OBSERVATIONS ON NITROGEN-FIXATION BY SOME BLUE-GREEN ALGAE AND REMARKS ON ITS POTENTIALITIES IN RICE CULTURE

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INTRODUCTION

IT is fairly well known that a large number of blue-green algae have the capacity to fix atmospheric nitrogen. The knowledge in this regard has been recently reviewed by Singh (1961), Fogg (1963) and Lund (1963).

The present work was undertaken as part of the study to assess the rôle of these algae in augmenting the fertility of rice-growing soils. The account reports the investigation in this regard of four species of blue-green algae isolated from the cultures of the rice field soil of Central Rice Research Institute.

MATERIAL

The material was obtained by culturing rice field soil from the Central Rice Research Institute campus. By repeated sub-culturing first in De's (1939) solution and later in Wolfe's (1954) solution *without* nitrogen, several species were isolated, species pure. Except for the culture media used, conditions were kept as near to nature as obtaining in the field. These algae were grown in the N-free medium for some time before being used for experiments to assess their nitrogen-fixing capacity. Known quantity of the material was inoculated into a definite volume of the medium in 100 ml. Kjeldhal digestion flasks. After a definite period the nitrogen was estimated in all the flasks including the blanks and distilled water. At the commencement of the experiment the nitrogen present in known quantities of dried material of each species and the dry weight ratio for each species were determined.

The results of the experiment with four species, *viz.*, *Tolypothrix campylonemoides* Ghose, *Nostoc sphaericum* Vaucher, *N. amplissimum* Setchell and *Westiella* sp. are reported here. These species are not known to have been tested for N-fixation earlier.

Only estimation of organic nitrogen was made; this was done by micro-Kjeldhal method.

As the aim of the investigation was utilization in the rice field of these blue-green algae which could fix nitrogen, an environment totally different from the laboratory, fastidious attention was not given to the cultures though aseptic conditions were maintained as far as possible. There was not any cognizable quantity of bacteria present in the cultures; and no organic chemical likely to promote their growth was employed in the cultures.

RESULTS

The results of the experiments with about ninety days incubation are presented in Table I. The table shows that organic nitrogen fixed during this period was 1·7 mgm. by *T. campylonemoides*, 1·3 mgm. by *Nostoc amplissimum*, 1·2 mgm. by *Nostoc sphaericum* and 1·0 mgm. by *Westiella* sp. It is also clear from the same table that *T. campylonemoides* fixes nitrogen 15 to 24 times and the others 2 to 4 times their respective original dry weights. The order of fixation is as follows:

T N_2 N_1 W [at C. D. (·05)]

T N_2 N_1 W [at C. D. (0·5)]

T—*Tollypothrix campylonemoides*
N_1—*Nostoc sphaericum*
N_2—*Nostoc amplissimum*
W—*Westiella* sp.

T. campylonemoides is the most efficient of all the four. *Nostoc amplissimum* and *Nostoc sphaericum* are at par in their nitrogen-fixing capacity but significantly inferior to *T. campylonemoides* and significantly superior to *Westiella* sp.

The results of another series of experiments with *T. campylonemoides* and *Nostoc sphaericum* conducted during the colder season are presented in Table II A and B. Here the total organic nitrogen (both extra- and intra-cellular) is only 0·29 mgm. and 0·71 mgm. in *Nostoc sphaericum* and *Tolypothrix campylonemoides* respectively, nearly ½ and 1½ times their own dry weight. Out of this, the extra-cellular nitrogen liberated is only 0·04 mgm.

TABLE I

Analytical data relating to organic nitrogen fixed by four blue-green aglal species—Expt. 1

Species of algae	No. of repli-cations	Weight of algae inocu-lated mgm.	Dry weight/ Fresh weight ratio	Calcu-lated dry weight mgm.	Amount of nitrogen initially present: mgm.	Amount of nitrogen initially present: Average %	Quantity of nitro-gen after 90 days incu-bation mgm.	Net quantity of nitro-gen fixed after 90 days incu-bation mgm.	Ratio of N fixed per dry weight
Westiella sp.	(i)	27·0	0·0209	0·56430	0·0298	5·3	1·04666	1·0169	3·5
	(ii)	29·0		0·60610	0·0321		1·04486	1·0128	2·7
	(iii)	12·5		0·26125	0·0138		1·106976	1·0922	3·0
Nostoc sphaericum	(i)	182·0	0·00187	0·340340	0·0170	5·1	1·20632	1·1893	2·6
	(ii)	259·5		0·485265	0·0243		1·33050	1·3062	3·5
	(iii)	202·0		0·377740	0·0189		1·24180	1·1229	2·7
Nostoc amplissimum	(i)	242·5	0·00195	0·472875	0·0236	5·1	1·25954	1·2359	1·8
	(ii)	183·0		0·356850	0·0178		1·25954	1·2417	1·7
	(iii)	275·0		0·536250	0·0268		1·45468	1·4279	4·2
Tolypothrix campylo-nemoides	(i)	48·0	0·00258	0·123840	0·00645	5·2	1·80948	1·8030	14·6
	(ii)	26·4		0·068112	0·00354		1·66756	1·6641	24·4
	(iii)	29·0		0·074820	0·00390		1·61434	1·6104	21·5

and 0·1 mgm. in *Nostoc sphaericum* and *Tolypothrix campylonemoides* respectively.

It is found that while the same species fixed more nitrogen during the period 5–3–1963 to 5–6–1963, they fix a lesser quantity during 2–10–1963 to 7–12–1963 (*Nostoc sphaericum*) and 10–12–1963 to 15–2–1964 (*Tolypothrix campylonemoides*). It is obviously due to the seasonal difference that while the first experiment fell in the summer months the later ones were during the winter. It is, therefore, seen that the amount of nitrogen fixed is dependent on external factors (*vide* Fogg, 1963), for N-fixation is a continuous process proportional to growth, and growth is less during the winter months.

DISCUSSION

As already stated elsewhere the purpose of these investigations was to test blue-green algal species with a view to utilising them in the field. As the four species mentioned above as well as others have been used in both pot and field cultures of paddy crop, it will be worthwhile to examine the rôle of these blue-green algae on the crop as well as to assess the quantum of nitrogen and organic matter added by them to the soil.

It is evident from the data presented in this account that the algal species concerned fix considerable quantities of nitrogen and a portion of this nitrogen is liberated during their growth; that their growth and the N-fixed are more during the warmer months, which are also the season for the growth of the main rice crop. It may also be seen that only a small proportion of the N-fixed is liberated as extra-cellular product, the remainder being locked up obviously in the cells of the algae, the spores or hormogones; and that nitrogen fixation is a continuous process proportional to the growth of the algae.

During the favourable period of growth, *T. campylonemoides* fixes nitrogen about 15 to 24 times its own original dry weight and the other species 2 to 4 times; as the amount of nitrogen in the algae concerned is only about 5% (Table I), this would mean a production of total organic matter of approximately 300–480 gm. of *Tolypothrix* for each gram of original matter inoculated, and in the other species 40–80 gm. for each gram of original matter, all within a span of ninety days.

It is well known that soils hold a rich algal flora several thousands of cells per gram, the bulk of the species belonging to the blue-green algae; in fact, at the Central Rice Research Institute one of us (R. S.) has isolated over fifty species of blue-green algae in cultures, the species appearing in

TABLE II

A. Analytical data relating to nitrogen fixation by Tolypothrix campylonemoides *and* Nostoc sphaericum—*Expt.* 2

Species of algae	No. of replications	Weight of algae inoculated mgm.	Dry weight/ Fresh weight ratio	Calculated dry weight mgm.	Amount of nitrogen initially present mgm.	Amount of nitrogen initially present Average %	Quantity of nitrogen after 60 days incubation mgm.	Net quantity of N-fixed after 60 days mgm.
Nostoc sphaericum	(i)	77·0	0·00693	0·5336	0·0188	3·51	0·2572	0·2384
	(ii)	78·0		0·5405	0·0190		0·2927	0·2737
	(iii)	81·0		0·5613	0·0197		0·3814	0·3617
Tolypothrix campylonemoides	(i)	7·0	0·05425	0·3798	0·02328	6·13	0·4315	0·4082
	(ii)	9·0		0·4883	0·02993		0·7504	0·7205
	(iii)	17·0		0·9223	0·05654		1·0693	1·0128

B. Analytical data relating to " extra-cellular nitrogen " fixed by Nostoc sphaericum *and* Tolypothrix campylonemoides

Species of algae	No. of repli-cations	Weight of algae inoculated mgm.	Dry weight/ Fresh weight ratio	Calcu-lated dry weight mgm.	Amount of extra-cellular N mgm.
Nostoc sphaericum	(i)	135·0		0·9356	0·0444
	(ii)	112·0	0·00693	0·7762	0·0444
	(iii)	99·0		0·6861	0·0266
Tolypothrix campylonemoides ..	(i)	25·0		1·3563	0·2064
	(ii)	8·0	0·05425	0·4340	0·1876
	(iii)	6·0		0·3255	0·1688

Date of inoculation : *Nostoc*, 2–10–1963. *Tolypothrix*, 10–12–1963.
Date of estimation : *Nostoc*, 7–12–1963, *Tolypothrix*, 15–2–1964.
Period of incubation : approx. 60 days.
Volume of media : 50 ml.

succession and the four species dealt with here are from such isolations. Therefore, given favourable conditions for their growth, the indigenous flora itself could be utilised to build up the N-fertility of the rice field soils, for as may be seen from the preceding paragraph the potentialities of the utilization of the blue-green algal flora could be very high indeed under the waterlogged conditions of rice culture.

In this connection, it needs to be emphasized that it is the *extra-cellular* nitrogenous matter released during *their growth period* that becomes immediately available to the crop in the field as well as the broken down organic matter including that of algal growth during the preceding season. The magnitude of this as well as the expression of indigenous and introduced algal forms may be gauged as reflected in the rice grain and straw yield in the experiments conducted at the C.R.R.I., some of which have been published (Relwani and Subrahmanyan, 1963; Subrahmanyan, Relwani and Manna, 1964 *a* and *b*). It may be seen from Table III, compiled from yield data, the very favourable rôle played by the indigenous flora as well as introduced forms on the rice yield. Nitrogen estimation of grain and straw are under progress to assess the total uptake of N by the crop and to compare it with experiments conducted with nitrogenous and other fertilisers and manures. Preliminary results (Dr. M. N. Sahay, Unpublished data) based on the analysis of 1962 main crop (Table III) indicate that the supply of nitrogen by indigenous algae (*Treatment* 2) is equivalent to 109 kg./ha. of ammonium sulphate (21·8 kg. N/ha.) on a par with 20 kg.N/ha. supplied as ammonium sulphate (*Treatment* 4) and, with introduced nitrogen-fixing alga (*Anabaena* sp., *Treatment* 3) the response went up to 180 kg. ammonium sulphate (36 kg. N/ha.) showing a linear trend in the uptake of N up to 40 kg. N/ha. as reported by some earlier workers (Anon, 1958–62).

In another experiment (Table III, *Treatments* 5, 6 and 7) where lime dose had been cut down to 500 kg./ha. assuming for the sake of rough assessment that the percentage of N in the grain and the straw are the same as in the earlier treatments (1962 main crop), it is found that the mixture of the four species of algae, dealt with in this paper, added at 5 gm./ha. has accounted for about 10 kg. N/ha. over indigenous flora while in the earlier experiments *Anabaena* contributed to 6 kg. N/ha. only over indigenous flora, which probably indicates the greater efficiency of a mixture of species.

Again, it may be mentioned here that a comparison of the nitrogen fixed by the algae in the laboratory experiments and the nitrogen uptake assessed leads us to surmise that the blue-green algae find their full expression in their

TABLE III

Quantity of above-ground dry matter at harvest and total nitrogen uptake in kg./ha.

Treatments	1962 Main season variety: T 141	1962 Second season variety: Ptb 10	1963 Main season Expt. 1 variety: T 141	1963 Main season Expt. 2 variety: T 141	Nitrogen uptake: Nitrogen Total	Nitrogen uptake: Nitrogen Over control	Nitrogen uptake: Response of treatments over no manure control in terms of ammonium sulphate
1. Control (No fertilizer)	6175	1412	3764	..	37	..	..
2. Lime 1000 kg./ha., superphosphate 20 kg. P_2O_5/ha., sodium molybdate 0·28 kg./ha.	7709	2652	6146	..	46	9	109
3. As in and above + algae	9003	3415	8306	..	52	15	180
4. Ammonium sulphate 20 kg. N/ha.	7828	2821	5093	..	46	9	100
5. Control (No fertilizer 1963)	..	..	..	3764	25	..	..
6. Lime 500 kg./ha. + superphosphate and sodium molybdate as in 2 above	..	..	..	5111	35	10	120
7. As in 5 above + algae (mentioned in this account)	..	..	..	7060	44	19	228

(Agronomical data after Subrahmanyan, Relwani and Manna, 1964, *a*, *b*.)

natural habitat, the rice soil. This view finds support in the observations of De and Sulaiman (1950 *a*) who also recorded that the nitrogen-fixing blue-green algae functioned better when they were associated with the rice plant, there being a sort of "symbiotic" relationship.

Some preliminary analyses of soils from the various treatments after harvest of crops have shown that algal inoculated plots held a higher organic carbon- and nitrogen-content as compared with the control (Mr. S. Prasad, personal communication). Plate Figs. VII1 and 2 show the appearance of the field after harvest, control and algae inoculated field respectively. The dark appearance of the field in Fig. 2 is due to the algal mat (*Scytonema*, *Westiella* and *Tolypothrix*, etc., marked A) left over which enriches the soil in the *succeeding* season by the growth of the spores in them and the decay of their own dead body matter. The results of the experiments of De and Sulaiman (1950 *b*) also point out that the fourth and the fifth year in a five-year course of pot culture experiments in the presence of algae gave a much higher yield than those in which no algae were present as well as than those at the start of the experiment. The results of the experiments on a field scale and in the pots carried out at the C.R.R.I. during four consecutive seasons since 1961 confirm a favourable response to inoculation of blue-green algae as reflected in the increased yield of grain and straw (Relwani, 1963; Relwani and Subrahmanyan, 1963; Subrahmanyan, Relwani and Manna, 1964, *a* and *b*). This clearly indicates a fertility build-up of the soil in the presence of blue-green algae and these algae may be used in combination with some fertilizer mixture (*excluding* nitrogen) such as lime for raising pH and supply of calcium, superphosphate for supply of phosphorus essential for growth and the trace element molybdenum which plays an essential rôle in the fixation of the element nitrogen, as a substitute for nitrogenous fertilizers which could be diverted to other crops.

Summary

The results of experiments in connection with the estimation of nitrogen fixed by four species of blue-green algae are presented and discussed. The species tested were *Tolypothrix campylonemoides*, *Nostoc sphaericum*, *N. amplissimum* and *Westiella* sp. Of these, *Tolypothrix* was the most efficient. The quantity of extra-cellular nitrogen liberated has also been estimated; this is only a fraction of the whole. The potentialities of using blue-green algal species in rice culture are discussed. It is indicated that blue-green algae could be employed as a substitute for nitrogen fertilizers such as ammonium sulphate, for rice culture in waterlogged conditions.

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REFERENCES

Anon .. *Annual Reports of Central Rice Research Institute*, 1958–62.

De, P. K. .. "The rôle of blue-green algae in nitrogen fixation in rice fields," *Proc. roy. Soc.* (*Lond.*), 1939, **127 B,** 121–39.

——— and Sulaiman, M. .. "Fixation of nitrogen in rice soils by algae as influenced by crop, CO_2 and inorganic substances," *Soil Sci.*, 1950 *a*, **70,** 137–51.

——— .. "Influence of algal growth in the rice fields on the yield of crop," *Indian J. agric. Sci.*, 1950 *b*, **20,** 27–42.

Fogg, G. E. .. "Nitrogen fixation" in: *Physiology and Biochemistry of Algae*, Ed. R. A. Lewin, Academic Press, N.Y., 1963, pp. 161–70.

Lund, J. W. G. .. "Soil algae" in: *Physiology and Biochemistry of Algae*, Ed. R. A Lewin, Academic Press, N.Y., 1963, pp. 759–70.

Relwani, L. L. .. "Rôle of blue-green algae on paddy yield," *Curr. Sci.*, 1963, **32,** 417–18.

——— and Subrahmanyan, R. "Rôle of blue-green algae, chemical nutrients and partial soil sterilization on paddy yield," *Ibid.*, 1963, **32,** 441-43.

Singh, R. N. .. *Rôle of Blue-Green Algae in Nitrogen Economy of Indian Agriculture, I.C.A.R. Monograph on Algae*, New Delhi, 1961.

Subrahmanyan, R., Relwani, L. L. and Manna, G. B. "Nitrogen enrichment of rice soils by blue-green algae and its effect on the yield of paddy," *Symposium on Land Fertility, etc.*, Natl. Inst. Sci. India, Allahabad, Feb. 1964.

——— .. "Observations on the rôle of blue-green algae on rice yield compared with that of conventional fertilizers," *F.A.O. I.R.C. Meeting, Manila*, March, 1964.

Wolfe, M. .. "The effect of molybdenum upon the nitrogen metabolism of *Anabaena cylindrica*," *Ann. Bot.* (*Lond.*), 1954, **18,** 299–308.

EXPLANATION OF PLATE VIII

Photograqh of rice field after harvest

FIG. 1. Control (untreated).
C, Crack in the soil.

FIG. 2. Plot inoculated with algae.
C Crack in the soil; *A*, Algal crust on surface.

FIG. 2

FIG. 1