FAO SOILS BULLETIN 46

blue-green algae for rice production

FOOD AND AGRICULTURE ORGANIZATION
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PREFACE

This manual is the first in a series planned by the Regional Project, designed to give detailed, practical advice on the various methodologies of organic recycling.

Blue-qreen algae form a self-sufficient system which is capable of fixing atmospheric nitrogen in organic forms and which grows upon a free water surface. It is thus ideally suited for propaqation in rice fields. However, the development and use of blue-green algae require special techniques and this manual is an attempt to present these techniques to agriculturists.

Although not an exhaustive treatise on blue-green algae, the manual summarizes the present status of algal biofertilizer technology for rice with practical information for its adoption by agricultural extension personnel and subject matter specialists. Early chapters present general information on the characteristics of nitrogen-fixing blue-green algae, their identification, ecology and agronomic role as background material. Since a considerable amount of information has been collected over the past several years in India where this technology is currently being used by many farmers in the rice belts, the manual largely projects the Indian experience and it is hoped that with suitable extension it will be shared by other rice growing regions in their efforts to harness low cost inputs to meet their ever-growing agricultural demands.

In the appendices is placed the more academic, scientific background data which will be of interest and use to the specialist in the subject; for the strictly practical aspects of the manual, readers are referred to Chapters 10 and 11.

If properly extended, the technology holds the promise of providing 25 - 30 kg N/ha every season to the growing crop. It also holds the promise of generating rural income and employment. The foremost need at present is to extend it to the farmers' fields in the shortest possible time. The ultimate success of the technology depends not only on creating an awareness but also on building a trained manpower reserve capable of applying the existing knowledge and carrying out further research.

The technical author of this manual, Dr. G.S. Venkataraman, is the Coordinator of the All India programme of blue-green algae and one of the foremost experts on the subject in Asia. The tremendous expansion and success of the technology in India is due largely to the dedication and activity of Dr. Venkataraman.

The early chapters contain many references to published literature on blue-green algae and although they slightly hinder the continuous reading of the text, they have been retained as a valuable source of further reading in this rapidly developing subject.

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The enthusiastic reception and adoption of the technology by a large number of farmers have helped in translating it from a concept into a reality.

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1. INTRODUCTION

One of the paradoxes in nature is the abundance of nitrogen in the atmosphere and its relative non-availability to plants and animals. Organized crop production has, therefore, to depend largely on synthetic nitrogen fertilizers, the production of which is based on non-renewable, fossil fuel resources. The escalating oil prices, the widening gap between supply and demand for this nutrient and the prevailing low purchasing power in the densely populated developing regions have necessitated the examination of alternate renewable organic resources to meet at least a part of the nitrogen demand of crops. Any saving in the consumption of chemical nitrogen without affecting the crop productivity will, therefore, be not only an economic advantage but also a strategic necessity. The present day global interest in biological nitrogen fixation is a direct consequence of this necessity to provide some economic assistance to the small and marginal farmers and to introduce a judicious combination of linear and cyclic fertilization of soils.

The great demand for nitrogen in agriculture can be seen from an investment of over ten thousand million US dollars in chemical fertilizer plants all over the world. It is estimated that the total energy required for the production of worldwide ammonium fertilizer is equivalent to two million barrels of oil per day and it takes the equivalent of 213 000 barrels of oil a day to meet the nitrogen fertilizer needs of the United States of America alone. By 1985 the global projection for the energy dependent nitrogen fertilizer input will be around 85 million metric tonnes, of which twenty-five percent is expected to be consumed in the developing regions.

Rice is the staple food of more than sixty percent of the world's population and is grown in an area of more than 135 million hectares. It is distributed geographically over a wide range of conditions between latitudes 45° N and 40° S. However, ninety percent of the total area under rice is situated in the tropical south and south-east Asia region. About ten percent of the nitrogen consumed globally is applied to rice crops. In most developing countriess the potential nitrogen requirement is often one third or one half of that of the developed nations and actual nitrogen requirement is even less. In terms of nitroqen consumption per hectare of agricultural land, developing countries consume only an average of 9 - l6 kg N/ha, with a world average of about 8 kg N/ha.

The triphasic rice crop presents special management problems of fertilization. There is an indication that wetland rice removes more nitrogen from the soil than can be accounted for from any recognized sources and there is qood reason to suspect that biological nitrogen fixation makes an important contribution to the nitrogen economy of wetland rice. About two thirds or more of the nitrogen in a rice crop comes from soil nitrogen even when inorganic nitrogen fertilizer is applied. This indicates the necessity of improving the soil nitrogen status and the productivity efficiency of the applied chimical nitrogen.

Although nitrogen occurs in the atmosphere in the form of molecular nitrogen in a concentration of 77 volume percent (about 77 000 ton/ha), agriculture is in a state of poverty amidst plenty with regard to this nutrient. Nitrogen fixation is a high energy budgeted process requiring an input of about 61.5 kJ (147 kcal) to reduce one molecule of nitrogen and this may explain why most higher plants have not evolved a nitrogen-fixing mechanism; they simply may not be able to afford the energy. Blue-green algae represent a self-supporting system capable of carrying out both photosynthesis and nitrogen fixation, the energy bill for the latter process being paid by the sun. Rice is perhaps the only major cereal crop to whose nitrogen economy the nitrogen-fixing blue-green algae make a significant contribution. If we could utilize these algae, particularly in rice fields which form an ideal environment for them, our costly reliance on the energy intensive nitrogen fertilizer input could be substantially reduced. Recent research has shown the feasibility of using these algae as a biological input in rice cultivation and of obtaining additional yields for which the incremental input cost will be low. The use of these regenerative biological sources may also minimize the environmental hazards and maximize the ecological benefits.

Unlike chemical fertilizers, algal fertilizer materials do not bring about spectacular visual changes in crop growth and production. Hence their development and use will require a different strategy from that adopted for chemical fertilizers. A good deal of extension work requires to be organized to create a better awareness among farmers of the benefits of algal fertilizer, and a consumer demand for the product has to be created. A real impact is possible only by mobilizing the available information and propagating it through a sustained extension network among small and marginal farmers.

2. DISTRIBUTION AND SUCCESSION OF BLUE-GREEN ALGAE IN RICE FIELD SOILS

Rice fields constitute artificial biotopes of a peculiar character. The biocenosis in them is the association of two micro-organizations, that of the water and that of the soil, although they are not mutually exclusive. Biocenotic life in the water of rice fields is, however, of a short duration extending over three to six months in all. After harvest, most of the aquatic micro-organisms either perish or perennate.

Although recurrent combinations of algal species appear, particularly in comparable habitats, the generalization that practically all cultivated soils and grasslands support an abundant nitrogen-fixing blue-green algal flora may not be universally applicable. A survey by Watanabe (1959a) suggests that they are present in only about four percent (34 out of 851) of the localities examined by him in south and east Asia. In Japanese rice soils, common species belong to the genera Nostoc, Anabaena, and Tolypothrix (Okuda & Yamaguchi 1952, 1956). Nitrogen-fixing blue-green algae form only a minor part of the rice field flora of Australian soils, presumably because of the heavy applications of ammonia nitrogen to the soil and addition of as much as 5 μg cm^{-3} of copper sulphate to the irrigation water. However, Bunt (1961) has recorded species of Nostoc and Anabaena from northern Australian soils. Species of Nostoc and Anabaenopsis are particularly common in Italian soils, where nitrogen-fixing species comprise about 35% of the flora under dry conditions and about 70% under waterlogged conditions (Materassi & Balloni 1965). Species of Calothrix, Anabaena, Hapalosiphon, Cylindrospermum, Nostoc, Scytonema, Symploca and Nodularia have been reported from Egyptian soils (El-Nawawy & Hamdi 1975).

In India, the rice field algal flora show a distinct periodicity during and just after the monsoon. Singh (1961) observed that in soils of Uttar Pradesh a general mixture of nitrogen-fixing species appear initially, which is soon followed by a 'huge and pure growth of thick brownish gelatinous mass of Aulosira fertilissima. As the soil dries, species of Cylindrospermum become dominant.' Pandey (1965) considered that about 70% of the algal species were blue-green algae. Potential nitrogen-fixing species of Aulosira, Anabaena, Anabaenopsis, Calothrix, Camptylonema, Cylindrospermum, Fischerella, Hapalosiphon, Microchaete, Nostoc, Westiella, Westiellopsis and Tolypothrix have all been recordea in the rice fields of many localities in India. Indigenous nitrogen-fixing blue-green algae in the soils show a seasonal periodicity and succession. Table 1 summarizes the monthly occurrence of some nitrogen-fixing blue-green algae in an unfertilized rice field.

A detailed comparative study of the algal flora of the rice field soils from seven districts of Kerala State in India was carried out by Aiyer (1965). The pH values of the soils in these areas ranged from 3.5 to 6.5, the percentage organic carbon from 0.3 to 4.25 and the

Table 1 SEASONAL OCCURRENCE OF INDIGENOUS NITROGEN-FIXING BLUE-GREEN ALGAE IN AN UNFERTILIZED RICE FIELD

Month	Blue-green algae
January	Nostoc muscorum (C)
February	Nostoc muscorum (C)
March	Nostoc muscorum (A),N. punctiforme(C), Anabaena variabilis(C), A. ambigua(C)
April	Nostoc muscorum(A), N. punctiforme(A) Anabaena variabilis(C), A. ambigua(C)
May	Nostoc muscorum(A), N. punctiforme(A), Anabaena variabilis(A), A. ambigua(C)
June	Nostoc muscorum(A), N. punctiforme(A), Anabaena variabilis(A), A. ambigua(A), Aulosira fertilissima (R)
July	All the above (A)
August	All the above (A), Cylindrospermum muscicola(C)
September	All the above (A), Westiella(C)
October	All the above (A)
November	Nostoc punctiforme(C), Westiella(C)
December	Nostoc muscorum(C), N. punctiforme(C)

C = common; A = abundant; R = rare

available phosphorus and potassium from 22.7 to 45.4 kg/ha and from traces to 349.5 kg/ha respectively. The total soluble salts expressed as conductivities varied from 0 to 4.5 mS cm^{-1}. Blue-green algae were found to constitute from 20 - 76% and dominated other forms of algae particularly in the rice fields of Trivandrum district. They were comparatively less in the districts of Kottayam, Ernakulam, Kozhikode and Palghat. Of special interest is the occurrence of many blue-green algae which are known to fix nitrogen. Species of Nostoc and Anabaena have been found even in peaty soils (Amma et al 1966). The species of Nostoc are almost ubiquitous in distribution (Figure 1).

An 'All India' survey showed that out of 2 213 soil samples from rice fields, only about 33% were found to harbour nitrogen-fixing forms of algae (Table 2). These algae were particularly rich in the soils of Uttar Pradesh (87%), West Bengal (60%) and Orissa (43%) and the dominant forms were Aulosira fertilissima and species of Nostoc. Soils of Tamil Nadu (lO%), Kashmir (7%) and Kerala (9%) were comparatively poor in these forms. Interestingly, regional differences could be seen in the distribution pattern of the dominant forms. While Uttar Pradesh soils were rich in Aulosira, Mastigocladus was dominant in Gujarat, Westiella in Maharashtra, Cylindrospermum in Karnataka and Calothrix in Punjab.

Figure 1

Floating colonies of *Nostoc* in a rice field

Table 2 DISTRIBUTION OF NITROGEN-FIXING BLUE-GREEN ALGAE IN INDIAN RICE FIELD SOILS (Venkataraman 1975)

State	Number of soil samples	Number of samples showing N-fixing forms	% of samples showing N-fixing forms	Dominant forms
Assam	17	3	17.6	*Nostoc*
Andhra Pradesh	273	64	23.1	*Anabaena*
Gujarat	14	3	21.5	*Mastigocladus*
Haryana	32	8	21.2	*Nostoc*
Himachal Pradesh	12	2	16.6	*Anabaena*
Kashmir	14	1	7.1	*Anabaena*
Kerala	352	32	9.1	*Nostoc*
Madhya Pradesh	72	9	12.5	*Anabaena*
Maharashtra	84	14	16.6	*Westiella*
Mysore (Karnataka)	192	32	11.4	*Cylindrospermum*
Orissa	178	87	43.2	*Aulosira*
Punjab	57	12	21.5	*Calothrix*
Tamil Nadu	401	40	9.9	*Nostoc*
Uttar Pradesh	450	392	86.9	*Aulosira*
West Bengal	65	39	60.0	*Nostoc*
TOTAL	2 213	738	32.9	

At the Paddy Experimental Station, Adutharai, extensive studies are being made on the distribution pattern and succession of blue-green algae in the rice fields as influenced by crop growth, fertilizer and manurial practices (Srinivasan 1979). Table 3 shows the succession of blue-green algae in relation to crop arowth.

Table 3 SUCCESSION OF THE INDIGENOUS BLUE-GREEN ALGAE IN RELATION TO CROP GROWTH (Srinivasan 1979)

Period	Blue-green algae
Before planting	*Chroococcus minimus*
After planting	
1 - 20 days	*Aphanothece pallida, Anabaena doliolum, Nostoc verrucosum, Microcoleus lacustris*
21 - 40 days	*Wollea bharadwajae, Anabaena circinalis, Nostoc sphaericum, Microcystis marginata*
41 - 55 days	*Plectonema boryanum, Anabaena sphaerica, Rivularia aquatica, Microcystis marginata*
56 - 78 days	*Cylindrospermum muscicola, Microcystis marginata*

The fertilizer schedules also influence the biomass of the indigenous blue-green algae both qualitatively and quantitatively (Tables 4 and 5).

A recent survey conducted in Tanjavur district in Tamil Nadu (20 villages) showed an abundance of indiqenous nitrogen-fixinq blue-green algae in a few rice fields where the village organic wastes accumulated (Table 6).

Few reports deal with the distribution and nitrogen fixation by blue-green algae in African ecosystems (e.g. Renault et al 1975; Reynaud & Roger 1978). In moist subtropical soils of South Africa, it was observed that the high temperature had an influence on the day-light nitrogen fixation in blue-green algal mats, while light intensities above 323 lux had only a marginal effect (Jones 1977). Reynaud and Roger (1978) examined the qualitative and quantitative variations in the algal flora in the rice fields of Senegal. They found that 60% of the soils had a nitrogen-fixing algal biomass between 100 and 500 kg wet weight per hectare, reaching an absolute maximum after heading and a relative maximum at the end of the cultivation cycle. They also observed a positive correlation between the nitrogen-fixing algal

Table 4 INFLUENCE OF FERTILIZER PRACTICES ON THE INDIGENOUS BLUE-GREEN ALGAL BIOMASS PRODUCTION IN $kg\ m^{-2}$ ON PERMANENT MANURIAL PLOTS (Srinivasan 1979)

Main treatments	Sub-treatments								Mean
	O	P	K	L	PK	PL	KL	PKL	
Control	0.82	5.43	2.43	0.83	6.05	3.27	1.95	4.09	3.11
Ammonium sulphate	0.74	2.95	0.46	0.32	2.96	0.46	0.45	2.18	1.37
Compost	2.24	5.92	2.37	0.88	4.76	3.77	1.48	4.29	3.21
FYM	1.92	4.48	1.67	1.24	4.02	2.82	1.94	3.66	2.72
Green manure	1.21	5.48	1.29	0.80	5.48	4.22	1.11	3.91	2.94
Mean	1.38	4.85	1.64	0.81	4.65	2.99	1.38	3.62	

O = no fertilizer; P = phosphorus; K = potassium; L = lime

Table 5 INFLUENCE OF FERTILIZER PRACTICES ON THE DISTRIBUTION OF INDIGENOUS BLUE-GREEN ALGAE (Srinivasan 1979)

Days after planting	Control	Ammonium sulphate	Compost	FYM	Green leaf
1 - 20	Ap, Ad, Nv, Ml	Ap, Ad, Nv, Ml	Ap, Ad, Nv, Ml	Ap, Ad, Nv, Ml	Ap, Ad, Nv, Ml
21 - 40	Wb, Ac, Ns, Mm	Ns, Ac, Mm	Wb, Ac, Ns, Mm	Wb, Ac, Ns, Mm	Wb, Ac, Ns, Mm
41 - 55	Mm, Pb, As, Ra	Mm	Mm, Pb, As	Mm, Pb, As, Cm	Mm, Ra, Cm

Ap = *Aphanothece pallida.* Ad = *Anabaena doliolum* Ac = *A.circinalis*
As = *A.sphaerica.* Cm = *Cylindrospermum muscicola.* Ml = *Microcoleus lacustria.* Mm = *Microcystis marginata.* Ns = *Nostoc sphaericum*
Nv = *N.verrucosum.* Pb = *Plectonema boryanum.* Ra = *Rivularia aquatica*
Wb = *Wollea bharadwajae*

Table 6 DISTRIBUTION OF INDIGENOUS NITROGEN-FIXING BLUE-GREEN ALGAE IN TANJAVUR DISTRICT, TAMIL NADU, INDIA (Srinivasan 1979)

Blue-green algae	*Number of villages*
Anabaena doliolum	7
A.oscillarioides	2
A.fertilissima	4
A.sphaerica	1
A.iyengarii	1
Nostoc verrucosum	2
N.linckia	3
N.carneum	1
Cylindrospermum muscicola	3
Gloeotrichia pisum	1
G.intermedia	1
Aphanothece pallida	20
Rivularia aquatica	5
Plectonema boryanum	15
Wollea bharadwajae	5
Gloeocapsa decorticans	5

biomass and the pH of the soil and the density of the plant cover. Blue-green algae have been reported to be ubiquitous in soils of Iraq and particularly abundant in rice fields, citrus orchards and under date palms (Hamdi et al 1978).

Nitrogen-fixing blue-green algae seem to constitute about 12% of the total algal flora south of latitude 35° N and nearly 2% of the flora north of it (Watanabe & Yamamoto 1971). The soils of central Sweden have been found to be an ecologically good habitat for nitrogen-fixing blue-green algae (Granhall & Henriksson 1969; Henriksson et al 1972; Granhall 1977; Englund 1977). Pantastico (1976) described blue-green algae from the Philippines.

3. ALGAL NITROGEN FIXATION IN RICE SOILS

Wetland rice can grow on the same land year after year without any fertilizer and without any decline in yield. Recent studies indicate that about 40-50% of the nitrogen in the crop comes from soil nitrogen, even when fertilizers are used. Thus rice seems to remove more nitrogen from the soil than can be accounted for from any recognized source. The classical Broadbalk experiments at Rothamsted have shown a steady gain of 34 kg N/ha every year from 1885 to 1967 (Jenkinson 1973) and most of the nitrogen fixation has been found to be associated with blue-green algal crusts which developed late in the growing season (Day et al1975). Long term continuous cropping without added nitrogen fertilizer at the International Rice Research Institute in the Philippines has shown no reduction in the yield of rice. Similar observations have been made at the Paddy Experiment Station at Aduthurai in India. There is good reason to suspect that biological nitrogen fixation makes an important contribution to the nitrogen economy of wetland rice. The balance sheet of soil nitrogen (Figure 2) for broadcast and transplanted rice fields in Japan indicates that the total amount of nitrogen fixation per crop may not be significantly reduced by a heavy dressing of fertilizer nitrogen (Matsuguchi 1977). Ethyne (acetylene) reduction assays indicate higher nitrogen fixation associated with blue-green algae than with other micro-organisms (Watanabe et al 1978; and Table 7). Removal of algae results in a sharp decrease in the nitrogen-fixing activity in the rice field from 117 µmol to 19 µmol of ethene (ethylene) per hour and both at the early and late growth stages, nitrogen-fixing activity after removal of the algae is very low, indicating that the contribution of nitrogen fixation in the rice root zone is minor. Moroccan rice fields have been found to show high rates of nitrogen fixing activity (Renaut et al 1975). In the rice fields of Bihar in India, algal nitrogen fixation has been estimated to be about 14 kg N/ha during the vegetation period and in West Bengal the algal contribution was from 15 - 49 kg N/ha and in soils fertilized with phosphorus it was from 18 - 69 kg N/ha (De & Mandal, 1956). In sugarcane and maize fields the fixation by Cylindrospermum licheniforme was of the order of 88 kg N/ha (Singh 1961). In Japanese soils Tolypothrix tenuis inoculation has been reported to contribute about 22 kg N/ha (Watanabe 1962). Using ^{15}N, MacRae and Castro (1967) demonstrated nitrogen fixation to the extent of 10 - 15 kg N/ha per year at the International Rice Research Institute and using a similar method, Yoshida and Ancajas (1973) obtained values of 40 - 80 kg N/ha per year. Algal fixation is generally found to be higher in presence of the rice crop than in its absence (De & Sulaiman 1950; Watanabe et al 1978).

Indirect evidence for the potentiality of algal nitrogen fixation and its contribution to soil fertility can be demonstrated by crop growth and yield (Table 8). In one of the trials, algae were grown in the fields for a period of two months prior to transplantation, during which period four harvests of algae were made. The crop raised in algal-grown plots registered an increase of 33.6% in the grain yield over the control in the absence of any added fertilizers (Table 8).

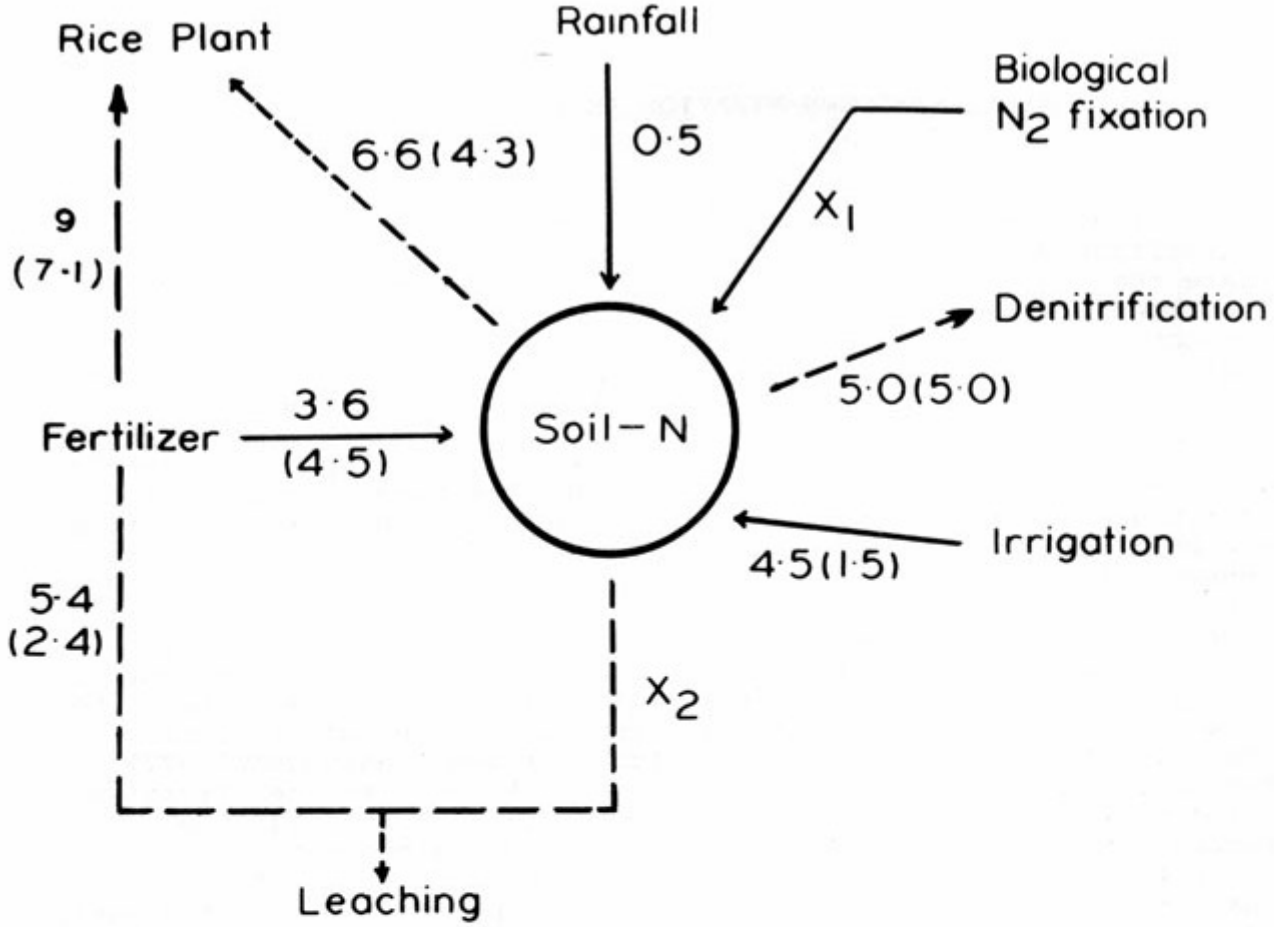


Figure 2 Flowsheet of nitrogen in broadcast and transplanted paddy fields in Japan. Nitrogen quantity is shown in terms of kg/10a/crop season; that of the transplanted field is given in parenthesis
x_1 amount of nitrogen fixed
x_2 leaching from soil N (Matsuguchi 1977)

Table 7 NITROGEN FIXATION DURING THE DRY AND WET SEASON (Watanabe 1978)

Season	Days	mmol C_2H_4 m^{-2}	
		Associated with algae	Associated with rice plants
Wet	163	204	90 (IR 26)
Dry	168	307	53 (IR 36)

Table 8 POTENTIALITY OF BLUE-GREEN ALGAE IN INCREASING CROP YIELD (ADT31) (Srinivasan 1979)

	Grain yield kg/ha	% increase over control
Control	3 450	-
Algalized	4 608	33.6

Even in temperate soils, nitrogen fixation by blue-green algae is substantial and an examination of over one thousand soil samples in Sweden by Henriksson (1971) showed an annual fixation of 15 - 51 kg N/ha per year in an agricultural field where Nostoc was abundant and from 4 - 44 kg N/ha per year in a lakeside meadow having Nostoc, Anabaena, Cylindrospermum and Calothrix species. Bortels (1940) observed a positive correlation between the fertility of the soil and nitrogen-fixing blue-green algae, and in the cultivated fields of the Soviet Union, an increase in soil fertility by the addition of nitrogen either singly or in combination with phosphate and potassium has been reported to result in the development of these algae (Shtina 1969). Even in polar and sub-polar regions, nitrogen fixation by blue-green algae is significant (Alexander 1975).

4. METHODS FOR THE ASSAY OF NITROGEN FIXATION

Many methods are used for estimating nitrogen and the following three have gained general acceptance:

The Kjeldahl method
The ^{15}N tracer method
The acetylene reduction assay

4.1 Kjeldahl Method

The Kjeldahl procedure is the easiest and most commonly used method for routine analyses and requires no special equipment. The basic principle is to convert the nitrogen in the sample to ammonium by digestion with concentrated sulphuric acid and to estimate the ammonium by alkaline distillation.

A full discussion of the procedure, its theory and experimental details can be found in Hesse's Textbook of Soil Chemical Analysis (1971) .

4.2 ^{15}N.Tracer Method

The ^{15}N isotopic method is at least fifty times more sensitive than the Kjeldahl method and involves a three step analytical procedure:

- conversion of the labelled nitrogen (^{15}N) in the material under investigation to ammonium;

- conversion of the ammonium to nitrogen by oxidation with alkaline sodium hypobromite in the absence of air;

- determination of the isotopic composition of the nitrogen using a mass spectrometer.

The procedure is complicated and time consuming, requiring special apparatus and facilities. Thus, in spite of its sensitivity, the procedure is not feasible for most laboratories. For details of the technique see Bremner (1965) and Burris (1972).

4.3 Acetylene Reduction Assay

Since Stewart et al (1967) and Hardy et al (1968) for the first time applied this assay technique to moist soil samples, it has been increasingly used to assay nitrogen fixation both under upland and lowland conditions. The procedure is simple, fast, sensitive and

correlates with nitrogen fixation. It is based on the fact that the nitrogen fixing enzyme nitrogenase can also reduce acetylene (C_2H_2) to ethylene (C_2H_4). 1/

The ethylene produced is quantitatively measured by gas chromatograpny and computed to nitrogen fixation using a factor of 3:1.

a. Assay procedure

The following procedure is for aerobic nitrogen-fixing micro-organisms like blue-green alqae. The assay is carried out in 7 cm^3 capacity glass vials provided with rubber serum stoppers. 2.0 cm^3 of algal suspension is added to each vial. The stopper is fitted and sealed by dipping in molten paraffin wax. The inside pressure is equilibrated to 101.325 kN m^{-2} (1 atm) with a syringe needle. 0.5 cm^3 (i.e. 10% gas phase) is removed from the vials (Figure 3) and replaced with 0.5 cm^3 of acetylene (final concentration of 10.133 kN m^{-2} (O.l atm) with a syringe. The vials are then incubated for 30 minutes in a light of about 5 000 lux for photosynthetic micro-organisms, on a rotary wheel at 11 rpm. The reaction is terminated by injecting 0.2 cm^3 of a 50% solution of trichloroacetic acid. The gas phase is then analysed for ethylene using a gas chromatograph.

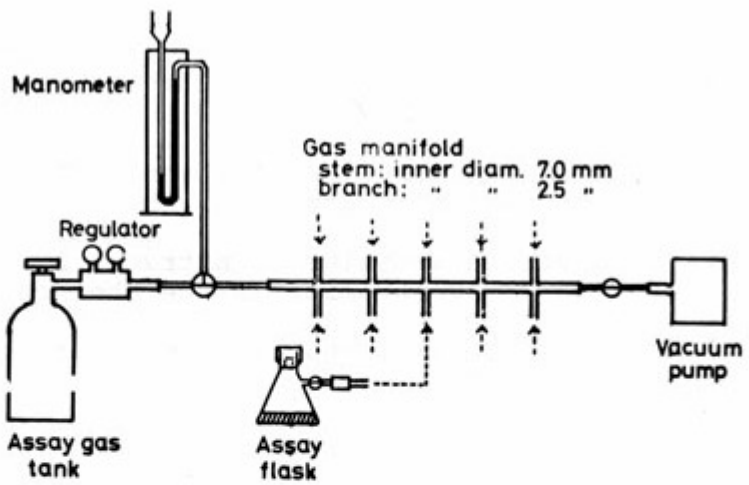


Figure 3 A gas exchange assembly for the acetylene reduction assay

1/ The modern, correct names for acetylene and ethylene are ethyne and ethene respectively, but the older terms have been retained here as they are probably more familiar.

b. Gas chromatography

Column:	Porapak Q, 2 m long, 3 mm diameter or Porapak T, 1 m long, 6 mm diameter.
Detector:	Flame ionization detector in conjunction with an electrometer amplifier at attenuation 10^{-10} ampere and to a 10 mV recorder.
Temperature:	Column (oven): 363 K (90 °C) Injection port: 383 K (110 °C) to avoid condensation of samples. Detector: 393 K (120 °C)
Carrier Gas:	Nitrogen at a flow rate of 30 cm^3 per minute (variable according to make of instrument). Oxygen and hydrogen gases are required for the flame; the flow rate of O_2 to H_2 is 1.5 to 2.0.

Under these conditions acetylene and ethylene are well separated with retention times of 46s and 30s respectively. The sensitivity for ethylene is about 200 picomoles under optimum conditions.

c. Preparation of ethylene standard

The ethylene standard should be prepared in a specially designed flask having a side-arm for evacuation and facility for mercury sealing at the main port with a rubber septum held in a brass cap.

i. Primary standard: Add exactly 5 cm^3 of mercury to the flask. Evacuate the flask and refill with high purity argon; repeat at least twice. Remove 25 cm^3 of the gas phase and replace with 25 cm^3 of pure ethylene (research grade, 99% pure). Add 10 cm^3 of argon to give a positive pressure.

Volume of flask	489.6 cm^3
Volume of mercury	5.0
Volume of gas phase	484.6
Volume of extra argon	10.0
Total gas phase	494.6

Thus 494.6 cm^3 of gas phase contains 25.0 cm^3 of ethylene; thus amount of ethylene per cm^3 of the gas phase is 0.0505 cm^3.

ii. Secondary (experimental standard: Add 5 cm^3 of mercury as before. Evacuate and fill a second flask of the same type with high purity argon; repeat several times. Adjust to 101.325 kN m^{-2} (1 atm). Remove 2 cm^3 of the gas phase and replace with 2 cm^3 of the gas phase from the primary standard. Add 10 cm^3 of argon to give a positive pressure.

Volume of flask	512.1 cm^3
Volume of mercury	5.0
Volume of gas phase	507.1
Volume of extra argon	10.0
Total gas phase	517.1

Thus 517.1 cm^3 contains 0.1010 cm^3 of ethylene from the primary standard and amount of ethylene per cm^3 of gas phase is 0.000195 cm^3 or 195 pm^3. 1/

From the gas laws, $\frac{P_1 V_1}{T_1} = \frac{P_2 V_2}{T_2}$

If the sample volume at room temperature (298 K or 25 °C) is 1.0 cm^3, then at STP

$$\frac{1 \times 1}{298} = \frac{1 \times V_2}{273}$$

$P_1 P_2$ = 101.325 kN m^{-2} (1 atm)
V_1 = 1.0 cm^3
T_2 = 273 K

thus V_2 = 273/298 = 0.92

and the volume of gas at STP is thus equal to the volume at room temperature x 0.92. Volume of ethylene in 1.0 cm^3 of gas phase from secondary standard = 195 x 0.92
= 179.4 pm^3

From Avagadro's hypothesis, 1 mole of an ideal gas at STP = 22.4 dm^3
= 1 nanomole (1 nmol)
= 22.4 pm^3

The molecular weight of ethylene is 28.0 and thus,
28 ng = 22.4 pm^3
or, 1 pm^3 of ethylene is equivalent to 28/22.4 = 1.25 ng.
179.4 pm^3 of ethylene therefore is equivalent to 223.75 ng.
Again, since the molecular weight of ethylene is 28,
223.75 ng = 223.75/28 nmol = 7.99 nmol

Hence 1 cm^3 of the gas phase in the secondary standard flask contains 7.99 nmol C_2H_4.

d. Calculations

i. Calculate peak height (cm) of C_2H_4 in the injection volume = a cm

ii. Calculate peak height for 1 cm^3 injection volume = b cm

1/ Note that in originally published figures the term 'nanolitre' or nl was used but use of the litre has now been abolished and all volumes are expressed in terms of the cubic metre. 1 pico-cubic metre (1 pm^3) is the same as 1 nl.

iii. Calculate nanomoles C_2H_4 corresponding to the 'b' cm peak height from a standard graph = c nmol

iv. Calculate volume of vial = d cm^3

v. Subtract volume of algal sample ('e' cm^3) from 'd' to get volume of gas phase in vial = f cm^3

vi. nmol C_2H_4 per vial = f x c for a 30 minute incubation.

vii. Calculate chlorophyll a in the sample = w mg chl a/ 2 cm^3 algae

viii. Express rates as nmol C_2H_4/mg chl a per hour = $\frac{f \times c \times 2}{w}$

For measuring the in situ activity in paddy fields, a unit with a plastic bag and a bottomless metallic frame developed by Lee et al (1973) is very useful. Matsuguchi et al (1979) have proposed the following set of procedures for waterlogged soils:

1) Take uniform portions of the soil sample into assay vials. The sample should be as undisturbed as possible.
2) After removing the surface water, stopper the vials with gas-tight rubber caps and attach them to a gas exchange apparatus (Figure 3).
3) Evacuate the vials gradually and keep them for one minute under a pressure of 1.01 kN m^{-2} or 0.01 atm. Gradually return the pressure to 101.325 kN m^{-2} (1 atm) by filling with acetylene-containing assay gas at pC_2H_2 of 10.1 kN m^{-2} (0.1 atm) and keep it for one minute. Repeat this gas exchange procedure four times.
4) Submit the vials to a three hour assay incubation.
5) After incubation, vibrate the vials strongly for one minute so that ethylene remaining in the soil can diffuse back to the headspace.
6) Take an aliquot of the headspace gas sample for gas chromatographic analysis of ethylene.

e. Comments

In the field assay of waterlogqed soils, gas transfer between the soil and water phases and vice versa may present difficulties. To ensure better gas transfer, Watanabe et al (1977) introduced a bamboo piece inside the assay chamber which could be manipulated from outside to stir the soil. The ratio of acetylene reduction to dinitrogen reduction is not constant. Consequently, unless the conversion ratio is tested under identical conditions for both acetylene reduction and 15N incorporation methods, acetylene assay results should be interpreted on a qualitative and relative basis. Normally the ratio is 3:1.

5. ALGAL APPLICATION AND SOIL PROPERTIES

The growth of blue-green algae in soil seems to influence the physical and chemical properties of that soil. For example, water-stable aggregates significantly increase as a result of algal growth (Roychoudhury et al 1979; Table 9) and this is important because soil aggregations and their arrangement influence infiltration rates, aeration and soil temperature and thereby can improve the physical environment of the crop.

Table 9 EFFECT OF BLUE-GREEN ALGAL INOCULATION ON WATER-STABLE SOIL AGGREGATES (Roychoudhury et al 1979)

Soil	% water-stable aggregates (50 µm)		
	Control	Algae inoculated	% increase
Sandy loam	2.2	4.1	85
Loam	2.6	6.0	130
Silty clay loam	3.5	9.1	160

The nitrogen content of soil has been reported to increase as a result of algal inoculation (Watanabe 1962; 1966). However, in a long term field trial in India, no appreciable increase in the residual soil nitrogen content was observed (Tables 10 and 11). Algal application has also been found to result in a continued maintenance of both total and organic nitrogen levels beyond the tillering stage of the crop and no depression on the addition of organic nitrogen due to algae was observed even in presence of chemically added nitrogen (Chopra & Dube 1971).

The bulk of the organic matter produced by algal growth is said to remain in the soil and become available to the next crop as organic enrichment (Sankaram 1971). However, in many trials no appreciable build up of organic matter could be observed. This is understandable because organic matter is readily broken down and lost in the warm humid regions.

Table 10 EFFECT OF ALGAL INOCULATION ON THE NITROGEN STATUS OF SOIL (Watanabe 1966)

	Amount of ammonification (mg/lOOg soil)			
	First Year		Third Year	
	0.2 cm	2.1 cm	0.2 cm	2.1 cm
Uninoculated	13.7	5.4	15.4	5.3
Inoculated	14.3	7.1	20.0	6.1

Table 11 EFFECT OF ALGAL INOCULATION ON SOIL PROPERTIES, BASED ON FOUR SEASONS (Aiyer et al 1972)

	- Algae	+ Algae	Increase/ Decrease	CD 5%
Soil nitrogen (%)	O.l	O.l	-	NS
Organic carbon (%)	0.98	1.125	+ 0.145	NS
Oxidizable organic matter	2.97	2.48	- 0.49	O.1
Total sulphide (µg g^{-1})	12.2	9.2	- 3.0	1.2
Iron (II) (µg g^{-1})	34.3	28.7	- 5.6	3.0

An interesting observation relates to the reduction in contents of reduced substances such as oxidizable organic matter, sulphides and iron (II) as a result of successive algal inoculation. This is of importance for areas where iron and sulphide toxicity is a common phenomenon. The reduction in the amount of reduced compounds is probably due to an increased oxygen tension in the medium resulting from algal photosynthesis.

6. PESTICIDES AND BLUE-GREEN ALGAE

The introduction of pest control measures in present agricultural practices has brought in many different pesticides, some of which are added to the irrigation water. These will have a bearing on the establishment and biological activity of the introduced, as well as the native, blue-green algae in the soil (Shtina 1957; Lundkvist 1970; Da Silva 1975). Gamma BHC application to rice fields stimulates the algal population by inhibiting the predators which feed on algae. MCPA and 2,4-D affect the nitrogen fixation by Nostoc muscorum, N. punctiforme and Cylindrospermum sp. at concentrations recommended for field application, but stimulate nitrogen fixation at concentrations of 10^{-4}M to 10^{-5}M (Inger 1970). Many blue-green algae show a high degree of tolerance to high levels of many agricultural chemicals although there exists a wide range of variation in their tolerance limits (Venkataraman & Rajyalakshmi 1971). While most of the strains of Anabaena could tolerate 100 µg g^{-1} of Ceresan, a few were sensitive to a concentration as low as O.l µg g^{-l}.

Tolypothrix tenuis and Aulosira fertilissima are of particular interest since these are the two commonly used forms in the algal biofertilizer mixture for field application. Both forms tolerate high concentrations of Ceresan M, 2,4-D and Delapon. However, Aulosira fertilissima is sensitive to substituted urea compounds such as Cotoron, Diuron, Linuron and also to the triazine herbicide Propazine. One should, therefore, be wary in recommending any one single strain of alga in the presence of different pesticides. The importance of using mixtures of different algal strains as seeding material is thus obvious and development of pesticide-tolerant strains will be a promising approach.

The growth and nitrogen fixation in Anabaenopsis raciborskii is little affected by up to 100 pg cm^{-3} of 2,4-D in the medium (Das & Singh 1977). The growth of Aulosira fertilissima continues unaffected up to a concentration of 100 µg g^{-1} of MCPA, MCPB, Stamm F34, the three herbicides commonly used in rice cultivation. Direct contact with higher concentrations than 10 µg g^{-1} of systematic and contact insecticides like Lindane, Parathion, Endrin, Diazinone and Sevin, drastically inhibits the growth of this alga; however, concentrations less than 10 µg g^{-1} stimulate growth (Ahmad & Venkataraman 1973).

7. CROP-ALGA ASSOCIATION

The crop plant may exert an influence on the qualitative and quantitative nature of algae occurring in the rhizosphere regions. The number of algae has been found to be much more in the rhizosphere of rice and cotton than outside the root zone (Shtina 1972). The rhizosphere effect seems to vary with crop plant and different species of algae are associated with different crop plants (Gonzalves & Yalavigi 1960). This aspect needs a critical examination to determine the occurrence of a possible associative symbiosis.

The nitrogen fixed by blue-green algae becomes available to the associated plant. When the algal crusts from desert grassland soils were labelled with ^{15}N and seeds of Artemesia germinated on these crusts, the seedlings became labelled with ^{15}N (Mayland & McIntosh 1966). Similarly when the sand dune slack soils rich in Nostoc were exposed to 15N, the shoots of the associated higher plants which grew through the soil became enriched with ^{15}N (Stewart 1967a). Direct proof for a transfer of nitrogen from algae to the rice plant has come from ^{15}N studies (Table 12). Using N, some of the nitrogen fixed and liberated by Westiellopsis prolifica has been shown to be assimilated by the rice plant(Renault)et al 1975).

Table 12 AVAILABILITY OF FIXED NITROGEN FROM Aulorisa fertilissima TO THE IR8 RICE PLANT (Venkataraman 1979)

Days	Mean atom % excess ^{15}N
7	0.033
14	0.185
21	0.387
28	0.479

In agronomic trials crop yields provide an indirect measure of nitrogen fixation in a particular soil and the algal fixation is estimated to range from 20 to 30 kg N/ha. The modern agricultural practices with high yielding, fertilizer-responsive rice varieties demand high levels of nitrogen fertilization. Under such conditions, increases in crop yield have been observed as a result of algal application. Such phenomena cannot be attributed solely to algal nitrogen fixation and presumably a variety of other biological substances synthesized and liberated by these algae may have a role to play. Production of vitamins and growth substances by Cylindrospermum muscicola has been shown to have a relation to crop growth and yield (Venkataraman & Neelakantan 1967). When the extract of Vitamin B_{12} compounds from the alga was tested on the root growth of rice seedlings and compared with twelve known standards, the algal extract was comparable to pure Vitamin B_{12} indicating that the alga provides this substance to rice plants.

Besides vitamins, algae also synthesize auxin-like substances which play an important role in crop growth. Cylindrospermum muscicola has been shown to synthesize inter-convertible auxin-like substances which stimulate the root growth of seedlinas (Table 13). Excretion of considerable amounts of ascorbic acid by blue-green algae is known and this may play a dual role: a) as an exudate from alaae in the rice field, it can accelerate growth and development of the plants directly and b), as a constituent of the algal cell, it may participate in processes of nitrogen fixation and nitrate reduction, ultimately influencing rice growth (Vaidya et al 1970).

Table 13 EFFECT OF AUXIN-LIKE SUBSTANCES PRODUCED BY Cylindrospermum muscicola ON THE ROOT GROWlTH OF RICE SEEDLINGS (Venkataraman & Neelakantan 1967)

	% Increase over control
Acid ether fraction chromatographed	
'X' zone	160
'Z' zone	210
'X' zone eluted and rechromatographed	
'X' zone	155
'Z' zone	202
'Z' zone eluted and rechromatographed	
'X' zone	162
'Z' zone	180

In the early period of growth, Nostoc exudates show the presence of IAA but in slightly older cultures the exudate contains mainly indole and sometimes IPA, anthrelinic acid and allied compounds (Vaidya, personal communication) .

The supply of growth substances and vitamins by algae raises the question whether non nitrogen-fixing blue-green algae will also be of agricultural importance (Rodgers et al 1979). Soil application of a non nitrogen-fixing blue-green alga Oscillatoria, has been found to increase substantially the yield of rice (Table 14). However, the amount of algal material needed is very high. Pre-treatment of seeds with extracts of Phormidium stimulates the growth of rice seedlings (Gupta & Lata 1964, Gupta & Shukla 1964).

Table 14 EFFECT OF APPLICATION OF THE NON NITROGEN-FIXING BLUE-GREEN ALGA Oscillatoria ON THE YIELD OF RICE ADT27

Treatment	Grain yield g/pot
Control	5.96
Alga	9.09
Ammonium sulphate at 60 kq/ha	12.59
Alga + ammonium sulphate	19.37
CD at 5%	2.32

8. ESTABLISHMENT OF BLUE-GREEN ALGAE IN RICE SOILS

The success of algal technology depends on the ability of the introduced algal forms to survive, establish and colonize the soil quickly. It is therefore important to select fast growing strains as seeding material. The trials conducted so far show that a continuous application of these algae at least for three consecutive cropping seasons results in an appreciable population build-up of the inoculated strains (Figure 4). In fact, this is the basis of the field multiplication of these algae (see Chapter 10). When there is a good establishment of the introduced algae, the soil surface can be seen covered extensively by algal growth after the harvest of the crop (Figure 5). Table 15 shows the establishment of the inoculated algal species in rice fields at four locations in India.

Table 15 ESTABLISHMENT OF ALGAL INOCULANTS IN RICE FIELD SOILS (examined after the harvest of the rice cron)

	Tirunelveli		Ambasamudram		Anakapalli		Kapoli	
	I	U	I	U	I	U	I	U
Tolypothrix tenuis 1/	D	-	D	-	D	-	D	-
Aulosira fertilissima 1/	D	-	D	-	D	-	D	-
Nostoc 1/	D	-	D	-	D	-	D	-
Anabaena 1/	D	-	D	-	D	-	D	-
Plectonema 1/	D	C	D	R	D	C	D	R
Chlorococcum	C	C	R	C	C	C	R	C
Chroococcus	R	R	C	C	C	C	R	C
Aphanothece	C	C	R	C	R	C	-	-

inoculants; I = inoculated U = uninoculated D = dominant
C = common R = rare

Crop yield is also an indirect indication for the establishment of the introduced algae. During 1975-1976, four rice crops were raised in succession at the Paddy Experiment Station at Aduthurai in Tamil Nadu, all of which received algal applications at the rate of 10 kg/ha. The fifth crop was raised in 1977 in the same plots without any further algal application. The yields in the fifth crop showed that in the absence of any added nitrogen the algalized field gave an additional yield of 342 kg/ha, in the presence of 25 kg N/ha an additional yield of 626 kg/ha and in the presence of 50 kg N/ha, an additional yield of 652 kg/ha (Table 16).

Microscopical identification of blue-green algae is fairly easy (see Appendix). However, it may be useful to develop an immune diffusion technique as in the case of bacteria. This is an aspect needing further research.

Figure 4 Algal growth in a farmer's field inoculated with algae for three successive cropping seasons

Figure 5 Harvested field showing algal crusts on the soil surface

Table 16 GRAIN YIELD OF THE FIFTH CROP OF RICE AS A RESULT OF CONTINUOUS ALGAL APPLICATION TO THE FOUR PRECEDING CROPS. THE FIFTH CROP DID NOT RECEIVE ANY ALGAE. (Srinivasan 1979)

Treatments	Grain yield kg/ha
No nitrogen	3 476
No nitrogen but with algae	3 818
25 kg N/ha	3 902
25 kg N/ha with algae	4 528
50 kg N/ha	4 732
50 kg N/ha with algae	5 384

Algal establishment may fail for one of several reasons:

a. Adverse conditions for multiplication and survival caused by direct contact with acid soils or fertilizers. Neutral or slightly alkaline conditions are generally preferred by most of the blue-green algae. Similarly, algae are affected when they come into direct contact with acid fertilizers. These hazards can be overcome by adopting such practices as liming the soil, applying neutralized fertilizers and scheduling the time of their application and algal inoculation. Development of acid-tolerant strains can also be attempted.

b. With slow growing forms, the problem will be the failure of the inoculated strains to colonize the soil rapidly. The answer therefore is to choose rapidly growing forms for field use. Most efficient forms may not be the most useful forms if they are slow growers.

c. Althou.gh blue-green algae generally tolerate high levels of different agricultural chemicals, certain pesticides are toxic to some strainso it is therefore desirable to choose strains having a wide tolerance. Development of pesticide resistant strains will be a profitable approach. It is always better to use a mixture of strains than a single one for field application.

d. Predators and cyamophages may affect the establishment of the inoculants under certain conditions. Predators can be controlled by chemicals and for phages the best remedy will be to develop resistant strains.

e. Nutrient deficiencies in the soil are also important factors. In Japanese soils, low pH and low available phosphorus have been found to be the limiting factors for blue-green algae (Okuda & Yamaguchi 1956). Probably liming and fertilization with phosphate would be sufficient to induce the growth of blue-green algae in most rice soils but the importance of trace elements such as molybdenum should not be overlooked.

9. ALGAL APPLICATION AND RICE YIELD

As early as 1939, P.K. De suggested that the fertility of tropical rice fields was due largely to the activity of the nitrogen-fixing blue-green algae and his suggestion has been validated by subsequent work. Based on long-term field trials, a progressive increase in the yield of rice was observed in Japan and the effect of algal application was found to be equivalent to that of 60 kg/ha of ammonium sulphate (Watanabe 1965). In the USSR, Burma, Egypt, China and the Philippines, increases from 10 - 24% in the grain yield of rice have been recorded as a result of algal inoculation. Field trials conducted in the Philippines with the blue-green alga, Nostoc commune, showed that the effect of the inoculation was comparable to that of an NPK fertilizer application (Table 17).

Table 17 RESPONSE OF DIFFERENT RICE VARIETIES TO THE APPLICATION OF Nostoc commune IN THE PHILIPPINES (Pantastico & Gonzales 1976)

	Grain yield - kg/ha			
	Tl	T2	T3	T4
First crop (C-168)	5 376	7 888	7 027	6 272
Second crop (IR-30)	4 704	4 352	5 248	4 832
Third crop (IR-28)	3 984	5 328	4 960	5 168
(IR-30)	3 536	6 144	5 200	6 256
(IR-30)	4 816	5 232	5 120	4 992
Pooled data	22 416	28 944	27 600	27 520
% increase over control	-	29.1	23.1	22.7

Tl = no fertilizer (control) T2 = NPK (429 kg/ha)
T3 = alga + NPK (0.5 kg dry alga + 214.5 kg NPK/ha)
T4 = alga alone (dry alga 1 kg/ha)

In Egypt increases of 16.6, 19.6, 16.7 and 16.6% in rice yield over control yields have been recorded due to the application of 10 kg N, 100 q alga (Tolypothrix tenuis), 20 kg N and 200 g alga respectively (Aboul-Fadl et al 1967) .

In India, the beneficial effect of algal application has been demonstrated in a number of localities in terms of grain yield with many rice varieties. In many areas, 10 - 20% increase in grain yield could;

be obtained through algal application in the absence of any added chemical nitrogen fertilizer (Table 18). This is of particular interest when one considers the small-scale farmers who are unable to invest in nitrogen fertilizers.

Table 18 AVERAGE GRAIN YIELD DUE TO ALGAL APPLICATION (10 kg/ha) IN THE ABSENCE OF ANY CHEMICAL NITROGEN FERTILIZER (Department of Agriculture, Madhya Pradesh, India, 1977/78)

Number of trials	Grain yield - kg/ha			
	ON	ON + BGA	% increase	CD 5%
160	2 079	2 541	22.2	335

9.1 Algal Application and Nitrogen Economy

A conservative estimate indicates that blue-green algae contribute about 25 - 30 kg N/ha per cropping season which means that to that extent the chemical nitrogen fertilizer could be saved through biological sources. The significance of this is obvious when one considers the present day position of mineral fertilizers. A number of field trials conducted in many parts of India have shown that by algal supplementation, about 25 - 30 kg N can be saved while achieving the •same crop yield obtainable with a full dose of nitrogen fertilizer (Table 19).

9.2 Algal Application at High Levels of Nitrogen

The use of algal biofertilizer in many developing regions should not be viewed solely from the angle of making substantial reductions in fertilizer use, especially when the present average fertilizer consumption in many of these areas is far below the recommended levels. We should view these biological sources from the point of view of giving additional yields for which the incremental input cost is low, so that the net profit to the farmer from the additional biofertilizer application is high. With this aspect in mind, a large number of field trials were conducted by complementing the recommended high levels of nitrogenous fertilizer with algal applications when an increase of 2.2 - 28%, with an average of 7.2%, in yields was obtained (Table 20). Besides nitrogen, these algae also provide to the crop plants many organic substances such as growth factors, vitamins, and so on (see Chapter 7).

9.3 Residual Effect of Algal Application

Algae are living systems and once they establish themselves in the soil their biological activity continues. Normally, continuous application for three or four consecutive cropping seasons results in an appreciable population build up of the algae. The algal effect can be seen in subsequent seasons without any further inoculation unless some unfavourable ecological conditions interact. A persistence of the algal effect has been clearly shown in many trials (Table 16).

Table 19 EFFECT OF ALGAL SUPPLEMENTATION AT REDUCED LEVELS OF NITROGEN FERTILIZER ON THE GRAIN YIELD OF RICE IN DIFFERENT STATES OF INDIA (Venkataraman 1979)

	Grain yield - kg/ha							
	Kerala	Orissa	Bihar	Madyha Pradesh	Maha-rashtra	Tamil Nadu	Uttar Pradesh	Andhra Pradesh
0 N		2 976	2 305	2 416	2 066		3 525	3 636
0 N + BGA		3 710	3 062	2 820	2 555		4 356	4 434
60 N				3 496				
40 N + BGA				3 630				
75 N					3 444	5 240		
50 N + BGA					3 444	5 115		
90 N	3 562							
60 N + BGA	3 838							
100 N					3 733	4 698		5 050
75 N + BGA					3 660	5 212		5 038
120 N							5 833	
60 N + BGA							5 761	

Table 20 COMPLEMENTATION EFFECT OF BLUE-GREEN ALGAL APPLICATION (Department of Agriculture, Tamil Nadu, India)

Number of trials	Grain yield - kg/ha (lOON : 50P : 50K)	(lOON:5OP:5OK + BGA)	% increase
118	5 112	5 485	7.29

The present evidence suggests that (a) in areas where chemical nitrogen fertilizers are not used for various reasons, algal application can give to the farmers the benefit of applying 25 - 30 N/ha and (b) where fertilizers are used, the dose can be reduced to an extent of 25 - 30 kg N/ha and supplemented by algal application to get the same crop yield and, (c) even at hiqh levels of nitrogen fertilization, algal complementation is beneficial and the incremental input cost is low (see Chapter 12). However, there are certain areas where the inoculated algae fail to establish and consequently do not register the beneficial effect. Soil conditions, aggressive indigenous microflora and other factors play a role and such areas need careful examination.

10. PRODUCTION OF ALGAE FOR FIELD APPLICATION

The introduction of algal biofertilizer as a package of practices in rice cultivation will depend largely upon an efficient and economical system of large-scale production of blue-green algae, its preservation and transport. A number of methods have been suggested for this (Venkataraman 1972).

A simple, rural-oriented, open-air method for producing these algae has been developed at the Indian Agricultural Research Institute at New Delhi and this method is being used by many farmers and agencies in India. The method is credited with simplicity of operation and adaptability by small and marginal farmers. The algae used in this system is a mixture of species of Tolypothrix, Aulosira, Anabaena, Nostoc and Plectonema. The same system is reported to be working well in Burma.

The procedure, starting in the laboratory and ending in the field, is described below. It may be noted that other efficient strains of algae may be selected and used, depending on the regions and locations.

10.1 Laboratory Culture

i. Maintain stock cultures of different nitrogen-fixing blue-green algae on 1 to 1.5% agar slants.

ii. Maintain the same cultures also in a soil extract medium (1 g of soil + 10 cm^3 of Fogg's medium, sterilized together in test-tubes). For Fogg's medium and soil extract medium see Appendix V.

iii. Grow the algae in 250 cm^3 flasks containing 100 cm^3 of Fogg's medium in the light.

iv. Scale up the cultures in aspirator bottles or carboys.

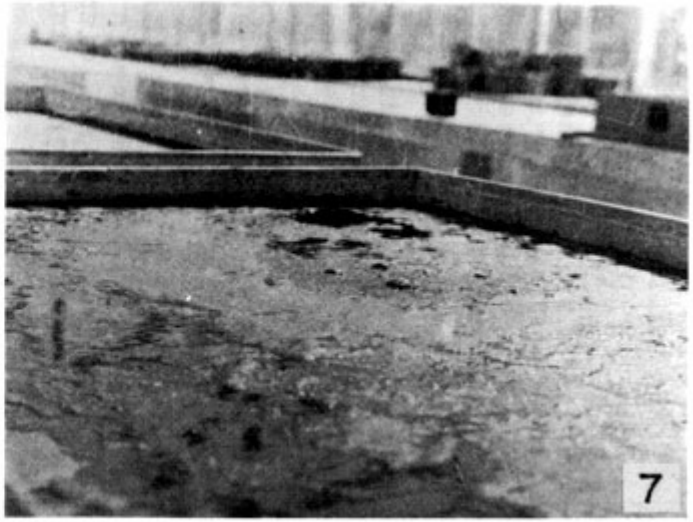
v. Transfer the algae to troughs to prepare soil-based starter material as described later under 'Trough Method'.

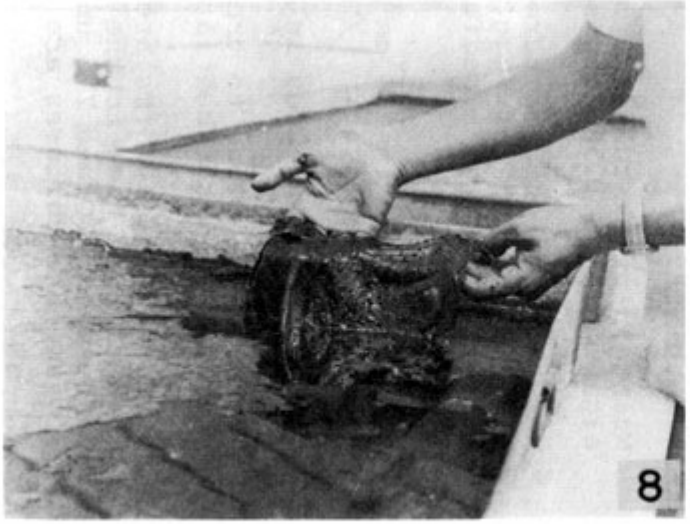
Different algal forms are cultured and maintained separately before transferring them to the troughs. The pH of all the cultures should be between 7.0 and 7.5 unless acid or alkali-tolerant forms are used. All the cultures are grown in light.

10.2 Trough Method (Figures 6-9, 10-17)

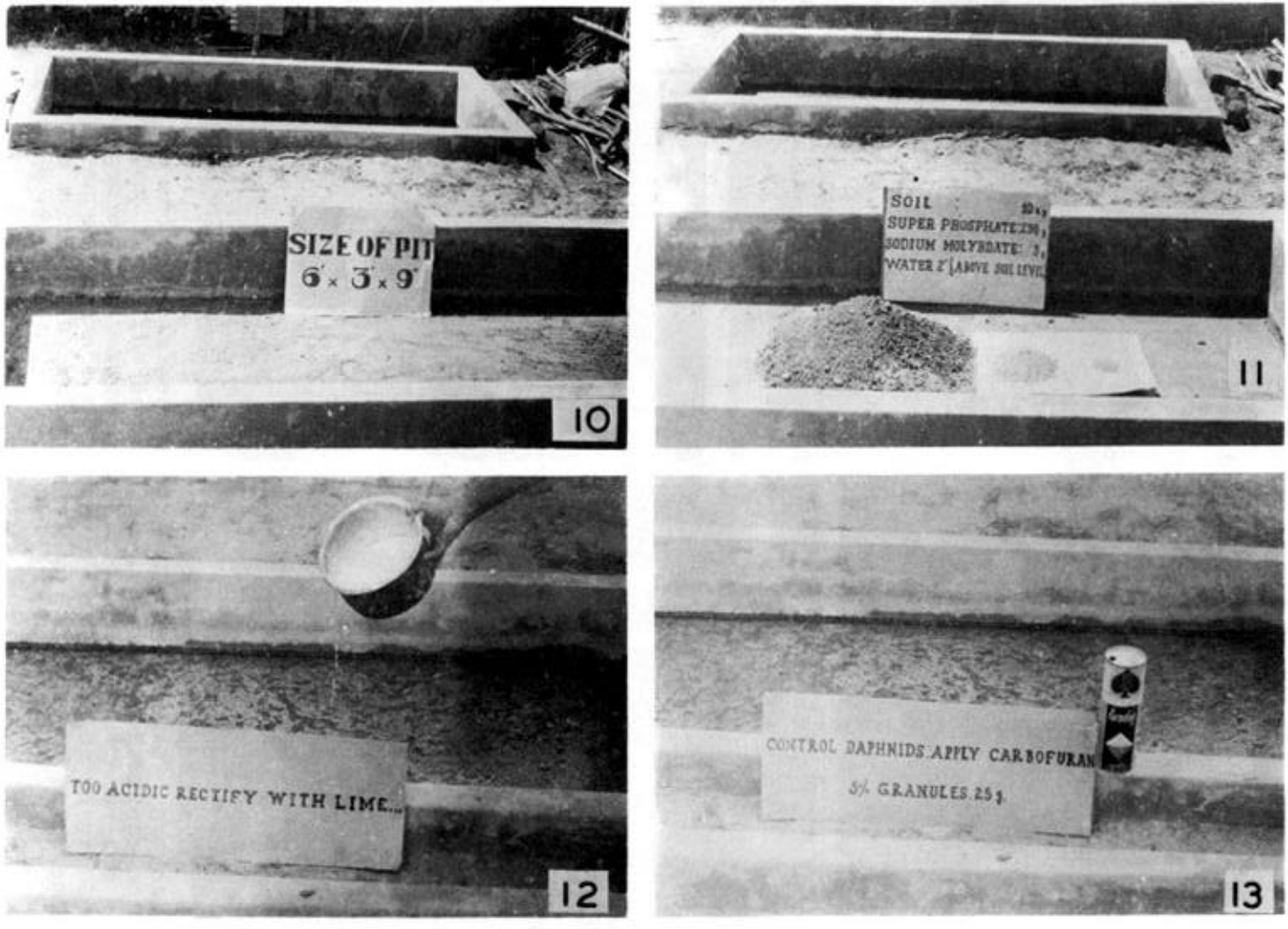
i. Prepare shallow trays (2 m x 23 cm) of galvanized iron sheet (Figure 6) or permanent tanks (Figure 10). The size can be increased if more material is to be produced.

ii. Introduce 8 - 10 kg of soil and mix well with 200 g of superphosphate (Figure 11).





Figures 6-9 Trough method of algal production



Figures 10-13 Tank method of algal production

Figures 14-17 Tank method of algal production

iii. Place from 5 to 15 cm of water in the trays (Figure 6) depending on the local conditions and rate of evaporation. The reaction of the soil should be about neutral; if acidic correct by adding lime (Figure 12 and Appendix X).

iv. To prevent insects, add Carbofuron (3% granules) at the rate of 25 g per tray (Figure 13) or BHC or other suitable insecticide.

v. After the soil has settled, sprinkle the algal culture on the surface of the standing water (Figure 14). Keep the units in the open air and completely exposed to the sun.

vi. In hot summer months, the growth of the algae will be rapid and in about seven to ten days they form a thick mat (Figures 7 and 8). If the daily rate of evaporation is high, add water intermittently. When the algal growth becomes sufficiently thick, stop watering.

vii . Allow the water to evaporate completely in the sun (Figures 9, 15, 16); the dry algae cracks into flakes.

viii . Collect the dry algal flakes from the trays and store in bags for use in the fields (Figure 17).

ix. Fill the troughs again with water and add a small amount of the dry algal flakes as further inoculum. Continue the process as above. Once the soil in the troughs is exhausted (usually after three or four harvests) replace it with fresh soil mixed with superphosphate and continue as above. A single harvest of surface algae from one trough of the given dimensions will give about 1.5 to 2.0 kg of material.

10.3 Pit Method (Figure 18)

This method does not differ from the trough method except in magnitude. Instead of troughs or tanks, shallow pits are dug in the ground and layered with a thick polythene sheet to hold the water (Figure 18). Other procedures are the same as in the trough method. This method is easy and less expensive to operate by small farmers.

The experiments conducted at the Paddy Fxperiment Station at Aduthurai in Tamil Nadu and at the Soil Testing Laboratory at Anakapalli in Andhra Pradesh in India show that addition of sawdust or rice husk to the troughs or pits at the rate of 200 g enhances the biomass production. This is presumed to be due to the availability of a greater surface area and since algae cling to these materials harvesting also becomes easier. Also the accidental collection of excess soil along with the algae during harvesting is avoided. Sometimes the addition of 200 g of superphosphate as a single dose results in problems of acidity. To avoid this, split the dose into two or three smaller doses; similarly the amount of soil can be split.

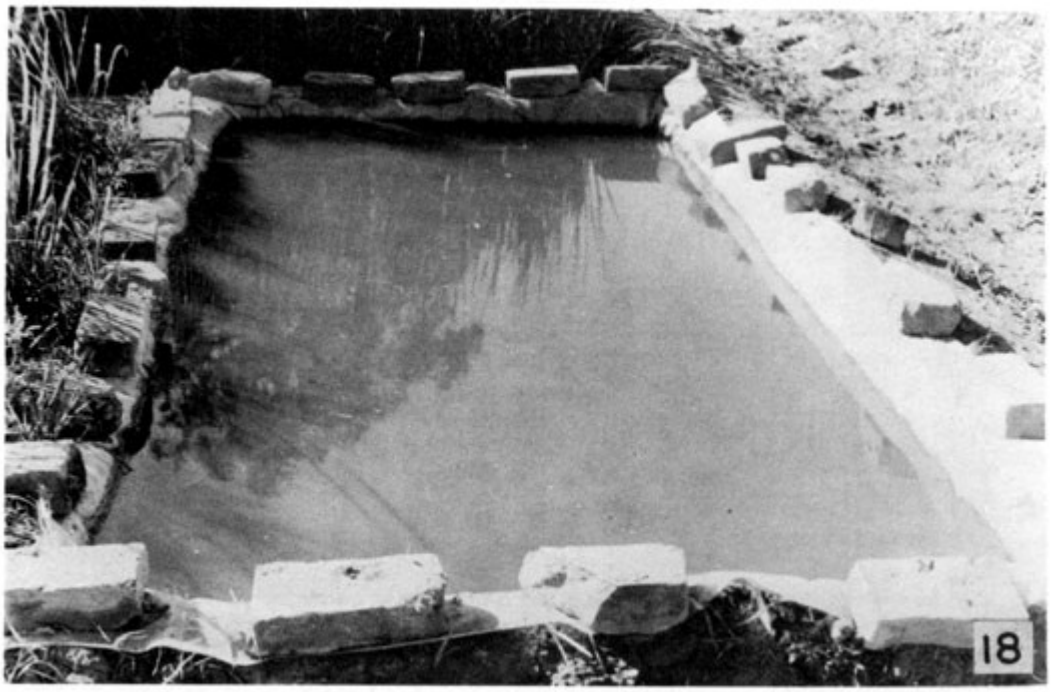


Figure 18 Polythene-lined pit method of algal production

10.4 Field Production (Figures 19-30)

The field production of algae is really a scaled up operation of the trough or pit methods to produce the material on a commercial scale: it is being adopted by a number of farmers in south India.

i. Demarcate the area in the field for algal production; the suggested area is 40 m^2. No special preparation is necessary although if algal production is envisaged immediately after crop harvest, the stubble is to be removed and if the soil is loamy it should be well puddled to facilitate waterlogging.

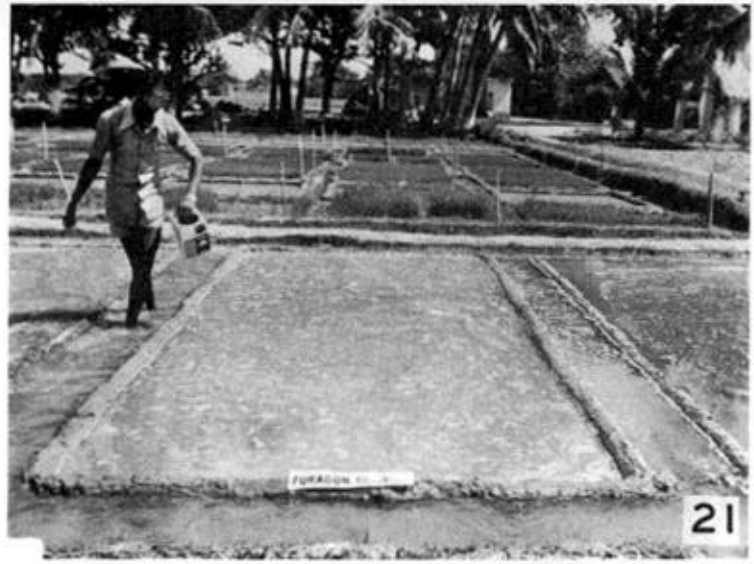
ii. Bund the area with strong 15 cm earth bunds (Figure 19).

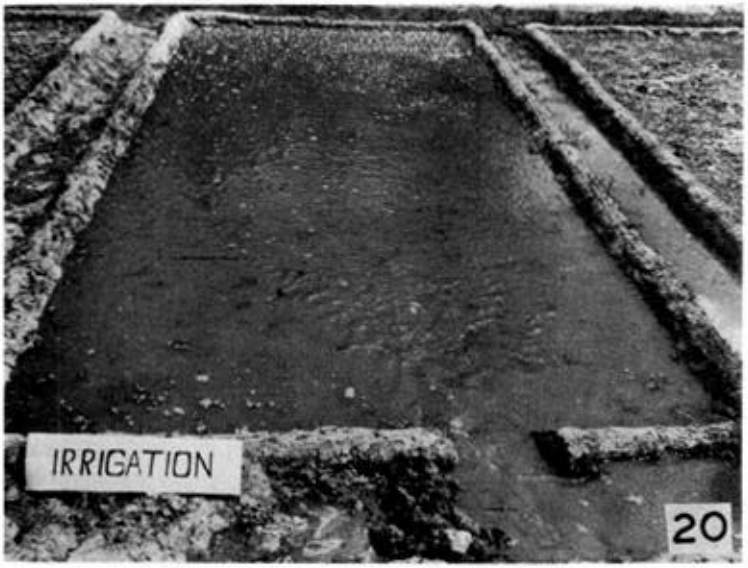
iii. Flood the area with water to a depth of about 2.5 cm (Figure 20). In the trough and pit methods, flooding is done only in the beginning; in the field, flooding is repeatedly needed to keep the water standing.

iv. Apply superphosphate at 12 kg 40 m^{-2} (Figure 22).

v. If the field has previously received algal application for at least two consecutive cropping seasons, no fresh algal application is required. Otherwise, apply the composite algal culture at 5 kg 40 m^{-2} (Figure 23).

vi. To control predators like daphnids, snails and mosquitoes, apply Carbofuron (3% granules) or Ekalux (5% granules) at 250 g 40 m^{-2} or BHC or Furadon (Figure 21).



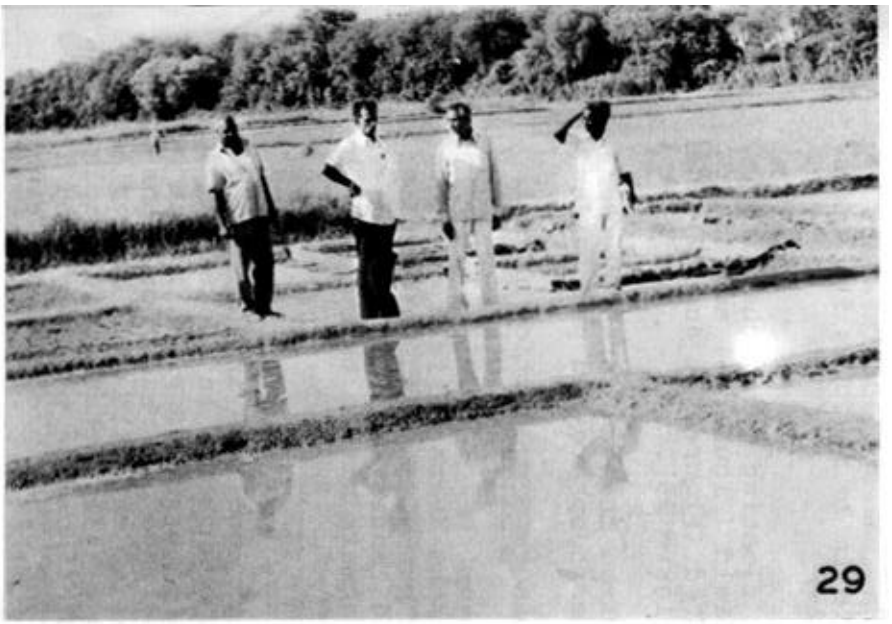


Figures 19-22 Production of algae in the field

Figures 23-26 Production of algae in the field

Figures 27-28

Production of algae in the field



Figures 29-30

Production of algae in the field at the Extension Training Centre, Bakshi-ka-Talab, Uttar Pradesh, India

vii. In clayey soils, good growth of algae takes olace in about two weeks in clear, sunny weather, while in loamy soils its takes about three to four weeks (Figure 24)

viii. Once the algae have grown and form floating mats, they are allowed to dry in the sun in the field (Figure 25) and the dried algal flakes are then collected (Figures 26 and 27) and stored in sacks for further use (Figure 28).

ix. One can continually harvest algae from the same area by re-flooding the plot and applying superphosphate and pesticides.

x. Addition of algal inoculum for subsequent production is not necessary.

xi. During summer months (April-June), the average yield of algae per harvest ranges from 16 - 30 kg per 40 m^2 . Adopting this method, a record production of 15.6 tonnes/ha of wet blue-green algae has been obtained by farmers within three weeks.

10.5 Nursery-cum-algal Production

Farmers can produce algae along with seedlings in their nurseries. If 320 m^2 of land are allotted to prepare a nursery, an additional 40 m^2 alongside can be prepared for algal production as described in the preceding section. By the time the rice seedlings are ready for transplantation, an amount of 15 to 20 kg of algal material will be available and sufficient to inoculate about one and a half hectares. Transplantation is made in the nursery and algal plots and in this way land is neither wasted nor locked up exclusively for algal production during the growing season.

In China, at the Agricultural Research Institute, Nanjing, a mixture of Anabaena sp. and Nostoc sp. is prepared for field application according to the following procedure (FAO 1977). The algae are first grown in flasks containing sterile medium and are then transferred to large glass bowls under non-sterile conditions. Nursery plots of 5-7 m x 1 m x 20 cm, containing 6-7 cm of water are inoculated at the rate of 150 g algae per m^2. After about seven days the algae attain a biomass of 500 - 1 000 g/m^2. The nursery plots are covered with transparent plastic sheets to protect them from low temperatures (Figure 31). The field inoculation is done by spreading the algae at the rate of 750 kg/ha, which grows to attain a biomass of 7.5 tonnes/ha within 10 - 15 days and if the temperature exceeds 303 kelvin (30 °C) 15 tonnes/ha can be reached.

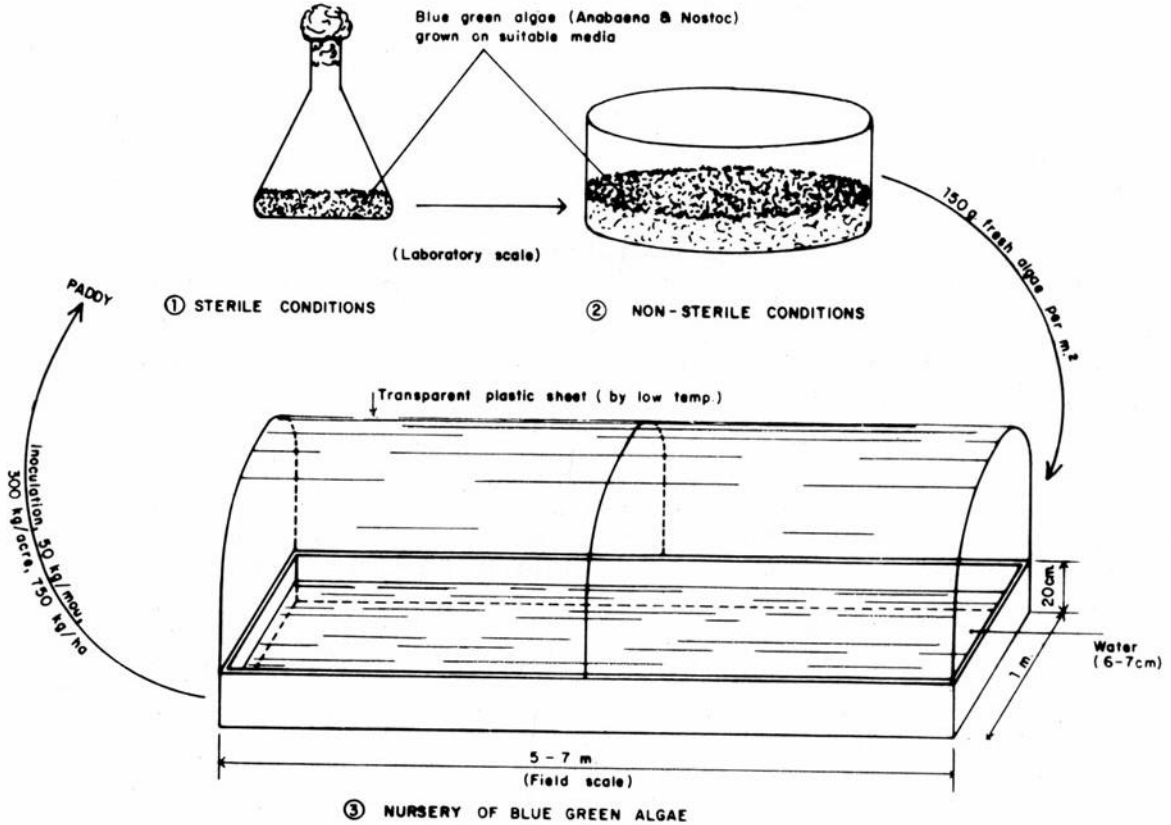


Figure 31 Algal multiplication in Nanjing, Jiangsu Province, China (from FAO Soils Bulletin No. 40)

11. RECOMMENDATIONS FOR FIELD APPLICATION OF BLUE-GREEN ALGAE

i. If mineral nitrogen fertilizers are not being used, apply blue-green algae in order to gain the benefit of from 20 - 30 kg N/ha.

ii. Broadcast the dry algal material over the standing water in the rice field at a rate of 10 - 15 kg/ha one week after transplanting the seedlings. Addition of excess algal material is not harmful and will accelerate the multiplication and establishment in the field. Providing that the field is not being used and that water facilities are available, algal application can be done well in advance of transplanting the rice.

iii. When mineral nitrogen fertilizer is used reduce its dose by 25 kg/ha and supplement with algal application.

iv. If so desired, algae can also be used along with high levels of nitrogen fertilizer.

v. The sun-dried algal material can be stored for a long time in a dry state without any loss in viability.

vi. Do not store the algal material in direct contact with fertilizers or other chemicals.

vii . Apply algae for at least three consecutive seasons.

viii . Recommended pest control measures and other management practices do not interfere with the establishmEnt and activity of the algae in the field.

12. ECONOMICS OF ALGAL PRODUCTION AND APPLICATION

The low cost algal technology has an income and employment-generating potential and can be integrated into rural development programmes.

12.1 Algal Production

Large-scale algal production is carried out in many experiment stations (Figure 32), State Seed Farms (Figures 33 and 34), village level 'Panchayat Unions' (Fiqure 35) and by many farmers by adopting the procedures described in Chapter 10.

At a State Seed Farm in Tamil Nadu, India, where algae are produced using a portion of the threshing floor (8.5 x 7.1 x 0.23 m), the cost of production and the income are as follows:

a. Raw materials

Blue-green algal culture	8.25 kg	US $	2.96
Superphosphate	8.25 kg		0.60
Sawdust	6.50 kg		0.09
Carbofuron	0.81 kg		1.29
Labour charges			1.32
			6.26

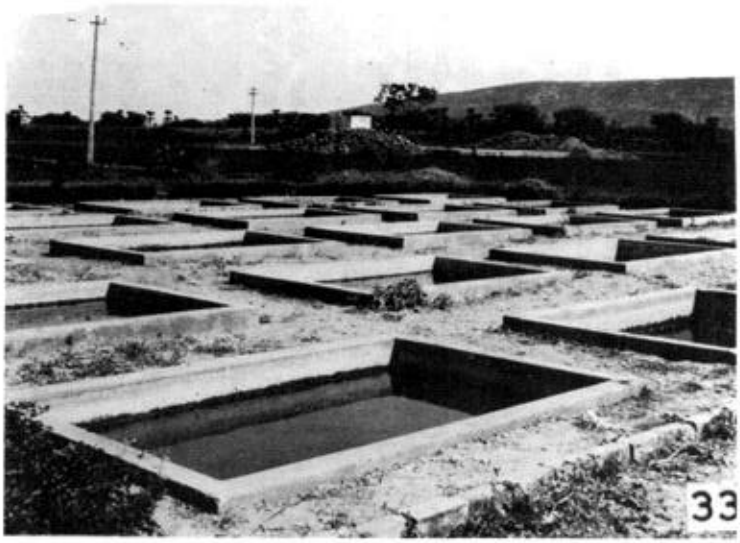
b. Produce obtained (algal harvest) 100 kg

c. Cost of harvest production 0.06

d. Return

Cost of material obtained per harvest (100)kg at rate of US $ 0.24 per kg	24.00
In one year of normal conditions the farm can produce 27 harvests; thus total income will be	478.96
Net profit per harvest	17.74

Many farmers use shallow pits layered with plastic sheets or use permanent tanks constructed sometimes on their roof-tops (Figure 37). Based on a village level unit where the farmer employs the pit method (1.5 x 0.3 x 0.2 m), the cost of production works out to about US $ O.O8 per kg of material at the rate of 10 kg per harvest in fifteen to twenty days, sufficient to inoculate one hectare.



Figures 32-35 Algal production units

Figure 36

Algal production unit at the Soil Testing Laboratory, Anakapalli, Andhra Pradesh, India

Figure 37

Farmer's algal production unit built on the roof-top of his house

Production of algae in the rice field yields about 2 tonnes per 2 000 m^2 in 25 - 30 days, for which the expenditure involved is about US $ 60. Thus the production cost is only about $ 0.03 per kg. The current sale price is $ 0.12 per kg and thus the net income during the short, non-cropping off season to the farmer is about $ 240

The cost-return for a typical farmer's field at Nasaratpet village in Chenglepet District of Tamil Nadu is as follows:

Area of production of algae = 2 000 m^2

Costs of production

Land preparation and labour	US $ 8.98
Superphosphate	7.18
BHC	1.44
Water, electricity	17.96
Algal inoculum (100 kg)	29.35
	59.51

Algal production = 2 600 kg/30 days

Production cost per kg	US $ 0.022
Sale of produce	311.00
Net total income	251.00

Table 21 shows the quantity of blue-green algae produced at Block Headquarters and in the farmers' fields in two Agricultural Divisions in Tamil Nadu during 1978/9.

Table 21 QUANTITY OF BLUE-GREEN ALGAE PRODUCED AT BLOCK HEADQUARTERS AND IN FARMERS' HOLDINGS IN TENKASI AND SANKARANKOIL AGRICULTURAL DIVISIONS IN TAMIL NADU, INDIA

Location	Quantity of algae in tonnes		
	Block Headquarters	Farmer's holdings	Total
Tenkasi Division			
Tenkasi	-	2.06	2.06
Kadayanallur	2.15	7.79	9.94
Keelapavoor	1.39	6.00	7.39
Alangulam	0.25	9.75	10.00
Kadayam	0.28	2.51	2.79
Ambasamudram	2.69	9.31	12.00
Mukkudal	0.32	3.13	3.45
Cherenmahadadevi	0.89	7.31	8.20
Shenchottah	0.O5	0.76	0.81
State Seed Farm, Melagaram	0.42	-	0.42
Sankarankoil Division			
Sankarankoil	0.002	0.19	0.19
Karivalamvandanallur	0.28	0:93	1.21
Vasudevanallur	1.00	1.06	2.06
Sivagiri	0.45	0.87	1.32
Puliangudi	1.12	0.68	1.80
Melaneelithanallur	-	1.20	1.20
Sendararam	-	1.20	1.20
Koilpatti	-	2.75	2.75
Kayathar	-	1.74	1.74
Ottapidaram	-	0.90	0.90
Vilangaikulam	0.03	0.84	0.87
Pudur	-	0.70	0.70
Kuruvikulam	-	0.20	0.20
Tiruvenkadam	0.02	0.37	0.39
Total	11.34	62.24	73.57

12.2 Algal Application

Twenty-five kilogrammes of nitrogen in terms of chemical fertilizer will cost around US $ 12.00. To provide this amount of nitrogen through algal inoculation and nitrogen fixation requires about 10 kg per hectare of algal material, which will cost US $ 1.20 to $ 2.40; if the farmer produces his own material, the cost will be less than one dollar.

Table 22 shows the complementation effect of algal application and additional income obtained by the farmers. The average increase in yield is around 10% and the net average additional income is about US $ 59.

Table 22 ADDITIONAL YIELD OF RICE AND INCOME OBTAINED BY FARMERS AS A RESULT OF ALGAL COMPLEMENTATION TO MINERAL FERTILIZATION WITH NPK AT 100:50:50 (District Agricultural Officer, Tenkasi 1978/9)

Location	Variety	Grain yield kg/ha		Percent increase	Additional income US $
		NPK	NPK/algae		
Urkadu	IR2O	4 485	4 950	10.3	53.05
Singampetti	IR2O	4 960	5 360	8.0	45.51
Brimmadesam	IR2O	5 535	6 125	10.6	67.66
Vikramasingapuram	ADT31	5 285	5 935	12.3	75.45
Aventhiruvaleswaram	ADT31	5 045	5 525	9.5	55.00
Kodarankulam	ADT31	4 000	4 935	14.7	73.65
Pappankulam	ADT31	4 065	4 502	10.9	50.90
Kallidaicurichi	ADT31	4 440	4 985	12.2	62.87
Mela Ambasamudram	ADT31	4 340	4 725	11.1	55.69
Keela Ambasamudram	ADT31	4 525	4 995	10.3	53.29
Mean		4 668	5 404	10.9	59.32

13. INDIAN STATE LEVEL ORGANIZATIONAL PATTERN FOR THE TRANSFER OF BLUE-GREEN TECHNOLOGY

Every country has to develop and fit in a pattern of organization for the transfer of technologies from laboratory to land. In agriculture, the clientele are the farmers and their immediate contact points for any innovation or recommendation are the village level workers (VLWs) who form a part of the extension machinery of the State. However an extension service cannot function for long without an effective research programme to support and sustain it. A scheme of organizational pattern is indicated in Figure 38, although the designations may vary from place to place. It is recommended that this programme be integrated into the overall extension system.

13.1 Headquarters

At the State level, the Director of Agriculture will be in charge of the programme and will be assisted by a Joint Director who would be responsible for the technical and operational aspects. He would in turn, be assisted by a Deputy Director of Agriculture in planning, execution and implementation of the work at various levels with constant monitoring and evaluation.

13.2 Research Stations

The responsibilities of the Research Stations will be to develop, refine and update a compact Research and Development schedule for the technology to fit into the overall management practices. Isolation of efficient strains of algae and devising economical methods for their multiplication, evaluation of agronomical responses and other similar aspects should form part of their activities. Research stations should also be responsible for training and building up a body of subject matter specialists (SMS). They should also conduct well-replicated trials on their farms for critical information and assessment and should jointly prepare with the extension personnel schedules for field trials on farmers' land to be carried out by the Agricultural Extension Officers (AEOs) and Village Level Workers (VLWs). These trials should be simple with small-sized plots, normally with no replications on the same farmer's land. By carrying out these trials in relatively large numbers, one can ensure the reliability of results.

The extension staff are not confined merely to showing the effectiveness of the technology in demonstration fields, but they establish. large, Compact Block Demonstrations in which several techniques are set up and contrasted under various natural and artificial conditions in a particular area, thereby educating the farmers to apply such techniques on their own farms.

13.3 Subject Matter Specialists (SMS)

The main purpose of having subject matter specialists is to give educational support to the extension personnel. They are normally located in research stations and also at district levels. They conduct experiments, relay information from the experimental stations or administrative authorities and give periodical subject matter training. Their advice is also directly available to extension personnel at various levels.

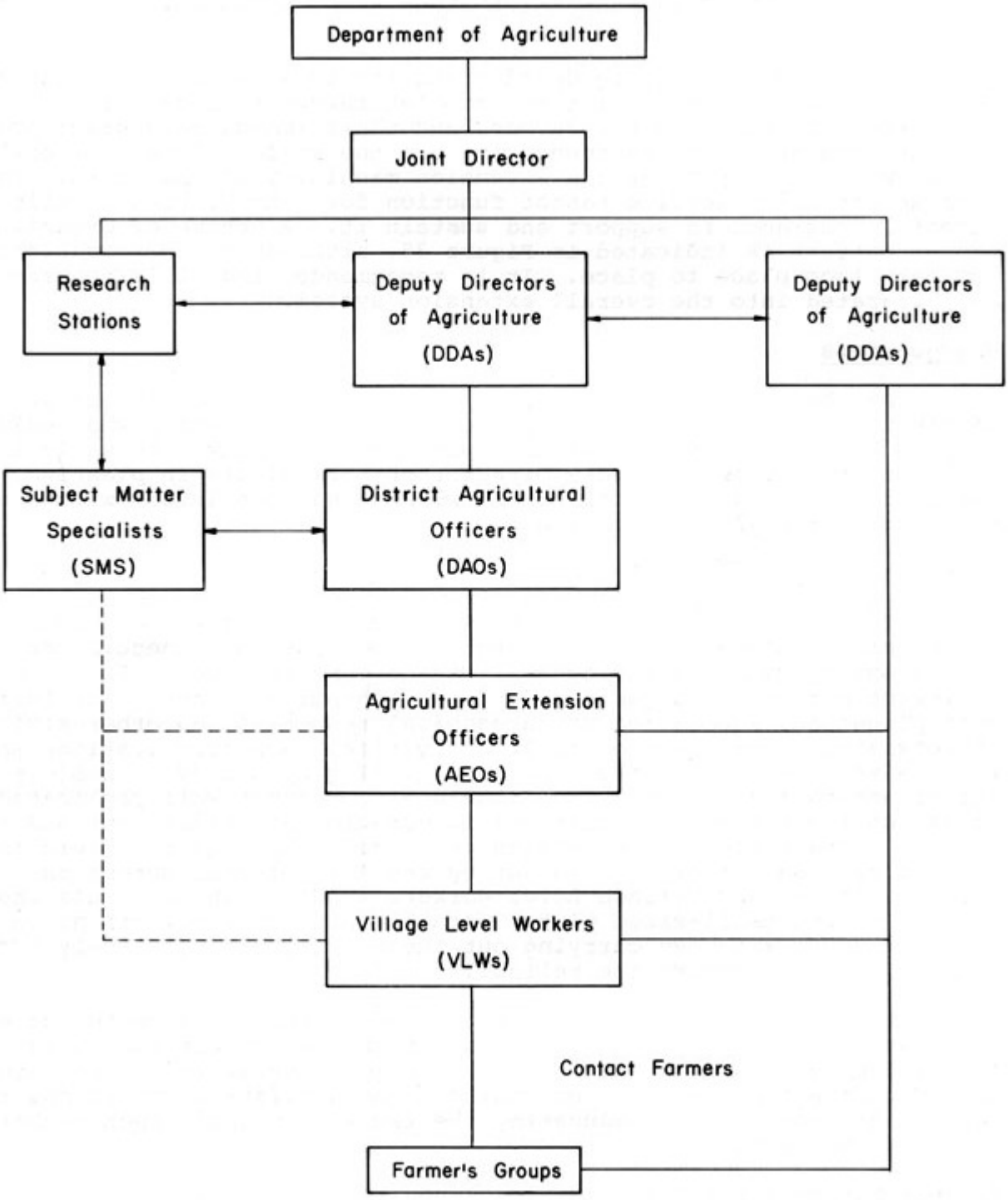


Figure 38 State level organization pattern for implementing blue-green algae technology transfer

13.4 District Level

At district level the Deputy Director of Agriculture (DDA) will interact with the farmers' training centres (FTCs) in organizing the training and extension programmes and will be assisted by the District Agricultural Officers (DAOs). One or more DAO will be the subject matter specialist(s) who are trained at the research stations. These subject matter specialists would be involved with the farmers' training centres in the regular training of the agricultural extension officers and village level workers as and when required.

13.5 Block Level

The agricultural extension officers at the block level will be supervised by the District Agricultural Officers. The AEOs will provide technical support for the village level workers and help them in carrying the message to the farmers.

13.6 Village Level

The village level workers play a key role in the actual transfer of technology to the farmers. The VLWs will live in the area and their ratio to farm families will depend upon local situations. In consultation with the village leaders, the VLWs will select about ten percent of the farmers as 'contact farmers'. The VLW will visit the fields demonstrate the recommended techniques and provide technical guidance. If there are any problems beyond his immediate ability to solve, he should bring them to the notice of those at the next in-service training session. He should also hold group discussions to appraise the farmers of the latest recommendations.

Periodic in-service training for VLWs at the farmers' training centres is an integral part of the exercise for they not only learn what to recommend and how to recommend it but they also can bring the farmers' problems to the notice of the trainers. The main purpose of the training sessions is to make the VLWs subject matter specialists on matters of immediate relevance.

Different kinds of extension methods are to be used taking into consideration various factors such as time, location, technical requirements, living standards and literacy of the farmers. For this purpose, audio-visual media such as slides, moving films, radio, television, graphical explanations and billboards (Figures 39 and 40) at vantage points are applied in addition to free distribution of pamphlets in local languages. Also farmers must have free access to the demonstration fields.

Figures 39-40 Billboards on algal fertilizers put up by the Department of Agriculture, Tamil Nadu, India.

14. ELEMENTS OF DEVELOPMENT PROGRAMMES

The algal technology developed in India could be initially adopted by other rice growing countries in the developing regions on an experimental basis and, if required, assistance could be arranged for the transfer of further information and other details. Ultimately every country has to draw from its own national expertise to introduce this technology to its agricultural development programme. Some of the following elements should be considered when initiating a Research and Development Programme.

A. Research

i. Since the ecological and edaphic conditions may vary in different regions, it is necessary to initiate active research programmes to isolate and identify different blue-green algal strains native to their own respective environments.

ii. Ecological and physiological characterization of the strains and establishment of an authentic National Culture Collection of Nitrogen-fixing Blue-green Algae must be done.

iii. It is necessary to standardize growth requirements, tolerance to stresses, competitive ability and multiplication techniques for suitable algal isolates.

B. Development

i. Algal mixtures should be multiplied under laboratory and field conditions.

ii . Algal multiplication units should be set up and possibly integrated with rural development programmes. The strategy should be to train farmers themselves to prepare the algal material; where this is not possible, the material will have to be prepared by other agencies and supplied to the farmers at an economical cost.

C. Extension

i. Algal material should be tested at experimental stations under different agroclimatic conditions.

ii. Large-scale demonstrations should be organized on government farms and on farmers fields, combined with other, related, extension methods.

iii . Popularization of the technology through mass media and other channels is essential.

D. Training

i. It is worthwhile organizing an intensive short-term training course in each country with the initial help of an expert.

ii. Periodic workshops and in-service training to extension personnel may be organized through the extension training centres.

iii. Similar training may also be given to the farmers to create a better awareness and a consumer demand.

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APPENDIX I

GENERAL FEATURES OF BLUE GREEN ALGAE

Blue-green algae are primitive micro-organisms characterized by a low state of cell organization. The ecological and agricultural importance of these organisms depends on the ability of certain forms to carry out both photosynthesis and nitrogen fixation. The cells of these algae lack a well defined nucleus and cell division is by division of the protoplast. They are generally blue-green in colour, the chief pigments being chlorophyll a, carotenes, xanthophylls, c-phycocyanin and c-phycoerythrin. The photosynthetic product is glycogen. These algae are characterized by the absence of flagellated reproductive bodies and sexual reproduction has not been recorded so far. They are ubiquitous in their distribution and commonly found in rice field soils.

Blue-green algae are unicellular (Fig. 41), colonial (Fig. 43) or filamentous (Fig. 44). The cells of the unicellular types are usually spherical, cylindrical or elliptical, with (Fig. 42) or without (Fig. 41) a well defined sheath. In others the cells remain aggregated after cell division to form well defined colonies (Fig. 43).

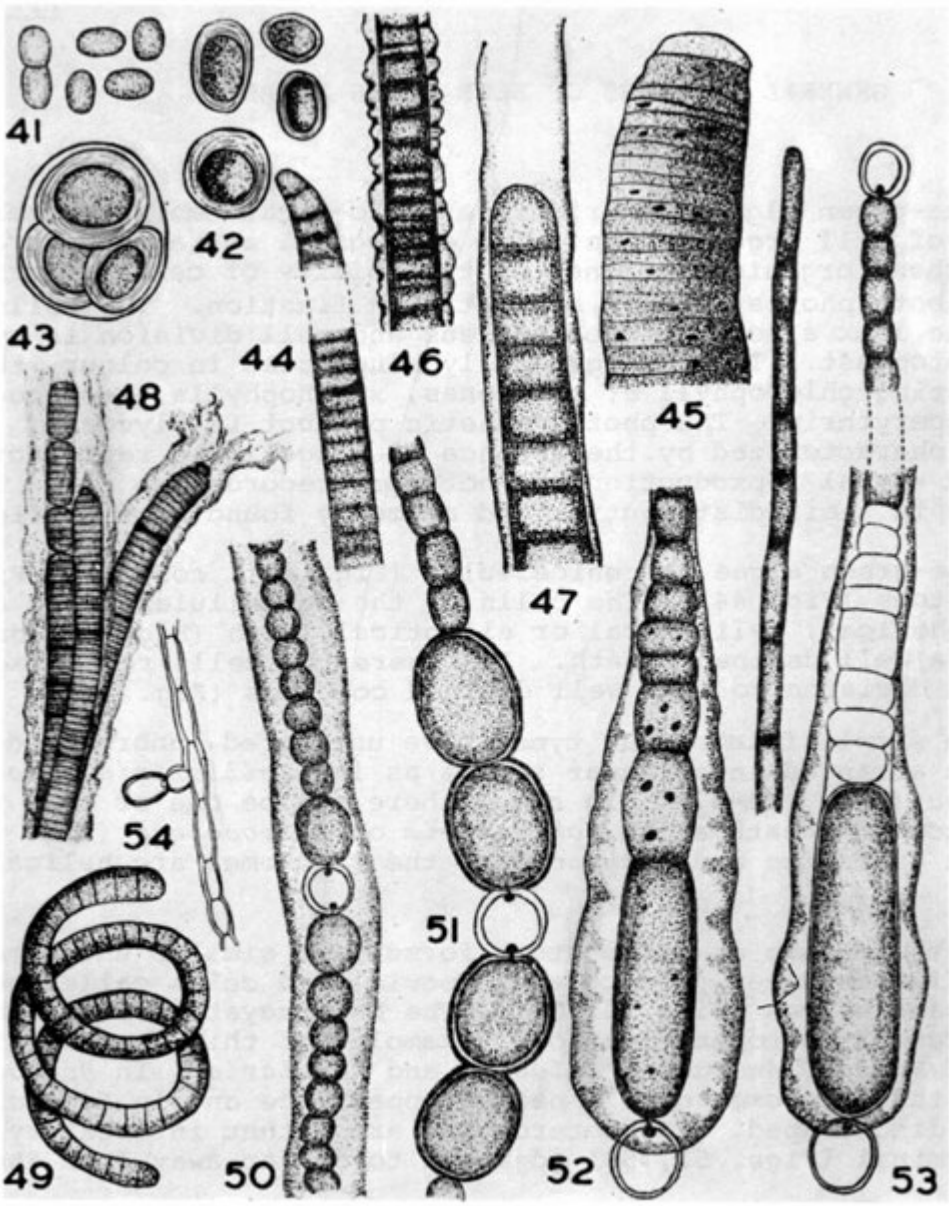
The simple filamentous types have untapered, unbranched filaments with cells arranged in a linear series as in Oscillatoria, Phormidium and Lyngbya (Figs. 44-47). In some, there may be one or more trichomes within a common sheath as in Schizothrix or Hydrocoleus (Fig. 48). In forms like Spirulina and Arthrospira, the trichomes are helical (Fig. 49.

Another group of filamentous forms have similar untapered unbranched filaments but produce some specialized cells called heterocysts (Fig. 50) and spores (Figs. 51-53). The heterocysts are considered to be the sites of nitrogen fixation. Examples of this type are the species of Nostoc, Anabaena, Aulosira and Nodularia. In Nostoc and Anabaena, the trichomes give a beaded appearance and in Nodularia the cells are disc-shaped. The heterocysts are either intercalary Fig. 50) or terminal (Figs. 52, 53) adjacent to or far away from the spores.

The third group of filamentous blue-green algae have false branches and untapered heterocystous filaments, typical examples being Scytonema and Tolypothrix· These false branches may either be single (Fig. 56) or geminate (Fig. 57).

The fourth group includes forms in which filaments may or may not show false branching, but are distinctly tapered often ending in a hair (Fig. 53). The filaments have a basal and sometimes intercalary heterocysts. Examples are Rivularia, Gleotrichia and Calothrix.

The fifth group of filamentous blue-green algae exhibits a complex organization. They are often differentiated into a prostrate and erect system with true branching (Fig. 55). The cells divide predominantly in two, but sometimes in three, directions. Forms belonging to this group are Mastigocladus, Stigonema, Hapalosiphon and other members of the order Stigonematales.



Figures 41-54 Thallus organization

Trichomes — The row of cells in filamentous blue-green algae is called a trichome (Figs. 44, 45).

Filament — The trichome along with the sheath is called a filament (Figs. 46, 47, 50).

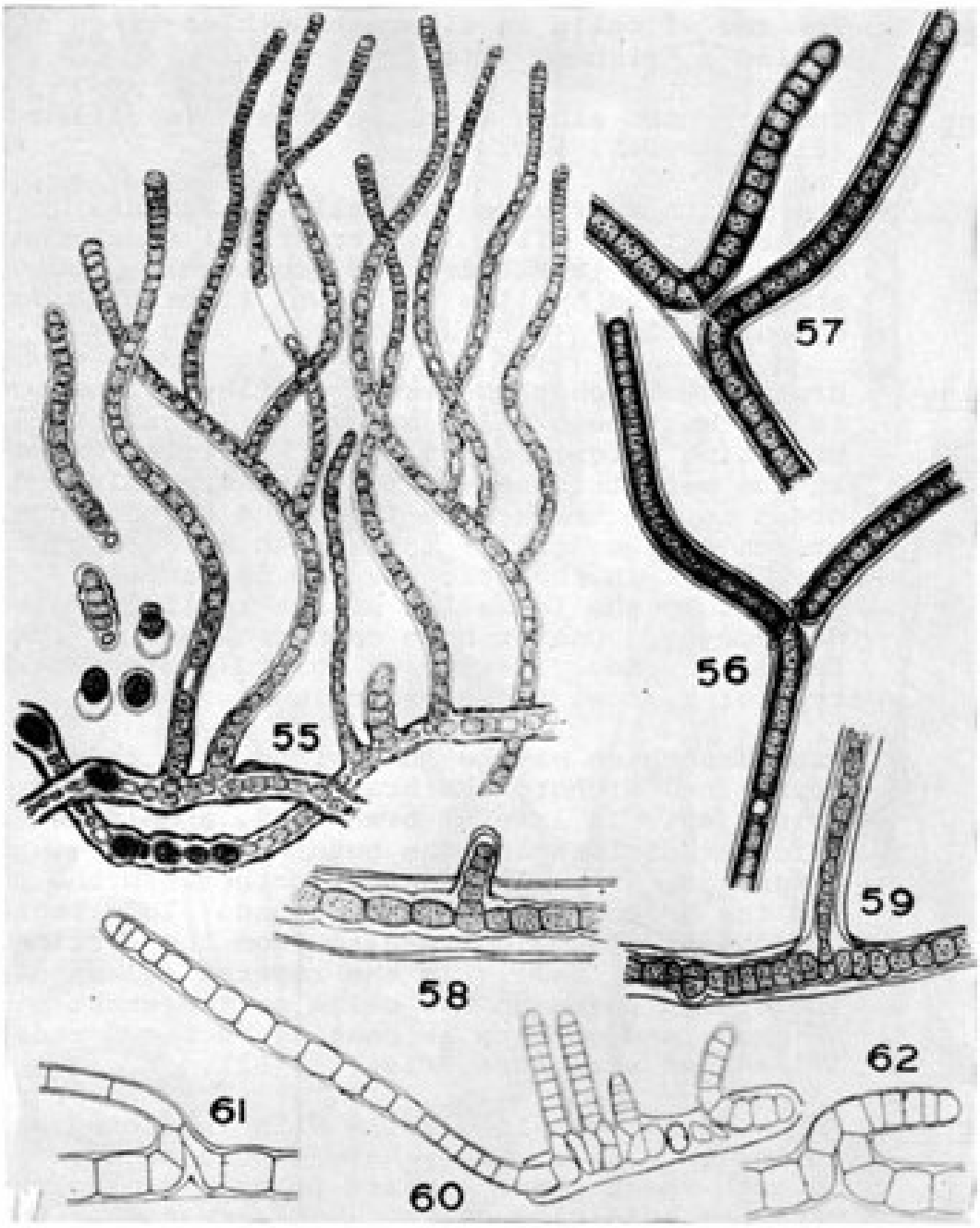
Sheath — The sheath surrounds the cells, filaments or colonies (Figs. 42, 43, 47). In terrestrial forms the sheath is often thick, lamellated and frequently pigmented. The sheath may be hyaline or brown, light blue or red in colour.

Branching — Branching is characteristic of many filamentous forms and is of two types, false branching (Figs. 56, 57) and true branching (Figs. 55, 58, 59). In false branching, a cell in the main trichome divides transversely and there is a break in the trichome between the daughter cells and the branch emerges out of the sheath at the point of the break. This break in the trichome may be caused by the death of a cell or by the formation of intercellular discs or a heterocyst. One or both ends grow out of the sheath as false branches. Scytonema and Tolypothrix exhibit this type of typical false branching.

True branching may be subdivided into three types, lateral branching, dichotomous branching and reverse 'V'-shaped branching. In lateral branching, a cell in the main trichome divides and the outer cell grows into a branch (Figs. 58, 59). The connection between the main filament and the branch is always retained. In dichotomous branching, branching results from the vertical division of the apical cell. In the reverse 'V'-shaped branching, the branch rests on two cells or two short pieces of trichome and appears as resting on the vertex of a reverse 'V'-shaped structure (Figs. 60-62).

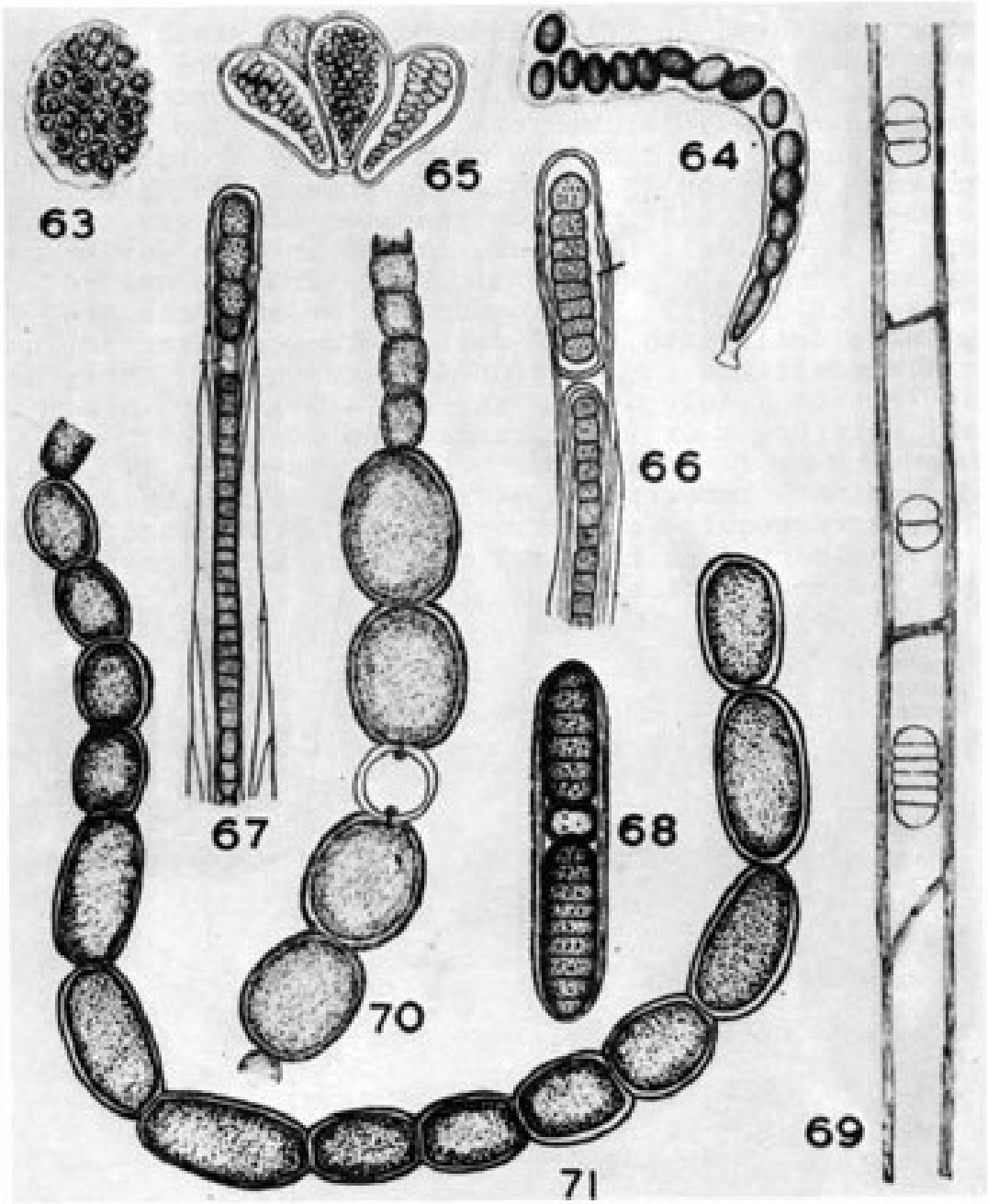
Heterocysts — These are specialized cells with thickened walls, usually yellow in colour. They have a pore at one or both ends through which there appears to be a protoplasmic connection with the adjoining cells. Heterocysts may be terminal (Fig. 53), intercalary (Fig. 50) or sometimes lateral (Fig. 54) with or without a pedicel. Many functions have been attributed to these cells such as reproductive cells, organs of attachment, regulators of spore formation and site of nitrogen fixation.

Reproductive bodies — The blue-green algae reproduce vegetatively either by hormogonia, hormocysts, endospores, exospores or akinetes. Hormogones are small pieces of trichome with one to many uniform cells (Fig. 69). Hormogone formation is one of the most common modes of reproduction and in some cases the only known mode of propagation in Nostocales and Stigonematales. In some forms, small pieces of trichome homologous to the hormogones enclose themselves in a thick lamellated pigmented sheath and become perennating bodies. These are called hormocysts or pseudo-



Figures 55-62 Types of branching

hormogonia (Figs. 66-68). Endospores are small spores formed endogenously within a cell by the quick succession of divisions in three planes. These are commonly formed in certain unicellular members (Fig. 65). Exospores are serially abstracted from the open ends of sporangia by transverse division (Fig. 64). In some forms like Gloeocapsa, the cells undergo repeated divisions so that groups of very small cells are formed in each parent cell. These are generally naked protoplasts and are called nanocytes (Fig. 63). Resting spores or akinetes are very large cells with thick walls. They are formed in specific positions in relation to heterocysts. They are formed either singly or one on each side of an intercalary heterocyst or in short or long chains either adjacent to or far away from heterocysts (Figs. 70, 71). Some germinate immediately giving rise to new trichomes while others require a resting period. They remain viable for a long period of time and can withstand high temperatures and desiccation.



Figures 63-71 Reproductive bodies

APPENDIX II

LIST OF NITROGEN-FIXING BLUE-GREEN ALGAE

The list of nitrogen-fixing blue-green algae is steadily expanding and there is a suspicion that the capacity to fix nitrogen may be more widespread in this group than hitherto thought. The unicellular and the non-heterocystous filamentous forms fix nitrogen under microaerophillic conditions, while the heterocystous, filamentous forms fix nitrogen aerobically.

Unicellular

- Gloeocapsa 795
- Gloeocapsa 6501
- Aphanothece pallida (?)

Non-heterocystous, filamentous

- Lyngbya 6409
- Oscillatoria 6407
- Oscillatoria 6412
- Oscillatoria 6506
- Oscillatoria 6602
- Microcoleus chthonoplastes
- Plectonema boryanum
- Plectonema 6306
- Plectonema 6402
- Phormidium foeveolarum
- Schizothrix sp.
- Trichodesmium (marine)

Heterocystous, filamentous

- Anabaena ambigua
- A. anomala
- A. azollae (endophytic in Azolla)
- A. azotica
- A. catanula
- A. circinalis
- A. cycadeae (endophytic in cycads)
- A. cylindrica
- A. doliolum

A. fertilissima
A. flos-aquae
A. gelatinosa
A. humicola
A. iyengarii
A. lavenderii
A. laxa
A. lemmermanii
A. minutissima
A. naviculoides
A. oryzae
A. scheremetrevi
A. sphaerica
A. spiroides
A. torulosa
A. variabilis
Anabaenopsis circularis
A. milleri
Aulosira fertilissima
Calothrix antartica
C. braunii
C. brevissima
C. clavata
C. crustacea
C. elenkenii
C. gracilis
C. membranacea
C. parietina
C. scopulorum
Camptylonema lahorense
Chlorogloea fritschii
Cylindrospermum alatosporum
C. gorakhpurense
C. lichenforme
C. majus
C. michailovskoense
C. sphaerica
Fischerella major

F. muscicola
Gloeotrichia natans
G. raciborskii
G. echinulata
Hapalosiphon delicatulus
H. fontinalis
H. intricatus
Mastigocladopsis jogensis
Mastigocladus Zaminosus
Nodularia harveyana
N. spumigena
Nostoc amplissimum
N. calcicola
N. commune
N. carneum
N. cycadeae (endophytic in cycads)
N. ellipsosporum
N. entophytum
N. humifusum
N. microscopicum
N. muscorum
N. paludosum
N. piscinale
N. punctiforme
N. sphaericum
N. spongaeforme
N. verrucosum
Nostochopsis lobatus
Rivularia aquatica
Scytonema arcangelli
S. bohneri
S. hofmanni
S. ocellatum
S. javanicum
Scytonematopsis sp.

Stigonema dendroideum
Tolypothrix campylonemoides
T. tenuis
T. polymorpha
T. rivularis
Westiella lanosa
Westiellopsis prolifica
Wollea bharadwajae

KEYS TO THE ORDERS AND GENERA OF SOME NITROGEN-FIXING BLUE-GREEN ALGAE

The blue-green algae (Myxophyceae) are divided into five orders and the keys given here are those of Desikachary (1969). The keys do not, however, cover all the families and genera but will help in identifying the forms for which there is evidence of nitrogen-fixing capacity. Not all species of blue-green algae have been tested for nitrogen fixation and the ability of some non-heterocystous forms to fix nitrogen under certain conditions (e.g. micro-aerophillic) suggests that this property may be widespread in this group of organisms. For complete keys, the reader is referred to Desikachary (1969).

KEY TO THE ORDERS

Plants unicellular or colonial, sometimes forming a pseudofilamentous colony, never with a 'trichome organization', no differentiation into base and apex, endospores not formed in sporangis; no exospores; nannocytes present.

Chroococcales

Plants unicellular, attached, typically differentiated into base and apex, reproduction by endospores or exospores.

Chmaesiphonales

Plants more or less distinctly filamentous, attached, arrangement very uniform, chrococcaceous structure, often forming parenchymatous thalli with prostrate and erect filaments, without differentiation into trichome and filament, no hormogones, no heterocysts, endospores in sporangia.

Plerocapsales

Plants filamentous with trichome and filament organized or 'hormogonalen organization', hormogones present, often with heterocysts, akinetes, exospores or endospores, pseudohormogonia present.

> Without true branching, unbranched, or with false branching.
>
> Nostocales
>
> With true branching or dichotomous branching and often with heterotrichous condition, i.e. with a differentiation of prostrate and erect portions.
>
> Stigonematales

KEY TO THE GENERA

ORDER CHROOCOCCALES

Cells unicellular or forming colonies, not forming filamentous growth; cells generally many in a single colony; cells without any regular or definite arrangement, cells with distinct individual envelopes or sheath; colonial, mucilage not homogenous; individual sheaths vesicular and broad and formed one in another; cells spherical.

ORDER NOSTOCALES — Gloeocapsa

FAMILY OSCILLATURIACEAE

Trichomes, cylindrical without a sheath or single within a sheath; and sheaths open always

1. Trichomes with a prominent sheath; sheath firm; filaments not in bundles — Lyngbya

2. Trichomes without prominent sheath; more or less straight, not regularly spirally coiled and not in bundles — Oscillatoria

3. Sheath mucilagenous, filaments forming a thallus with more or less confluent sheath — Phormidium

FAMILY NOSTOCACEAE

1. Trichomes without firm sheath, generally not endophytic; heterocysts present — 2

1. Trichome with a firm sheath, single within a sheath; heterocysts present — 6

2. Intercalary heterocysts generally in pairs — Anabaenopsis

2. Intercalary heterocysts generally single — 3

3. Heterocysts commonly terminal with a single large spore adjoining — Cylindrospermum

3. Heterocysts terminal rarely; generally intercalary — 4

4. Filaments single or in formless gelatinous mass — Anabaena

4. Filaments generally in definite colonies — 5

5. Thallus finger shaped, attached at first — Wollea

5. Thallus otherwise — Nostoc

5. Cells of thallus arranged in a linear series forming pseudofilamentous growth; cells in more than a single row; thallus not cylindrical; cells with sheaths not like those of Gloeocapsa; with a thin sheath or without individual sheaths in a more or less homogenous gelatinous thallus; cells in distinct radial rows — Chlorogloea

6. Cells very short, discoid — Nodularia

6. Cells not discoid — Aulosira

FAMILY SCYTONEMATACEAE

l. Heterocysts absent; apices of trichomes as broad as the rest — Plectonema

l. Heterocysts present; single trichome in a sheath — 2

2. Apex not tapering — Scytonematopsis

2. Apex not tapering: sheath mostly with parallel lamellation — 3

3. False branches usually geminate — Scytonema

3. False branches usually single and often arising next to a terminal heterocyst — Tolypothrix

FAMILY RIVULARIACEAE

l. With terminal heterocysts; filaments in a spherical or hemispherical thallus — 2

l. With terminal heterocysts; filaments free, simple or distinctly false branched dichotomously; corymbose thallus — Calothrix

2. Spores not known — Rivularia
2. Spores commonly found, single, large — Gloeotrichia

ORDER STIGONEMATALES

Families: Nostochopsidaceae, Mastigocladopsidaceae, Mastigocladaceae, Stigonemataceae

l. With lateral or reverse ‘V’ shaped branching, pedicellate heterocysts present — 2

l. With lateral or reverse ‘V’ shaped branchinq, pedicellate heterocysts absent — 3

2.	Branching true and lateral	Nostochopsis
2.	Branching reverse ‘V’ shaped	Mastigocladopsis
3.	Branching reverse ‘V’ shaped, filaments not ending in a hair	Mastigocladus
3.	Branching true and lateral	4
4.	Older filaments with many rows of cells; filaments prostrate without a distinct erect system	Stigonema
4.	Older filaments with a single row of cells or many rows only for short portions	5
5.	Lateral branches not much different from the main filament	6
5.	Lateral branches distinct from the main filament	Pischerella
6.	Hormocysts present	Westiella
6.	Hormocysts absent	7
7.	Hormogones present	Hapalosiphon
7.	Hormogones not known, endospores present	Westiellopsis

DESCRIPTION OF THE NITROGEN-FIXING BLUE-GREEN ALGAL GENERA

The species of blue-green algae which are known to fix nitrogen are more than fifty and the list is steadily increasing. The nitrogen-fixing forms can be grouped into three main categories, heterocystous forms fixing nitrogen aerobically (see Fogg et al 1973), unicellular forms fixinq nitrogen aerobically (Wyatt & Silvey 1969; Rippka et al 1971) and filamentous non-heterocystous forms fixing nitrogen under micro-aerophillic conditions (Stewart & Lex 1970; Stewart 1971a; Kenyon et al 1972). Most of the nitrogen fixing species belong to the genera Gloeocapsa, Lyngbya, Oscillatoria, Plectonema, Anabaena, Anabaenopsis, Aulosira, Calothrix, Chlorogloea, Cylindrospermum, Fischerella, Hapalosiphon, Mastigocladus, Nostoc, Scytonema, Stigonema, Tolypothrix, and Westiellopsis. The descriptions of the various genera given below are from Desikachary (1969).

GLOEOCAPSA (Fig. 72)

Cells spherical, 2-8 in colonies, seldom many, with a number of concentric envelopes; colonies single or many together forming an expanded mass, individual sheaths lamellated or unlamellated, cell division very regular in three directions, cells in large colonies often with thick secondary colonies, arranged irregularly; occasionally with nannocytes; spores with firm thick walls often formed in a number of species.

Twenty three species are known from the Indian sub-continent.

Two strains (Gloeocapsa 795 & 6501) have been found to be nitrogen fixers (Wyatt & Silvey 1969; Stewart 1971a; Rippka et al 1971).

LYNGBYA (Fig. 73)

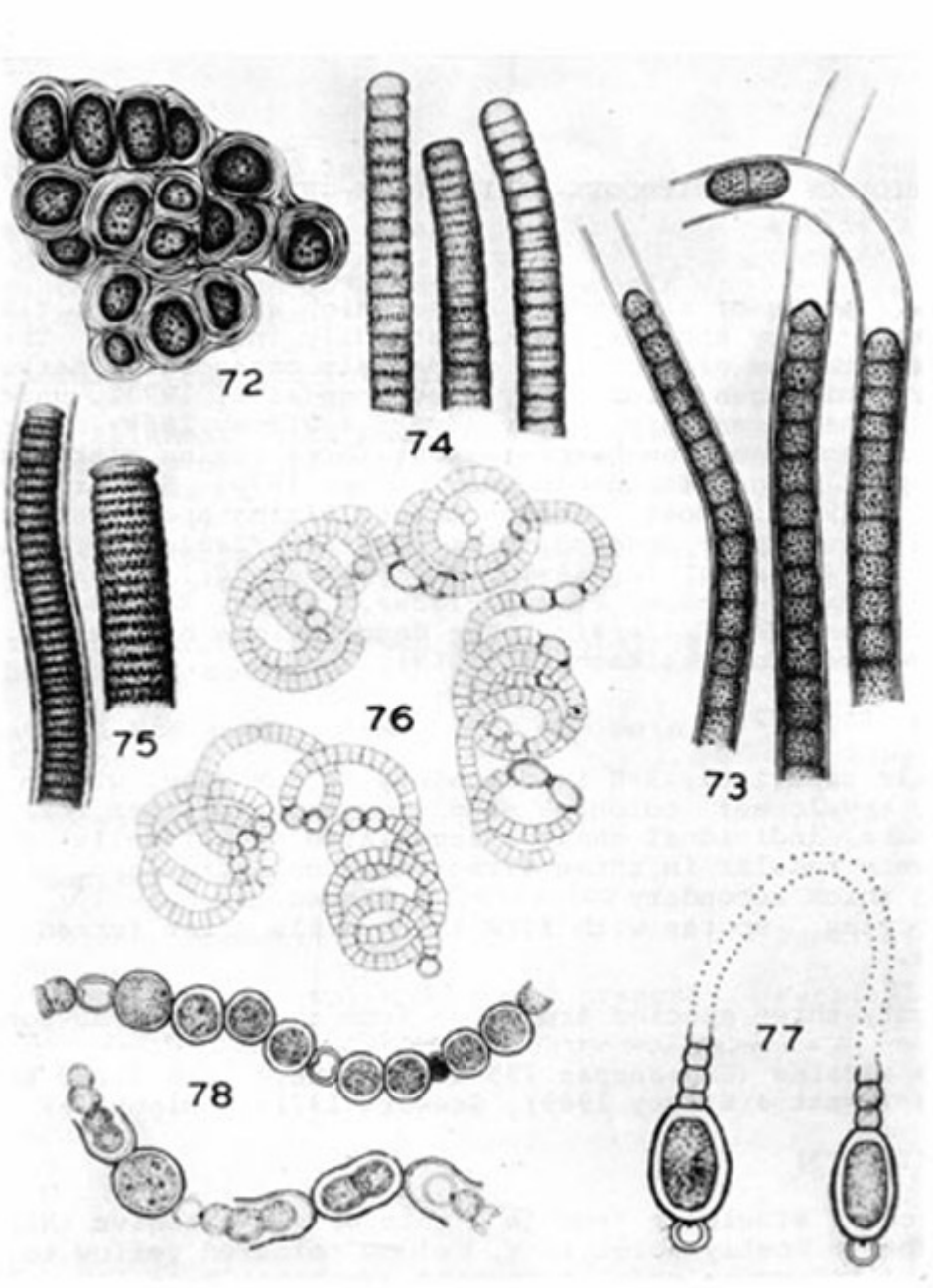
Trichome single or free in a thin or very massive thick, firm sheath; sheath mostly colourless, seldom coloured yellow to brown or red, blue to purple red; filaments sometimes spirally coiled or attached at the base or in the middle or the entire filament attached, mostly without such attachment or free swimming or forming free thallus.

Sixty five species are known from the Indian sub-continent.

One strain (Lyngbya 6409) is a nitrogen fixer (Kenyon et al 1972).

OSCILLATORIA (Fig. 74)

Trichome single or forming a flat spongy, free swimming thallus, sheath absent, rarely with a more or less delicate sheath, motile, mostly by a creeping movement causing rotation on the longitudinal axis; end of trichomes distinctly marked, pointed, bent like a sickle or coiled more or less like a screw. Hormogones formed by a division of the trichome.



Figures 72-78 Different types of blue-green algae

Eighty five species are known from the Indian sub-continent.

Four strains (Oscillatoria 6407, 6412, 6506, 6602) have been found to be nitrogen fixers (Kenyon et al 1972).

PHORMIDIUM (Fig. 75)

Filaments many forming a gelatinous or leathery stratum, thallus attached by the lower side; sheath present, more or less firm, sometimes agglutinated, sometimes partly diffluent, thin, colourless; trichomes of cylindrical shape, in some constricted at the joints, apices often attenuated, straight or bent, never regularly spirally coiled, capitate or non-capitate, apical cells in many species with a calyptra; sometimes false branchina.

Forty eight species known from the Indian sub-continent.

One species (P. foveolarum) is capable of fixing nitrogen under micro-aerophillic conditions.

ANABAENOPSIS (Fig. 76)

Trichome free swimming, short spirally coiled like Anabaena; heterocysts terminal and intercalary, the intercalary ones in pairs formed by the unegual division of two adjoining vegetative cells; spores intercalary and formed away from the heterocyst.

Three species are known from the Indian sub-continent.

A. circularia and A. sp. are nitrogen fixers (Watanabe, A. 1951, 1959).

CYLINDROSPERMUM (Fig. 77)

Thallus mucilagenous, mostly dull blue-green; trichome uniformly broad, short, without sheath, but in a common, mostly very delicate and often imperceptible mucilage of thin consistency; cells cylindrical, constricted at the cross walls; heterocysts terminal, at both ends or at one end only, sometimes intercalary; spores single, rarely in series, next to the heterocyst on one side, much bigger than the vegetative cells.

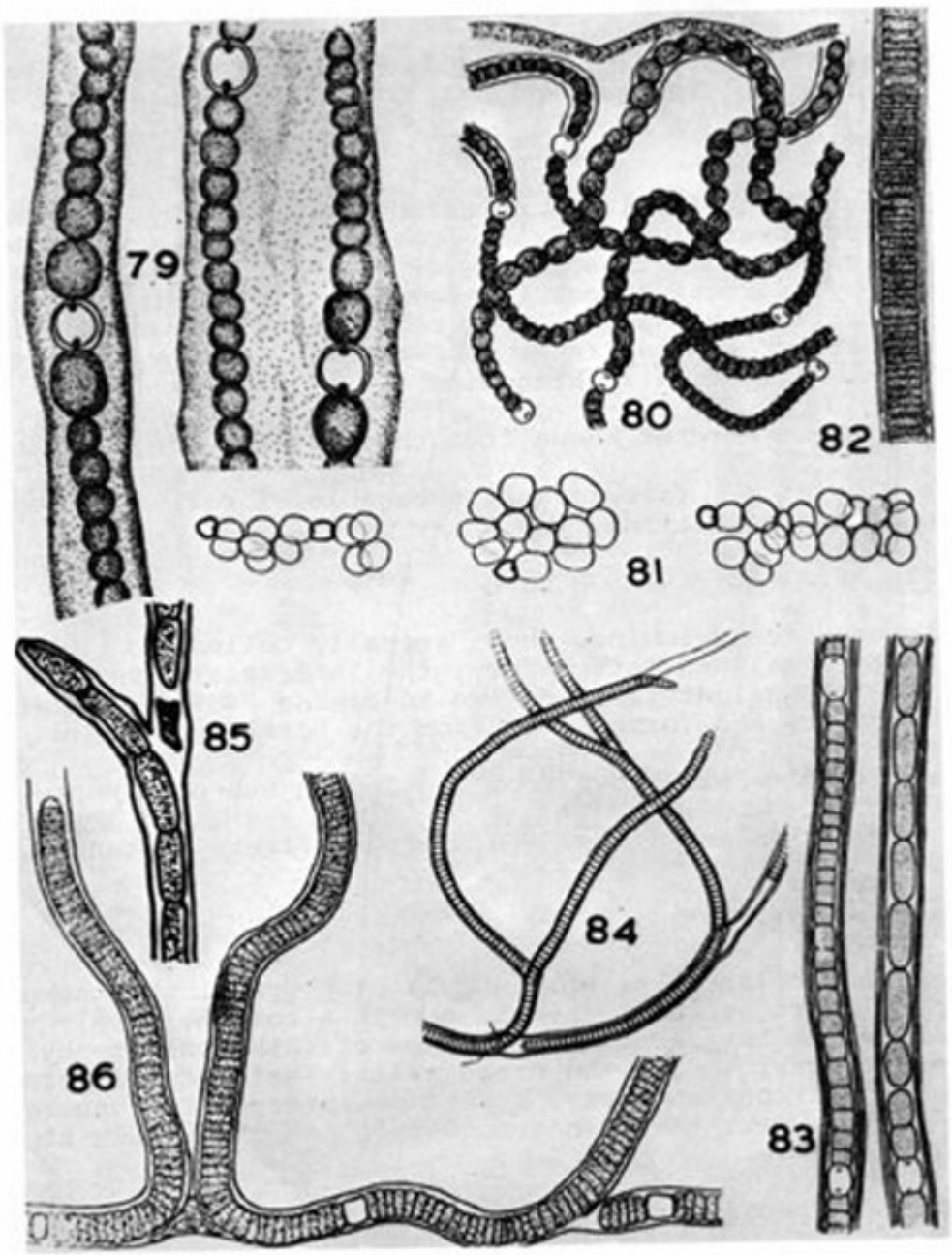
Fourteen species are known from the Indian sub-continent.

Nitrogen fixation is known to occur in many species (Fogg et al 1973) .

ANABAENA (Fig. 78)

Trichomes uniformly broad throughout or apices alone somewhat attenuated, sheath absent or more or less diffluent, forming a free, torn or floccose or soft mucilagenous thallus; heterocysts generally intercalary; spores single or in long series, formed from near the heterocysts or in between the heterocysts.

Twenty five species are known from the Indian sub-continent and most of them can fix nitrogen.



Figures 79-86 Different types of blue-green algae

WOLLEA (Fig. 79)

Thallus tubular, cylindrical, soft; filaments sub-erect, parallel, to sliahtly curved, agglutinated; sheath confluent; heterocysts intercalary; spores in chains continuous with the heterocysts or remote from them.

Only one species (W. bharadwajae) is known from the Indian sub-continent and there is evidence of nitrogenase activity.

NOSTOC (Fig. 80)

Thallus mucilagenous, gelatinous or coriaceous, first globose to oblong, later globose, foliose, filiform, bullose, solid or hollow, free or attached, the periphery dense and darkly coloured; filaments flexuous, curved or entangled; sheath sometimes distinct, generally diffluent; trichome torulose; cells depressed, spherical, barrel-shaped or cylindrical; heterocysts intercalary and in young condition terminal; spores spherical or oblong, formed centrifugally in series between the heterocysts.

Twenty five species are known from the Indian sub-continent and most of them are nitrogen fixers.

CHLOROGLOEA (Fig. 81)

Heterocystous; cells spherical or ellipsoidal mostly without individual envelopes or with a thin unlamellated sheath, in a common mucilage, in straight erect or radial rows, rows sometimes indistinct, forming more or less hemispherical or flat irregularly lobed thalli, sometimes with daughter colonies; cell division in three directions but generally in a single determined direction; gonidia and nannocytes present.

Two species are known from the Indian sub-continent and one (C. fritschii) is a nitrogen fixer (Fay & Fogg 1962). Desikachary (1969) includes this aenus under Entophysalidaceae in Chroococcales.

NODULARIA (Fig. 82)

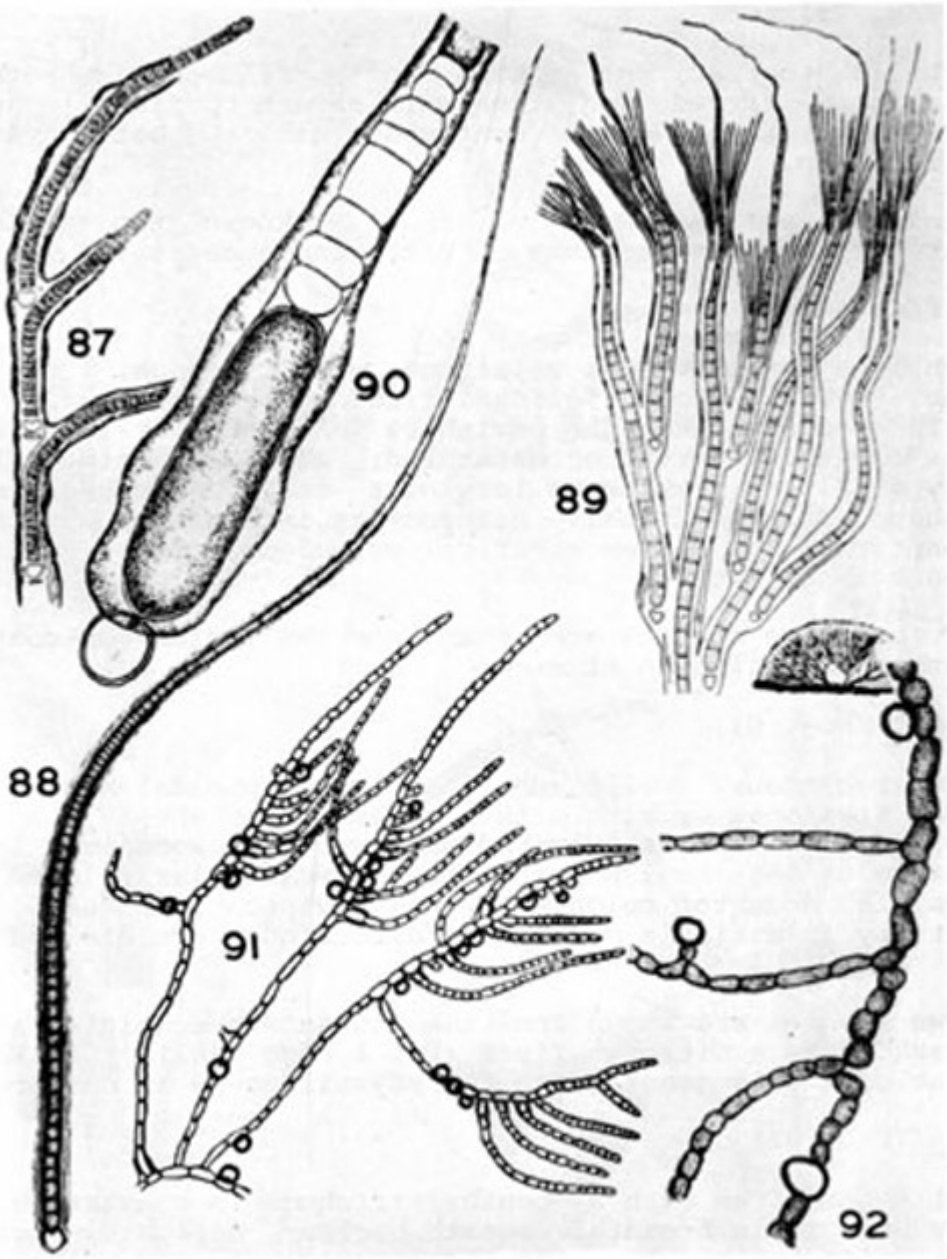
Filaments free with a sheath; trichome in vegetative condition extremely uniform in breadth; sheath hyaline, delicate, mucilagenous, sometimes diffluent; cells short, depressed, discoid; heterocysts intercalary, compressed; spore globose, sub-globose to discoid, formed singly or in series between the heterocysts, epispore smooth.

One species (N. spumigena and var. major) is known from the Indian sub-continent and the species shows nitrogenase activity.

AULOSIRA (Fig. 83)

Filaments free, sparse or in fascicles, generally uniformly broad, without differentiation of base and apex; trichomes with sheath, indefinite; heterocysts intercalary; spores often in series, formed near a heterocyst or away from it, cylindrical.

Eight species are known from the Indian sub-continent.



Figures 87-92 Different types of blue-green algae

A. fertilissima is a good nitrogen fixer in rice fields and is one of the components of algal biofertilizer.

PLECTONEMA (Fig. 84)

Trichomes variously bent, with a thin firm sheath; false branched, branches single or geminate; heterocysts absent; hormogones present; spores not known.

Eleven species are known from the Indian sub-continent.

P. boryanum (Stewart & Lex 1970) and two strains of Plectonema (6306, 6402) have been shown to fix nitrogen under micro-aerophillic conditions (Kenyon et al 1972). This species also forms one of the components of algal biofertilizer. It grows adpressed to the soil in rice fields.

SCYTONEMATOPSIS (Fig. 85)

Filaments branched, branching false, gradually attenuated towards the tip, growth by intercalary division; sheath present; heterocysts and spores present.

Two species known from the Indian sub-continent and are suspected to fix nitrogen.

SCYTONEMA (Fig. 86)

Filaments false branched, false branches single or geminate, formed laterally generally in between heterocysts; trichomes single in each sheath, straight; hormogones terminal, solitary; pseudohormogonia present; spores known only in a few species, spherical or ovate, exospore thin and smooth.

Forty nine species are known from the Indian sub-continent.

S. arcangelii and S. hofmanni are nitrogen fixers (Cameron Fuller 1960).

TOLYPOTHRIX (Fig. 87)

Filaments with a generally firm, or thick sheath with a single trichome in each sheath; false branches, mostly free, prostrate or erect; false branches single mostly subtending a heterocyst, occasionally geminate as in Scytonema; hormogonia formed from the tips; trichome with apical growth, apices often broader with shorter cells; spores known in some species.

Nineteen species known from the Indian sub-continent.

T. tenuis is a good nitrogen fixer in rice fields (Watanabe, A. 1951) and is one of the constituents of algal biofertilizer.

CALOTHRIX (Fig. 88)

Filaments single or in small bundles, caespitose, tomentose, pulvinate or penicellate; filaments arranged more or less parallel, mostly erect, unbranched or in some species false branched; sheath mostly firm, sometimes seen only at the base; heterocysts mostly basal, seldom intercalary; spores wher formed single or in series, next to the basal heterocysts.

Twenty nine species known from the Indian sub-continent.

C. scopulorum (Stewart 1962), C. parietina (Williams & Burris 1952), C. elenkinii (Taha 1963, 1964) and C. brevissima (Watanabe, A. 1951, 1959) are known nitroqen fixers.

RIVULARIA (Fig. 89)

Trichome unbranched, more or less irregularly false branched; filaments more or less radial or parallel in a hemispherical or spherical mucilagenous colony, hollow or solid; sheath more or less gelatinizing; trichomes ending in a hair, often with distinct trichothallic growth; heterocysts basal or intercalary; often false branching at the base; hormogonia single or in series gradually progressing towards the base from the meristematic zone; spores absent.

Seven species are known from the Indian sub-continent.

R. aquatica is found in some rice fields of south India and shows nitrogenase activity.

GLOEOTRICHIA (Fig. 90)

Thallus spherical or hemispherical, solid, sometimes when old inflated and hollow; filaments radial more or less parallel, often with false branches; sheath at the base firm, only gelatinizing on the outside, soft to mostly diffluent; trichomes with distinct trichothallic growth; heterocysts basal; spores at the base of the trichome, single or few next to the heterocyst; hormogones present.

Ten species are known from the Indian sub-continent.

G. natans is frequently seen in rice fields and shows nitrogenase activity.

NOSTOCHOPSIS (Fig, 91)

Thallus attached at first, later free floating with free filaments with soft, diffluent sheath, mucilagenous, more or less hemispherical, at first solid, later hollow, torn and expanded; trichome a single or two rows of branches, branches of two types, one long and many celled, the other with limited growth with a heterocyst at the end (pedicellate heterocysts), pedicells made of one to three cells; heterocysts intercalary or terminal or pedicellate or lateral and sessile; hormogones present; spores not known.

Three species known from the Indian sub-continent.

N. lobatus is found in rice fields and shows nitrogenase activity.

MASTIGOCLADOPSIS (Fig. 92)

Filaments sheathed and branched profusely, branching both reverse 'V' shaped and single; branches generally thinner than the main filaments; sheaths thin, hyaline and unlamellated; trichome somewhat torulose in the main filaments; heterocysts intercalary, lateral and pedicellate or terminal at the end of very short branches. This alga has a very close resemblance to Nostochopsis in its general appearance and in the presence of lateral pedicellate heterocysts.

Only one species (M. jogensis) is known and it is very rarely found in rice fields in Mysore, India. It shows nitrogenase activity.

MASTIGOCLADUS (Fig. 93)

Plants with a single series of cells with reverse 'V' shaped short branches arising on one side, true lateral branching as well as false branches often present; sheath thin and firm or diffluent; heterocysts intercalary; hormogones not known.

One species (M. laminosus) and one variety (var. indicus) are known. M. laminosus is a nitrogen fixer (Fogg 1951).

STIGONEMA (Fig. 94)

Thallus or free irregularly laterally branched, variously curved filaments in older parts with two to many rows of cells; sometimes with apical growth and division; lateral branches with as many rows of cells as the main filament; sheath when young close to the trichome, in older ones broader; old filaments with Gloeocapsa-like sheath and cell grouping; heterocysts intercalary or lateral; hormogonia formed at the end of young branches, two to a few celled, seldom many celled.

Twelve species are known from the Indian sub-continent.

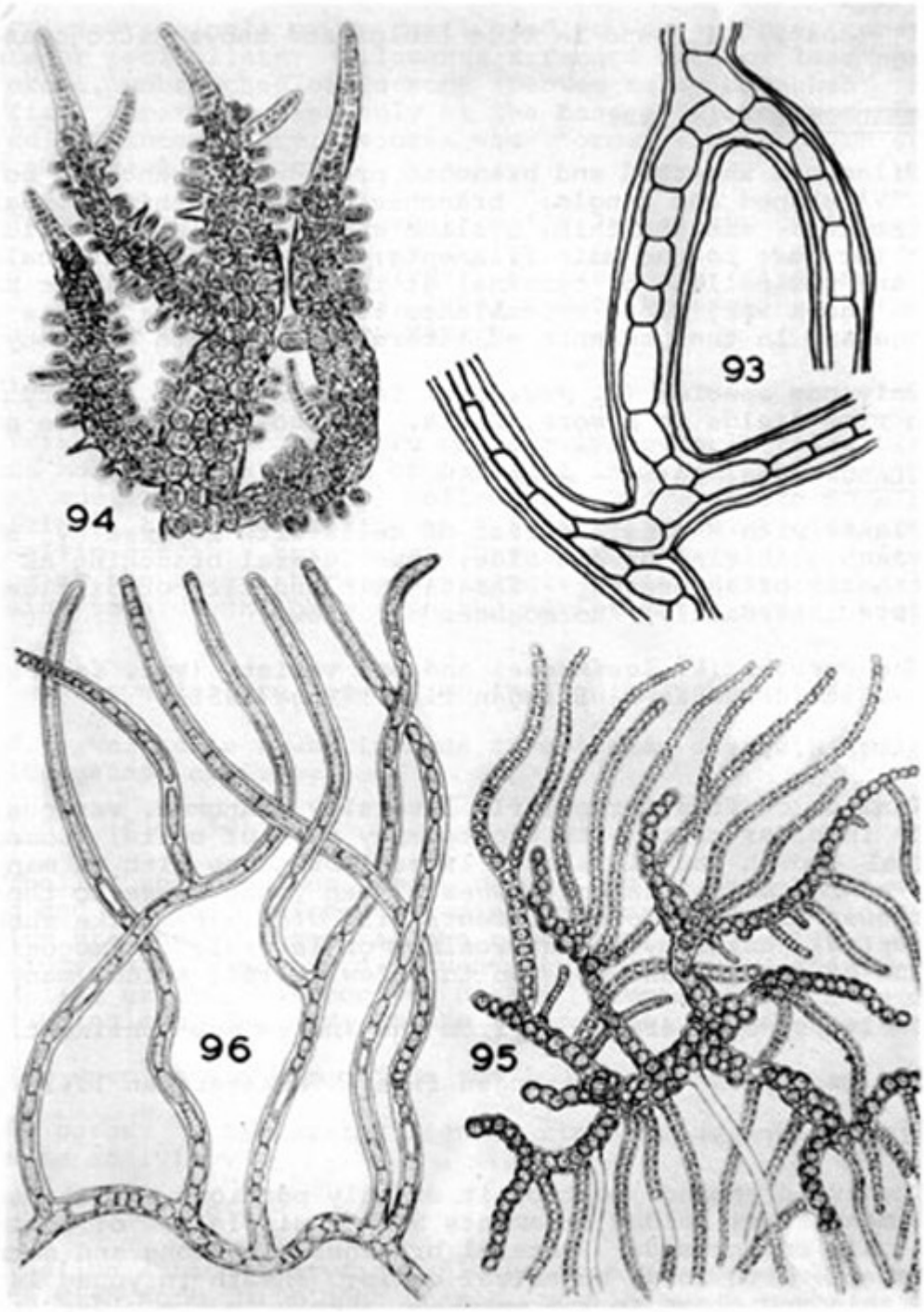
S. dendroideum is a nitrogen fixer (Venkataraman 1961b).

FISCHERELLA (Fig. 95)

Thallus creeping, most of it or only portions of filaments with cells in many rows, seldom filaments with a single row of cells, erect branches only on one side; lateral branches with long and narrow cells, main filaments with large spherical cells; sheath in young lateral branches thin and close to the trichome, sheath in the older filaments thicker; cells of old filaments often with Gloeocapsa-like sheath; heterocysts intercalary or lateral; hormogones from the ends of side branches; spores known in some species.

Three species are known from the Indian sub-continent.

F. major and F. muscicola are nitrogen fixers (Pankow 1964; Mitra 1961).



Figures 93-96 Different types of blue-green algae

HAPALOSIPHON (Fig. 96)

Thallus caespitose, floccose, thin, aquatic or in submerged soils; filaments free, not coalescing laterally; cells in one or two rows, sheath present, continuously branched; branches irregularly lateral, true, often arising only on one side of the filaments, false branches present, branches erect from the primary prostrate filaments, erect branches as broad as and similar to the main filament; heterocysts intercalary, only occasionally lateral; hormogones formed mostly from the side branches; spores present.

Ten species are known from the Indian sub-continent.

H. fontinalis is a nitrogen fixer (Taha 1963, 1964). In some paddy fields H. delicatulus is frequently seen and shows nitrogenase activity.

WESTIELLA (Fia. 97)

Thallus made up of free, requlary laterally branched filaments; trichomes with a single series of cells; lateral branches sometimes attenuated, main filaments uniformly cylindrical; sheath close to the trichome, homogenous; heterocysts intercalary; hormogones formed at the ends of branches; hormocysts terminal and intercalary, single or few together, 2-12 celled; spores present.

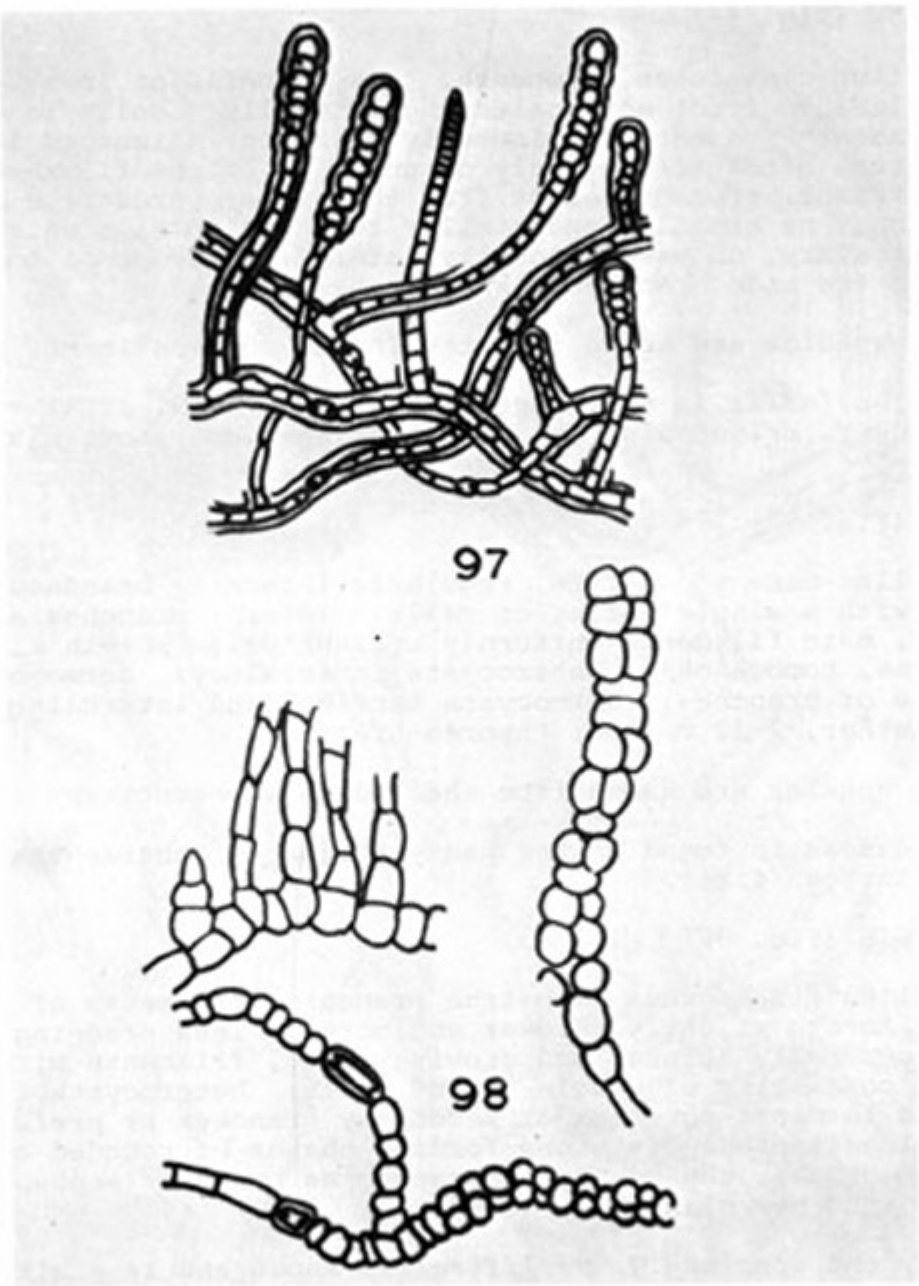
Two species are known from the Indian sub-continent.

W. lanosa is found in the paddy fields of Maharashtra in India and is a nitrogen fixer.

WESTIELLOPSIS (Fig. 98)

Thallus filamentous with true branching filaments of two kinds, primary filaments slightly thicker and more or less creeping, secondary filaments generally thinner and growing erect, filaments without a sheath and consisting of single row of cells; heterocysts intercalary; the dilated terminal portions of secondary branches by profuse transverse and longitudinal divisions forming chains of rounded cells (pseudohormocysts), the contents escaping as gonidia (endospores) and developing into new plants.

Only one species (W. prolifica) is known and is a nitrogen fixer (Pattnaik 1966).



Figures 97-98 Different types of blue-green algae

APPENDIX V

CULTURE MEDIA

1. A_5 Solution

H_3BO_3	2.5 g dm^{-3}
$MnCl_2.4H_2O$	1.8 "
$ZnSO_4.7H_2O$	0.2 "
$CuSO_4.5H_2O$	0.05 "

2. Fe - EDTA Solution

Dissolve 26.1 g of ethylenediaminetetra-acetic acid in 1M potassium hydroxide solution and add 24.9 g $FeSO_4.7H_2O$. Make the volume to one litre. Aerate the solution overnight to produce the stable complex marked by a change in colour to brown. Again make up the volume when aeration is over.

1 cm^3 of this solution in 1 dm^3 of the culture medium will give 5 µg cm^{-3} of iron.

3. Fogg's Nitrogen-free Nutrient Medium

K_2HPO_4	0.2 g dm^{-3}
$MgSO_4.7H_2O$	0.2 "
$CaCl_2.7H_2O$	O.l "
A_5 solution	1.0 cm^3 dm^{-3}
Fe-EDTA solution	1.0 "

Adjust the pH of the medium to 7.5 using dilute solutions of HCl or NaOh. Where nitrogen is also required, add 0.25 g dm^{-3} $NaNO_3$. For preparing the solid medium, add 1.0 - 1.5 g agar per 100 cm^3 of liquid medium.

4. Soil Extract

Mix 1 kg of garden soil with one litre of water and boil for one hour. Cool and allow to settle overnight. Decant and filter. Add 50 cm^3 of this soil extract to 950 cm^3 of Fogg's medium.

5. Soil-water Medium

Add 1 g of garden soil to 10 cm^3 of Fogg's nitrogen-free medium and steam for one hour in a domestic pressure cooker without the lid, for three consecutive days. Inoculate the algal strains and maintain in low light (200-500 lux) by adjusting the distance of the illumination source (incandescent of fluorescent lamps) and using a lux meter.

ISOLATION AND QUANTIFICATION PROCEDURES

The isolation of an alga from its natural habitat aims at transferring it to artificial conditions conducive to its growth with a view to obtaining a pure or unialgal culture. Unlike bacteria and fungi, the majority of the algae can be isolated and maintained on simple inorganic media, although maintenance of bacteria-free cultures is exacting. The conventional micro-biological methods for the isolation of micro-organisms can be used for algae also (Venkataraman 1969).

l. Direct Microscopic Examination of Algae

A really accurate quantitative measurement of algal abundance is rather difficult to obtain and will be time-consuming. Qualitatively, it is enough simply to recognize microscopically the types of algae in a particular sample and record their relative proportion as: Dominant (D), Common (C), Rare (R) or Absent (A).

2. Soil Plate Method

Take 1 g of soil and suspend it in Fogg's medium (Appendix V), the degree of dilution depending on the population density of the organisms in the soil. Place suitable aliquots in Petri dishes and disperse. Add 10 cm^3 of molten agar medium to the dishes, rotating to allow uniform dispersion. Incubate the dishes in light and observe for algal growth. One should be careful in picking up the fast-growing forms as soon as they appear and sub-culture them; otherwise they will overgrow the plate.

3. Surface Plating

Pour 15 to 20 cm^3 molten agar medium (1-1.5%) in Petri dishes and allow to set. Inoculate the surface of the agar with 0.5-1.0 cm^3 of the diluted soil suspension in Fogg's medium (Appendix V). Spin the plates or spread the inoculum with a sterile, flattened glass rod. Incubate the dishes in light. This method enables colonies to be picked off without digging into the agar.

4. Dilution Plate Method

Take 1 g of soil and suspend it in 100 cm^3 of Fogq's medium. Make ten-fold dilutions by pipetting 1 cm3 aliquots into 9 cm^3 of medium. It is usually enough to prepare up to one thousand-fold dilutions. Transfer 1 cm^3 aliquots of each dilution to Petri dishes containing a thin layer of solidified agar medium. 10 cm^3 of molten agar medium are poured over the inoculum which is dispersed by rotating the dishes while pouring the agar. After the agar sets, the dishes are incubated in light. Colonies may be counted, quantified, picked up and sub-cultured.

5. Most probable Number Method

This is also known as the Extinction Dilution method and is useful to determine the total population in a given soil. The dilutions prepared for the Dilution Plate method are used. A series of tubes containing Fogg's medium are inoculated with 1 cm^3 aliquots of a dilution series. After fifteen days incubation in light, the tubes are scored for growth or no growth. The observations are referred to Probability Tables and the most probable number is calculated. One should, however, bear in mind that the precision of this method is poor.

Example After dilutions, 1 cm^3 is inoculated from the 10^{-4}, 10^{-5} and 10^{-6} dilutions into five tubes for each dilution. After incubation, the numbers of tubes showing algal growth at each of the dilutions are 5, 5 and O respectively. Table 23 shows that the most probable number is 24 per inoculum taken from the 10^{-4} dilution, or 24 x 10^4 per cm^3 of the undiluted suspension. If the soil suspension has been made by suspending 1 g of soil in 100 cm^3 of medium, the number of algae per gramme of soil would be 24 x 10^6.

Table 23 VALUES OF THE MOST PROBABLE NUMBER FOR FIVE TUBES
(Meynell & Meynell 1965)

Numbers of turbid tubes observed at three successive dilutions			MPN (per inoculum of the first dilution)
O	1	O	0.18
1	O	O	0.20
1	1	O	0.40
2	0	O	0.45
2	O	1	0.68
2	1	O	0.68
2	2	O	0.93
3	O	O	0.78
3	O	1	l.l
3	1	O	l.l
3	2	O	1.4
4	O	O	1.3
4	O	1	1.7
4	1	O	1.7
4	1	1	2.1
4	2	O	2.2
4	2	1	2.6
4	3	O	2.7
5	O	O	2.3
5	O	1	3.1
5	1	O	3.3
5	1	1	4.6
5	2	O	4.9
5	2	1	7.0
5	2	2	9.5
5	3	O	7.9
5	3	1	11.0
5	3	2	14.0
5	4	0	13.0
5	4	1	17.0
5	4	2	22.0
5	4	3	28.0
5	5	O	24.0
5	5	1	35.0
5	5	2	54.0
5	5	3	92.0
5	5	4	160.0

APPENDIX VII

PROFORMA FOR COMPILING INFORMATION ON THE PRODUCTION AND PERFORMANCE OF ALGAL FERTILIZERS

1. Name and address of the farmer/institution :
2. Date of receipt of the starter culture :
3. Location of the production units :
4. Type and size of the units
   a) Galvanized trays
   b) Cement tanks
   c) Field multiplication
5. Date of installation and number of units :
6. Factors which motivated setting up production :
7. Periodicity of harvesting the algal material :
8. Total algal material produced (kg) :
   May - June
   July - October
   November - February
   March - April
9. Land owned under rice : hectares upland/lowland
10. Normal fertilizer schedule adopted (kg/ha) : N p K
11. Normal plant protection measures adopted :
12. Number of crops raised in a year and average yield (kg/ha) :
13. Since when has algal material been used :
14. Amount of algal material applied (kg/ha) and schedule of chemical nitrogen added :
15. Savings in chemical nitrogen fertilizer :
16. Yield response to algal application :
17. Is algal material sold? If so, at what rate :
18. Is there any difficulty in producing algal material? :
19. Is there any difficulty in storing the algal material? :
20. Is the material obtained used regularly in the production units? If not, what kind of difficulty is encountered? :
21. Is the algal material being used regularly in the fields? For what reasons? :
22. Is there any trouble with mosquitoes and flies because of the production units? If so, what control measures are taken? :
23. Are other members of the locality interested in the use of algae?

24. Is the experience with algal fertilizer shared with other farmers in the locality? :

25. Any special suggestions :

For institutions only

26. Has any field trial been conducted with algae? If so, give the following details (use additional sheets if necessary) : Yes/No

   a) Number and location
   b) Crop variety
   c) Treatment schedule
   d) Yield data with statistical analysis
   e) Any other information

27. Has any extension been done? If so : Yes/No

   a) Number and location
   b) Response in terms of,
      i) Yield
      ii) Nitrogen fertilizer savings
   c) Recommendations made, if any

Signature:
Address :

Please mail the completed forms to:

Table 24 AVERAGE ANALYSIS OF IMPORTANT FERTILIZERS AND ORGANIC MANURES
(adapted from the Handbook of Manures and Fertilizers, ICAR 1964)

Material	Nitrogen N	Phosphorus P	Potassium K
Potassium nitrate (70%)	8-10	-	25.2
Ammonium phosphate (60%)	17-18	9	-
Urea	46	-	-
Ammonium sulphate	20.6	-	-
Calcium cyanamide	18-20	-	-
Diammonium phosphate	21	23 (watar-	-
Superphosphate (single)	-	soil) 7-9	-
Superphosphate (concentrated)	-	l8-22	-
Basic slag	-	6-8	-
Potassium chloride	-	-	50
Potassium sulphate	-	-	40-43
Liquid ammonia	82.2	-	-
Castor cake	4.3	0.8	l.l
Cottonseed cake (undecorticated)	3.9	0.8	1.3
Linseed cake	5.5	0.6	l.l
Farmyard manure	0.5-1.5	0.2-0.4	0.4-1.6
Compost (urban)	1.0-2.0	0.4	1.2
Compost (rural)	0.4-0.8	0.1-0.2	0.6-0.8
Green manure (various)	0.5-0.7	0.04-0.08	0.7-1.3

APPENDIX IX

Table 25 EQUIVALENT ACIDITY OF SOME OF THE COMMONLY USED FERTILIZERS (From the Handbook of Manures and Fertilizers, ICAR 1964)

Fertilizer	% Nitrogen	Equivalent Acidity
Ammonia, anhydrous	82.0	148
Ammonium chloride	24.0	128
Ammonium nitrate	33.5	60
Ammonium nitrate-limestone	20.5	0
Ammonium sulphate	20.5	110
Ammonium sulphate-nitrate	26.0	93
Urea	45.0	80

Equivalent acidity is the number of parts by weight of calcium carbonate ($CaCO_3$) required to neutralize the acidity resulting from the use of 100 parts of the fertilizer material. For example, ammonium sulphate has an equivalent acidity of 110; it takes therefore 110 kg of calcium carbonate to neutralize the acidity developed in the soil by the use of 100 kg, of ammonium sulphate fertilizer.

APPENDIX X

Table 26 APPROXIMATE AMOUNTS OF FINELY GROUND LIMESTONE NEEDED TO RAISE THE pH VALUE OF AN 18 CM LAYER OF SOIL (From the Handbook of Manures and Fertilizers, ICAR 1964)

Soil texture:	Limestone requirement (kg/ha) to raise pH		
	from 3.5 to 4.5	from 4.5 to 5.5	from 5.5 to 6.5
Sandy and loamy sand	243	243	364
Sandy loam		446	610
Loam		683	890
Silt loam		1 100	1 300
Clay loam		1 375	1 740
Muck	2 260	3 040	3 480