almost 100% higher than the corresponding yields in absence of blue-green algae.

Finding *Tolypothrix tenuis* inoculation superior to some other species of blue-green algae in increasing rice yield in Japanese rice soils (Watanabe *et al*, 1951), Watanabe (1956, 1959, 1962 and 1965) mass cultured this species and conducted inoculation trials in fields for four successive years and observed a progressive increase in rice yield ranging from 2 to 20 percent during the entire period. Since then, attempts have been made to increase rice yield by inoculating soils with nitrogen fixing blue-green algae. The reported increase in yield in most of the experiments has brought to light the possibility of utilizing blue-green algae as bio-fertilizer in rice cultivation. Singh (1961) reported an increase in paddy yield over the control by 368 percent in pots and 114 percent in fields, due to inoculation with *Aulosira fertilissima*. Similar increase in rice yield due to inoculation of the said species was also reported by Sundara Rao *et al*, (1963), Singh (1978) and Saha and Mandal (1980), but Watanabe (1973) could not find any such beneficial effect of this species in Japanese rice soils. Such diversity in the results was attributed to various factors, one of which was pH values of the soils. Soils used for rice cultivation in various parts of India are mostly neutral to alkaline, whereas those in Japan are in general acidic in nature.

Konishi and Seino (1961) conducted inoculation trials at Hokuriku Experimental station in Japan for a period of six years and found that the algal inoculation was significantly effective in increasing rice yield when supplemented with $CaCO_3$.

Hosoda and Takata (1955) observed that in one field, the effect of algal inoculation was comparable to that of the application of $(NH_4)_2SO_4$ at the rate of 71.74 kg N/ha.

In the U.S.S.R., Burma, Egypt, China and Philippines, increases from 10-24% in the grain yield of rice have been reported as a result of algal inoculation (Venkataraman, 1981).

The proliferation of inoculated algae in an area may be influenced by climatic conditions, geographical features and physical and chemical characteristics of the soil. So the efficiency of an inoculum in a particular area may vary with the variation of the above mentioned factors. Besides, a small quantity of added algal inoculum must struggle for survival and for proliferation with the autochthonous organisms, some of which have N_2-fixing ability. The composition of inoculum is also an important factor to ensure efficient inoculation.

Algal inoculation experiments with rice in soils of different agroclimatic regions in India showed differential response.

Subrahmanyan et al, (1965 a-c) inoculated seven different soils with a mixture of the nitrogen fixing species of the genera such as Nostoc, Anabaena and Scytonema of blue-green alga in presence or absence of lime, superphosphate, $Na_2M_0O_4$ and $(NH_4)_2SO_4$ (20-40 kg N ha^{-1}) and observed an increase in nitrogen content and growth of rice crop in all the soils, but the magnitude of such response varied in different soils.

In co-ordinated field trials conducted at 6 centres in India, Sankaram _et al_, (1967) found that inoculation with blue-green algae (mixture of _Nostoc_, _Anabaena_ and _Scytonema_ with river sand as carrier) along with the application of non-nitrogenous fertilizer mixtures enhanced rice yield over control at four of the centres. The effect was equivalent to an application of 20 kg N ha^{-1} as sulphate of ammonia. Negative results recorded at two of the centres (Maruteru and Hyderabad) were attributed to the initial high fertility of the soil and the use of a variety of rice not responsive to high fertilization.

Sankaram (1971) and Subrahmanyan (1972) evaluated the effect of algal inoculation, in a large number of rice-growing soils obtained from different states of India, on the yield of rice crop under green house conditions and observed that in majority of the soil types the response was nearly equivalent to an application of 20 kg N/ha as sulphate of ammonia.

Goyal and Venkataraman (1971) recorded the increase in rice yield due to algal inoculation to the extent of 13.4, 14.1, 11.7, 19.1 and 19.5 per cent over that in the control in Delhi, Ambasamudrum, Pusa, Coimbatore and Kottayam, respectively.

Algal inoculation in presence of phosphorus found to be more efficient in increasing the rice yield.

In a field experiment conducted at Agricultural College, Sabour (Bihar), Jha *et al*, (1965) recorded an increase of about 40 per cent in the grain yield due to inoculation with *Tolypothrix tenuis*. Addition of phosphate along with algae recorded the highest yield. Such beneficial effect of P has also been reported by other workers (Ibrahim *et al*, 1971, Roger and Kulasooriya, 1980). The general conclusion was that benefits from algal inoculation could be maximised through soil conditioning by way of application of superphosphate.

A number of pot and field experiments with algal inoculation superimposed with lime, phosphate and molybdate in presence of different doses of organic matter and popular nitrogenous fertilizers have been conducted at various centres located in different agro-climatic regions in India.

Subrahmanyan *et al*, (1964 a,b) observed in a field experiment that inoculation with a mixture of blue-green algae of *Nostoc sphaericum*, *N. amplissimum*, *Tolypothrix campylonemoides* and *Westiella* Sp. increased grain yield by about 30% over the control when the algal treatments were superimposed over a basal dressing of nutrient mixture of lime at 500 kg/ha, superphosphate at 20 kg P_2O_5/ha and sodium molybdate at 0.28 kg/ha. The effect was equivalent to that of 20 kg N/ha applied as farm yard manure, $(NH_4)_2SO_4$ or urea.

Relwani and Subrahmanyan (1963) showed that partial sterilization of soil followed by algal inoculation in conjunction with nutrient mixture brought about an increase in the yield of rice grain and straw to the extent of 275 and 236 percent and 53 and 58 percent in the pot and field experiment respectively.

Venkataraman and Goyal (1968) applied a dry mixture (2.3 kg ha^{-1}) of blue-green algae consisting of Aulosira fertilissima Tolypothrix tenuis, Cylindrospernum muscicola and Nostoc sp. through broadcast in a paddy field at Vasudevanallur in the erstwhile Madras State, supplemented with a fertilizer mixture of super phosphate (112.1 kg ha^{-1}) and sodium molybdate (0.25 kg ha^{-1}) and observed an increase in grain yield of ASD 5 rice variety to the extent of 15.7 percent over that in the uninoculated one. Algal inoculation also increased significantly the N content of grains and the N uptake by crop plants to the extent of 19.62 kg ha^{-1} in one cropping season.

Ramaswami et al, (1964) studied the effect of algal inoculation (T. tenuis) alone and in combination with green manure, superphosphate and $(NH_4)_2SO_4$ on the yield of rice in a field trial conducted for three successive years (1962-65) at the Central Farm of the Agricultural College at Coimbatore and observed that inoculation alone had increased the grain yields by 3.4 per cent over the unmanured control, while inoculation along with superphosphate, with green leaf and superphosphate, and with green leaf, $(NH_4)_2SO_4$ and superphosphate had increased the yield over their respective controls by 3.2, 3.3 and 15.3 per cent respectively.

Venkataraman and Goyal (1972) examined in detail the efficiency of algal inoculation in presence of ammonium sulphate, superphosphate and farm yard manure in various combinations and observed the efficiency in the following order : Ammonium sulphate + Superphosphate $>$ FYM + Superphosphate $>$ FYM + ammonium sulphate $>$ ammonium sulphate. The beneficial effect of urea on inoculation of blue-green algae has also been observed by various workers. (Relwani and Manna, 1964; Mudholkar et al, 1968; Subrahmanayan, 1972).

Relwani and Manna (1964) observed that the blue-green algae alone increased yield of grain and straw by 109.0% and 108.9% respectively. Urea at 0-20 kg N ha^{-1} in combination with blue-green algae generally produced additional response in the instance of soil application whereas in combination with foliar spray the response was inferior.

Mudholkar et al, (1968) compared the effect of urea spray in graded doses of 25, 50, and 75 kg N/ha superimposed over algal inoculation on a high yielding, nitrogen-responsive variety of rice 'Taichung Native-1' against foliar spray of urea alone. All treatments excepting control received a basal application of a mixture of lime, superphosphate and sodium molybdate. The results showed that in all the seasons maximum grain yield was obtained when 75 kg N/ha was applied as urea spray in addition to algal inoculation. The results suggested that nitrogen gain through algae and urea spray could be made additive to meet the high nitrogen requirement of the crop.

Goyal and Venkataraman (1972) observed a significant effect of algal inoculation irrespective of whether the urea fertilizer was applied to the soil or as foliar spray in a pot experiment with TN-1 rice variety.

Algal inoculation was also found to have a significant additive effect at all levels of nitrogen fertilization, applied in the form of ammonium sulphate.

Aiyer et al, (1972) conducted a long term field trial, covering four seasons to study the effect of algal inoculation on the high yielding rice varieties at different levels of ammonium sulphate (30, 60, 90 and 120 kg N/ha) and observed a significant increase in the number of productive tillers as well as the total grain yield upto the maximum dose of 120 kg N/ha, the overall increase in the grain yield being about

586 kg/ha due to inoculation. Whereas Sahay (1972) summarised the data of the yield of grain and straw and nitrogen uptake of rice plant in sand culture conducted for seven seasons with the inoculation of algal sp. Anabaena, Scytonema, Aulosira and Wollea separately or in a mixture, in combination with 0,5, 10 and 50 ppm inorganic nitrogen added through NH_4NO_3. The inoculation of algal sp. separately resulted in a significant increase in grain yield in all cases except the inoculation of Wollea sp. There were indications of continued nitrogen fixation by algae at lower concentrations of extraneous nitrogen. However, the yields obtained were very much less compared to application of higher rates of N with or without algae. At the higher rate of nitrogen the algae did not appear to play any important role. Nitrogen uptake pattern followed more or less similar trend as that of grain yield. Goyal (1985) observed in a pot culture experiment that the additive effect of algae was better in presence of nitrate nitrogen than ammonium nitrogen.

Irrespective of the varietal differences among the recently introduced high yielding, fertilizer responsive rice genotypes, all of them were found to respond positively to algal inoculation even in presence of high levels of nitrogen fertilizers (Venkataraman and Goyal, 1969 a,b; Goyal and Venkataraman, 1970). In a field experiment, when algal inoculation was superimposed over a basal dressing of phosphate (89.68 kg P_2O_5/ha) and potash (50.45 kg K_2O/ha), the grain yield of high yielding paddy variety IR-8 was increased by about 22% over the corresponding uninoculated control, the increase being comparable with that of application of 112 kg of N/ha as $(NH_4)_2SO_4$.

Goyal and Venkataraman (1970) in a pot experiment observed that algal inoculation in presence of high dose of N fertilizer (100 kg N ha^{-1}) increased the yield of 4 high yielding varieties of rice by 10-15 percent over that in the uninoculated control.

Biological nitrogen fixation is known to be suppressed by exogenous nitrogen. Venkataraman (1975), however, suggested that in presence of high level of nitrogenous fertilizers the production of growth substances and vitamins by algae might increase which may partly be responsible for the greater plant yield. Gupta and Shukla (1967) reported that the nitrogen and free amino acids content in the rice grain increased as a result of algal inoculation. Roger and Kulasooriya (1980) compiled the results of inoculation experiments at different locations in six countries (30 in India; 5 in Japan; 3 in China; 3 in Egypt; 1 in Phillippines, 1 in USSR) and concluded that algal inoculation on an average could increase the rice yield to the extent of 14% over the uninoculated control.

This review on the effect of algalization on rice yield shows that the results obtained from pot and field experiments in different agroclimatic regions in India, are encouraging for utilization of blue-green algae for higher crop yield and as a supplementary source of nitrogen. But these experiments emphasized that benefits of algal inoculation as judged by higher rice yields are limited in magnitude and confined to certain soil conditions and favourable seasons. As the efficiency of a high-nitrogen fixing species of blue-green algae varies with the variation of the localities, experiments are to be conducted in a more intensive way to provide answers for such questions like (1) what soil properties are decisive for the establishment, multiplication and performance of the inoculated algae, so as to formulate an agronomic schedule for adaptation in the field; (ii) how best nitrogen fixation by blue-green algae could supplement other nitrogenous sources to meet high nitrogen requirement of certain varieties of rice.

Very little information is available on the efficiency of the inoculation of nitrogen fixing blue-green algae in rice soils of West Bengal, one of the major rice growing states in India. So the different rice growing centres of West Bengal need extensive trials on this problem for the utilization of proper nitrogen fixing algal species for higher crop yield.

CHAPTER NO. 3

MATERIALS AND METHODS

3.0 MATERIALS AND METHODS

The entire work was distributed into four main sections. Each section comprises one or more experiments. The materials used and methods followed for each of these studies are described below :

3.1 Distribution of nitrogen fixing blue-green algae (Cyanobacteria).

3.1.1 Materials:

Thirty two number of surface (0-15 cm) soil samples were collected from the rice-fields in the districts of Coochbehar and Jalpaiguri during the period March-April when the fields remained dry and fallow. The samples were representative of a composite sample from five randomly selected spots in each area. They were air dried, powdered, sieved and carefully preserved in clean jars for further use.

3.1.2 Methods :

3.1.2.1 Physico-chemical analysis of the soil samples :

a) Soil reaction - pH value of the soil samples was determined in a soil water suspension of 1 : 2.5 using electrical pH meter.

b) Electrical conductivity (E.C.) - The specific conductance of soil suspension was determined by conductivity bridge using 1 : 2 soil : water ratio.

c) Organic C - Organic carbon was determined by wet digestion following the method of Walkley and Black as described by Jackson (1967).

d) Total N - This was determined by modified Kjeldahl method as described by Jackson (1967).

e) Inorganic N - The ammonium - N and Nitrite + Nitrate - N was estimated by steam distillating the KCl - extract of the soil with ignited MgO and then using Devarda's alloy as described by Bremner and Keeney (1966).

f) Available P - This was estimated colorimetrically in 2.85 : 20 soil to Bray and Kurtz No.1 solution extract by chlorostanous reduced molybdophosphoric acid method as described by Jackson (1967).

g) C.E.C. - This was determined following the method of Schollenberger and Simon as Described by Jackson (1967).

h) Exchangeable Ca^{++} and Mg^{++} - Exchangeable calcium and magnesium was determined by leaching the soil with neutral normal ammonium acetate and estimating Ca and Mg by complexo - metric titration with Na-Salt of ethylene-diamine - tetra acetic acid using Eriochrome Black-T and calcon indicator (Black, 1965).

i) Clay content - Clay content was determined following International pipettee method (Piper, 1950).

3.1.2.2 Preparation of culture medium :

Modified Chu-10 medium (Safferman and Morris, 1964) with trace elements (Allen and Arnon, 1955) was used for growing the culture of blue-green algae. The composition of the medium was -g l^{-1} (1) $MgSO_4$, $7H_2O$: 0.025; (2) Na_2CO_3 : 0.02, (3) Na_2SiO_3, $5H_2O$: 0.044; (4) $Ca(NO_3)_2$, $4H_2O$: 0.232; or $CaCl_2$, H_2O : 0.0555; (5) K_2HPO_4 : 0.01; (6) Ferric citrate : 0.0035 + Citric acid : 0.0035; (7) 1.0 ml of the micro-nutrient stock solution l^{-1} of the medium. The composition of the micronutrient stock solution (g l^{-1}) : (I) H_3BO_3 : 0.5; (II) $MnSO_4$, $7H_2O$: 0.05; (III) $ZnSO_4$, $7H_2O$: 0.05, (IV) $CuSO_4$, $5H_2O$: 0.02; (V) MoO_3 : 0.01 & (VI) $COCl_2$: 0.04. The medium was solidified by 1.5 percent agar as and when required for the experimental purposes.

3.1.2.3 Conditions for culturing blue-green algae :

For culturing blue-green algae, the culture medium was always maintained at a temperature of 25 ± 2°C in the culture room which was illuminated with approximately 3,000 lux light intensity by day light fluorescent tubes for 24 h/day.

3.1.2.4 Enumeration of blue-green algae :

The most probable number (MPN) technique as described by Clark and Durrell (1965) was adopted for enumerating the initial population of blue-green algae in air dry soil using the medium as described before.

3.1.2.5 Composite blue-green algal culture of different soils :

The algal growth appeared in the tubes from each of the soils in MPN technique was transferred aseptically into 250 ml conical flask, containing 100 ml sterilized N -free culture medium. The flasks were then kept under illumination by day light fluorescent tubes for 24 hours in the culture room as described. The algae were subcultured at every 15 days interval by transferring aliquots of 1.0 ml of the culture into 100 ml conical flasks containing a fresh medium. The flasks were shaken intermittently with the help of a rotary shaker. The growth of algae in the flasks were described as the 'composite culture of blue-green algae' of different soils.

3.1.2.6 Identification of blue-green algae :

Ten such culture flasks of composite blue-green algae obtained from each soil were then examined under the Kerl Zeiss research microscope after mounting the algal mass in a drop of 10 percent glycerine solution on the microscopic slide and covering them with a thin cover slip. For characterization of the algal species upto generic level most of the microscopic characters of prominance were taken into consideration and they were identified according to Desikachary (1959).

3.1.2.7 Isolation of unialgal culture :

A small portion of algal growth in N-free medium as described was transferred by means of bacteriological loop into several glass tube, each containing 10 ml sterilized distilled water, the mouth of the tubes being closed with rubber stopper. The contents of the tubes were shaken for 5 to 10 minutes to break the algal filaments in the tubes. A drop of algal suspension was then streaked over the surface of previously prepared agar plates with N-free medium by inoculating loop. The plates were then illuminated by day light fluorescent tubes in the culture room. The colonies on the surface of agar plates and the growth of blue-green algae that moved out from the point of streaking were marked and examined under the microscope. The selected portions of the growth on agar plate containing mostly one type of filament were then subcultured into N-free liquid medium and exposed to light in the culture room. At the exponential growth stage, 1 ml of algal suspension was taken by fine pipette and spread on to the surface of a solid medium, then a second agar layer was poured on the top. A shallow layer of sterilized culture solution was placed on top of the 2nd agar layer and illuminated from above in the culture room. After appearance of the growth of blue-green algae through the upper agar layer a small portion of the growth from the edge was taken out with the help of inoculating loop and subcultured into agar slant of N-free medium. These agar slants were illuminated until good growth appeared and then examined microscopically. This process of repeated subculturing and intensive microscopic examination was continued until filaments of only one type was obtained in one agar slant. These cultures were transferred to the liquid medium; Morphological characteristics were noted and photo-micrographs of some of the unialgal cultures were taken (Fig.1-3).

3.1.2.8 Determination of N_2-fixation in soils :

Soils in 5 g portions were taken in 6" x 1" flat bottomed specimen tubes and waterlogged by distilled water, the thickness of the soil layer and height of the standing water on the soil column being 1.3 $\pm$ 0.1 and 2 $\pm$ 0.1 cm respectively. In order to simulate field conditions as closely as possible five or six sample tubes were placed in a 500 ml beaker containing a 3 cm layer of waterlogged soil. By applying gentle pressure, each sample tube was pushed into the soft soil layer in the beaker maintaining the same soil and water level both in the beaker and inside the sample tubes. The beakers containing the sample tubes were then placed in a big tray containing soil and water at the same level with that in inside the beakers. The tray was then kept in the green house in open sunlight. The tubes were protected from dust and rain water by placing another 500 ml inverted glass beaker on each beaker containing the sample tubes. A similar set was also maintained with the tubes covered with glazed black paper to avoid any growth of algae. Each soil was replicated four times for each set. The level of standing water inside the sample tubes was maintained constant throughout the incubation period by periodic addition of distilled water. The minimum and maximum temperature ranged from 23° - 35°C during the incubation period. After six weeks of incubation the contents of the tubes were completely transferred into Kjeldahl digestion flask by washing thoroughly, at first by using minimum amount of distilled water and then by H_2SO_4 of requisite volume and were analysed for total N by Kjeldahl method as described earlier.

3.1.2.9 Assessment of N_2-fixation by composite algal culture in N-free medium :

A loopful of the mixed cultures from each soil was inoculated in 100 ml conical flask containing 50 ml sterilized N -free medium, each being replicated four times. Similarly there was an inoculated sterilised series. All the flasks were then incubated under day light fluorescent tubes in the culture room with intermittent shaking, for a period of 42 days after which the contents were analysed for total-N by semi-micro Kjeldahl method as described by Jackson (1967). From the difference of the results in the inoculated and'inoculated - sterilized' series the magnitude of N_2-fixed by algal cultures was calculated.

3.1.2.10 Assessment of C_2H_2 reduction activity and N_2-fixation by unialgal cultures in N-free medium.

One ml of the unialgal culture from exponential growth phase was transferred by means of fine pipette into 100 ml conical flasks containing 50 ml of sterilized N -free medium. This was replicated four times for each of the culture used. There was a similar sterilised series which served as control. The flasks were incubated for a period of three weeks under day light fluorescent tubes in the culture room, after which the C_2H_2reduction (N_2ase) activity and total N contents of the cultures were assessed by the methods as described below :

a) C_2H_2 reduction (N_2-ase) activity of the cultures :

The culture flasks were sealed with rubber septa. The air inside the flasks was evacuated by vacuum and flushed with argon, 10 percent of the gas phase was replaced by acetylene and incubated for 3 hours at 25 $\pm$ 1°C. The amount of C_2H_2 reduced in the flasks were measured by withdrawing 0.5 ml gas samples through septum into gas tight glass syringes and injecting them onto a Hewlet Packard Gas Chromatograph fitted with a flame ionization detector and glass column (6 ft) packed with porapak R. The oven temperature and carrier gas (N_2) flow rate were 80°C and 60 ml mt^{-1} respectively. The peak height of sample flasks was checked against control series. The results were calculated against standard and expressed as nM C_2H_4 50 ml^{-1} culture h^{-1}.

b) N_2-fixation in the culture :

Immediately after the C_2H_2 reduction assay, 5 ml conc. H_2SO_4 was added to each of the culture flasks and total-N content in the cultures was determined by the conventional semi-micro Kjeldahl method.

3.2 Influence of carbofuran on the growth and nitrogen accretion by blue-green alga, *Aulosira fertilissima*.

Two experiments were conducted under this section, one in culture medium and the other in two rice soils.

3.2.1 Experiment on the influence of carbofuran on the growth and accumulation of intra-and extra cellular nitrogen in culture medium inoculated with *Aulosira fertilissima*.

3.2.1.1 Materials :

a) Inoculum - Pure cultures of *Aulosira fertilissima*, was obtained from Dr. P.K.Singh, Division of Soil Science and Microbiology, Central Rice Research Institute, Cuttack. The alga was grown in combined N-free modified Chu-10 medium (Safferman and Morris, 1964) with trace elements (Allen and Arnon, 1955) in the culture room. To prepare inocula, late exponential phase cultures were centrifuged at 8000 rpm for 15 minute, washed twice with sterile distilled water. The cells were resuspended in water to obtain an approximate optical density 0.065 and was used for inoculation.

b) Pesticide - The commercial grade of granular insecticide, carbofuran (2,3-dihydro-2, 2-dimethyl-7-

benzofuranyl-N-methyl carbamate), containing 3 percent active ingredient was used.

3.2.1.2 Methods :

a) Experimental set up in culture medium - Fifty ml. portions of medium containing 0, 10, 25, 100 and 200 μg/ml of carbofuran were distributed into 100 ml conical flasks which were inoculated with 1 ml of the inoculum. The treatments were replicated twelve times, four for the analysis of each item. The flasks were incubated at a temperature of 25 ± 2°C with an approximate illumination of 1500 lux provided by fluorescent tubes, in the culture room. The contents were shaken inter-mittently by hand. After 45 days of incubation, the cultures were centrifuged for 15 minutes at 8000 rpm and the cells of alga were finally separated from the medium through filtration. the alga was washed once with sterile distilled water and the washing were added to the cell-free medium.

b) Chlorophyll content of the algal cells - Chlorophyll contents of the algal pallets, obtained by centrifugation of the cultures, were extracted in 10.0 ml of 80 percent of acetone. The optical density of pigments was measured colorimetrically at 655 nm.

c) Dry weight of the algal cells - The dry weight of the algal cells was taken after oven drying at 80°C for 48 h.

d) Estimation of intra-and extra cellular - N : The N content in the algal cells (intra-cellular-N) and in the medium (extra-cellular-N) were estimated separately by conventional micro-Kjeldahl method.

3.2.2 Experiment to study the effect of carbofuran on nitrogen fixation and the contents of inorganic, hydrolysable and non-hydrolysable-N in rice soils inoculated with Aulosira fertilissima.

3.2.2.1 Materials :

Two soil samples used in this experiment were collected from traditional rice fields in the alluvial and laterite tract of West Bengal. They were airdried, powdered and passed through 2 mm sieve and used for experimental purposes. The inoculam and the insecticide was same as described earlier.

3.2.2.2 Methods :

a) Hydrolysable and non-hydrolysable-N content of the soil - Hydrolysable nitrogen of the soil was determined by hydrolysing the soil (after extraction of

$NH_4^+ + NO_3^- + NO_2^- - N$) with 6(N) HCl (1 : 5) and distillating the hydrolysate as described by Bremner (1965). Non-hydrolysable nitrogen was determined by digesting the residual soil with conc. H_2SO_4 and distillating the digest as described by Bremner (1965). The other physico-chemical properties were determined following the methods described earlier.

b) Experimental procedure : - Ten gram portion of soil samples were taken in 50 ml conical flasks, to which P at the uniform basal dose of 30 ppm as KH_2PO_4 and carbofuran at the rate of 0, 25, 100 and 200 μg per gram of soil were added. They were inoculated with 1 ml of inoculum and waterlogged with 20 ml distilled water. Each of the treatments were replicated eight times. The flasks were then incubated for 42 days under open sunlight with proper measures for their protection from dust and rain. Simultaneously, a control series which received the same amount of sterilized inoculum, was also maintained covering the flasks with glazed black paper to avoid the growth of blue-green algae. After the incubation period was over, the soil samples in the flasks were analysed for- (i) total N following Kjeldahl method, (ii) $NH_4^+ + NO_3^- + NO_2^- - N$ by extracting the soil with 2 N KCl and distilling the extract with MgO and Devarda's alloy as described earlier: (iii) total hydrolysable-N by hydrolysing the soil (after extraction of NH_4^+ + NO_3^- - N) with 6N HCl (1:5) and distilling the hydrolysate as described by Bremner (1965) and (iv) Non-hydrolysable-N by digesting the residual soil with concentrated H_2SO_4 and distilling the digest as described by Bremner (1965). The changes in the contents of all the forms of nitrogen in soil due to inoculation was calculated by taking the difference in the contents in the light and dark series.

3.3 Effect of inoculation with N_2-fixing blue green algae on the nitrogenase activity (C_2H_2 reduction) in soil, on root surface and the yield of rice.

3.3.1 Materials :

3.3.1.1 Inoculum :

Four species of blue-green algae viz. Aulosira fertilissima, Nostoc muscorum, Nostoc sp. and Anabaena sp. isolated from neutral low land rice fields of West Bengal and having N_2-fixing capacities of 8.87, 6.48, 6.73 and 4.06 mg 100 ml^{-1} culture media, respectively, were used as inoculum. All these species were cultured separately in modified N-free Chu-10 media under fluorescent day light tube. At late exponential growth stage, the cells were separated from liquid media through filtration and repeatedly washed with sterile distilled water. The cultures thus collected on filter papers were then suspended and mixed in distilled water in a culture flask. The contents in culture flasks were then rotated reciprocally to make it homogeneous. Further, dilution was done to make it contain approximately 0.15 $\pm$ 0.02 g (dry weight) algal cells 100 ml^{-1}. This was used as soil inoculant.

3.3.1.2 Soil - Soil used in this experiment was collected from a traditional rice field situated under the extension project area of the Viswavidyalaya. Surface soil (0-22 cm depth) was collected following the usual procedure.

3.3.1.3 Variety of rice :

The rice variety IR-50 was used in this experiment.

3.3.2 Methods :

3.3.2.1 Physico-chemical analysis of the soil :

The physico-chemical properties of the soil were determined following the methods described earlier with the exception that available-N content was determined by modified alkaline permanganate method as described by Sahrawat and Burford (1982).

3.3.2.2 Treatments :

The treatments used were -

1. Control (N_0P_0)
2. N at the level of 60 kg ha^{-1} ($N_{60}P_0$)
3. P at the level of 40 kg ha^{-1} (N_0P_{40})
4. Combination of treatment (2) and (3) ($N_{60}P_{40}$)

Each treatment was divided into two series, viz.(a) Inoculated (b) Uninoculated. Each pot of the inoculated series received 25 ml of live algal suspension whereas the uninoculated ones received equal volume of sterilized algal suspension. There were eight possible treatment combinations, each being replicated four times for a single assay period.

3.3.2.3 Experimental set-up :

The soil sample was air dried, mixed thoroughly and sieved through a 20-mesh screen. 8.5 kg of soil was then placed in a number of porcelin pots, (having a height of 25.48 cm and diameter of 21.32 cm). The soil occupied an average depth of 18.84 cm in pots. All pots were kept flooded with deionized water. Nitrogen in the form of urea (analytical grade) and P as KH_2PO_4 were added in the form of solutions.

The entire amount of P and half the amount of N was applied as basal and the remaining half of N was applied in two equal splits, one at tillering stage (TS) and the other at panicle initiation stage (PIS) of the crop. An uniform basal dose of potassium, in the form of muriate of potash was applied to each pot at the rate of 30 kg ha^{-1}. Transplanting was done with two 'three weeks old seedlings' of rice in each pot and was followed by inoculation after 7 days. Uninoculated pots as mentioned before received sterilized inoculating material. The pots were then arranged randomly in the green house, and the level of water was maintained at 3.0 $\pm$ 0.5 cm with deionized water.

3.3.2.4 Assay of Nitrogenase (C_2H_2- reduction) activity :

a) N_2-ase (C_2H_2 - reduction) activity in soil - water system -

Soil core along with water was collected from pots first by pushing open ended glass - tubes (12.5 cm in length and 1.8 cm in inner diameter) into the soil to a depth of 6 cm and then cutting the bottom by sharpened sterilised stainless steel spatula. Immediately after collection of sample the bottom and open end of the tube was sealed with rubber septa and suba-seal respectively. Altogether 4 samples were collected at an interval of 30 days after transplanting, which was corresponding to tillering (TLS), maximum tillering (MTLS), panicle initiation (PIS) and harvesting stage (HS).

Each of the tubes was injected with acetylene gas to make the atmosphere saturated with approximately 10% acetylene and incubated for 24 hours at 20 $\pm$ 1°C under fluorescent day light. At the end of the incubation period the gas samples were analysed for ethylene by flame ionization gas chromatography as described earlier. The results were calculated against standard and expressed as n moles C_2H_4 sq.cm^{-1} soil day^{-1}.

b) N_2-ase (C_2H_2-reduction) activity associated with rice roots - Following the analysis of N_2-ase activity in soil-water system, the plants from the pots were carefully uprooted. The soil adhering to roots were washed with running tap water followed by sterilized distilled water and the excess water was carefully blotted out. About 2 cm long segments of roots from top, middle and end portions were taken for analysis. Two grams of such root segments were transferred into 50 ml capacity glass tubes and then sealed with rubber septa. The volume occupied by the roots in assay tubes was measured and the contents were incubated in approximately 10 per cent acetylene atmosphere for 24 hours in the incubator. Each of the samples was replicated thrice. The N_2-ase activity of excised roots was measured gas chromatographically as described. The dry weight of root samples in each tube and the total volume of roots obtained from each pots were taken. The results were calculated on the dry weight basis of the root samples in each assay tube and expressed as mole C_2H_2 g^{-1} dry root day^{-1}. Any C_2H_2 response by the similar amounts of roots without C_2H_2 atmosphere were always deducted from the results.

c) Grain and straw - The crop was harvested at maturity and the yield of grain and straw from each of the pots was taken.

3.4 Effect of inoculation of blue-green algae (Cyanobacteria) on the nitrogen status in soil and rice productivity under sub-merged condition.

Under this section, two field experiments were conducted at the District Seed Farm, Kalyani, under the Viswavidyalaya, during kharif season, 1984 and 1985, with very closely related objectives.

3.4.1 Field experiment on the effect of algal inoculation on the inorganic nitrogen (NH_4^+ + NO_2^- + NO_3^- - N) contents in soil and yield of rice in presence of P and N application.

3.4.1.1 Materials :

a) Inoculum - A soil based mixed culture of *Aulosira fertilissima*, *Nostoc commune* and *Nostoc muscorum* was prepared as described by Roger and Kulasooriya (1980) in shallow trays (6' x 3' x 9") of galvanized iron sheet using the soils of the experimental field. The dry algal flakes were used as inoculum.

b) Seedlings - Three weeks old rice seedlings (variety IR-36) were used for transplantation.

3.4.1.2 Methods :

a) Properties of soil of the experimental site - The physico-chemical properties of the soil were determined using methods described earlier.

b) Treatment combinations - There were eight treatment combinations as follows :

1. N_0P_0 ; 2. N_0P_0 + BGA ; 3. N_0P_{40} ; 4. N_0P_{40} + BGA
5. $N_{40}P_0$; 6. $N_{40}P_0$ + BGA; 7. $N_{40}P_{40}$; 8. $N_{40}P_{40}$+ BGA

c) Experimental layout - The experiment was conducted following a randomised block design with 5 x 5m plot size. In all the plots potassium at the rate of 30 kg K_2O ha^{-1} in the form of muriate of potash was applied as basal. Nitrogen (40 kg ha^{-1}) in the form of urea was applied in two equal splits, one at the time of transplanting and the other as top dressing at maximum tillering stage of the crop. Phosphorus (40 kg P_2O_5 ha^{-1}) in the form of single super phosphate was applied as basal. The plots were puddled and transplanted with seedlings in rows with the spacing of 20 cm x 10 between the rows and between the hills. There were three replications of each treatment. Inoculation was made by applying algal flakes as top dressing in the respective plots at the rate of 10 kg ha^{-1} after seven days of transplanting. Water levels on the soil surface was maintained approximately at 3 $\pm$ 1 cm upto the grain formation period of the crop.

d) Soil samples - From the date of inoculation, three representative soil samples were taken from each of the plots at monthly intervals and also one after harvest with the help of a soil augur specially fabricated by CRRI, Cuttack for collection of wet undisturbed sample, from a depth of 0-15 cm and were analysed for total inorganic (NH_4^+ + NO_2^- + NO_3^-) nitrogen content excepting the soil sample collected after crop harvest which was analysed for total nitrogen.

e) Grain and straw - The crop was harvested at maturity and dry weights of grain and straw were recorded and their samples were analysed for total N following the method as described by Jackson (1967).

3.4.2 Field experiment on the effect of inoculation of blue-green algae (Cyanobacteria) on the changes of different forms of nitrogen in soil, yield and N uptake by rice in presence of chemical and organic fertilizers.

3.4.2.1 Materials :

Inoculum, physico-chemical properties of soils of experimental site, plot size and rice variety etc. were same as in the previous experiment. The organic inputs used were -

i) Dhaincha twigs (Sesbania aculata L. 21 days old, 3.58% N)

ii) Neemcake (3.09% N)

iii) FYM (0.61% N)

3.4.2.2 Methods :

a) Treatment combinations :- There were ten treatment combinations as follows :

i) No fertilizer + No inoculation (No)

ii) No fertilizer + Inoculation (No + BGA)

iii) 30 kg N ha^{-1} as urea + No inoculation (N_{30} urea)

iv) 30 kg N ha^{-1} as urea + Inoculation (N_{30} urea + BGA)

v) 30 kg N ha^{-1} as Dhaincha + No inoculation (N_{30} Dhaincha)

vi) 30 kg N ha^{-1} as Dhaincha + Inoculation (N_{30} Dhaincha + BGA

vii) 30 kg N ha^{-1} as Neemcake + No inoculation (N_{30} Neemcake)

viii) 30 kg N ha^{-1} as Neemcake + Inoculation (N_{30} Neemcake + BGA)

ix) 30 kg N ha^{-1} as FYM + No inoculation (N_{30} FYM)

x) 30 kg N ha^{-1} as FYM + Inoculation (N_{30} FYM + BGA).

b) Experimental layout - The experiment was laid out following the randomised block design with each treatment being replicated three times. In all the plots both P and K at the rate of 30 kg P_2O_5 and K_2O ha^{-1} as single superphosphate and muriate of potash respectively were applied as basal. The amounts required for (i) Dhaincha (Sesbania aculata L.) twigs (ii) Neemcake and (iii) FYM, in each plot were calculated on the basis of their N contents; all of them were applied and incorporated into soil seven days before transplantation of seedlings. Half of the urea-N was applied at the time of transplanting and the remaining half as top dressing at maximum tillering stage of the crop. All other experimental details were the same as in the previous experiment.

c) Soil sample - Representative soil samples were collected from each plot at 30, 60 and 90 days after inoculation and analysed for Inorganic (NH_4^+ + NO_2^- + NO_3^-) - nitrogen. The first two samples were further analysed for (i) available (alkaline $KMnO_4$) - N; (ii) Hydrolyzable (HL-N)-N and (iii) non-hydrolysable (NHL-N)-N following the methods as described earlier.

d) Soil solution - Soil solutions from each plot were collected at 30 and 60 days after the inoculation with the help of peizometers using a suction pump and analysed for inorganic (NH_4^+ + NO_2^- + NO_3^-)-N following the method used for estimation of such content in soil sample.

e) Grain and straw - Grain and straw yields were recorded from each plot and their samples were analysed for total-N.

CHAPTER NO. 4

RESULTS AND DISCUSSION

4.0 RESULTS AND DISCUSSIONS

4.1 Distribution of nitrogen fixing blue-green algae (Cyanobacte

4.1.1 Physico-chemical properties of the soils :

The distribution of nitrogen-fixing blue-green algae and their nitrogen-fixing ability were studied in respect of 32 soils collected from the rice fields in different blocks in the districts of Coochbehar and Jalpaiguri in North Bengal. The physico-chemical properties (Table-1) of the soils show that the soils of both the districts are generally acidic in reaction low in clay content, medium in organic carbon, total nitrogen an available phosphorus. The soils are of low cation exchange capacity, the calcium plus magnesium saturation being hardly 50% of the exchange capacity.

4.1.2 Most probable number of blue-green algae :

The results of most probable number of blue-green algae (Table-2) show that the soils of Coochbehar district in general harboured a larger population of blue-green algae than those of the district of Jalpaiguri, the number varying from 490 to 54,000 and 700-54,000 with mean values of 15011 and 8527 g^{-1} soil respectively. The soils of the former district are slightly less acidic than those of the latter, the pH varying from 5.0 to 6.8 and 4.3 to 6.1 with mean values of 5.77 and 5.33

Table-1 : Some important physico-chemical characteristics of rice field soils.

Location Block	Place	pH	E.C. dS m^{-1}	Organic carbon (%)	Total N (%)	Inorganic Nitrogen NH_4-N (ppm)	Inorganic Nitrogen NO_3-N (ppm)	Available P (ppm)	C.E.C. C.mol(P^+) kg^{-1}	Exchangeable Ca^{++} C.mol(P^+) kg^{-1}	Exchangeable Mg^{++} C.mol (P^+) kg^{-1}	Clay (%)
COOCHBEHAR :												
Dinhata-I												
	Putimari :	5.5	0.061	0.480	0.065	26.04	12.15	7.5	8.211	4.216	1.258	14.0
	Baranachina	5.8	0.041	0.375	0.051	32.98	5.21	2.5	16.619	3.552	1.824	12.0
	Dinhata	5.1	0.061	0.473	0.067	24.30	8.68	24.0	11.030	4.704	2.016	15.9
Dinhata-II												
	Borosakdal	6.4	0.224	1.193	0.119	22.57	5.21	4.6	7.191	3.632	1.465	14.4
	Sahebganj	5.6	0.153	1.020	0.110	24.30	3.47	14.6	18.920	8.890	0.890	16.6
	Khambanj	6.1	0.418	1.234	0.115	24.30	46.87	18.6	16.730	7.369	2.128	18.8
Coochbehar-II												
	Chakchaka	6.7	0.357	0.953	0.093	15.62	43.40	12.0	10.478	5.402	1.442	14.0
	Khagrabari	5.6	0.173	0.968	0.088	26.04	36.46	14.5	17.299	4.458	1.666	18.4
	Guriahati	6.5	0.489	1.388	0.119	19.10	27.78	5.0	15.584	8.256	2.542	16.8
Mathabhanga-I												
	Prachim - Katerbari	5.3	0.112	1.163	0.093	27.78	22.57	19.0	11.161	2.976	1.824	14.0
	Baradola	5.5	0.122	1.013	0.090	38.19	5.21	13.5	19.478	3.84	1.632	13.3
	Dungkopa	5.7	0.133	1.088	0.102	20.83	19.10	11.0	17.059	4.708	1.080	11.2
Mathabhanga-II												
	Bhubaneswara	5.8	0.112	1.335	0.090	34.72	20.83	12.5	10.33	5.194	1.372	14.2
	Subdumniguri	6.8	0.418	0.945	0.074	15.62	17.36	8.5	10.81	5.782	2.646	9.6
Toofangang												
	Vandigeleus	5.4	0.082	1.380	0.134	29.51	19.10	7.5	11.801	4.899	2.263	13.4
	Barokodali	5.3	0.092	1.065	0.113	22.57	10.42	13.5	9.950	2.94	1.274	15.0
	Thinapurni	5.0	0.184	0.720	0.096	27.78	22.57	6.5	10.730	4.606	1.274	16.0
	Range	5.0-6.8	0.041-0.489	0.375-1.388	0.051-0.134	15.62-38.19	3.47-46.87	2.5-24.0	7.191-19.478	2.94-8.89	0.89-2.646	9.6-18.8
	Mean	5.77	0.190	0.988	0.095	25.43	19.20	11.49	13.14	5.025	1.682	14.57

Contd.....

Location Block	Location Place	pH	E.C. $dS\ m^{-1}$	Organic carbon (%)	Total N (%)	Inorganic Nitrogen NH_4- N (ppm)	Inorganic Nitrogen NO_3- N (ppm)	Available P (ppm)	C.E.C. C.mol (P^+) kg^{-1}	Exchangeable Ca^{++} C.mol (P^+) kg^{-1}	Exchangeable Mg^{++} C.mol(P^+) kg^{-1}	Clay
JALPAIGURI :												
Alipurduar-I												
	Birpara	6.0	0.178	1.013	0.130	39.93	22.57	12.5	15.249	4.128	1.920	11.6
	Pararpar	4.3	0.214	1.687	0.163	38.19	34.72	8.0	16.681	3.822	1.372	11.7
	Pakurtala	5.3	0.102	1.028	0.089	27.78	17.36	18.5	15.969	2.592	1.728	16.1
Alipurduar-II												
	Karghipara	5.9	0.112	0.450	0.069	19.10	15.62	13.5	15.161	3.920	1.800	14.0
	Chandir-jhar	4.9	0.082	0.833	0.096	27.78	24.30	17.0	12.040	3.628	1.175	16.6
	Chaparer-par	5.2	0.102	0.623	0.088	26.89	14.34	4.5	11.900	3.168	1.248	14.4
Dhupguri												
	Dhakshin-Altagram	5.3	0.082	1.508	0.165	29.51	10.42	8.0	15.913	4.410	0.686	11.6
	Purba-Altagram	5.5	0.112	1.568	0.146	26.04	32.98	11.5	10.19	5.376	2.208	11.2
	Dhupguri	5.2	0.204	0.930	0.086	22.57	12.15	19.5	10.391	3.570	1.248	13.0
Moynaguri												
	Tarirbari	5.0	0.102	0.720	0.084	19.10	6.94	23.0	16.748	4.214	1.574	21.6
	Khagrabari	6.1	0.122	0.848	0.099	15.62	3.47	29.0	13.776	8.160	3.264	11.2
	Dhakshin-khagra-bari	5.3	0.102	0.945	0.110	20.83	15.62	6.0	10.74	4.512	1.440	17.0
Malbazar												
	Metali	5.1	0.153	1.035	0.116	38.19	10.42	4.5	16.811	3.360	2.016	15.5
	Tisimla	5.6	0.184	1.133	0.109	26.04	29.51	6.5	15.248	5.088	1.824	19.2
	Malbazar	5.2	0.133	2.048	0.153	20.83	27.78	8.5	14.250	3.360	2.016	16.3
	Range -	4.3-6.1	0.082-0.214	0.450-2.048	0.069-0.165	15.62-39.93	3.47-34.72	4.5-29.0	10.19-16.811	2.592-8.160	0.686-3.264	11.2-21.6
	Mean	5.33	0.132	1.091	0.114	26.56	18.55	12.7	14.070	4.221	1.701	14.73

Table-2 : Most probable number (MPN) of blue-green algae in rice field soils.

Location: Block	Place	MPN of blue-green algae/1 g soil (dry wt. basis)
COOCHBEHAR :		
Dinhata-I :	Putimari	2400
	Baranachina	2200
	Dinhata	24000
Dinhata-II :	Borosakdal	11000
	Sahebganj	35000
	Knambanj	54000
Coochbehar-II :	Chakchaka	24000
	Khagrabari	4600
	Guriahati	35000
Mathabhanga-I :	Praschim Katerbari	17000
	Baradola	7900
	Dungkopa	3300
Mathabhanga-II	Bhubaneswara	13000
	Subdumniguri	17000
Toofanganj	Vandigelous	3500
	Barokodali	490
	Thinapurni	790
	Range :	490-54,000
	Mean :	15011

Contd.... Table-2.

Location: Block	Location: Place	MPN of blue-green algae/1 g soil (dry wt. basis)
JALPAIGURI :		
Alipurduar-I :	Birpara	11000
	Pararpar	54000
	Pakurtala	4900
Alipurduar-II :	Karghipara	17000
	Chandirjhar	1300
	Chaparerpar	1300
Dhupguri :	Dhakshin Altagram	13000
	Purba Altagram	7000
	Dhupguri	7900
Moynaguri :	Tarirbari	3500
	Khagrabari	2400
	Dhakshin Khagrabari	1300
Malbazar :	Metali	700
	Tisimla	1300
	Malbazar	1300
	Range :	700-54,000
	Mean	8527

respectively, which may be the reason for greater pre-ponderance of blue-green algae (which prefers neutral to slightly alkaline environment) in the former than in the latter group of soils. The highest population (54000 g^{-1} soil) was recorded in the Khambang soil in Dinhata-II block in the district of Coochbehar as well as in the pararpar soil in Alipurduar-I Block in the district of Jalpaiguri, whereas the lowest number (490 and 700) was observed in the Barokodali and Metali soils in the districts of Coochbehar and Jalpaiguri respectively. The highest number of blue-green algae in the Khambanj soil may be ascribed to its being nearly neutral in reaction (pH 6.1), fairly rich in organic matter (organic carbon 1.234%), NO_3- N (46.87 ppm), fairly good in available P (18.6 ppm) and exchangeable Ca^{2+}(7.37 C.mol (P^+) kg^{-1}), while the lowest population in the soils of Barokadali and Metali may similarly be attributed to their having comparatively lower values in respect of all the above physico-chemical properties. The pararpar soil inspite of being highly acidic (pH 4.3) also recorded highest population, which may possibly be due to its fairly high organic matter (Organic Carbon 1.687%) and NO_3-N (34.72 ppm). A good supply of readily assimilable source of nitrogen (NH_4^+ and NO_3^-) is likely to encourage the initial growth of algae. Sardeshpande and Goyal (1981) reported abundance of blue-green algae in some of the acid soils (pH 6.5) of Ratnagiri district in Maharastra, which were rich in organic matter. MacRae and Castro (1967) also observed that high soil organic matter counteracted to some extent the adverse effect of low soil pH on the population of blue-green algae. The high soil organic matter leads to the increased concentration of CO_2 and HCO_3^- in the soil-water environment due to its microbial decomposition and thus encourages the photosynthetic activity of blue-green algae and hence their growth.

4.1.3 Correlation with soil properties :

Attempt was made to find out if the most probable number of blue-green algae in soil has any significant correlation with the physico-chemical properties of the soil. It was observed that although this was positively correlated with soil pH (r = 0.1572) organic carbon (r = 0.2613), total N (r = 0.2360) available P (r = 0.1120), C.E.C. (r = 0.2477), clay content (r = 0.0162), none of these correlations was statistically significant. However, the most probable number of blue-green algae showed a significant correlation with electrical conductivity ($r = 0.5926^{**}$), NO_3^- - N ($r = 0.4162^{*}$) and exchangeable Ca^{2+} ($r = 0.4798^{**}$). Electrical conductivity is an index of the amount of soluble salts in soils. Some of the soluble salts are needed for the growth of algae. Similarly high calcium content is helpful for the growth of blue-green algae. These explain the significant correlation, as has been observed between the most probable number of blue-green algae and the electrical conductivity and exchangeable calcium contents of the soils.

4.1.4 Nitrogen fixation in soil culture :

The amount of nitrogen fixed (Table-3) by indigenously occurring blue-geeen algae in 5.0 g soils after 6 weeksperiod of incubation under submergence (soil : water = 1 : 2.5) show that it varied from 0.42 to 1.65 and from 0.19 to 1.75 with mean values of 1.19 and 1.15 mg in the soils of Coochbehar and Jalpaiguri districts respectively. These values are comparatively lower than the values (27.66 to 73.33 μg N g^{-1} $week^{-1}$) reported by Saha and Mandal (1979) in respect of neutral to slightly alkaline alluvial soils of West Bengal and (4.51 to 8.28 μg N g^{-1} soil day^{-1}) reported by Rinaudo et al, (1971) in Ivory coast paddy soils, as determined by acetylene reduction technique. The lower amount of nitrogen fixation in these soils may be ascribed to the acidic reaction, low available P and low exchangeable Ca^{2+} content of the soils. One interesting point observed is this that two soils (e.g. Khagrabari and Pakurtala) inspite of having almost similar pH (5.6 and 5.3), available P (14.5 to 18.5 ppm) and similar low mpn (4600, 4900) showed widely different amounts (1.18 and 0.19 mg) of nitrogen fixation by blue-green algae. This might be due to the

Table-3 : Algal N_2-fixation in rice field soils.

Location: Block	Location: Place	Amount of nitrogen fixed (mg N 6 wk^{-1} $5g^{-1}$ soil)
COOCHBEHAR :		
Dinhata-I :	Putimari	0.87 (± 0.08)
	Baranachina	0.42 (± 0.08)
	Dinhata	1.39 (± 0.76)
Dinhata-II :	Borosakdal	1.40 (± 0.15)
	Sahebganj	1.47 (± 0.45)
	Khambanj	0.87 (± 0.29)
Coochbehar-II :	Chakchaka	1.62 (± 0.08)
	Khagrabari	1.18 (± 0.11)
	Guriahati	1.49 (± 0.16)
Mathabhanga-I :	Paschimkaterbari	0.89 (± 0.31)
	Baradola	1.65 (± 0.78)
	Dungkopa	1.20 (± 0.21)
Mathabhanga-II :	Bhubaneswara	1.21 (± 0.08)
	Subdumniguri	0.99 (± 0.15)
Toofanganj :	Vandigelors	1.08 (± 0.13)
	Barokodali	1.26 (± 0.16)
	Thinapurni	1.27 (± 0.15)
	Range :	0.42 - 1.65
	Mean :	1.19

Contd..... Table-3.

Location Block	Place	Amount of nitrogen fixed (mg N 6 wk^{-1} 5 g^{-1} soil)
JALPAIGURI :		
Alipurduar-I :	Birpara	1.02 (± 0.18)
	Pararpar	0.76 (± 0.10)
	Pakurtala	0.19 (± 0.07)
Alipurduar-II:	Karghipara	0.97 (± 0.07)
	Chandirjhar	1.26 (± 0.16)
	Chaparerpar	1.02 (± 0.23)
Dhupguri :	Dhakshin Altagram	1.62 (± 0.18)
	Purba Altagram	1.41 (± 0.21)
	Dhupguri	1.03 (± 0.26)
Moynaguri :	Tarirbari	1.45 (± 0.60)
	Khagrabari	1.36 (± 0.19)
	Dhakshin Khagrabari	1.75 (± 0.18)
Malbazar :	Metali	1.05 (± 0.36)
	Tisimla	0.91 (± 0.10)
	Malbazar	1.45 (± 0.07)
	Range :	0.19 - 1.75
	Mean :	1.15

± indicate standard deviation of the mean.

variation in the composition of the blue-green algae occuring in the soils and their nitrogen fixing ability.

4.1.5 Correlation with soil physico-chemical properties :

The results (not presented) of simple correlation studies revealed that the amount of nitrogen fixed by indigenous blue-green algae in soil culture although showed positive or negative correlation with most of the physico-chemical properties of the soils, none of the correlations was statistically significant. It neither showed any significant correlation with the most probable number of blue-green algae in soils. This suggests that none of the physico-chemical properties of the soils, which have been determined to the present case, can alone significantly influence the nitrogen fixation. Similar was the observation of Saha and Mandal (1979). Henrikson et al, (1975) expressed the view that the difference of algal fixation of nitrogen in different soils could hardly be explained by the differential content of available nutrient in soils. Wilson and Alexander (1979), however, observed a significant correlation between nitrogen fixation by indigenous blue-green algae and pH, levels of extracable K, Ca and Mg in soils. The non-existence of any significant co-rrelation between the nitrogen fixation and the most probable number of blue-green algae suggests that the nitrogen fixation depends more on the nitrogen fixing ability of the different species present rather than on the total algal population.

4.1.6 Composition of blue-green algae :

The enrichment cultures of all the 32 soils from both the districts showed the growth of blue-green algae represented by 26 forms (Table-4A). The heterocysts bearing forms were represented by the genera Anabaena, Nostoc, Cylindrospermum, Aulosira, Scytonema, Calothrix, Microchaete, Rivularia, Gloeotrichia, Hapalosiphon, Stigonema, Camptylonema Mastigocladus and Westiellopsis, whereas the non-heterocystous filamentous forms belonged to the genera Oscillatoria, Lyngbya and Phormidium. Besides the heterocystous and non-heterocystous filamentous forms, abundant growth of unicellular blue-green algae was also observed. Out of the 14 heterocystous filamentous forms found, five namely Aulosira, Scytonema, Camptylonema, Mastigocladus and Westiellopsis were totally absent in the soils of Coochbehar district, where Anabaena, Nostoc and Cylindrospermum were of common occurrences. Sardeshpende and Goyal (1981) encountered majority of these filamentous forms excepting Aulosira, Microchaete, Rivularia, Gloeotrichia, Stigonema, Camptylonema and Mastigocladus in acid soils of Ratnagiri district of Maharastra. Aiyer (1965) reported presence of Aulosira and Calothrix in acid soils of Kerala.

Table-4A : Distribution of blue-green algae in rice field soils.

Location		Blue-green algae		Most dominant genus	
Block	Village	Filamentous	Unicellular	Filamentous	Unicellula
COOCHBEHAR :					
Dinhata-I					
	Putimari :	Phormidium Lyngbya Oscillatoria Very few filaments of Nostoc	Aphanothece Aphanocapsa Gloeocapsa Chroococcus	Phormidium	Aphanocapsa
	Baranachina :	Cylindrospermum	Aphanothece Aphanocapsa Microcystis Gloeocapsa Chroococcus	Cylindrospernum	Aphanothece Aphanocapsa
	Dinhata :	Anabaena Nostoc Microchaete Calothrix	Gloeocapsa Aphanocapsa	Anabaena	—
Dinhata-II					
	Borosakdal :	Rivularia Cylindrospermum Anabaena Gloeotrichia	Gloeocapsa Gloeothece Microcystis Aphanothece	Rivularia	—
	Sahebganj :	Anabaena Cylindrospermum	Microstis Gloeoopsa Chroococeus Synechocystis Chlorogloea	Anabaena	Microstis

Contd.... Table-4A.

Location			Blue-green algae		Most dominant genus	
Block	Village		Filamentous	Unicellular	Filamentous	Unicellular
Dinhata-II						
	Khambanj	:	Anabaena Nostoc Cylindrospermum	Aphanothece	Anabaena	—
Coochbehar-II						
	Chakchaka	:	Anabaena Nostoc Phormidium	Gloeocapsa Aphanothece Aphanocapsa	Anabaena	—
	Khagrabari	:	Nostoc Calothrix	Gloeocapsa Aphanocapsa Aphanothece Chroococcus	Nostoc	—
	Guriahati	:	Anabaena Cylindrospermum	Gloeocapsa Aphanocapsa Aphanothece Microcystis	Anabaena	Gloeocapsa
Mathabhanga-I						
	Praschim-katerbari	:	Nostoc Anabaena Gloeotrichia Cylindrospermum (few)	Aphanothece Gloeocapsa	Nostoc	Aphanothece
	Baradola	:	Nostoc(few)	Gloeocapsa Aphanotheae Aphanocapsa	Nostoc	Gloeocapsa Aphanocapsa Aphanothece

Contd.... Table-4A.

Location Block	Village	Blue-green algae Filamentous	Blue-green algae Unicellular	Most dominant genus Filamentous	Most dominant genus Unicellular
Mathabhanga-I					
	Dungkopa :	Nostoc Anabaena Cylindro-spermum	Apahnothece Gloeocapsa	Nostoc	—
Mathabhanga-II					
	Bhubaneswara:	Haphaloshi-pon Stigonema Cylindro-spermum	Aphanothece	Haphalosi-phon	Aphanothece
	Subdumni-guri :	Oscillatoria Anabaena Nostoc Haphalosiphon	—	Oscillatoria	—
	Vandige-lous :	Anabaena Oscillatoria Nostoc	Aphanothece Aphanocapsa	Anabaena	—
Toofanganj					
	Barokodali :	Anabaena Nostoc	—	Anabaena	—
	Thinapurni :	Nostoc Oscillatoria	Gloeocapsa	Nostoc	—

Contd.... Table-4A.

Location: Block	Village		Blue-green algae: Filamentous	Unicellular	Most dominant genus: Filamentous	Unicellular
JALPAIGURI :						
Alipurduer-I						
	Birpara	:	Nostoc Anabaena Cylindro-spermum	Aphanothece	Nostoc	Aphanothece
	Pararpar	:	Haphalosiphon Calothrix Microchaete Gloeotrichia	Aphanothece	Haphalosi-phon	—
	Pakurtala	:	Scytonema Anabaena Calothrix (very few) Microchaete	Glocoeapsa Aphanocapsa Chroococcus Microcystis	Scytonema	—
Alipurduar-II						
	Karghipara	:	Anabaena Nostoc Camptylonema	Chroococcus	Anabaena	—
	Chandir-jhar	:	Haphalosi-phon Anabaena Scytonema Westeillop-sis Mastigocla-dus Gloeotrichia (few) Oscillatoria	Gloeocapsa Aphanothece Aphanocapsa Chroococcus	Haphalosi-phon	—

Contd.... Table-4A :

Location		Blue-green algae		Most dominant genus	
Block	Village	Filamentous	Unicellular	Filamentous	Unicellular
Alipurduar-II					
	Chaparerpar :	Oscillatoria Anabaena	Aphanothece Aphanocapsa Chroococcus Microcystis Dermocarpa	Oscillatoria	Aphanothece
Dhupguri					
	Dhakshin Altagram :	Anabaena Nostoc Haphalosiphon Lyngbya Microchaete Gloeotrichia Oscillatoria	Aphanothece Chroococeus	Anabaena	—
	Purba-Altagram :	Stigonema Camptylonema Anabaena Gloeotrichia (few)	Aphanothece Aphanocapsa Chroococeus	Stigonema	—
	Dhupguri :	Anabaena Nostoc Cylindrospermum Oscillatoria	Chroococcus	Anabaena	—

Contd.... Table-4A :

Location: Block	Village	Blue-green algae: Filamentous	Blue-green algae: Unicellular	Most dominant genus: Filamentous	Most dominant genus: Unicellular
Moynaguri :					
	Tarirbari :	Anabaena Nostoc Scytonema (few)	Aphanothece Aphanocapsa	Anabaena	—
	Khagrabari :	Anabaena Nostoc Cylindro-spermum	Aphanothece Aphanocapsa Gloeocapsa Chroococcus	Anabaena	—
	Dhakshin-Khagrabari :	Anabaena Nostoc Aulosira Cylindro-spermum Microchaete (few)	Aphanothece Aphanocapsa Chroococcus Microcystis	Anabaena Nostoc Aulosira Cylindro-spermum	—
Malbazar :					
	Metali :	Anabaena Westiello-psis Microchaete Haphalosi-phon Scytonema Oscilla-toria	Aphanothece Chroococcus	—	Aphanothece
	Tisimla :	Anabaena Haphalosi-phon	—	—	—
	Malbazar :	Anabaena Nostoc	Glococapsa	Anabaena	—

4.1.7 .Percentage occurrence and dominance :

The results (Table-4B) as computed from those in Table-4A show that amongst the filamentous heterocyst bearing forms of blue-green algae, Anabaena is most widely distributed which is followed by Nostoc. They occurred in 78 and 62% and dominated in 44 and 19 per cent of the soils respectively. Although the distribution of Cylindrospermum, was more wide (37%) than that of Haphalo-siphon (22%), but the latter was found to be dominant in greater number of soils. The distribution of most of the other heterocystous forms of algae was found to be highly localised, occurring in only 1 or 2 out of the 32 soils examined. The filamentous non-heterocystous forms of blue-green algae were represented by the three genera namely Ostillartoria, Phormidium and Lyngbya of which the latter two occured only in 2 out of the 32 soils. Amongst the unicellular forms the genus Aphanothece was found to be most widely distributed followed by Gloeocapsa, Aphanocapsa and Chroococcus, occurring in 22, 16, 15 and 14 soils respectively out of the 32 soils examined. Occurrence of most of the other genera was highly localised, being found only in 1 out of the 32 soils. The results (Table-4B), therefore, clearly show that the filamentous heterocysts bearing forms of blue-green algae are more common than the non-heterocystous and unicellular forms in these soils. Since most of the species having nitrogen-fixing ability are known to belong to the

Table-4B : Percentage distribution of different genus of blue-green algae in rice soils.

Genus of blue-green algae.	Percentage occurrence	Percentage dominance
Filamentous heterocyst bearing.		
1. *Anabaena*	78.13	43.75
2. *Nostoc*	62.5	18.75
3. *Cylindrospermum*	37.5	6.25
4. *Haphalosiphon*	21.88	9.38
5. *Rivularia*	3.13	3.13
6. *Aulosira*	3.13	3.13
7. *Scytonema*	12.5	3.13
8. *Glocotrichia*	18.75	-
9. *Microchaete*	18.75	-
10. *Calothrix*	12.5	-
11. *Stigonema*	6.25	3.13
12. *Camptylonema*	6.25	-
13. *Westeiollopsis*	6.25	-
14. *Mastigocladus*	3.13	-
Filamentous non-heterocystous.		
1. *Oscillatoria*	28.13	6.25
2. *Phormidium*	6.25	3.13
3. *Lyngbya*	6.25	-

Contd.... Table-4B.

Genus of blue-green algae.		Percentage occurrence	Percentage dominance
<u>Unicellular</u>.			
1.	*Aphanothece*	68.75	25.00
2.	*Gloeocapsa*	50.00	6.25
3.	*Aphanocapsa*	46.88	9.38
4.	*Microcystis*	21.88	3.13
5.	*Chroococcus*	43.75	-
6.	*Gloeothece*	3.13	-
7.	*Chlorogloea*	3.13	-
8.	*Synechocystis*	3.13	-
9.	*Dermocarpa*	3.13	-

heterocysts bearing filamentous group the wider distribution of this form of blue-green algae in the soils of the districts of Coochbehar and Jalpaiguri is of practical significance from the point of view of recuperation of soil nitrogen in these soils particularly under rice growing condition. The composition of the blue-green algal population differed in different soils. This may explain the variation in the amount of nitrogen fixed in different soils with similar physico-chemical properties.

4.1.8 Nitrogen fixation by composite cultures of blue-green algae in N-free medium.

The results (Table-5) of fixation of nitrogen by composite cultures of blue-green algae from different soils show that the amount of nitrogen fixed on 50 ml of N-free media after 6 weeks of incubation varied from 0.64 $\pm$ 0.05 to 2.36 $\pm$ 0.18 mg and from 1.19 $\pm$ 0.12 to 2.34 $\pm$ 0.09 mg with mean values of 1.66 and 1.64 mg in the soils of Coochbehar and Jalpaiguri districts respectively.

The composite cultures obtained from the soils of Sahebganj, Chakchaka, Guriahati, Bhubaneswara, Karghipara of Coochbehar district and of Khagrabari and Dhakshin Khagrabari of Jalpaiguri district exhibited comparatively high amount of N_2-fixation. All the cultures except that from Bhubaneswara were dominated either by the species of genera Anabaena alone or Anabaena with unicellular species of Microcystis and

Table-5 : Nitrogen fixation by composite cultures of blue-green algae of different acidic rice soils in N-free media after 42 days of incubation in light

LOCATION Block	Place	Amount of N_2-fixed (mg N 50 ml^{-1} media)
COOCHBEHAR :		
Dinhata-I		
	Putimari	1.04 (± 0.09)
	Baranachina	0.64 (± 0.05)
	Dinhata	1.80 (± 0.04)
Dinhata-II		
	Borosakdal	1.59 (± 0.17)
	Sahebganj	2.35 (± 0.17)
	Khambanj	1.52 (± 0.16)
Coochbehar-II		
	Chakchaka	2.00 (± 0.09)
	Khagrabari	1.58 (± 0.04)
	Guriahati	2.09 (± 0.18)
Mathabhanga-I		
	Praschim Katerbari	1.89 (± 0.42)
	Baradola	1.76 (± 0.07)
	Dungkopa	1.23 (± 0.11)
Mathabhanga-II		
	Bhubaneswara	2.36 (± 0.18)
	Subdumniguri	1.57 (± 0.07)
Toofanganj :		
	Vandigelous	1.58 (± 0.14)
	Barokodali	1.60 (± 0.15)
	Thinapurni	1.62 (± 0.05)
	Range :	0.64 ± 0.05 - 2.36 ± 0.18
	Mean :	1.66

Contd.... Table-5 :

LOCATION Block	Place	Amount of N_2-fixed (mg N 50 ml^{-1} media)
JALPAIGURI :		
Alipurduar-I		
	Birpara	1.51 (± 0.06)
	Pararpar	1.19 (± 0.12)
	Pakurtala	1.62 (± 0.06)
Alipurduar-II		
	Karghipara	2.26 (± 0.15)
	Chandirjhar	1.48 (± 0.17)
	Chaparerpar	1.60 (± 0.22)
Dhupguri		
	Dhakshin Altagram	1.78 (± 0.05)
	Purba Altagram	1.22 (± 0.02)
	Dhupguri	1.85 (± 0.04)
Moynaguri		
	Tarirbari	1.35 (± 0.12)
	Khagrabari	2.34 (± 0.09)
	Dhakshin Khagrabari	1.97 (± 0.07)
Malbazar		
	Metali	1.39 (± 0.09)
	Tisimla	1.29 (± 0.24)
	Malbazar	1.71 (± 0.11)
	Range :	1.19 ± 0.12 - 2.34 ± 0.09
	Mean :	1.64

± = Standard deviation of the mean.

Gloeocapsa or *Anabaena* with other heterocysts bearing filamentous forms like *Nostoc*, *Aulosira* and *Cylindrospermum*. They also recorded a fairly good amount of N_2-fixation in soil culture. This may be due to the occurrence of species of the above mentioned genera of blue-green algae in those soils. The composite algal population in the soils of Dinhata, Baradola, Dhakshin Altagram, Malbazar, Borosakdal, Khagrabari, Dungkopa, Barokodali, Thinapurni, Chandirjhar, Purba Altagram and Tarirbari which were found to be dominated by *Anabaena*, *Gloeocapsa*, *Aphanothece* and *Aphanocapsa* with few *Nostoc*, *Rivularia* *Haphalosiphon*, and *Stigonema* although recorded comparatively high fixation in soil culture, but did not do so in liquid medium culture. This may possibly be due to the some beneficial factor which is likely to be present in the soil medium, but not in the liquid culture medium. This suggests that nitrogen fixation in liquid medium cannot correctly assess the N_2-fixing potentiality of blue-green algae in soils.

4.1.9 Nitrogenase (C_2H_2 reduction) activity of unialgal species.

The results (Table-6) of C_2H_2 reduction activity of twenty unialgal species of blue-green algae isolated from composite cultures show that six strains of *Anabaena* (viz. *Anabaena* sp-1, 2, 5,7, 8 and 11) four strains of *Cylindrospermum* (viz. *Cylindrospermum*-1, 2,3,4) and four strains of *Nostoc* (viz.*Nostoc*

Table-6 : Nitrogenase (C_2H_2 - reduction) activity (i
C_2H_4 hr^{-1} 50 ml^{-1} culture) and nitrogen fix
mg N 50 ml^{-1} culture) by different unialga
of blue-green algae isolated from differen
soils after 21 days of incubation in light

Species		C_2H_2- reduction activity	Nitrogen
1. Anabaena	sp-1	262.6 (± 37.5)	3.68 (± 0
2. Anabaena	sp-2	248.8 (± 17.8)	4.56 (± 0
3. Anabaena	sp-3	17.2 (± 4.6)	2.61 (± 0
4. Anabaena	sp-4	19.4 (± 5.3)	2.57 (± 0
5. Anabaena	sp-5	347.5 (± 52.2)	6.79 (± 0
6. Anabaena	sp-6	47.1 (± 9.7)	3.41 (± 0
7. Anabaena	sp-7	182.4 (± 69.3)	3.83 (± 0
8. Anabaena	sp-8	420.4 (± 82.8)	6.29 (± 0
9. Anabaena	sp-9	22.4 (± 1.8)	3.83 (± 0
10. Anabaena	sp-10	26.9 (± 22.5)	3.57 (± 0
11. Anabaena	sp-11	367.2 (± 12.8)	3.50 (± 0
12. Cylindro-spermum	sp-1	217.2 (± 29.6)	6.84 (± 0
13. Cylindro-spermum	sp-2	142.1 (± 25.9)	7.02 (± 0
14. Cylindro-spermum	sp-3	142.1 (± 25.9)	6.52 (± 0
15. Cylindro-spermum	sp-4	197.4 (± 24.4)	7.56 (± 0
16. Nostoc	sp-1	408.7 (± 21.1)	6.48 (± 0
17. Nostoc	sp-2	205.3 (± 20.1)	7.54 (± 0
18. Nostoc	sp-3	181.6 (± 14.8)	7.91 (± 1
19. Nostoc	sp-4	165.8 (± 23.7)	8.77 (± 0.
20. Nostoc	sp-5	27.6 (± 13.7)	2.71 (± 0.

± = Standard deviation of the mean.

FIG.-1 : Photomicrograph of <u>Anabaena</u> Sp. 7 isolated from the algal culture of Dungkopa soil of Coochbehar district (X 600).

FIG.-2 : Photomicrograph of <u>Nostoc</u> Sp. 1 isolated from the algal culture of Dhakshin Khagrabari soil of Jalpaiguri district (X 600) sp-1, 2, 3 and 4) possessed efficient N_2-fixing activity of which Anabaena sp-8 and Nostoc sp-1 were found to be the most efficient. Both the species had shown reduction activity higher than 400 nM C_2H_2 hour^{-1} 50 ml^{-1} of cultures at 21 days' growth stage. Five strains of Anabaena viz. Anabaena sp-3,4, 6, 9 and 10 and Nostoc sp-5 showed very low reduction activity, which varied from only about 17 to 47 nM C_2H_2 h^{-1} 50 ml^{-1} culture.

4.1.10 Nitrogen fixation by unialgal species in culture media;

Results (Table-6) show that two strains of Anabaena, (viz., Anabaena sp-5, and 8), all the four strains of Cylindrospermum and four strains of Nostoc. (viz. Nostoc sp-1, 2, 3 & 4) fixed considerably high amounts of nitrogen, Nostoc sp-4 being the most efficient one. All the above mentioned strains were also found to possess high C_2H_2 reduction activities but the amount of nitrogen fixed by the strains did not appear to have any correlation with their C_2H_2 reduction activity. This becomes evident from the observation that Anabaena sp-1, 2, 7 and 11 with fairly good C_2H_2 reduction activities recorded comparatively low fixation in the medium. This may be due to the variation of the growth rate of the strains. Shtina et al, (1968) observed a correlation between the amount of nitrogen fixed and the rate of growth of blue-green algae. The remaining four strains of Anabaena viz. Anabaena sp-3, 4, 6 and 9 and one of

Nostoc viz. *Nostoc* sp-5, which showed low C_2H_2 reduction activity also fixed comparatively very low amounts of nitrogen in medium.

4.1.11 Conclusion :

The results of this investigation, therefore, indicate that the slightly acidic rice fields soils of the districts of Coochbehar and Jalpaiguri in West Bengal can harbour the growth of nitrogen fixing blue-green algae and suggests the possibility of their algalization by isolating and mass culturing the acid tolerant forms.

4.2 Influence of carbofuran on the blue-green alga (*Cyanobacterium*) *Aulosira fertilissima* :

4.2.1 Growth in liquid culture media :

Carbofuran at the level of 10 and 25 μg ml^{-1} stimulated the growth of blue-green alga, *Aulosira fertilissima* as evidenced from the increase in optical density of the chlorophyll content as well as the dry weight of algal biomass (Table-7) over that in the control, the effect being most prominent at the 10 μg level. An adverse effect was, however, observed at the level of 100 μg and above per ml. Similar stimulatory effect of this insecticide (carbofuran) was also observed by Kar and Singh (1977) at 25 ppm level in culture medium on *Nostoc muscorum*. This suggests that the carbofuran itself at low concentration, or

Table-7 : Influence of carbofuran on the growth of *Aulosira fertilissima* in culture medium. (Incubation period 45 days)

Carbofuran ($\mu g. ml^{-1}$)	Optical density of chlorophyll 50 ml^{-1} culture	Dry wt of biomass (mg 50 ml^{-1} culture)
0	0.11 ± 0.008	14.50 ± 0.94
10	0.13 ± 0.016	18.00 ± 1.38
25	0.12 ± 0.016	16.00 ± 1.05
100	0.09 ± 0.006	9.50 ± 1.13
200	N.D.	4.94 ± 0.57
C.D. at 5%	0.023	2.04

± = Standard deviation of the mean of 4 replicates its hydrolysis products might have some direct or indirect beneficial effect on the growth of the blue-green alga, Aulosira fertilissima in liquid culture medium.

4.2.2 Nitrogen fixation in liquid culture medium :

Carbofuran at the level of 10 and 25 $\mu g\ ml^{-1}$ encouraged the nitrogen fixation by Aulorisa fertilissima (Table-8) as evidenced by the increase in the intracellular as well as the extracellular nitrogen content in the medium over their respective values under the control, the effect, however, being highest at the 10 μg level. Higher levels of carbofuran caused a decrease in the contents of both. Carbofuran at all levels caused a relatively higher proportion of total nitrogen fixed to be in the extracellular form.

4.2.3 Nitrogen fixation in soils inoculated with Aulosira fertilissima :

This was studied with one alluvial and one laterite rice field soils (Physico-chemical properties presented in Table-9) of West Bengal. In both the cases inoculation with the alga, Aulosira fertilissima caused considerable fixation of nitrogen, being higher in the alluvial (pH 7.5) than in the laterite (pH 6.5) soil (Table-10). Application of carbofuran did not show any effect on the amount of nitrogen fixed in the alluvial soil, whereas in the laterite soil such application caused an increase

Table-8 : Influence of carbofuran on accretion of intra-and extracellular nitrogen in culture medium inoculated with <u>Aulosira fertilissima</u>. (Incubation period 45 days, N in mg)

Carbofuran ($\mu g. ml^{-1}$)	Intracellular -N 50^{-1} ml culture	Extracellular-N 50 ml^{-1} culture	
		Actual	% Total
0	2.04 ± 0.13	0.36 ± 0.029	14.99 ± 0.24
10	2.59 ± 0.17	0.68 ± 0.065	20.78 ± 1.28
25	2.26 ± 0.18	0.46 ± 0.051	16.88 ± 0.63
100	1.56 ± 0.10	0.35 ± 0.031	18.30 ± 0.68
200	0.73 ± 0.05	0.25 ± 0.029	25.44 ± 1.00
C.D. at 5%	0.23	0.084	

± Standard deviation of the mean of 4 replicates.

Table-9 : Physico-chemical Characteristics of Soils

	Alluvial	Laterite
pH (1:2.5)	7.50	6.50
E.C. dS m^{-1}	0.51	0.48
Organic carbon (%)	1.04	0.80
C.E.C. [C mol (P+) kg^{-1}]	9.90	6.74
Total-N (%)	0.08	0.05
Hydrolysable-N (%)	0.05	0.04
Non-hydrolysable-N (%)	0.03	0.01
Available P (ppm)	10.50	3.00

Table-10 : Influences of carbofuran on N_2-fixation in soils inoculated with *Aulosira fertilissima* (N in mg/flask containing 10 g soil, incubation period 45 days).

Carbofuran $\mu g. g^{-1}$ soil	Alluvial Soil			Laterite Soil		
	Control	+ *Aulosira fertilissima*		Control	+ *Aulosira fertilissima*	
	N Content	N Content	Amount of N fixed	N Content	N Content	Amount of N fixed
0	8.02	9.90 (23.44)	1.88 ± 0.025	4.94	5.81 (17.61)	0.87 ± 0.130
25	7.81	9.70 (24.20)	1.89 ± 0.065	4.71	6.32 (34.18)	1.61 ± 0.200
100	7.71	9.52 (23.48)	1.81 ± 0.075	4.71	6.00 (27.39)	1.29 ± 0.230
200	7.51	9.32 (24.10)	1.81 ± 0.046	4.80	6.05 (26.04)	1.25 ± 0.38
C.D. at 5%			N.S			0.35

(Figures in parenthesis denotes the percentage increase over control)
± = Standard deviation of the mean of 4 replicates.

in the amount of nitrogen fixed, the effect being highest at 25 $\mu g\ g^{-1}$ soil level. The results further show that the application of carbofuran even at the level as high as 200 $\mu g\ g^{-1}$ of soil did not show any adverse effect on the nitrogen fixation in the alluvial soil while it continued to have beneficial effect in the laterite soil. The tolerance limit of blue-green alga, Aulosira fertilissima to carbofuran therefore, seem to be much higher in the soil than in the liquid culture medium. This is probably due to the rapid bio-degradation of carbofuran in the flooded soil environment to carbofuran phenol and its subsequent metabolism by soil microorganisms (Venkateswaralu and Sethunathan, 1978). Similar beneficial effect of carbofuran application upto 2 kg ha^{-1} in flooded rice-fields on the growth of particularly filamentous blue-green algae was observed by Megharaj et al, (1988).

4.2.4 Inorganic ($NH_4^+ + NO_3^- + NO_2^-$) - N content in soils inoculated with Aulosira fertilissima :

Results (Table-11) show that there was a marked increase in the contents of $NH_4^+ + NO_3^- + NO_2^-$ - N in both the alluvial and laterite soils due to inoculation, the magnitude of increase being about, 174 and 24 per cent respectively over the corresponding uninoculated controls. Application of carbofuran at all the concentrations upto 200 $\mu g\ g^{-1}$ soil in presence of inoculation, further increased significantly these contents over their no

Table-11 : Influence of carbofuran on the inorganic ($NH_4^+ + NO_3^- + NO_2^-$) - N content in soils inoculated with the blue-green alga *Aulosira fertilissima* (N in g. g^{-1} soil, Incubation period 60 days)

Carbofuran µg. g soil	Alluvial Soil			Laterite soil		
	Control	+ *Aulosira fertilissima*	Net amount increase of $NH_4 + NO_3$-N $+ NO_2$	Control	+ *Aulisira fertilissima*	Net amount increase of $NH_4 + NO_3$ - N $+NO_2$
	N Content g^{-1}	N Content g^{-1}		N Content g^{-1}	N Content g^{-1}	
0	8.30	22.76 (174.22)	14.46 ± 1.83	17.18	21.29 (23.92)	4.11 ± 0.78
25	11.79	33.24 (181.93)	21.45 ± 1.62	10.25	16.09 (56.98)	5.84 ± 0.81
100	12.06	33.69 (179.35)	21.63 ± 2.85	11.11	16.42 (47.79)	5.31 ± 0.51
200	13.17	33.09 (151.25)	19.92 ± 2.80	9.09	19.13 (110.45)	10.04 ± 2.08
C.D. at 5%			2.64			1.80

(Figures in Parenthesis denote the percentage increase over control)

± = Standard deviation of the mean of 4 replicates.

pesticide control. The results in Table-8 indicated that carbofuran caused a relatively higher proportion of fixed nitrogen to be released in the extracellular form. The higher amount of inorganic nitrogen in presence of carbofuran is possibly due to the rapid mineralisation of extra cellular nitrogenous compounds. However, the extent of increase was always much greater in the alluvial than in the laterite soil. This is due to the greater N_2-fixation by blue-green algae in the former than in the latter soil resulting in more release of fixed N as extracellular product and its subsequent mineralisation to the inorganic form.

4.2.5 Hydrolysable and non-hydrolysable N content in soil inoculated with *Aulosira fertilissima*:

The results (Table-12) show that the growth of blue-green alga resulted in an increase in the hydrolysable-N content while the non-hydrolysable **N** content remained more or less unchanged in both the soils. The increase in the hydrolysable N content was almost equal to the amount of increase in total nitrogen content in soils due to inoculation, which suggests that most of the products of N_2 fixation by blue-green algae are in hydrolysable forms. These products undergo mineralisation and the nitrogen is released gradually into available form as was evidenced by the increase in the contents of inorganic N in soil (Table-11). Application of carbofuran at the concentrations of

Table-12 : Influence of carbofuran on hydrolysable and non-hydrolysable-N contents in soils* inoculated with <u>Aulosira fertilissima</u> (N in mg/flask containing 10 g soil, Incubation period 60 days)

Carbofuran $\mu g. g^{-1}$ soil	Alluvial Soil		Laterite Soil	
	Net amount increase of Hydrolysable-N	Net amount increase of Non-hydrolysable-N	Net amount increase of Hydrolysable-N	Net amount increase of Non-hydrolysable-N
0	1.86 ± 0.11	-0.01	1.28 ± 0.14	-0.02
25	1.95 ± 0.07	+0.02	1.83 ± 0.11	-0.02
100	1.42 ± 0.11	±0.00	1.84 ± 0.09	-0.02
200	1.45 ± 0.05	-0.03	1.64 ± 0.08	+0.01
C.D. at 5%	0.15		0.22	

(± Results given after deduction from control series)

± = Standard deviation of the mean of 4 replicates.

100 and 200 $\mu g\ g^{-1}$ soil caused a significant decrease in the hydrolysable-N content in the alluvial soil, although it did not show much influence in the laterite soil. This might be due to the differential effect of carbofuran on the nitrogen fixation and the release of fixed nitrogen in the extracellular form in the two soils.

4.2.6 Conclusion :

The results, indicate that carbofuran addition profoundly influenced the growth and N_2-fixation by blue-green alga Aulosira fertilissima as well as the release of fixed N into available pool in soil.

4.3 Effect of inoculation with N_2-fixing blue-green algae (Cyanobacteria) on the nitrogenase activity (C_2H_2 - reduction) in soil, on root surface and the yield of rice.

4.3.1 Physico-chemical properties of the soil -

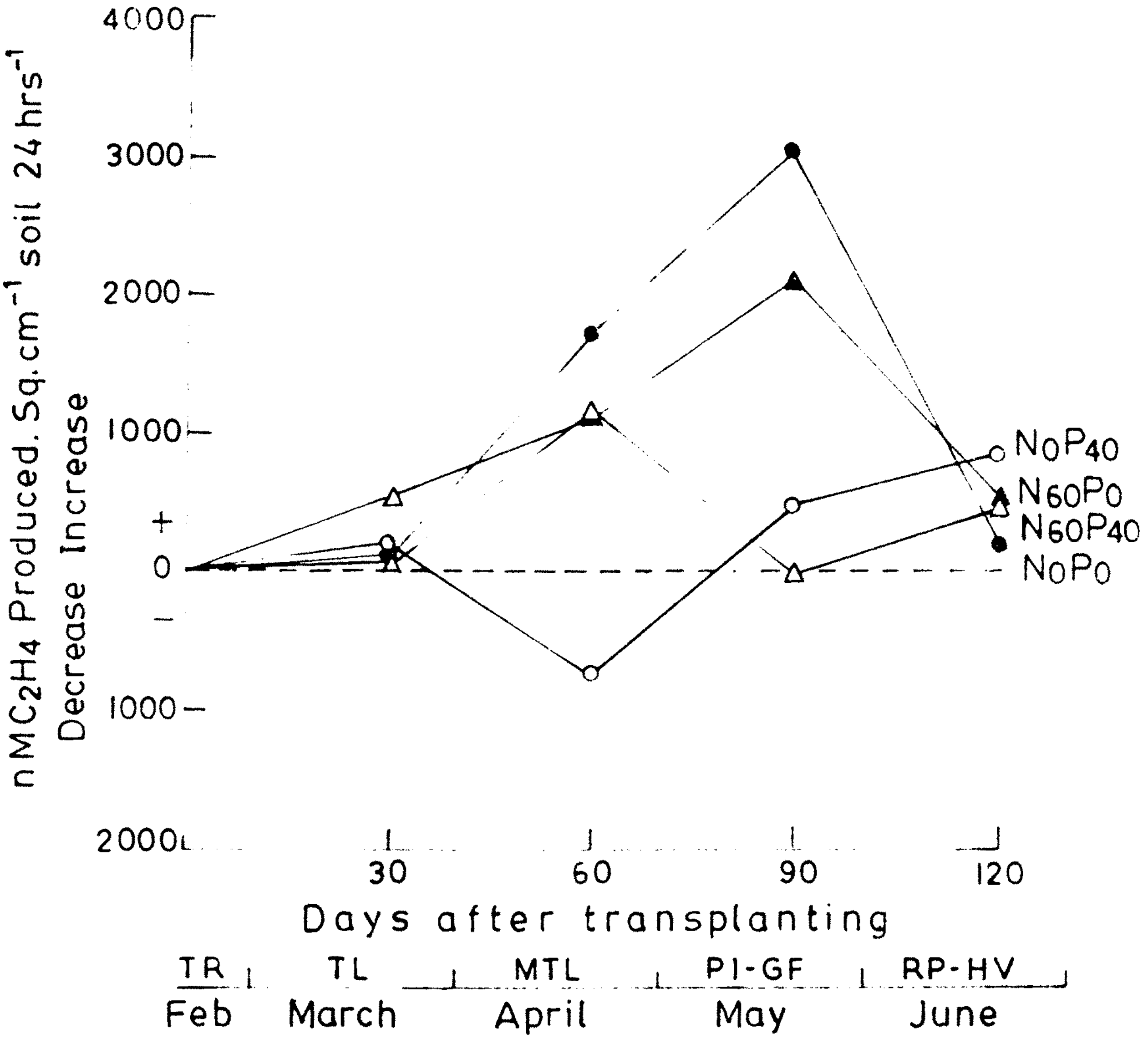
Physico-chemical properties show (Table-13) that the soil was slightly alkaline in reaction and low in available phosphorus. The soil was also rich in indigenous growth of blue-green algae (Cyanobacteria) dominated by Nostoc sp.

Table-13 : Physico-chemical properties of the soil used in pot experiment.

pH (1 : 2.5)	...	7.5
E.C.(dSm^{-1})	...	0.06
Organic carbon (%)	...	0.85
Total N (%)	...	0.095
Available N (ppm) ($KMnO_4$ oxidizable)	...	97.5
Available P (ppm)	...	2.66
C.E.C. [C.mol (P^+) kg^{-1}]	...	24.29
Exchangeable Ca^{++} [C.mol (P^+) kg^{-1}]	..	16.46
Exchangeable Mg^{++} [C.mol (P^+) kg^{-1}]	..	3.84
Mechanical composition :		
Clay (%)	...	38.9
Silt (%)	...	17.3
Fine sand (%)	...	33.2
Course sand (%)	...	8.0

4.3.2 N_2-ase activity in soil :

Results (Figure-4) indicate that the inoculation of blue-green algae (Cyanobacteria) generally resulted in a considerable increase in the N_2-ase activity in soil system during the period of rice growth. The extent of increase, however, varied significantly at different growth stages of the crop and with fertilization of the soil with P and N. Although inoculation increased the N_2-ase activity in soil at the early growth stage of rice plants the magnitude of increase was significantly higher at 60 days period (MTLS), and attained its' peak at 90 days after transplanting (GFS). The activity, however, gradually declined thereafter and reached to a very low value at harvesting stage (HVS) of the crop. This trend was observed in both the unfertilized and N-fertilised soil system, although the activity in the latter case was comparatively lower than that in the former at most of the stages of crop growth. The increase in the N_2-ase activity was obviously due to succession of inoculated blue-green algae (Cyanobacteria) over the uninoculated ones while the decline at the later part of plant growth may be attributed to the initiation of decomposition of old cyanobacterial cells. Apparently the increase in N_2-ase activity due to inoculation was much less in soil which received P alone or in combination with N. In some of the stages, this activity was even lower than the corresponding uninoculated one. This may be due to the encouragement of growth and activity of indigenous blue-green algae



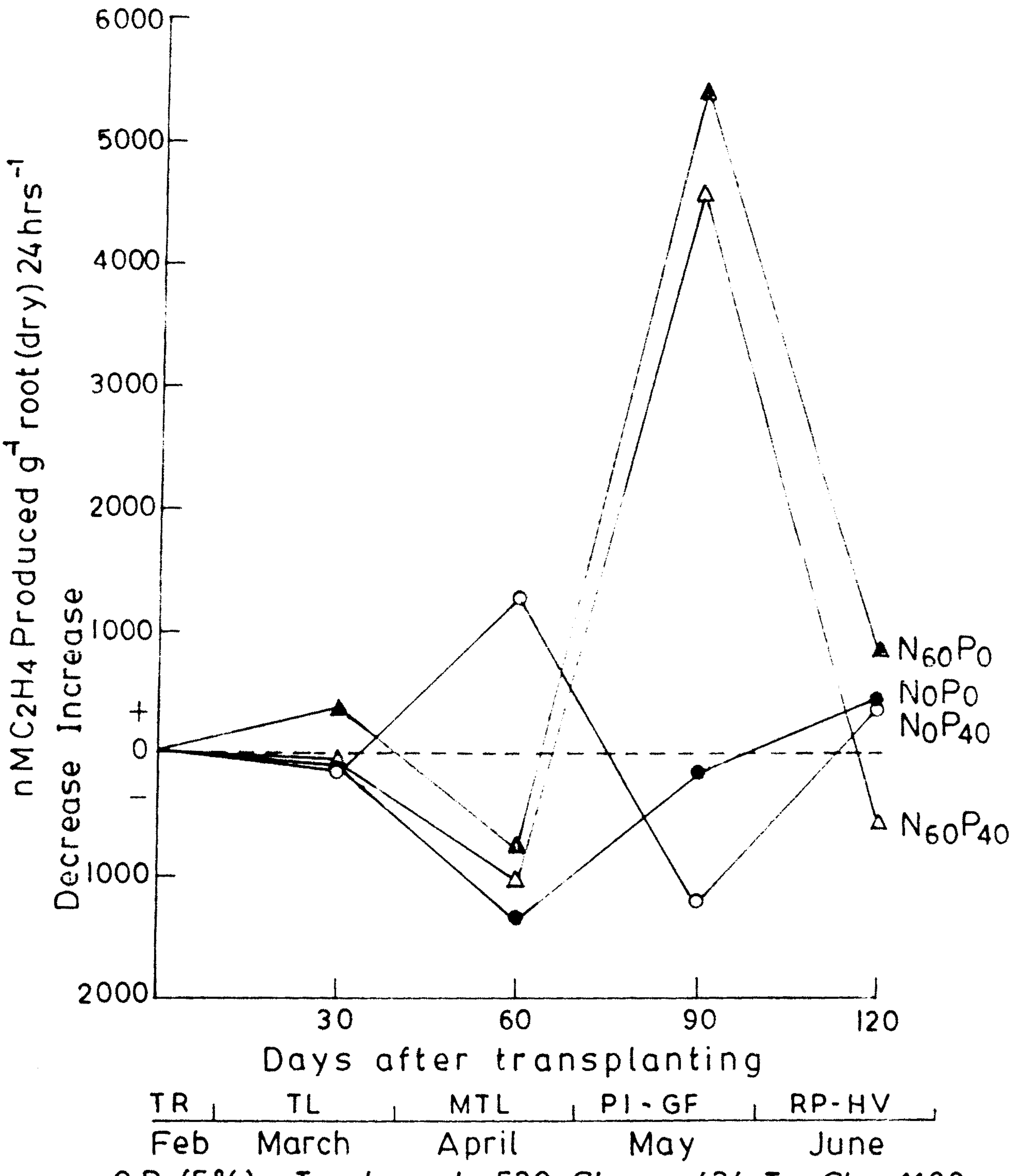
C.D. (5%) - Treatments 437, Stages 376, Tr × Stgs 1063

Fig. 4: Effect of blue-green algal inoculation on the nitrogenase activity in soil (values obtained after deduction from corresponding uninoculated control)

(Cyanobacteria) present in soil due to P application (Table-14).

4.3.3 N_2-ase activity associated with roots :

Results (Figure- 5) reveal that during the early reproductive stage of rice plants there was a repression in root associative N_2-ase activity due to blue-green algal (Cyanobacterial) inoculation, although the duration of such repression varied under the different fertilizer treatments. This indicates that the initial active N_2-fixing phase of blue-green algal (Cyanobacterial) growth in soil discourages the root associative N_2-fixation with rice. Towards the later period of plant growth, however, the results in most of the treatments showed a reverse trend. In unfertilized soil, inoculation did not show any favourable effect on root associative N_2-ase activity at any of the growth stages of rice plants excepting at the harvesting stage. Similar trend was also observed in P-fertilized soil with the exception at 60 days after transplantation of rice (MTLS), when an increase in the activity was observed. But such increase was always associated with the decrease in blue-green algal (Cyanobacterial) nitrogen fixation in soil (Fig- 4). Inoculation in presence of applied N alone or with P, caused a tremendous increase in the root associative N_2-ase activity at 90 days (GFS) after transplanting and thereafter it declined sharply. The magnitude of



C D. (5%) - Treatments 530, Stages 424, Tr × Stgs 1199

Fig. 5: Effect of blue-green algal inoculation on nitrogenase activity associated with roots (values obtained after deduction from corresponding uninoculated control)

Table-14 : Mean values of soil and root N_2-ase activities as influenced by the blue-green algal (Cyanobacterial) inoculation, at different growth stages of rice

Levels of P and N kg ha^{-1}	Cyanobacterial inoculation	Soil N_2-ase activity (nM C_2H_4 produced. Sq.cm^{-1} soil 24 hrs^{-1})	Root associative N_2-ase activity(nM C_2H_4 produced. g^{-1} root. 24 hrs^{-1})
Control N_0P_0	Uninoculated	600.22 ± 105.25	1402.82 ± 296.16
	Inoculated	1873.42 ± 460.18	1115.60 ± 343.21
N_0P_{40}	Uninoculated	2006.69 ± 372.89	1637.52 ± 374.91
	Inoculated	2195.36 ± 586.86	1729.29 ± 430.58
$N_{60}P_0$	Uninoculated	460.15 ± 68.24	1815.32 ± 484.88
	Inoculated	1540.57 ± 285.20	3310.97 ± 531.46
$N_{60}P_{40}$	Uninoculated	1799.60 ± 486.16	2402.90 ± 654.80
	Inoculated	2212.19 ± 242.92	3148.84 ± 622.24
C.D. at 5%		429.00	528.62

± Standard deviation of 4 replicates

increase was, however, higher where the soil was fertilized with N alone than with both N and P (Table-14). This suggests that the inoculation of blue-green algae (Cyanobacteria) encouraged the rice root associative N_2-fixation particularly when N was applied to the soil. This might be due to increased growth and nutrition of the rice plant resulting from fertilization.

4.3.4 Yield :

The results (Table-15) show that inoculation of blue-green algae (Cyanobacteria) in absence of any fertilizer brought about a significant increase in grain yield over that in the uninoculated control, the magnitude of increase being about 27 per cent. Inoculation in presence of applied N did not show any beneficial effect over the uninoculated counterparts, whereas the same in presence of P or P and N together brought about an increase in yield by about 15 and 7 per cent respectively over that in the corresponding uninoculated counterparts.

The straw yield showed more or less similar trend of increase due to inoculation of blue-green algae (Cyanobacteria) in presence of applied P or N either alone or in combination, as compared to their corresponding uninoculated control series.

4.3.5 Relation between N_2-ase activity and yield :

Results (Table-16) indicate that the N_2-ase activity in

Table-15 : Influence of blue-green algal (Cyanobacterial) inoculation on the grain and straw yield of rice (Var. IR-50)

Levels of P and N kg ha^{-1}	Cyanobacterial	Grain (g pot^{-1})	Straw (g pot^{-1})
Control N_0P_0	Uninoculated inoculation	11.77 ± 0.31	13.83 ± 0.24
	Inoculated	15.00 ± 0.70	18.50 ± 1.22
N_0P_{40}	Uninoculated	15.80 ± 0.73	19.17 ± 0.62
	Inoculated	18.13 ± 2.33	21.17 ± 3.09
$N_{60}P_0$	Uninoculated	20.30 ± 1.63	31.83 ± 9.49
	Inoculated	21.50 ± 1.74	28.33 ±10.02
$N_{60}P_{40}$	Uninoculated	22.70 ± 0.62	30.83 ± 7.33
	Inoculated	24.23 ± 0.87	33.50 ± 8.44
C.D. at 5%		2.77	13.30

± Standard deviation of 4 replicates

Table-16 : Relationship between straw and grain yield and estimates of N_2-ase activity in soil and root systems at different growth stages of rice.

	Days after transplanting	Grain(Y_1)		Straw (Y_2)	
		Correlation coefficient(r)	Regression equation	Correlation coefficient(r)	Regression equation
Soil N_2-ase activity	30(TLS)	0.583**	Y_1= 14.661 + 0.008X (t = 3.369)	0.498*	Y_2=16.937 + 0.015X (t = 2.691)
	60(MTLS)	0.245	-	0.005	-
	90(GFS)	-0.273	-	-0.251	-
	120(HVS)	0.521*	Y_1= 15.269 + 0.003X (t = 2.861)	0.347	-
Root associative N_2-ase activity	30(TLS)	0.101	-	-0.116	-
	60(MTLS)	0.600**	Y_1=14.607 + 0.002X (t = 3.521)	0.561**	Y_2=16.084 + 0.004X (t = 3.181)
	90(GFS)	0.610*	Y_1=15.514 + 0.001X (t = 3.612)	0.385	-
	120(HVS)	-0.113	-	0.009	-

* Significant at 5% level;
** Significant at 1% level.

Table-17 : Multiple regression analysis relating grain (Y_1) and straw (Y_2) yield with N_2-ase activity in soil and associated with roots

Dependent variable	Intercept (a)	N_2-ase activity in soil (days after transplanting)				N_2-ase activity associated with root (days after transplanting)				R^2
		30 (TLS)	60 (MTLS)	90 (GFS)	120 (HVS)	30 (TLS)	60 (MTLS)	90 (GFS)	120 (HVS)	
Y_1	11.5673	0.0058	0.0004	-0.0002	0:0005	-0.0041	0.0015	0.0003	-0.0001	0.7166**
Y_2	11.9714	0.0167	-0.0003	0.0001	-0.0003	-0.0286	0.0034	0.0006	0.0009	0.6505**

Equation of the type $Y = a + bx_1 + bx_2 \;\ldots\ldots\; bx_8$

** Significant at 1% level

soil system at 30 days (TLS) and that associated with roots at 60 (MTLS) and 90 (GFS) days assay period after transplanting correlated significantly with grain yield. This suggests that N_2-fixation by these two sources at those stages influence the grain yield significantly. Similarly, a significant relationship was also found in case of straw yield with N_2-ase activity in soil system at TLS and that associated with root at MTLS stage assay periods. The yield of grain and straw was further predicted by employing N_2-ase activity in soil system (X_1 - X_4) and that associated with roots (X_5 - X_8) as independent variables in multiple regression analysis (Table-17). The equations explain 71.66 and 65.05 per cent of variability in grain and straw yield respectively. The sign and magnitude of coefficients in regression equation suggest that the N_2-ase activity in soil system at TLS and that associated with roots at MTLS and GFS stage assay periods respectively are although important in predicting the yield but not statistically significant.

4.3.6 Conclusion :

The results of this investigation clearly demonstrate that the cyanobacterial inoculation increased the N_2-ase activity in soil system and such increased activity particularly during the early reproductive to grain formation stage is beneficial in increasing the grain and straw yield of rice.

4.4 Effect of inoculation of blue-green algae (Cyanobacteria) on nitrogen status in soil and rice productivity under submerged condition.

Two experiments were conducted by inoculating blue-green algae in presence (i) chemical fertilizers like urea and super-phosphate and (ii) organic and green manures like (Sesbania aculata L. twigs (Dhaincha), Neemcake and Farm-yard-manure (FYM), at the same site of a farm under the Viswavidyalaya, in kharif 1984 and 1985. The physico-chemical properties of the soil of the experimental site are presented in Table-18. Some of the analytical parameters were common in both the experiments, therefore, for brevity and for better understanding of the influence of blue-green algal inoculation, on the nitrogen status of soil and rice yield, the results are presented itemwise.

4.4.1 Effect of BGA inoculation on the inorganic-N ($NH_4^+ + NO_3^- + NO_2^-$ - N) content in soil.

Inorganic form of nitrogen in soils estimated from both the experiments are presented in Table-19 and 20. The results reveal that this form of nitrogen accumulated gradually from 30 DAI onwards with the age of the plants irrespective of any treatments in both the seasons. The rate of absorption of this form of nitrogen by the plants was probably slower than its rate of release from the soil organic matter and the added fertilizers/manures. This imbalance which became more prominant

Table-18 : Physico-chemical properties of the soil of the experimental field

pH	...	8.3
Electrical conductivity (dSm^{-1})	...	0.02
Organic carbon (%)	...	0.98
Total nitrogen (%)	...	0.11
NH_4-N (ppm)	...	4.48
NO_3-N (ppm)	...	0.83
Available phosphorus (ppm)	...	3.12
Hydrolyzable - N (ppm)	...	856
Non-hydrolyzable-N (ppm)	...	385
C.E.C. [C.mol (P^+) Kg^{-1}]	...	24.5
Exchangeable Ca^{++} [C.mol (P^+) kg^{-1}]	..	20.7
Exchangeable Mg^{++} [C.mol (P^+) kg^{-1}]	..	2.4
Mechanical composition :		
Clay (%)	...	30.4
Silt (%)	...	18.6
Sand (%)	...	51.0

Table-19 : Influence of BGA inoculation on the periodical changes in inorganic nitrogen (NH_4^+ + NO_2^- + NO_3^{-1} -N) content of rice soil (kharif 1984) (N in ppm)

Treatments	NH_4^+ + NO_2^- + NO_3^- - N (N in ppm)			
	30 days after inoculation	60 days after inoculation	90 days after inoculation	Mean
N_0P_0	2.99 ($\pm$ 0.68)	3.09 ($\pm$ 0.16)	6.55 ($\pm$ 0.99)	4.21
N_0P_0+ BGA	4.28 ($\pm$ 1.02)	4.81 ($\pm$ 0.37)	9.36 ($\pm$ 2.68)	6.15
N_0P_{40}	3.08 ($\pm$ 0.21)	3.17 ($\pm$ 0.26)	8.18 ($\pm$ 0.53)	4.81
N_0P_{40} + BGA	4.43 ($\pm$ 0.64)	6.05 ($\pm$ 2.54)	8.74 ($\pm$ 1.48)	6.41
$N_{40}P_0$	3.39 ($\pm$ 1.14)	3.64 ($\pm$ 0.50)	7.16 ($\pm$ 1.92)	4.73
$N_{40}P_0$ + BGA	3.28 ($\pm$ 0.58)	5.14 ($\pm$ 1.17)	7.91 ($\pm$ 0.22)	5.44
$N_{40}P_{40}$	3.38 ($\pm$ 0.51)	4.09 ($\pm$ 0.52)	7.35 ($\pm$ 1.02)	4.94
$N_{40}P_{40}$ + BGA	4.84 ($\pm$ 0.29)	5.08 ($\pm$ 0.24)	7.38 ($\pm$ 0.57)	5.77
Mean	3.71	4.38	7.83	

C.D. (5%) Treatment - 1.33

Stage - 0.77

$\pm$ = Standard deviation of the mean of three replicates.

Table-20 : Changes in (NH_4^+ + NO_2^- + NO_3^-) -N content in soil as influenced by BGA inoculation in presence of urea and organic matter (kharif, 1985).

(N in ppm)

Treatments	NH_4^+ + NO_2^- + NO_3^- - N			Mean
	30 days after BGA inoculation	60 days after BGA inoculation	90 days after BGA inoculation	
N_0	2.94(±0.79)	8.60(±1.59)	14.87(±2.78)	8.80
N_0 + BGA	3.08(±0.30)	13.40(±2.54)	16.35(±0.68)	10.94
N_{30} (Urea)	7.93(±1.80)	15.36(±1.95)	18.07(±2.96)	13.79
N_{30} (Urea) + BGA	4.12(±1.25)	11.28(±2.43)	18.75(±4.66)	11.38
N_{30} (Dhaincha)	4.51(±0.61)	12.24(±3.43)	20.69(±3.23)	12.48
N_{30} (Dhaincha) + BGA	3.95(±0.93)	15.57(±2.80)	22.32(±1.43)	13.95
N_{30} (Neem cake)	5.32(±0.52)	12.46(±4.23)	19.62(±2.62)	12.46
N_{30} (Neem cake) + BGA	4.65(±0.97)	13.23(±1.42)	20.19(±1.07)	12.69
N_{30} (FYM)	3.84(±1.01)	10.42(±2.90)	18.60(±0.76)	10.95
N_{30} (FYM) + BGA	6.98(±2.13)	14.04(±0.53)	18.89(±1.21)	13.30
Mean	4.73	12.66	18.84	

C.D. Treatment - 2.66 (at 5%)
3.65 (at 1%)

Stage - 1.46 (at 5%)
1.95 (at 1%)

± Standard deviation of the Mean of the three replicates with age of the crop may explain the accumulation of inorganic nitrogen in soil. In most of the cases the magnitude of accumulation of inorganic nitrogen was greater in inoculated than in the corresponding uninoculated treatment. During early stage (30 DAI) this form of nitrogen in soil increased marginally from 4 (Table-20) to 40% (Table-19), due to inoculation alone but inoculation in presence of P, P + N (Table-19) and FYM (Table-20) yielded a significant increase of this form of nitrogen in soil over their corresponding uninoculated controls. Such increase due to inoculation under these treatments persisted upto 60 DAI when inoculation in presence of Sesbania aculata (Dhaincha) also showed a significant increase (Table-20) of this form of nitrogen over its uninoculated control. Later, at 90 DAI, the efficiency of inoculation in releasing inorganic nitrogen in all the treatments was found to be minimum, excepting those in inoculation alone where the release of nitrogen was significantly higher over their respective uninoculated controls. Inoculation of blue-green algae in presence of Neemcake (Table-20) and Urea-N (Table-19,20) was less effective in this respect. The initial decrease in the inorganic nitrogen content in soil due to inoculation in presence of Urea-N in both the experiments may be attributed to the immobilization of the added N by the inoculated blue-green algae. Shioiri and Misui (1935) in a pot experiment recovered in the algal biomass 10-30% of N added as urea. Vlek and Craswell (1979) reported that urea fertilization stimulated algal growth and led to a net immobilization of 18-30% of N from fertilizer three weeks after application.

Blue-green algae are known to release part of their fixed N as extracellular compounds as well as decomposition products of aged cells. These are then converted to inorganic form of nitrogen through the process of mineralization. Therefore, the amount released is directly related to their growth and N_2-fixation i.e. C : N ratio. Application of phosphorus increases the growth and N_2-fixation by blue-green algae. This may explain the increased accumulation of inorganic nitrogen due to inoculation in presence P application. Decomposition of organic matter, on the other hand, increases the partial pressure of CO_2, and the availability of some beneficial elements particularly P and may decrease the mineral nitrogen content in soil. All these factors favour the growth of N_2-fixing blue-green algae and thus increase their N_2-fixing activity. The variation in the rate of decomposition of different organic manures added may be responsible for variation in the amount of accumulation of inorganic nitrogen in soil through blue-green algae at different stages of plant growth. The reduction in the efficiency of inoculation in most of the cases at later stages of crop growth may be attributed to the ceasation of growth and N_2-fixation of the inoculated blue-green algae due to lower incident light.

4.4.2 Inorganic nitrogen content in soil solution :

Besides nitrogen content in soil, the inorganic nitrogen content in soil solution is also important so far as plant uptake of nitrogen is concerned. Inorganic nitrogen content in soil solution was estimated from the experiment conducted in 1985. The results (Table-21) show a significant increase of this form of nitrogen in soil solution due to inoculation alone. The magnitude of increase was 1175 per cent over uninoculated control at 30 DAI. Inoculation in presence of Urea-N, Sesbania aculata (Dhaincha), Neem cake also showed an increase in this respect at 30 DAI. But while the increase was statistically significant in case of Urea-N, it was insignificant in case of the latter two. Inoculation in presence of FYM did not show any effect at 30 DAI. Later, at 60 DAI inorganic nitrogen content in soil solution decreased excepting in Neemcake and FYM treated plots where a significant increase over that of 30 DAI was observed in both inoculated and uninoculated plots.

The accumulation of inorganic nitrogen in soil and soil solution in a particular period is the result of many interacting processes like mineralization - immobilization, plant uptake, loss through various means. Significant increase of this form of nitrogen, due to inoculation alone may be attributed to the slow

Table-21 : Influence of BGA inoculation on the periodical changes of (NH_4^+ + NO_2^- + NO_3^-) -N content of soil solution. (Kharif 1985)

Treatments	30 days after inoculation	60 days after inoculation	Mean
N_0	0.08(± 0.00)	0.19(± 0.03)	0.14
N_0 + BGA	1.02(± 0.17)	0.28(± 0.14)	0.65
N_{30} (Urea)	0.17(± 0.08)	0.15(± 0.00)	0.16
N_{30} (Urea) + BGA	0.79(± 0.03)	0.28(± 0.24)	0.54
N_{30} (Dhaincha)	0.35(± 0.07)	0.15(± 0.00)	0.25
N_{30} (Dhaincha) + BGA	0.50(± 0.19)	0.15(± 0.00)	0.33
N_{30} (Neem cake)	0.20(± 0.09)	0.68(± 0.22)	0.44
N_{30} (Neem cake) + BGA	0.35(± 0.07)	0.71(± 0.07)	0.53
N_{30} (FYM)	0.08(± 0.00)	0.30(± 0.00)	0.19
N_{30} (FYM) + BGA	0.08(± 0.00)	0.40(± 0.07)	0.24
Mean	0.36	0.33	

C.D. Treatment - 0.17 (at 5%)

0.23 (at 1%)

Stage - 0.14 (at 5%)

0.20 (at 1%)

± Standard deviation of the mean of three replicates.

release of algal fixed nitrogen due to slow establishment of the inoculant, which resulted in low plant uptake and minimum loss through various means. The increase due to inoculation in presence of Urea nitrogen may be explained on the basis of rapid hydrolysis of urea and its subsequent conversion to biomass-N of the inoculated and indegenous algae which act as slow release nitrogenous fertilizer and prevent the loss by denitrification, volatilization etc. The decrease of this form of nitrogen in soil solution at 60 DAI may be due to its assimilation by plants and/or losses through leaching, volatilization and denitrification. Neemcakes are known to act as nitrification inhibitors and prevent losses of nitrogen through leaching and denitrification. This may explain the increase of inorganic nitrogen in soil solution at 60 DAI in Neemcake treated plots but the reason for increase due to FYM remains obscure.

4.4.3 Available nitrogen content ($KMnO_4$-oxidizable) in soil :

Available nitrogen content in soil estimated from the experiment of 1985 are presented in Table-22. The results show that available nitrogen content in soil generally increased due to inoculation excepting in presence of urea-N at 30 DAI. The extent of increase varied between 6-17 per cent. Inoculation in presence of Sesbania aculata (Dhaincha) and Neemcake was more effective in this respect, however the increase was always

Table-22 : Influence of BGA inoculation on the periodic changes in available (alkaline permanganate oxidizable) nitrogen in soil (Kharif, 1985)

(N in ppm)

Treatment	30 days after inoculation	60 days after inoculation	Mean
N_0	96.62(±6.16)	97.26(±14.74)	96.94
N_0 + BGA	102.43(±11.84) (6.01)	105.83(± 3.43) (8.81)	104.13
N_{30} (Urea)	119.58(±13.82)	114.19(± 10.48)	116.89
N_{30} (Urea) + BGA	110.81(±15.07) (-7.33)	116.17(± 18.17) (1.73)	113.49
N_{30} (Dhaincha)	98.03(±8.08)	113.24(± 6.32)	105.64
N_{30} (Dhaincha) + BGA	114.58(±20.99) (16.88)	119.64(± 1.02) (5.65)	117.11
N_{30} (Neem cake)	96.08(± 5.86)	107.73(± 6.81)	101.91
N_{30} (Neem cake) + BGA	111.29(±15.33) (15.83)	114.63(± 9.13) (6.40)	112.96
N_{30} (FYM)	101.12(±7.48)	119.87(± 7.29)	110.50
N_{30} (FYM) + BGA	103.85(±15.19) (2.70)	121.25(± 9.46) (1.15)	112.55
Mean	105.44	112.98	

C.D. (5%) Treatment - N.S.; Stage - N.S.

± Standard deviation of the mean of three replicates
Figures in parenthesis indicate percentage increase over the respective control.

statistically insignificant. At 60 DAI the available nitrogen content in soil improved marginally, particularly where organic inputs were added.

Alkaline permanganate in presence of reducing agents like Devarda's alloy releases easily oxidizable organic-nitrogen fraction from soil organic-N pool by both oxidation and hydrolysis in addition to already present inorganic-N of soil. The initial decrease (at 30 DAI) in available-N content due to inoculation in presence of Urea-N may be due to immobilization of the urea hydrolysis product by the algal mass in soil, which was not accounted for in the estimation. The variation in the magnitude of increase in the available nitrogen content in soil by inoculated blue-green algae was obviously due to variation in their release of oxidizable and hydrolysable products either extracellularly or through decomposition in addition to that released from applied organic manures.

4.4.4 Hydrolyzable (HL-N) and non-hydrolyzable (NHL-N) nitrogen content in soil :

Results (Table-23) of the hydrolyzable-N content (determined from the experiment of 1985) show that the content increased due to inoculation, more prominently at later stage i.e. at 60 DAI. However, the increase was not statistically significant. While inoculation in presence of Sesbania aculata (Dhaincha) and Neemcake recorded an increase in hydrolyzable-N content in soil

Table-23 : Changes in Hydrolyzable and non-hydrolyzable - N content in soil as influenced by BGA inoculation in presence of Urea and organic matter (kharif, 1985)

(N in ppm)

Treatments	Hydrolyzable N content		Mean
	30 days after BGA inoculation	60 days after BGA inoculation	
N_0	713.0(±55.4)	814.8(±27.8)	763.9
N_0 + BGA	730.0(±13.8) (2.38)	905.4(±42.3) (11.12)	817.7
N_{30} (Urea)	758.2(±136.7)	984.7(±55.5)	871.5
N_{30} (Urea) + BGA	746.7(±138.4) (-1.52)	1007.2(±89.1) (2.28)	877.0
N_{30} (Dhaincha)	763.9(±69.3)	933.7(±13.8)	848.8
N_{30} (Dhaincha) + BGA	814.8(±73.4) (6.66)	961.9(±16.0) (3.02)	888.4
N_{30} (Neemcake)	746.9(±110.9)	905.4(±16.0)	826.2
N_{30} (Neemcake) + BGA	780.6(±138.6) (4.51)	1018.5(±35.4) (12.49)	900.0
N_{30} (FYM)	780.9(±73.4)	950.6(±0.00)	865.8
N_{30} (FYM) + BGA	690.3(±42.3) (-11.60)	1052.5(±73.3) (10.72)	871.4
Mean	752.53	953.47	

C.D. Treatment - N.S.

Stage - 57.65 (at 5%)

- 78.62 (at 1%)

± = Standard deviation of the mean.

Contd... Table-23. Periodical changes in non-hydrolysable nitrogen content (NHL-N) of soil under different treatments (kharif,1985)

Treatments	30 days after inoculation	60 days after inoculation	Mean
N_0	198.0(±5.7)	153.6(±31.9)	175.80
N_0 + BGA	230.4(±19.8) (16.36)	181.9(±9.9) (18.42)	206.15
N_{30} (Urea)	212.2(±34.7)	194.0(±0.00)	203.10
N_{30} (Urea)+ BGA	214.2(±5.7) (-0.94)	190.1(±20.8) (-2.01)	202.15
N_{30} (Dhaincha)	202.1(±5.8)	175.9(±4.9)	189.00
N_{30} (Dhaincha) + BGA	214.2(±30.2) (5.99)	169.8(±9.9) (-3.47)	192.00
N_{30} (Neemcake)	224.3(±14.9)	173.8(±24.9)	199.05
N_{30} (Neemcake) + BGA	224.3(±5.0) (0.00)	177.8(±15.1) (2.30)	201.05
N_{30} (FYM)	206.1(±9.9)	165.7(±11.5)	185.90
N_{30} (FYM) + BGA	238.5(±11.4) (15.72)	161.7(±11.5) (-2.41)	200.10
Mean	216.46	174.43	

C.D. Treatment - N.S

Stage - 12.324 (at 5%)

16.805 (at 1%)

± = Standard deviation of the mean.

Figures in parenthesis indicates percentage increase over respective controls.

over controls, that in presence of FYM was not effective in this respect at 30 DAI. This may be due to different immobilization rates associated with these organic manures. Inoculation in presence of Urea-N caused an initial depression followed by small increase (2.28%) over its control.

In Section-2 of this thesis (Table-12) it has been found that the major portion of N_2-fixed by blue-green algae could be accounted for in hydrolyzable-N content in soil which subsequently enriches the available pool. The increase in HL-N content can be explained simply due to N-accretion by blue-green algae. The initial decrease followed by subsequent recovery due to inoculation in presence of urea-N may be attributed to initial suppression of algal N_2-fixation by urea-N which disappeared later on due to fall in its concentration. However, the same phenomena in presence of FYM remains obscure. The results (Table-23) of changes in non-hydrolyzable-N content in soil show that all the treatments recorded a decrease at later stage from their respective values at 30 DAI, indicating transformation of this form of nitrogen to other forms. Inoculation of blue-green algae in presence of chemical or organic N sources could not bring about any significant change and consistent influence in this respect.

4.4.5 Post-harvest total nitrogen content in soil :

Post-harvest total nitrogen content in soil (Table-24) was estimated from the experiment of 1984. Inoculation of blue-green algae in presence or absence of N,P and N + P always increased the total nitrogen content of the post-harvest soil, but none of the increase was statistically significant. The highest increase equivalent to 61.6 kg N ha^{-1} was recorded in absence of any fertilizer. The quantity of nitrogen added through inoculation of blue-green algae in rice fields was estimated to be about 13 to 70 lb $acre^{-1}$ (Prasad, 1949; Watanabe et al, 1951; Nishigaki et al, 1951; 1953; Singh, 1961). This may be attributed to that fraction of fixed nitrogen which remains within the ambit of algal cells. The lowest increase in total nitrogen content due to inoculation in presence of P alone may be explained on the basis of its stimulatory effect on the native flora which minimized the efficiency of the inoculated blue-green algae. This is evident from 3.42% increase in total nitrogen content in P treated soils over no fertilizer control. The significant increase in presence of N + P and the marginal increase in presence of N alone over no fertilizer control also coroborate this view. The beneficial effect of P in increasing nitrogen fixation by blue-green algae was reported by De and Suliaman (1950), De and Mandal (1956).

Table-24 : Influence of BGA inoculation on total nitrogen (in ppm) content of rice soil (post harvest) under different treatments (kharif, 1984)

Treatments	Total nitrogen (in ppm)
N_0P_0	1049.77(± 34.62)
N_0P_0 + BGA	1080.57(± 15.82)
N_0P_{40}	1085.70(± 0.00)
N_0P_{40} + BGA	1093.40(± 32.67)
$N_{40}P_0$	1052.33(± 3.63)
$N_{40}P_0$ + BGA	1067.73(± 18.15)
$N_{40}P_{40}$	1098.53(± 3.63)
$N_{40}P_{40}$ + BGA	1116.50(± 12.57)

C.D. Treatment - 40.6 (at 5%)

56.3 (at 1%)

± = standard deviation of the mean of three replicates.

4.4.6 Yield :

The yields of grain and straw for two experiments are presented in Table-25 and 26. The results (Table-25) show that inoculation of blue-green algae alone brought about an increase in grain yield by about 13 per cent over that with uninoculated control. Application of P or N and their combination alone without inoculation increased the yield upto significant level, highest in the latter case. Inoculation in presence of all those treatments, improved the grain yield, but the efficiency of inoculation in this respect was much higher when P was applied. The results in Table-26 also show an increase in grain yield due to inoculation in presence of all the organic N-sources but none of such increase reached level of significance over their corresponding uninoculated counterparts. However, inoculation in presence of FYM had an edge over other organic inputs in this respect.

Increase in straw yield due to inoculation in presence of any of the treatments showed more or less similar trend as that of the grain yield.

Rice yield depends largely on the availability of N in soil system particularly at early tillering and panicle initiation stages, when the crop takes up major portion of N of their total requirement. Significantly higher release of inorganic N particularly at an early stage in soil system, due to inoculation in

Table-25 : Effect of algal inoculation in conjunction with nitrogenous and phosphatic fertilizers on the grain and straw yield of rice (kharif,1984)

Treatments	Grain yield (q/ha)	Straw yield (q/ha)
N_0P_0	22.11(±3.0)	40.01(±5.44)
N_0P_0 + BGA	25.05(±1.57) (13.30)	43.34(±2.72) (8.32)
N_0P_{40}	26.46(±0.46)	44.45(±0.78)
N_0P_{40} + BGA	30.68(±1.67) (15.95)	50.01(±2.72) (12.51)
$N_{40}P_0$	31.14(±3.50)	48.90(±5.50)
$N_{40}P_0$ + BGA	32.56(±2.00) (4.56)	51.12(±3.15) (4.54)
$N_{40}P_{40}$	36.28(±3.66)	55.56(±5.67)
$N_{40}P_{40}$ + BGA	38.52(±2.09) (6.17)	57.78(±3.14) (4.00)
C.D. (at 5%) .	5.29	8.84
(at 1%) .	7.33	12.26

± = Standard deviation of the mean.

Figure in parenthesis indicate percentage increase over respective controls.

Table-26 : Grain and straw yield of rice (Var. IR-36) as influenced by BGA inoculation in presence of Urea and organic matter (kharif 1985)

Treatments	Grain yield ($q\ ha^{-1}$)	Straw yield ($q\ ha^{-1}$)
N_0	35.13(±0.52)	45.88(± 7.74)
N_0 + BGA	37.86(±1.88) (7.77)	50.78(± 1.61) (10.68)
N_{30}(Urea)	39.47(±0.11)	58.05(±4.94)
N_{30} (Urea) + BGA	41.11(±2.13) (4.16)	61.54(±1.43) (6.01)
N_{30} (Dhaincha)	39.36(±1.91)	57.28(±1.16)
N_{30} (Dhaincha) + BGA	41.04(±1.33) (4.27)	59.75(±2.06) (4.31)
N_{30} (Neem cake)	38.80(±0.65)	57.80(±2.79)
N_{30} (Neemcake) + BGA	39.21(±2.38) (1.06)	59.65(±2.41) (3.20)
N_{30} (FYM)	37.92(±1.67)	55.03(±7.32)
N_{30} (FYM) + BGA	40.63(±1.54) (7.15)	61.24(±3.45) (11.28)
C.D. (at 5%)	3.46	8.30
(at 1%)	4.74	11.64

± = Standard deviation of the mean of three replicates.

Figures in parenthesis indicate percentage increase over respective controls.

presence of P and FYM application, may be attributed to the higher yield response under these two treatments. Simple correlation studies (Table-27) showed a positive relationship between inorganic-N content in soil and grain and straw yields, but the values of correlation coefficient (r) were insignificant. It was interesting that 'r' values declined with the age of the crop indicating the importance of early accumulation of that form of N to the yield. Both available-N (Permanganate oxidizable) and HL-N content at 60 DAI estimation period, were found to be significantly correlated with the yields while that for NHL-N content was insignificant.

4.4.7 N-content and uptake :

The results of N-content in and uptake by grain and straw for two experiments are presented in Table-28 and 29. The N content in grain and straw although increased due to inculation in different treatments but the same was inconsistent in terms of significance over that in corresponding uninoculated controls. The uptake of N was increased by inoculation and followed more or less similar trend like that of the yield of grain and straw under all the treatments excepting that where inoculation was made in presence of urea application. In this treatment, the increase in yields due to inoculation was accompanied with comparatively low uptake of N particularly in the second experiment.

Table-27 : Influence of BGA inoculation on the **Nitrogen** content in and uptake rice grain and straw (kharif 1984)

Treatments	N content (%) and uptake (kg/ha) in grain and straw				
	Grain		Straw		
	Content	Uptake	Content	Uptake	Total
N_0P_0	1.26(±0.09)	27.86	0.55(±0.02)	22.01	49.87
N_0P_0 + BGA	1.28(±0.05)	32.06 (15.08)	0.56(±0.07)	24.27 (10.27)	56.33
N_0P_{40}	1.22(±0.00)	32.27	0.50(±0.03)	22.23	54.50
N_0P_{40} + BGA	1.22(±0.05)	37.43 (15.99)	0.76(±0.08)	38.01 (70.99)	75.44
$N_{40}P_0$	1.26(±0.09)	39.24	0.78(±0.02)	38.14	77.38
$N_{40}P_0$ + BGA	1.32(±0.07)	42.98 (9.53)	0.72(±0.15)	36.81 (-3.49)	79.79
$N_{40}P_{40}$	1.26(±0.03)	45.71	0.62(±0.05)	34.45	80.16
$N_{40}P_{40}$ + BGA	1.28(±0.05)	49.31 (7.88)	0.70(±0.05)	40.45 (17.42)	89.76
Mean	1.26	38.36	0.65	32.05	
C.D (at 5%)	N.S		0.16		

± = Standard deviation of the mean

Figures in parenthesis indicate percentage increase over respective controls.

Table-28 : Nitrogen content in and uptake by grain and straw of rice as incluenced by algal inoculation in presence of Urea and organic matter (kharif 1985)

Treatment	N content (%) and uptake (kg/ha) in grain and straw				
	Grain		Straw		
	Content	Uptake	Content	Uptake	Total
N_0	1.00(±0.00)	35.13	0.59(±0.05)	27.07	62.20
N_0 + BGA	1.21(±0.02)	45.81 (30.40)	0.63(±0.02)	31.99 (18.18)	77.80
N_{30} (Urea)	1.37(±0.04)	54.07	0.83(±0.03)	48.18	102.25
N_{30} (Urea) + BGA	1.31(±0.02)	53.85 (-0.47)	0.67(±0.06)	41.23 (-14.43)	95.08
N_{30} (Dhaincha)	1.23(±0.03)	48.41	0.73(±0.01)	41.81	90.22
N_{30}(Dhaincha) + BGA	1.29(±0.01)	52.94 (9.36)	0.83(±0.03)	49.59 (18.61)	102.53
N_{30} (Neemcake)	1.21(±0.02)	46.95	0.68(±0.02)	39.30	86.25
N_{30} (Neem cake) + BGA	1.24(±0.03)	48.62 (3.56)	0.68(±0.06)	40.56 (3.21)	89.18
N_{30} (FYM)	1.25(±0.05)	47.40	0.63(±0.01)	34.67	82.07
N_{30} (FYM) + BGA	1.28(±0.04)	52.01 (9.73)	0.68(±0.05)	41.64 (20.10)	93.65
Mean	1.24	48.52	0.70	39.60	
C.D. at 5%	0.06		0.08		
at 1%	0.08		0.11		

± = Standard deviation of the mean of three replicates.

Figures in parenthesis indicate percentage increase over respective controls.

Table-29 : Simple correlation Co-efficients relating different nitrogen fractions at different growth stages with grain and straw yield of rice.

Kharif, 1984

Nitrogen Fractions	Correlation coefficients	
	Grain yield	Straw yield
Total KCl extractable nitrogen		
30 days after inoculation	(+) 0.3437	(+) 0.3534
60 days after inoculation	(+) 0.2552	(+) 0.2413
90 days after inoculation	(+) 0.0721	(+) 0.1319

Kharif, 1985

KCl-extractable nitrogen		
30 days after inoculation	(+) 0.3278	(+) 0.1582
60 days aftar inoculation	(+) 0.1398	(+) 0.3366
$KMnO_4$ oxidizable nitrogen		
NH_4^+-N 30 days after inoculation	(-) 0.0059	(+) 0.0602
60 days after inoculation	** (+) 0.4795	*(+) 0.3918
NO_3^--N		
30 days after inoculation	(+) 0.2311	**(+) 0.4423
60 days after inoculation	(+) 0.2119	(-) 0.2536
$(NH_4^+ + NO_3^-)$-N		
30 days after inoculation	(+) 0.2593	(+) 0.3371
60 days after inoculation	**(+) 0.5496	(+) 0.1030

Contd.... Table-29.

Nitrogen Fractions	Correlation co-efficients	
	Grain yield	Straw yield
Hydrolyzable nitrogen		
30 days after inoculation	(+) 0.0704	(+) 0.1200
60 days after inoculation	** (+) 0.5275	**(+) 0.5688
Non-hydrolyzable nitrogen		
30 days after inoculation	(+) 0.1989	(+) 0.0410
60 days after inoculation	(-) 0.0971	(+) 0.2173

* Significant at 5% level

** Significant at 1% level

This indicates that the influence of inoculation of blue-green algae on the yield of rice in presence of N application is partially caused by some factors other than N input.

4.4.8 Conclusion :

Results indicate that inoculation of blue-green algae improved the N-fertility status of the soil and increased the grain and straw yield which was more pronounced in presence of phosphorus application as compared to all other inorganic and organic input.

CHAPTER NO. 5

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

With the advent of high yielding, short duration, photo-insensitive varieties of rice, rice based cropping system has become highly intensive particularly in those areas where assured irrigation facilities are available. In order to sustain high productivity in such intensive cropping system, besides many other things supply of adequate quantity of nutrients in soils is required to be ensured through addition of fertilizers and manures. Of all the nutrients nitrogen is the most limiting one in tropical agriculture. With the increasing price of fossil fuel, the cost of nitrogenous fertilizers has gone very high and has become more and more difficult for the poor rice farmers of the tropical Asia including India to use adequate quantity of nitrogenous fertilizer for rice farming. Moreover, the nitrogen use efficiency in wet land rice is very low hardly exceeding 35% of the applied amount, because of considerable losses through leaching, denitrification, NH_3 volatilization etc. In this context the use of blue-green algae as biofertilizer in paddy fields as a supplementary source of nitrogen has assumed a great deal of importance.

West Bengal is one of the major rice growing States in India. The soils of a large part of the rice-growing tract in this state situated both in the alluvial and laterite zone are acidic in reaction. It is known that N_2-fixing species of blue-green algae do not prefer acidic soil environment. Accordingly the distribution of N_2-fixing blue-green algae in 32 alluvial acidic low land rice field soils of the district of Jalpaiguri and Coochbehar in West Bengal was studied and their potentiality for nitrogen fixation was assessed. Results showed that the most probable number (MPN) of the blue-green algae varied from 490-54000 g^{-1} of dry soil while the algal nitrogen fixation ranged from 0.19 to 1.75 mg 5 gm^{-1} soil WK^{-1}. Although the MPN showed significant positive correlation with EC, NO_3^- - N and exchangable Ca^{2+} content of the soils but N_2-fixation did not show significant correlation with any of the soil properties.

The species of 26 genera of blue-green algae, comprising heterocytous and non-heterocystous filamentous and unicellular forms were recorded in these soils by enrichment culture technique. Most of the soils showed the growth of filamentous heterocystous type, in which the species of genera Anabaena and Nostoc were most dominating. The species belonging to the genera Cylindrospermum, Haphalosiphon, Rivularia, Aulosira, Scytonema and Stigonema were also found in some of the soils. The non-heterocystous type found in these soils mostly belonged to the genera Oscillatoria, Lyngbya and Phormidium. An abundant growth of unicellular blue-green algae was also observed in these soils, in which the most dominating genus was Aphanothece. Nitrogen fixation by the composite algal culture obtained from different soils was determined in modified Chu-10 N-free medium. The fixation varied from 0.64 to 2.36 mg N/50 ml of the medium during 42 days of growth. The magnitude of fixation in the medium, however, differed from that in the soil culture.

A total of 20 unialgal species viz. 11 strains of Anabaena, 5 strains of Nostoc and 4 strains of Cylindrospermum, were isolated from the composite algal cultures in N-free medium. All the isolates were tested for their C_2H_2 reduction (N_2-ase) activity and N_2-fixation in N-free medium after 3 weeks' of growth. Six of Anabaena and four of Nostoc and all the four strains of Cylindrospermum showed comparatively better C_2H_2 reduction activity in culture medium. But this was not always correlated with the nitrogen fixed in the medium. The results indicated that the slightly acidic rice field soils of Coochbehar and Jalpaiguri in West Bengal could harbour the growth of nitrogen fixing blue-green algae and suggested the possibility of their 'algalization' by isolating and mass culturing the acid tolerant forms.

Use of pesticides, has become imperative in modern rice culture. In an incubation experiment the influence of carbofuran (2,3-dihydro-2, 2-dimethyl-7-benzofuranyl-N-methyl carbamate), one of the most widely used insecticide in rice cultivation, on blue-green alga, Aulosira fertilissima was studied in relation to growth, N_2-fixation and the pattern of accumulation of fixed-N into different forms in culture medium and in two rice soils viz. alluvial and laterite under waterlogged condition. In culture medium, carbofuran at the concentrations of 10 and 25 $\mu g\ ml^{-1}$ significantly increased the chlorophyll content and bio-mass (dry weight) of the organism, with a simulatneous increase in the intra-and extra-cellular nitrogen content. However, higher doses of carbofuran were found to be toxic in this respect. In soil cultures, the tolerance limit of this alga to carbofuran was found to be much higher. Inoculation with Aulosira fertilissima caused considerable fixation of nitrogen in both alluvial and laterite soil. Application of carbofuran even upto higher level (200 $\mu g\ g^{-1}$ soil) significantly encouraged the N_2-fixation in latter soil while the magnitude of fixation in case of former soil was not hampered. Such algal N_2-fixation equally benefitted the soils in accumulating hydrolyzable and inorganic ($NH_4^+ + NO_3^- + NO_2^- - N$) forms of nitrogen. The net mineralization of nitrogen due to the growth of blue-green alga had also shown an increase in presence of carbofuran. The results indicated that application of carbofuran profoundly influenced the growth and N_2-fixation by blue-green alga Aulosira fertilissima and the release of fixed-N into available pool in rice soil.

To study the algal N_2-fixation in soil system and the influence on their growth of heterotrophic root associative N_2-fixation at different growth stages of wet land rice plant, a green house pot experiment was conducted by inoculating the soil with the mixed cultures of Aulosira fertilissima, Nostoc *muscorum* and *Anabaena* sp. in presence or absence of phosphorus and nitrogen. The results showed that inoculation of blue-green algae increased the nitrogenase activity in soil during tillering, maximum-tillering, Grain formation and harvesting stages both under unfertilized (110%, 400%, 250%, 30% respectively) and fertilized condition. The effect was prominent at the maximum tillering and the grain formation stage. Inoculation in presence of phosphorus was less effective in this respect, due to stimulation of the nitrogenase activity in soil by phosphorus alone. Inoculation, on the otherhand, remarkably increased the root-associative nitrogenase activity after maximum tillering stage of the crop particularly under N-fertilized (60 kg N ha^{-1}) condition. Inoculation also increased the grain and straw yield of rice (var. IR 50) excepting in combination with N (60 kg ha^{-1}). N_2-ase activity in soil system at tillering stage and that associated with roots at maximum tillering, grain filling stage was significantly correlated with grain yield. The results indicated that inoculation of N_2-fixing blue-green algae in soil might have an indirect influence on the bacterial N_2-fixation associated with the roots of rice plant, while increasing the N_2-ase activity in soil and yield of rice.

Two field experiments were conducted with the inoculation of the soil based cultures of blue-green algae (*Aulosira fertilissima* + *Nostoc muscorum* + *Nostoc commune*) in presence of (i) chemical fertilizers like urea and super phosphate and (ii) organic and green manures like Sesbania aculeata, Neem cake and FYM in kharif 1984 and 1985, with the objectives to understand how far such fixation of nitrogen by algae can benefit the nitrogen status of soil and yield of rice. The results obtained are summarized below :

i) Inoculation of blue-green algae brought about a persistent increase (4 - 40%) of inorganic forms of N (NH_4^+ + NO_2^+ + NO_3^- -N) in soil. The efficiency of inoculation in presence of various inorganic and organic N inputs and superphosphate was in the order of Superphosphate > Superphosphate + Urea-N > FYM Sesbania (Dhaincha) > Urea-N.

ii) Inorganic nitrogen content in soil solution generally decreased with the age of the crop excepting in Neemcake and FYM treated plots. Inoculation of blue-green algae alone or in presence of Urea-N significantly increased This form of nitrogen in soil solution at the initial stage of crop growth (30 DAI). The insignificant increase due to inoculation in presence of Sesbania was short lived, but the same under Neemcake continued upto later stage (60 DAI) of crop growth. Almost the same trend was observed with FYM.

iii) Available nitrogen content of the soil generally increased (6-17%) due to inoculation of blue-green algae but the increase was always statistically insignificant. Among the inorganic and organic N inputs, Sesbania (Dhaincha) and Neemcake was more effective in this respect.

iv) An increase of 3-12.5% in hydrolysable-N (HL-N) content of soil due to inoculation of blue-green algae either alone or inpresence of organic and inorganic N-sources showed almost a similar pattern of changes as that of the available nitrogen content. The non-hydrolyzable-N (NHL-N) content of the soil decreased at later stage (60 DAI) from their respective values at 30 DAI. No consistent influence of inoculation was observed on this form of nitrogen in soil.

v) Inoculation of blue-green algae always brought about an increase in post harvest total nitrogen content of the soil. However, the magnitude of increase was highest, equivalent to 61.6 kg N ha^{-1}, in case of inoculation alone. The increase due to inoculation in presence of other inputs was insignificant.

vi) Inoculation of blue-green algae increased grain yield by about 13 percent. Superphosphate encouraged while urea-N suppressed the efficiency of inoculation in this respect. Among the organic N-sources used, inoculation was effective in the order of FYM $>$ Sesbania

(Dhaincha) > Neemcake, in increasing the grain yield. Increase in the straw yield also showed the similar trend.

vii) Simple correlation studies between inorganic-N content in soil and grain and straw yield of rice showed positive but insignificant values. However, the significant correlations were established between the yields and available and HL-N content in soil at 60 DAI.

viii) The uptake of nitrogen was increased due to inoculation and followed almost the similar trend with that of grain and straw yield, excepting that with urea-N. The low uptake of N associated with increased yield due to inoculation in presence of urea N indicated beneficial effect of inoculation other than nitrogen input.

The results indicated that inoculation of blue-green algae was beneficial for the improvement of N-fertility status of the soil as well as for the increase in grain and straw yield of rice (var. IR 36). These benefits were more pronounced in presence of phosphorus application.

CHAPTER NO. 6

FUTURE SCOPE OF RESEARCH

FUTURE SCOPE OF RESEARCH

The questions which occured and remained unanswered during the course of the investigation and which should be taken into consideration for further studies are -

1. What are the factors/combination of factors govern the presence and activity of nitrogen fixing blue-gren algae in a soil ?
2. What is the mechanism that protects blue-green algae from acidic soil pH ? Whether it is due to a favourable microniche in soil or related with physiology of blue-green algae or a combination of both ?
3. How to determine algal bio-mass in soil more accurately and easily ?
4. How does carbofuran (insecticide) stimulates growth and nitrogen-fixation by blue-green algae ? Radio tracer studies may be employed.
5. How does the growth of blue-green algae interact with other different nitrogen-fixing groups of organisms in wetland paddy ecosystem ?
6. What is the role of blue-green algae in total balance sheet of nitrogen in paddy soils.
7. What are the agronomic practices apart from phosphorus application favour growth of and nitrogen fixation by blue-green algae.

CHAPTER NO. 7

BIBLIOGRAPHY

B I B L I O G R A P H Y

Abdullah, H.A.; Al-Mousawi and Brian A. Whitton.(1983) Influence of environmental factors on algae in rice-field soil from the Iraqi marshes. Arab Gulf J. Sci. Res., 1(1) : 237-253.

Abu-el-Fadl, M.; El-Nawawy, A.S.; El-Mofty, M.; El-Nady, M. and Farag, F.A. (1964) Nitrogen fixation by the blue-green alga, Tolypothrix tenius, as influenced by ammonium sulphate, compost, straw and superphosphate with special reference to their effect on rice yield. J. Soil Sci., UAR, 4 : 31-104.

Ahmad, M.H. and Venkataraman, G.S. (1973) Tolerance of Aulosira fertilissima to pesticides. Curr. Sci., 42 : 108.

Aiyer, R.S. (1965) Comparative algological studies in rice fields in Kerala State. Agric. Res. J. Kerala, 3 : 100-104.

Aiyer, R.S.; Salahudeen, S. and Venkataraman, G.S.(1972) Long-term algalization field trial with high-yielding varieties of rice (Oryza sativa L.). Indian J. Agric. Sci., 42 : 380-383.

Alimagno, B.V. and Yoshida, T. (1977) In-situ acetylene-ethylene assay of biological nitrogen fixation in low land rice soils. Pl. & Soil, 47 : 239-244.

Allen, M.B. (1956) Photosynthetic nitrogen fixation by blue-green algae. Sci. Monthly, 83 : 100-106.

Allen, M.B. and Arnon, D.I. (1955) Studies on nitrogen-fixing blue-green algae I.Growth & nitrogen fixation by Anabaena cylindrica Lemm. Pl. Physiol., 30 : 366-372.

Amma, P.A.; Aiyer, R.S. and Subramoney, N. (1966) Occurrence of blue-green algae in acid soils of Kerala. Agri. Res. J. Kerala, 4 : 141-143.

Anonymous (1965) Final report of the ICAR scheme for the study of nitrogen fixing blue-green algae in rice soils of Madras State.

Anonymous (1985) Report of the Industrial Toxicology Research Centre on pesticide, environment and safety. Quoted from Debacle : II(1) : 21-31.

Batterton, J.C.; Bousch, S.M. and Matsumura, M. (1971) Growth responses of blue-green algae to aldrin, endrin and their metabolites. Bull. Environm. Toxicol., 6 : 589-594.

Black, C.A. (1965) Methods of Soil Analysis, Am. Soc. Agron., Madison, Wisconsis, USA.

*Bortels, H. (1940) Ueber die Bedeutung des Molybdans fur Stickstoffbindende Nostocaceen. Arch. Microbiol., 2 : 155-186.

Bottomley, P.J.; Grillo, J.F.; Van Ballen, C. and Tabita, F.R. (1977) Synthesis of nitrogenase and heterocyst by Anabaena sp. CA in the presence of high levels of Ammonia. J. Bacteriol., 140 : 938-943.

Bremner, J.M. (1965) Organic forms of nitrogen, In : Methods of Soil Analysis, Part-2, (Ed. C.A. Black), Agron. Ser. No.9, Am. Soc. Agron. Inc. Madison, Wisconsin, U.S.A. pp.1238-1254.

Bremner, J.M. and Keeney, D.R. (1966) Determination of exchangeable ammonium, nitrate, nitrite by extraction distillation method. Soil Sci. Soc. Amer. Proc., 30 : 577-587.

Bunt, J.S.(1961) Nitrogen fixing blue-green algae in Australian rice soils. Nature, 192 : 479-480.

Burris, R.H.; Eppling, F.J.; Wahlin, H.B. and Wilson, P.W.(1943) Detection of nitrogen fixation with isotopic nitrogen. J. Biol. Chem., 148 : 349-357.

Cameron, R.E. and Fuller, W.H. (1960) Nitrogen fixation by some soil algae in Arizona Soils. Proc. Soil Sci. Soc. Amer. 24 : 353-356.

Chopra, T.S. and Dube, J.N. (1971) Changes of nitrogen content of a rice soil inoculated with Tolypothrix tenius. Pl. & Soil., 35 : 453-462.

Clark, F.E. and Durrell, L.W. (1965) In :Methods of soil Analysis (Part-2), Agronomy series No.9 (Black, C.A. ed.), Amer. Soc. Agron, P. 1506.

Das, B. and Singh, P.K. (1977a) Detoxication of the pesticide benzenehexachloride by blue-green algae. Microbios Letters., 4 : 99-102.

Das, B. and Singh, P.K. (1977b) Relative tolerance of a bloom forming blue-green alga Microcystis flos-aquae to pesticides. Nova Hedwigia.

Das, B. and Singh, P.K. (1977c) Pesticide (hexochlorocyclohexane) inhibition of growth and nitrogen fixation in blue-green algae Anabaenopsis raciborskii and Anabaena aphanizomenoides. Z. Allg. Mikrobiol., 18 : 161-167.

Davis, E.B.; Tischar, R.G. and Brown, L.R.(1966) Nitrogen fixation by the blue-green alga Anabaena flosaquae A-37. Physiol. Plantarum., 19 : 823-826.

De, P.K. (1936) The problem of the nitrogen supply of rice. I. Fixation of nitrogen in the rice soils under water-logged conditions. Indian J. Agric. Sci., 6 : 1237-1245.

De, P.K. (1939) The role of blue-green algae in nitrogen fixation in rice fields. Proc. R. Soc. Lond., 127 B : 121-139.

De, P.K. and Mandal, L.N. (1956) Fixation of nitrogen by algae in rice soils. Soil Sci., 81 : 453-458.

De, P.K. and Sulaiman, M. (1950) The influence of algal growth in rice fields on the yield of crops. Indian J. Agric. Sci., 20 : 327-342.

Desikachary, T.V. (1959) Cyanophyta, ICAR, New Delhi.

*Drewes, K. (1928) Ueber die Assimilation des Luftstickstoffs durch Blaualgen, Zentr. Backt. Parasi Tenk, Abstr., 111 76 : 88-101.

*Dugdale, R.C. and Goering, J.J. (1967) Uptake of new and regenerated forms of nitrogen in primary productivity, Limnol. Oceanogr., 12 : 196-206.

El-Ayouty, E.Y.M. (1966) Cited by El-Nawawy, A.S. and Hamdi, Y.A. (1975).

El-Borollosy, M.A. (1972) Cited by El-Nawawy, A.S. and Hamdi, Y.A. (1975).

El-Nawawy, A.S.; Abou-el-Fadl, M. and Nada, M.M.(1962) Economical studies on algae in Egypt (Part-1). Effect of new isothiouronium derivatives of arylmercaptoalkane carboxylic acids on the paddy soil flora of algae in Egypt.Soil Sci., UAR, 2 : 3-14.

El-Nawawy, A.S. and Hamdi, Y.A.(1975) Research on blue-green algae in Egypt, 1958-72. In : Nitrogen fixation by free living microorganisms, (ed. W.D.P.Stewart). Cambridge Univ. Press., London, pp. 219-228.

Fay, P. and Fogg, G.E.(1962) Studies on nitrogen fixation by blue-green algae III. . Growth and nitrogen fixation in Chlorogloea fritschii Mitra. Arch. Mikrobiol., 42 : 310-321.

Fogg, G.E. (1942) Studies on nitrogen fixation by blue-green algae, I. Nitrogen fixation by Anabaena cylindrica.Lemm. J.Exp. Biol., 19 : 78-87.

Fogg, G.E. (1951) Studies on nitrogen fixation by blue-green algae II. Nitrogen fixation by Mastigocladus laminosus.J. Exp. Bot. 2 : 117-120.

Fogg, G.E.; Stewart, W.D.P. and Walsby, A.E. (1973) The Blue-green algae. Academic Press, London, England.

Gangawane, L.V. (1979) Tolerance of thimet by nitrogen-fixing blue-green algae. Pesticides., 13(3) : 33-34.

Gangawane, L.V., and Saler, R.S. (1979) Tolerance of certain fungicides by nitrogen fixing blue-green algae. Curr. Sci., 48 : 306-308.

Gilmour, J.T.; Gilmour, C.M. and Johnson, T.H. (1978) Nitrogenase activity of rice plant root systems. Soil Biol. Biochem., 10 : 261-264.

Gonzalves, E.A. and Gangala, K.S. (1949) Observations of the algae of paddy soils. Bombay, U.J. Sec. B. Biol. Sci. (n.s.) 18 : 51-59.

Goyal, S.K. (1985) Effect of different sources of combined nitrogen on algalization. Phykos,, 24 : 149.

Goyal, S.K. and Venkataraman, G.S.(1970) Effect of algalization on high yield rice varieties Part-I. Response of rice varieties. Phykos,, 9 : 137-138.

Goyal, S.K. and Venkataraman, G.S. (1971) Response of high yielding rice varieties to algalization, Part-2. Phykos,, 10 : 32-33.

Goyal, S.K. and Venkataraman, G.S. (1972) Cited by Venkataraman, 1975, In : Alcal Biofertilizers and Rice cultivation, To-day and To-morrow's Printers and Publishers, New Delhi.

Grant, I.F. and Seegers, R. (1985) Tubificid role in soil mineralization and recovery of algal nitrogen by low land rice. Soil. Biol. Biochem., 17 : 559-563.

Gupta, A.B. and Shukla, A.C. (1967) Studies on the nature of algal growth promoting substances and their influence on growth, yield and protein content of rice plants. Labdev. J. Sci. Technol., Kanpur, 5 : 162-163.

Habte, M. and Alexander, M. (1980) Nitrogen fixation by photosynthetic bacteria in low land rice culture. Applied and Environmental Microbiology., 39 : 342-347.

Henriksson, E.; Henriksson, L.E. and Dasilva, E.J. (1975) A comparison of nitrogen fixation by algae of temperate and tropical soils. In : Nitrogen Fixation by Free-living Micro-organisms, Intl. Biol. Programme-6 (Stewart, W.D.P. ed.), Cambridge Univ Pres, Cambridge.

*Herisset, A.(1952) Influence de la lumiere sur la fixation biologique de l' azote par le Nostoc commune, Bull. Soc. Chem. Biol., Paris, 34 : 532.

*Hosoda, K. and Takata, H. (1955) Effect of nitrogen fixing blue-green algae Tolypothrix tenius on the growth of rice plants (in Japanese). Trans. Tottori Soc. Agr.Sci., 10 : 1-15.

*Ibrahim, A.N. (1972) Effect of certain herbicides on growth of nitrogen-fixing algae and rice plants. Symp. Biol. Hung., 11 : 445-448.

*Ibrahim, A.N.; Kamel, M. and El-Sherbeny, M. (1971) A Tolypothrix tenius algaval torteno oltas hatasa a rizs termesere es a talaj nitrogen - merlegere (Effect of inoculation with alga Tolypothrix tenius on the yield of rice and soil nitrogen balance) (English Summary). Agrokem. Talajtan, 20 : 389-400.

*Inger, L. (1970) Effect of two herbicides on nitrogen fixation by blue-green algae. Sven. Bot Tidskr., 64 : 460-461.

Ishizawa, S. and Matsuguchi, T. (1966) Effects of pesticides and herbicides upon microorganisms in soil and water under waterlogged condition. Bull. Nat. Inst. Agric. Sci., B. 16 : 1-90.

Jackson, M.L. (1967) Soil Chemical Analysis, Prentice Hall, Inc., New Jersey, USA.

Jha, K.K.; Ali, M.A.; Singh, R. and Bhattacharya, P.B. (1965) Increasing rice production through the inoculation of *Tolypothrix tenius*, a nitrogen fixing blue-green alga. J. Indian Soc. Soil Sci., 13 : 161-166.

Jutono (1973) Blue-green algae in rice soils of Jog-jakarta, Central Java.,Soil Biol. Biochem., 5 : 91-95.

Kaushik, B.D. (1987) Response of Cyanobacterial nitrogen fixation to exogenous nitrogen. Acta. Botanica. Indica. 15 : 80-85.

Kar, S. and Singh, P.K. (1977) Toxicity of carbofuron to the blue-green alga *Nostoc muscorum*. Bull. Enviorn. Contam. Toxicol. 20; 707.

Khurana, A.S. and Venkataraman, G.S.(1968) Algal and fungal flora of paddy soils. Indian. J. Microbiol. 8; 91-94.

* Konishi, C. and Seino, K. (1961) Bull. Hokuriku agric. Expt. Sta. 2; 41, Cited by Watanabe (1965).

Lakshmi Kumari, M.; Kavimandan, S.K. and Subba Rao, N.S.(1976) Occurence of N_2-fixing Spirillum in roots of rice, sorghum, maize and other plants. Indian J. Exp. Biol., 14 : 638-639.

Lal, S.; Saxena, D.M. and Lal, R. (1987) Effects of fenitrothion and chloropyrifos on growth, photosynthesis and nitrogen fixation in Anabaena (Arm 310) and Aulosira fertilissima. Agriculture. Ecosystems and Environment, V., 19(3) : 197-209.

MacRae, I.C. and Castro, T.F. (1967) Nitrogen fixation in some tropical rice soils. Soil Sci., 103 : 277-280.

Mandal, S.C.; Sinha, M.K. and Sinha, H. (1982) Acid soils of India and liming. ICAR, New Delhi, pp.-126.

*Materasi, R. and Balloni, W. (1965) Quelques observations sur la presence de microorganisms autrotrophes fixateurs d'azote dans les rizieres (in French, English Summary). Ann. Inst. Pasteur, Paris 3, Suppl., 218-223.

Mayland, H.F. and McIntosh, T.H. (1966) Availability of biologically fixed atmospheric 15_N to higher plants. Nature., 209 : 421-422.

Megharaj, M.; Venkateswarlu, K & Rao, A.S. (1988) Tolerance of algal population in rice soil to carbofuran application. Curr. Sci., 57 : 100-102.

Mian, M.H. and Stewart, W.D.P. (1985) Fate of nitrogen applied as Azolla and Blue-green algae (Cyanobacteria) in waterlogged rice soils - a 15_N tracer study. Pl.& Soil., 83 : 363-370.

Mitra, A.K. (1951) The algal flora of certain Indian soils. Indian J. Agric. Sci., 21 : 357.

Mitra, A.K. (1961) Some aspects of fixation of elementary nitrogen by blue-green algae in the soil. Proc. Natl. Acad. Sci. India, 31 : 98-99.

Mudholkar, N.J.; Sahay, M.N. and Sankaram, A. (1968) Effect of blue-green algae and urea spray on the high yielding varieties. Oryza., 5 : 59-62.

Muralikrishna, P.V.G. and Venkateswarlu, K. (1984) Effect of insecticides on soil algal population. Bulletin of Environmental contamination and Toxicology., 33 : 241-245.

Nayak, D.N. and Rao, V.R. (1977) Nitrogen fixation by *Azospirillum* spp. from rice roots. Arch. Microbiol., 115 : 359-360.

*Nishigaki, S.; Shibuya, M. and Kodaihara, K. (1951) J. Sci. Soil Manures., 22 : 69, Cited by Watanabe, 1965.

*Nishigaki, S., Shibuya, M. and Kodaira, K. (1953) J.Sci. Soil and Manures., 23 : 150, Cited by Watanabe, 1965.

Okuda, A. and Yamaguchi, M.(1952) Algae and atmospheric nitrogen fixation in paddy soils. II Relation between the growth of blue green algae and physical or chemical properties of soil and effect of soil treatments and inoculation in nitrogen fixation. Mem. Res. Inst. Food Sci. Kyoto Univ., 4 : 1-11.

Okuda, A. and Yamaguchi, M. (1955) Nitrogen fixing micro-organisms in paddy soils. I. Characteristics of nitrogen fixation in paddy soils. Soil and Plant Food., 1 : 102-104.

Okuda, A. and Yamaguchi, M.(1956a) Distribution of nitrogen fixing microorganisms in paddy soils in Japan. VI Cong. Int. Sci. Sol. Rap. C., pp.521-526.

Okuda, A. and Yamaguchi, M. (1956b) Nitrogen fixing microorganisms in paddy soils. II. distribution of blue-green algae in paddy soils and the relationship between the growth of them and soil properties. Soil and Plant Food., 2 : 4-7.

Pandey, A.K. (1985) Effects of propanil on growth and cell constituents of Nostoc calcicola. Pesticide Biochemistry and physiology., 23 : 157-162.

Pandey, D.C. (1965a) A study of the algae from paddy field soils of Balia and Ghazipur districts of Uttar Pradesh, India. I. Culture and ecological conditions. Nova Hedwigia., 9 : 299-334.

Pandey, D.C. (1965b) A study on the algae from paddy field soils of Balia and Ghazipur districts of Uttar Pradesh, India. II. Taxonomic considerations of Cyanophyceae. Nova Hedwigia., 10 : 177-209.

*Pankow, H. (1964) Die Bindung Von Luftstickstoff durch Zwei Weitere Blauaigen - Arten, Fischrella muscicola and Und Fischrella major. Naturwissenschaften, 51 : 274-275.

*Pankow, H. and Martens, B. (1964) Ueber Nostoc sphaericum Arch. Microbiol., 48 : 203.

Pattanaik, H. (1966) Growth and nitrogen fixation by Westiellopsis prolifica Janet. Ann. Bot. N.S., 30 : 231-238.

Pattanaik, U. and Singh, P.K. (1977) Effect of nitrate nitrogen on the growth, heterocyst and nitrogen fixation of rice field's blue-green alga Gloeotrichia sp., Algological studies, cited by Singh (1978).

Piper, C.S. (1950) Soil and Plant Analysis, Interscience Inc., New York.

Prasad, S. (1949) Nitrogen recuperation by blue-green algae in soils of Bihar and their growth on different types. J. Proc. Instn. Chemists India, 21 : 135-140.

Raghu, K. and MacRae, I.C. (1967) The effect of gamma isomer of benzene hexchloride upon the microflora of submerged rice soils. I. Effect upon algae. Can. J. Microbiol., 13 : 173-180.

Rai, A.N.; Rowell, P. and Stewart, W.D.P. (1980) Ammonia assimilation and N_2-ase regulation in the lichen, New Phytol., 85 : 545.

*Ramaswami, K.; Rajagopalan, S. and Jagadeesan, M. (1964) Madras agri. J. 51 : 84 cited from Venkataraman, 1972.

Reddy, K.R. and Patrick (Jr.), W.H. (1979) Nitrogen fixation in flooded soil. Soil Sci., 128 : 80-85.

Relwani, L.L. and Manna, G.B. (1964) Effect of blue-green algae in combination with urea on rice yield. Curr. Sci., 33 : 687.

Relwani, L.L. and Subrahmanyan, R. (1963) Role of blue-green algae, chemical nutrients and partial soil sterilization on paddy yield. Curr. Sci., 32 : 441-443.

Renaut, J.;Sasson, A.; Pearson, H.W. and Stewart, W.D.P.(1975) Nitrogen fixing algae in Morocco. In : (W.D.P.Stewart, ed.) Nitrogen Fixation by Free Living Microorganisms. Cambridge University Press, Cambridge, pp. 229-249.

Rinaudo, G.; Balandreau, J. and Dommergues, Y. (1971) Algal and bacterial non-symbiotic nitrogen fixation in paddy soils. Plant & Soil spec. Vol., 471-479.

Rippka, R.; Neilson, A.; Kunisawa, R. and Cohen - Bazire, G. (1971) Nitrogen fixation by unicellular blue-green algae. Arch. Mikrobiol., 76 : 341-348.

Rippka, R. and Waterbury, J.B. (1977) The synthesisof nitrogenase by non-heterocystous cyanobacteria. FEMS Microbiology Letters., 2 : 83-86.

Rogger, P.A.; Grant, I.F.; Reddy, P.M. and Watanabe, I. (1987) The photosynthetic aquatic biomass in wetland rice fields and its effect on nitrogen dynamics. In : Efficiency of Nitrogen Fertilizers for Rice. Proc. of the Meeting of the International Net work on soil Fertility and Fertilizer Evaluation for Rice, New South Wales, Australia (1985). IRRI, Los Banos, Philippines. pp. 43-68.

Roger, P.A. and Kulasooriya, S.A. (1980) Blue-green algae and Rice. International Rice Research Institute, P.O.Box 933, Manila, Philippines, 112 p.

Safferman, R.S. and Morris, M.E. (1964) Growth characteristics of the blue-green algal virus LPP-1. J. Bacteriol., 88 : 771-775.

Saha, K.C. and Mandal, L.N. (1979) Distribution of nitrogen fixing blue-green algae in some rice soils of West Bengal. J. Indian. Soc. Soil Sci., 27(4) : 470-477.

Saha, K.C. and Mandal, L.N. (1980a) Fixation of nitrogen by blue-green algae in acidic and lateritic rice soils of West Bengal. J. Indian. Soc. Soil Sci., 28(1) : 98-102.

Saha, K.C. and Mandal, L.N. (1980b) Influence of urea on the nitrogen accretion due to blue-green algae. India J. Agric. Sci., 50(5) : 431-433.

Saha, K.C. and Mandal L.N. (1980c) A greenhouse study on the effect of inoculation of N-fixing blue-green algae in an alluvial soil treated with P and Mo on the yield of rice and changes in the N-content of soil. Pl. & Soil., 57 : 23-30.

Saha, K.C.; Panigrahi, B.C. andSingh, P.K. (1982) Blue-green algae or Azolla additions on the nitrogen and phosphorus availability and red-ox potential of a flooded rice soil. Biol. Biochem., 14 : 23-26.

Saha, K.C.; Sannigrahi, S.; Bandyopadhyay, S.K. and Mandal, L.N. (1984) Effect of phorate on nitrogen fixation by blue-green algae. J. Indian Soc. Soil Sci., 32 : 79-83.

Sahay, M.N. (1972) Comparative efficiency of algal species in conjunction with inorganic nitrogen on the growth and yield of rice. Annual Tech. Report, CRRI, Cuttack, India, pp. 155-156.

Sahrawat, K.L. and Burford, J.R. (1982) Modification of the alkaline permanganate method for assesing the availability of soil nitrogen in upland soils. Soil Sci., 133(1) : 53-57.

Sankaram, A. (1971) Work Done on Blue-green algae in Relation to Agriculture. Indian Council of Agricultural Research, New Delhi, p.28.

Sankaram, A.; Mudholkar, M.J. and Sahay, M.N. (1967) Inoculation of blue-green algae on the yield of rice under field conditions. Indian. J. Microbiol., 7 : 57-62.

Sardespande, J.S. and Goyal, S.K. (1981) Distributional pattern of blue-green algae in rice field soils of Konkan region of Maharastra State. Phykos, 20 : 102-106.

Shakeeb, M.A. (1970) Nitrogen fixation in some cyanophycean algal members, University of Cairo, A.R.E., cited by El. Nawawy and Hamdi, Y.A. (1975) In : Nitrogen Fixation by free living Micro-organisms. Int. Biol. Programme-6 (ed. W.D.P. Stewart) Cambridge Univ. Press, Cambridge.

* Shioiri, M and Misui, S. (1935) On the chemical composition of some algae and weeds developing in the paddy field and their decomposition in the soil (in Japanese). J. Sci. Soil Manure, Japan 9 : 261-268. Quoted from Roger et al, (1987).

Shtina, E.A.; Pankratova, E.M.; Perminova, G.N.; Tretjakova, A.N. and Young, L.A. (1968) The distribution and the role of nitrogen fixing blue-green algae in the soils of the temperate zone of the USSR. 9th Int. Cong. Soil Sci. Trans., 2 : 151-158.

*Silva, M.E.S. and Dobereiner, J. (1978) Occurence of Azospirillum spp. in soils and roots. Basic Life Sciences., 10 : 372.

Sims, G.K. and Dunigan E.P. (1984) Diurnal and Seasonal variations in nitrogenase activity (C_2H_2-reduction) of rice roots. Soil Biol. Biochem., 16 : 15-18.

*Singh, D.N.; Venkataraman, G.S.; Rao, S.B.P.; Bhattacharya, A. and Goyal, S.K. (1972) Effect of algal inoculation on Jaya rice variety under field conditions. Laldev, J.Sci. Technol., 10-13 : 107-108.

Singh, H.N. and Vaishampayan, A. (1978) Biological effects of rice-field herbicide "Machete" on various strains of the nitrogen-fixing blue-green alga Nostoc muscorum. Environ. Expt. Bot., 18 : 87-94.

*Singh, H.N.; Singh, H.R. and Vaishampayan, A. (1979) Toxic and mutagenic action of the herbicide Alachlor (Lasso) on various strains of the N_2-fixing blue-green alga Nostoc muscorum and characterization of the herbicide induced mutants resistant to Methylamine and L-methionine -DLO sulfoximine. Environ. Expt. Bot., 19 : 5-12.

Singh, P.K. (1971) Blue-green algae. CRRI, Annual Report CRRI, Cuttak, Orissa. pp.120-127.

Singh, P.K. (1973) Effects of pesticides on blue-green algae. Arch. Mikrob., 89 : 317-320.

Singh, P.K. (1974) Algicidial effect of 2,4-dichlorophenoxy acetic acid on blue-green alga Cylindrospermum sp. Arch. Mikrobiol., 97 : 69-72.

Singh, P.K. (1975) Fertilizers tolerance of blue-green algae and their effect on heterocyst differentiation. Phykos., 14 : 81-88.

Singh, P.K. (1978) Nitrogen economy of rice soils in relation to nitrogen fixation by blue-green algae and Azolla. In : Proc. National Symposium on increasing rice yield in kharif. Central Rice Research Institute, Cuttack, India. pp. 121-239.

Singh, R.N. (1942) The fixation of elementary nitrogen by some of the commonest blue-green algae from the paddy field soil of the United provinces and Bihar. Indian J. Agric. Sci., 2 : 743-756.

Singh, R.N. (1961) The Role of Blue-green algae in Nitrogen Economy of Indian Agriculture. ICAR, New Delhi, p175.

Stewart, W.D.P. (1962) Fixation of elemental nitrogen by marine blue-green algae. Ann. Bot. NS., 26 : 439-445.

Stewart, W.D.P. (1964) The effect of available nitrate and ammonium nitrogen on the growth of two nitrogen fixing blue-green algae. J. Exp. Bot., 15 : 138-145.

Stewart, W.D.P. (1967) Transfer of biologically fixed nitrogen in sand dune slack region. Nature, 214 : 603-604.

Stewart, W.D.P. (1973) Nitrogen Fixation. In : The Biology of Blue-Green Algae (eds. N. Carr and B.A. Whitton), Blackwell Scientific Publications, Oxrofd. pp.260-278.

Stweart, W.D.P. (1980) Systems involving blue-green algae. In : Methods for Evaluating Biological Nitrogen Fixation. (ed. F.J. Bergorson), John Willey & Sons Ltd., pp.584-635.

Stewart, W.D.P. and Lex, M. (1970) Nitrogenase activity in the blue-green algae Plectonema boryanum strain 594. Arch. Mikrobiol., 73 : 250-260.

Stewart, W.D.P.; Rowell, P.P.; Ladha, J.K. and Sampaio, J.A.M. (1979) Blue-green algae (Cyanobacteria) some aspects related to their role as sources of fixed nitrogen in paddy soils. In : Nitrogen and Rice, IRRI, Los Banos, Philippines, pp. 263-386.

Subrahmanyan, R. (1972) Some observations on utilization of blue-green algal mixtures in rice cultivation in India. In : Proceedings of the 1st International Symposium on Taxonomy and Biology of Blue-green algae. Madras, India, 1970. pp. 281-293.

Subrahmanyan, R.; Relwani, L.L. and Manna, G.B. (1964a) Observations on the role of blue-green algae on rice yield compared with that of conventional fertilizers. Curr. Sci., 33 : 485-486.

Subrahmanyan, R.; Relwani, L.L. and Manna, G.B. (1964b) Role of blue-green algae and different methods of partial sterilization on rice yield. Proc. Indian Acad. Sci. Sect., 60B : 293-297.

Subrahmanyan, R.; Manna, G.B. and Patnaik, S. (1965a) Preliminary observations on the interaction of different rice soil types to inoculation of blue-green algae in relation to rice culture. Proc. Indian Acad. Sci. Sect., 62B : 171-175.

Subrahmanyan, R.; Relwani, L.L and Manna, G.B. (1965b) Fertility build-up of rice field soils by blue-green algae. Proc. Indian. Acad. Sci., 62B : 252-277.

Subrahmanyan, R.; Relwani, L.L and Manna, G.B. (1965c) Nitrogen enrichment of rice soils by blue-green algae and its effect on the yield of paddy. In : symposium on land fertility improvement by blue-green algae. Proc. Nat. Acad. Sci. India, Sect. A, 35 : 382-386.

Sundara Rao, W.V.B. (1964) Soil inoculants - bacterial and algal. In : Handbook of Manures and Fertilizers., ICAR, New Delhi., pp. 240-252.

Sundara, Rao, W.V.B.; Goyal, S.K. and Venkataraman, G.S.(1963) Effect of inoculation of *Aulosira fertilissima* on rice plants. Curr. Sci., 32 : 366-367.

Taha, M.S. (1963) Isolation of some nitrogen fixing blue-green algae from the rice fields of Egypt, in pure culture. Mikrobiologiya, U.S.S.R., 32 : 397-403.

Taha, M.S. (1964) The effect of nitrogen compounds upon growth of blue-green algae and fixation of molecular nitrogen by them. Mikrobiologiya, U.S.S.R. 33 : 397-403.

Tirol, A.; Roger, P.A. and Watanabe, I. (1982) Fate of nitrogen from a blue-green alcae in a flooded rice Soil. Soil Sci. Plant Nutrit., 28 : 559-569.

*Vaishampayan, A.; Singh, H.R. and Singh, H.N. (1978) Biological effects of rice field herbicide "Stam F-34" on various strains of N_2-fixing blue-green alga *Nostoc muscorum*. Biochem. Physiol. Pflanz., 173 : 410-419.

Venkataraman, G.S. (1961a) Nitrogen fixation by *Stigonema dendroideum* Fremy. Indian J. Agric. Sci., 31 : 213-215.

Venkataraman, G.S. (1961b) Studies on nitrogen fixation by blue-green algae. II. Nitrogen fixation by *Cylindrospermum sphaerica* Prasad. Under various conditions. Proc. Nat. Acad. Sci., 31A : 100-104.

Venkataraman, G.S. (1962) Studies on nitrogen fixation by blue-green algae. III. Nitrogen fixation by *Anabaena azollae*. Indian. J. Agric. Sci., 32 : 22-24.

Venkataraman, G.S. (1972) Algal Biofertilizers & Rice Cultivation. To-day and Tomorrow's Printers and Publishers, New Delhi p.75.

Venkataraman, G.S. (1975) The role of blue-green algae in tropical rice cultivation. In : Nitrogen Fixation by Free Living Microorganisms (ed. W.D.P. Stewart), Cambridge University Press, Cambridge. pp.207-218.

Venkataraman, G.S.(1977) Blue-green algae as a biological N-input in rice cultivation, Proc. Nat. Symp. Nitrogen Assimilation and crop productivity, Hissar, India, pp. 132-141.

Venkataraman, G.S. (1979) Algal inoculation of rice fields. In : Nitrogen and Rice. IRRI Los Bonos. Philippines. pp. 311-321.

Venkataraman, G.S. (1981) Blue-green algae for Rice Production - A mannual for its promotion, FAO soils Bull. No.46, p.102.

Venkataraman, G.S. and Goyal, S.K. (1968) Influence of blue-green algal inoculation on crop yields of rice plants. Soil Sci. Plant Nutrit., 14 : 249-251.

Venkataraman, G.S. and Goyal, S.K. (1969a) Influence of blue-green algae on the high yielding paddy variety IR-8, Sci. and Cult., 35 : 58-59.

Venkataraman, G.S. and Goyal, S.K. (1969b) Some recent observations on the effect of nitrogen fixing blue-green algae on crop plants. Mikrobiologiya., 38 : 709-712.

Venkataraman, G.S. and Goyal, S.K. (1972) Cited from Venkataraman (1972) p.42.

Venkataraman, G.S. and Rajyalakshmi, B. (1971) Tolerance of BGA to pesticides. Curr. Sci., 40 : 143-144.

Venkataraman, G.S. and Rajyalakshmi, B. (1972) Relative tolerance of nitrogen fixing blue-green algae to pesticides. Indian J. Agric. Sci., 42 : 199.

Venkateswarlu, K. and Sethunathan, N. (1978) Degradation of Carbofuran in rice soils as influenced by repeated applications and exposure to aerobic conditions following anaerobsis J. Agric. Food Chem., 25 : 1148-1151.

Vlek, P.L.G. and Craswell, E.T. (1979) Effect of nitrogen source and management on ammonia volatilization losses from flooded rice-soil systems. Soil. Sci. Soc. Am. J. 43; 352-358.

Wada, H.; Panichsakpatana, S.; Kimura, M. and Takai, Y. (1978) Nitrogen fixation in paddy soils. I. Factors affecting N_2-fixation. Soil Sci. Plant Nutrit., 24 : 257-363.

Watanabe, A. (1951) Production in cultural solutions of some amino acids by the atmospheric nitrogen fixing blue-green algae. Arch. Biochem. Biophys., 34 : 50-54.

* Watanabe, A. (1956) On the effect of the atmospheric nitrogen fixing blue-green algae on the yield of rice (In Japanese). Bot. Mag. Tokyo.; 69 : 530-535.

Watanabe, A. (1959a) Distribution of nitrogen fixing blue-green algae in various areas of south and East Asia. J. Gen., Appl Microbiol., 5 : 21-29.

Watanabe, A. (1959b) On the mass culturing of a nitrogen fixing blue green algae Tolypethrix tenius.J. Gen. Appl. Microbiol., 5 : 85-91.

Watanabe, A. (1962) Effect of nitrogen fixing blue-green alga Tolypothrix tenius on the nitrogenous fertility of paddy soil and on the crop yield of rice plant. J. Gen. Appl. Microbiol., 8 : 85-91.

Watanabe, A. (1965) Studies on blue-green algae as green manure in Japan. Proc. Nat. Acad. Sci. India, 35 : 361-369.

Watanabe, A. (1967) The blue-green algae as the nitrogen fixators. Proc. IX. Inter. Congr. Microbiol., Moscow'66 pp.77-86.

Watanabe, A. (1973) On the inoculation of paddy fields in the Pacific area with nitrogen fixing blue-green algae. Soil Biol. Biochem., 5 : 161-162.

Watanabe, A. and Kiyohara, T. (1960) Decomposition of blue-green algae as affected by the action of soil bacteria. J. Gen. Appl. Microbiol., 5 : 175-179.

Watanabe, A. and Kiyohara, T. (1963) Symbiotic blue-green algae of lichens, liverworts and Cycads. In : Studies on Micro algae and Photosynthetic Bacteria (ed. Jap. Soc. Pl. Physiol). Tokyo, Japan, pp. 189-196.

Watanabe, A.; Nishigaki, S. and Konishi, C. (1951) Effect of nitrogen fixing blue-green algae on the growth of rice plants. Nature, 168 : 748-749.

Watanabe, A. and Yamamoto, Y. (1971) Algal nitrogen fixation in the tropics. Pl. and Soil., (Special Volume) 403-413.

Watanabe, I. (1986) Nitrogen fixation by non-legumes in tropical agriculture with special reference to wetland rice. Pl. & Soil., 90 : 343-357.

Watanabe, I. and Cholitkul, W. (1979) Field studies on nitrogen fixation in paddy soils. In : Nitrogen and Rice. International Rice Research Institute. Los Banos, Philippines, pp. 223-239.

Watanabe, I. and Lee, K.K. (1975) Non-symbiotic nitrogen fixation in rice paddies. In : Biological Nitrogen in Farming Systems of the Tropics. (Eds. A. Ayanaba and P. Dart.). John Wiley and Sons.pp. 289-305.

Watanabe, I.; Lee, K.K.; Alimagno, M.; Sato, M.; Deb Rosario, D.C. and DE Guzman, M.R. (1977) Biological N_2-fixation in paddy field studied by in situ acetylene - reduction assay. IRRI Res. Pap. Ser., 3 : 1-16.

Watanabe, L, Lee, K.K. and Alimagno, B.V. (1978a) Seasonal changes of N_2-fixing rate in rice field assayed by in situ acetylene reduction technique. I. Experiments in long-term fertility plots. Soil Sci. Plant Nutrit., 24 : 1-3.

Watanabe, I.; Lee, K.K. and DE Guzman, M. (1978b) Seasonal changes of N_2-fixing rate in rice field assayed by in situ acetylene reduction technique. II. Estimation of nitrogen fixation associated with rice plants. Soil Sci. Plant Nutrit., 24 : 465-471.

Watanabe, I.; DE Guzman, M. and Cabrera, D. (1980) Effect of nitrogen fertilizer on N_2-fixation in paddy field, measured by in situ acetylene reduction assay. Pl. & Soil.

Williams, A.E. and Burris, R.H. (1952) Nitrogen fixation by blue-green algae and their nitrogenous composition. Amer J. Bot., 39 : 340-342.

Willis, W.H. and Green, V.E. (1948) Movement of nitrogen in flooded soils planted to rice. Soil Sci. Soc. Amer. Proc., 13 : 229-237.

Wilson, J.T. and Alexander, M. (1979) Effect of soil nutrient status and pH on nitrogen-fixing algae in flooded soils. Soil Sci. Soc. Amer J., 43 : 936-939.

Wilson, J.T.; Eskew, D.L. and Habte, M. (1980) Recovery of nitrogen by rice from blue-green algae added in a flooded soil. Soil Sci. Soc. Am. J., 44 : 1330-1331.

*Winter, G. (1935) Uber die Assimilation des Luftstickstoffs durch endophytische Blaualgen. Biet. Biol. Pfl., 23 : 295-335.

Wright, S.J.L.; Stainthorpe, A.E. and Downs, J.D. (1977) Interactions of the herbicide Propanil and a metabolite 3,4-dichloroaniline, with blue-green algae. Acta. Phytopathol. Acad. Sci., 12 : 51-60.

*Wyatt, J.T. and Silvey, J.K.G. (1969) Nitrogen fixation by Glococapsa. Science, 165 : 908-909.

Yamaguchi, M. (1976) Nitrogen fixation by microorganisms in paddy soils in relation to their fertility. In : The Fertility of Paddy Soils and Fertilizer Applications for Rice. Food and Fertilizer Technology centre for the Asian and Pacific Region, Taipei, Taiwan, pp.60-75.

Yoshida, T. and Ancajas, R.R. (1971) Nitrogen fixation by bacteria in the root zone of rice. Soil Sci. Soc. Amer. Proc., 35 : 156-159.

Yoshida, T. and Ancajas, R.R. (1973) Nitrogen-fixing activity in upland and flooded rice fields. Soil Sci. Soc. Amer. Proc. 37 : 42-46.

Yoshida, T.; Roneal, R.A. and Bautista, E.M. (1973) Atmospheric nitrogen fixation by photosynthetic microorganisms in a submerged Philippine Soil. Soil Sci. Plant Nutrit., 19 : 117-123.

Zimmerman, W.; Metting, B. and Rayburn, W. (1980) The occurrence of blue-green algae in silt loams of Whitman county, Washington. Soil Sci. 130 : 11-18.

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* Original not seen.