5. Free - living blue - green algae in tropical soils

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1. Introduction

One result of the fertilizer price increase during the last decade is a renewed interest in biological N_2 fixation as a means of reducing the use of N fertilizer. However, biological N_2 fixation requires energy generally obtained by the catabolism of photosynthetically fixed carbon (photosynthate). Among the N_2-fixing micro-organisms, only blue-green algae (BGA) are able to generate their own photosynthate from CO_2 and water. This trophic independence makes BGA especially attractive as a biofertilizer. The agronomic potential of BGA was recognized in 1939 by De [14], who attributed the natural fertility of tropical paddy fields to N_2-fixing BGA. Since rice forms the staple diet of a high proportion of the world's population in areas where N fertilizer is rarely available, research on BGA in tropical soils has been focussed mainly on the paddy field ecosystem. Relative to the amount known about paddy soils, very little is known about other soils.

2. Occurrence of BGA in tropical soils

Blue-green algae (BGA) are photosynthetic prokaryotic microorganisms, some of which are capable of N_2 fixation. This resulting trophic independence which has already been stressed, and a great adaptability to environmental factors should enable BGA to be ubiquitous. However Watanabe [81] and Watanabe and Yamamoto [84] found that N_2-fixing BGA are not present in every environment: of 911 samples only 46 (5%) harbored N_2-fixing species. This surprisingly low value is probably due to unsuitable methodology and to the small size of the samples. Watanabe's results suggested that N_2-fixing BGA grow more abundantly in tropical and subtropical regions than in temperate and subtemperate regions.

2.1. Upland soils

Upland soils in arid climates are probably very inhospitable to many microorganisms because the temperatures are high and water is severely limited. BGA are especially resistant to such adverse conditions; thus they are the dominant components of the microflora in many cases [17]. A study of the savanna soils in the Congo indicated that the flora was almost entirely composed of BGA [10]; N_2-

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fixing BGA have been reported to develop profusely in sugar cane and maize fields in India [63]. Large populations of *Calothrix* sp. in pearl millet fields and of *Gloeotrichia* sp. in sugarcane fields were found by the authors in Senegal.

A qualitative study of the algal flora of dried soil samples from experimental upland fields (pH 7.8–8.3) at IARI, New Delhi, indicated that BGA were dominant in all the soil samples, Chlorophyceae were poorly represented and Xantophyceae were absent. Among the BGA, numerous N_2-fixing forms were observed [16]. Soil algae from regions around the Gulf of Mexico and areas in Ecuador and Colombia were studied by Durrell [15]. In 120 samples he found 62 species of algae; 46 of these species belonged to BGA. About half of the samples contained N_2-fixing species; *Nostoc muscorum* was observed in 21% of the samples and *Nostoc paludosum* in 13%. Other N_2-fixing species were observed in less than 4% of the samples.

2.2. Paddy soils (submerged soils)

The paddy field ecosystem provides a favourable environment for the growth of BGA with respect to their requirements for light, water, high temperature and nutrient availability. This could be the reason BGA grow in higher abundance in paddy soils than in upland soils [84] as reported in the widely different climatic conditions of India [35] and Japan [36]. By pooling data obtained in Senegal it appears that N_2-fixing BGA were recorded in 86 out of 89 paddy soils [48]. However, Venkataraman [78] pointed out that 'contrary to general belief, N_2-fixing BGA are not invariably present in tropical rice soils, and that an all India survey showed that out of 2213 soil samples from rice fields, only about 33% harboured N_2-fixing forms'. The heterogeneous and sometimes limited distribution of N_2-fixing BGA is still not well understood because no systematic analysis has correlated the presence or absence of BGA with environmental factors [30].

2.3. Quantitative evaluations

The lack of satisfactory methods for estimating biomasses of the different algal groups [17] has certainly limited ecological studies of soil algae. Plating techniques, most frequently used, are advantageous in providing qualitative and quantitative results simultaneously; however the accuracy of the count depends on the reliability of the particular dilution method. Filamentous forms are hard to separate into individual cells whereas moniliform filaments, which are easily separated may give inflated figures of abundance [17].

Algal enumerations are often limited by an inadequate sampling methodology. Most of the results are expressed as numbers of algae per gram of soil, which do not take into account algae present in the floodwater of submerged soils and do not permit any extrapolation at the field level (what is the dry weight of soil colonized by algae in one hectare of a paddy field?). A more satisfactory way to evaluate algal population is to determine the number of algae per cm^2 by using core samples with a well-defined diameter, each core sample including the first centimeter of soil and the corresponding floodwater column [52]. Such a procedure allows comparisons and extrapolations at the field level. Roger and Reynaud [55] found that the distribution of soil algae is log-normal (logarithms of numbers are normally distributed) and that many samples are required to obtain a significant evaluation. For example, the mean value of *Anabaena* sp. biomass based upon 40 samples (each sample being obtained by mixing 10 sub-samples) taken in a 0.25 ha paddy field still exhibited a confidence interval of + 32% and − 27% of the mean. One fact at least is well established: in paddy soils (see Roger and Kulasooriya [58]), BGA numbers vary within large limits, from a few to 10^7 units g^{-1} dry soil. In upland soils lower values ranging from a few to 10^6 units g^{-1} dry soil have been generally reported [71, 25, 3, 69]. Reports on biomass are scarce. In paddy fields the biomass of BGA can reach values of several tons per hectare [58]. The little data available suggest that BGA seem to develop more produsely in submerged soils than in other cultivated soils. An exceptionally high total algal biomass of about 40 t ha^{-1} was recorded by Reynaud and Roger [47] in a sandy soil in Senegal that was spontaneously watered by a permanent spring; such a soil exhibited a water regime related more to that of submerged soils than to that of upland soils.

3. Ecology

3.1. Physical factors

3.1.1. Light. Algae, as phototrophic microorganisms, are restricted to the photic zone and usually located in the upper 0.5 cm horizon. Yet algae also exist in deeper horizons, in a dormant condition as spores or filament fragments [10]. Light availability for soil algae depends upon the season and latitude, the bud cover, the plant canopy, the vertical location of the algae in the photic zone and the turbidity of the water. Light intensity reaching the soil may vary from too low to excessive levels (10 to 110,000 lux).

In cultivated soils the screening effect of a growing crop canopy appears to cause a rapid decrease of light reaching the algae. Thus the canopy of transplanted rice decreased light by 50% when plants were 15 days old, 85% after one month and 95% after two months [29]. In Senegal, diatoms and unicellular green algae developed first and BGA developed later when the plant cover was dense enough to protect them from excessive light intensities, higher than 80 klux at 13:00 h (Fig. 1); the N_2-fixing algal biomass and the density of the plant cover were positively correlated [52].

In the laboratory, after one month of incubation of a submerged unplanted soil under a range of screens BGA were dominant in the most heavily shaded one, and green algae and diatoms were dominant in the soil exposed to full sunlight [45]. A

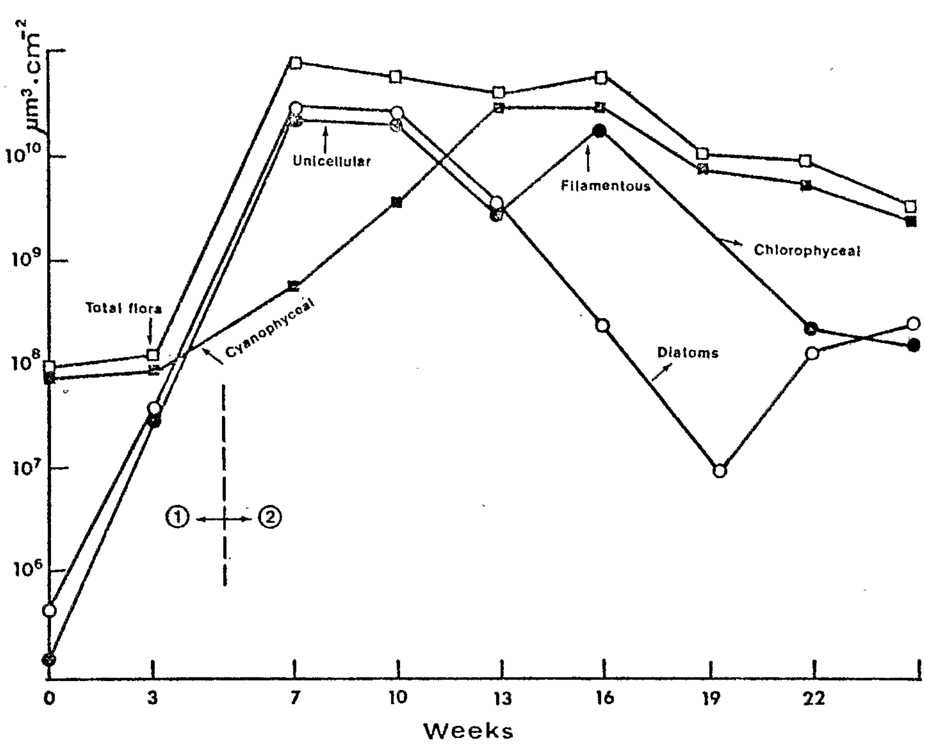

Fig. 1. Variation in biomass of different components of the algal flora during a vegetative cycle of rice in Senegal [52].

beneficial effect of the plant canopy shading the algae was also reported by Singh [63] in sugarcane fields, maize fields and grasslands in India.

As BGA are generally sensitive to high light intensities, they develop various protective mechanisms against it, namely:

- vertical migrations in the water of submerged soil;
- preferential growth in more shaded zones like embankments, under or inside decaying plant material [27] or a few millimeters, below the soil surface [17];
- migration into shaded zones (photophobotaxis) and aggregation providing a self-shading effect (photokinesis [46]);
- stratification of the strains in algal mats where N_2-fixing strains grow under a layer of eukaryotic algae more resistant to high light intensities [47].

However, some strains of BGA seem more resistant to high light intensities. *Cylindrospermum* sp. developed large biomasses in a harvested paddy field in Mali where light intensity was higher than 100 *k*lux at 13:00 [72]. *Oscillatoria princeps* was also reported to grow profusely in full sunlight [46].

On the other hand, light deficiency may also be a limiting factor. In Japan, available light under the canopy was below the compensation point of the phytoplankton during the second part of the cycle [22]. In the Philippines, during the wet season when light was moderate acetylene reducing activity (ARA) was higher in bare soil than in planted soil [85].

3.1.2. Temperature. The optimal temperature for BGA growth is about 30–35 °C which is higher than that for the growth of eukaryotic algae. In submerged soils daily variations in the temperature are moderated by the buffering effect of floodwater; temperature is rarely a limiting factor for BGA in paddy fields, because the range of temperatures permitting the growth of BGA is larger than that required by rice; however, it influences both algal biomass composition and productivity. Low temperatures decrease productivity and favour eukaryotic algae. High temperatures favour both the phytoplankton productivity and BGA [56].

Daily changes in the temperature are more drastic in terrestrial habitats than in aquatic environments [42]. An inhibitory effect of high temperature was observed by Jones [24] in the Kikuyu grasslands in Africa where algal N_2 fixation was higher on overcast days than on hot sunny days. Stewart [65] indicated a correlation between the algal ARA response to temperature and the temperature of the habitats from which the algae were collected. For many tropical species, ARA is optimum between 30–35 °C, but a *Nostoc* sp. isolated from the algal crust on a sandy soil in Senegal still exhibited significant ARA at 60 °C (Reynaud, unpublished). High temperatures occurring in the surface of tropical upland soils may have a selective action on the algal flora, favouring BGA which are more tolerant to high temperatures than eukaryotic algae. For example, the dry spores of *Nostoc* sp. can tolerate 2 minutes of 100 °C, the wet spores 20 minutes at 60–70 °C, and the vegetative filaments 10 minutes at 40 °C [10].

3.1.3. Desiccation and remoistening. Algal growth is hindered by intermittent desiccation periods which occur during the dry season and even during drought periods that occur in the rainy season. BGA have a high capacity to withstand desiccation. *Nostoc muscorum* and *Nodularia harveyana* were isolated from a soil that had been dry for 79 years [10]. Resistance to desiccation has been attributed to various characteristics [41], namely, with respect to fatal plasmolysis, the lack of cell vacuoles, the ability of some genera to quickly take on an encysted form, the presence in some genera of a mucilaginous sheath that absorbs water quickly and retains it. This latter characteristic could explain the dominance of mucilaginous colonies of *Nostoc* spp. and *Cylindrospermum* spp. in the paddy field during the last part of the cultivation cycle when the soil dries [38, 45, 72]. The dominance of BGA in tropical soils is partly due to their better resistance to dry conditions than other soil algae. This resistance seems to be more related to an ability to support long periods of desiccation than to the ability to remain active at low humidities [8]. The floristic composition of desiccated soils can be related to the dryness of the biotope. In a paddy in Italy, where the dry period is relatively short, N_2-fixing BGA comprised only about 30% of the algal flora [32], whearas in Senegal, where the dry season lasts about 8 months spores of heterocystous BGA constituted more than 95% of the algal flora at the end of the dry period. In Uttar Pradesh (India), a large number of Chlorophyceae occurred in low-lying fields, whereas BGA were found in larger numbers in paddies at higher elevations [38]. In arid soils, BGA have been reported as dominant species [31] and sometimes as the only species present [5, 10].

3.2. Biotic factors

Organisms that limit BGA growth are pathogens, antagonistic organisms and grazers. Of these, only grazers have been documented. The development of zooplankton populations, especially cladocerans, copepods, ostracods, mosquitoe larvae, etc. prevented the establishment of algal blooms within one or two weeks [73]. Snails form another group of algal grazers in submerged paddy fields: the biomass of snails can be as high as 1.6 t ha^{-1} in certain rice fields in the Philippines which explains the low population of algae [58].

3.3. Soil properties

Among the soil properties, pH is the most important factor determining the algal flora composition. In culture media the optimal pH for BGA growth seems to range from 7.5 to 10.0 and the lower limit is about 6.5 to 7.0 [20]. Under natural conditions BGA grow preferentially in environments that are neutral to alkaline; which explains that in paddies correlations occur between:

- water pH and BGA number [37];
- soil pH and number of spores of N_2-fixing BGA in the soil during the dry season [18];
- soil pH and BGA growth [36];
- soil pH and the N_2-fixing algal biomass (however, this relationship was conspicuous only in samples-homogenous for stage of rice development, fertilization and plant cover density [53]).

The beneficial influence of high pH on BGA growth is further demonstrated by the fact that the addition of lime increases BGA growth and N_2 fixation [58]. However, the presence of certain strains of BGA in soils with pH values between 5 and 6 have been reported. Durrel [15] demonstrated the presence of *Nostoc muscorum* and *Anabaena torulosa* in soils with pH ranging from 5 to 7. *Aulosira fertilissima* and *Calothrix brevissima* have been reported to be ubiquitous in Kerala rice fields with pH from 3.5 to 6.5 [1]. The development of a dense algal bloom on an acidic soil (pH 5.5) was observed after the surface application of straw [33]. Stewart [66] also reported that some tropical BGA exhibited ARA even at pH 4. The poor growth of N_2-fixing BGA, frequently observed in acidic soils, is probably due to the inability of BGA to compete with Chlorophyceae, which are favoured by acidic conditions.

3.4. Algal successions

The factors mentioned above are responsible for the algal successions that have been reported. An example of algal succession is provided by a study conducted in paddies in Senegal [52, 53]. Soils are acidic, and have an average pH value of 5.0 at the beginning of rice cultivation and 6.2 after 2 months of submersion. The rainy season is short (15 July–15 November) and rice fields are dry the rest of the year. High light intensities (70–80 klux) occur throughout the year. During the early part of the cultivation cycle (planting and tillering), the algal biomass increased and consisted mainly of diatoms and unicellular green algae (Fig. 1). From tillering to panicle initiation, the algal biomass reached its highest values, and filamentous green algae and non-N_2-fixing BGA were dominant. After panicle initiation the total biomass decreased; if the plant cover was sufficiently dense, heterocystous BGA developed, but if it was thin, filamentous green algae and homocystous BGA remained dominant. The observed variations in the algal flora were attributed to a decrease in light intensity and the N level resulting from the rice growth, and to an increase in pH value favouring BGA growth.

4. N_2 fixation

BGA are the only N_2-fixing microorganisms that exhibit a higher plant type of photosynthesis. Their N_2-fixing ability was first related to the presence of specialized non O_2-evolving cells called heterocysts in which the nitrogenase, a highly O_2-sensitive enzyme, is protected from O_2. It is now clearly demonstrated that the ability to fix N_2 is not confined to heterocystous BGA, but is also found in a number of BGA, which were not known to fix N_2 a few years ago, and which can fix N_2 under microaerobic or anaerobic conditions [68]; over 125 strains are presently known to fix N_2.

ARA is a reliable method for quantitative studies provided that one takes into account the following facts:

- the distribution of ARA is log-normal [54];
- N_2 fixation by BGA occurs not only in aerobic but also in anaerobic conditions [67];
- there are diurnal variations in ARA and also variations in ARA throughout the cultivation cycle. In submerged soils the variations exhibit typical patterns with one or two maxima. The occurrence of two maxima results from the inhibitory effect of excessive light during the middle of the day [45]. Similar variations were observed in upland soils but the inhibitory decrease of ARA during the middle of the day was attributed to an increase in temperature of the surface soil [24, 44]. Moreover, one should note that the duration of the incubation should be short [13] and that the conversion factor $C_2H_4:N_2$ reduced should be measured in each situation [40].

In submerged soils, light intensity appears to be the main factor governing variations in photosynthetic N_2 fixation, whereas in upland soils water availability becomes the main factor. Studying photosynthetic N_2 fixation in a sandy soil colonized by a thick algal mat, Reynaud and Roger [47] observed that the variations of both total algal biomass and ARA were governed not only by light but also by the distribution of precipitations.

The average of 38 quantitative evaluations of N_2 fixation in paddies recorded in the literature was 27 kg N ha^{-1} per crop [58]. The highest recorded value was 50–80 kg N ha^{-1} per crop [72].

In the savanna lands of southwest Nigeria, N_2-fixing Cyanobacteria such as species of *Scytonema, Tolypothrix* and *Nostoc* develop during the rainy season, particularly in previously burnt areas and may contribute significantly to the input of total nitrogen. Similarly in the open 'campina' areas among parts of the rain forests of tropical Brazil, N_2-fixing growth of blackish Stigonematales may be found [66]. In some conditions N_2-fixing BGA may significantly contribute to the N status of cultivated upland soils. Singh [63] reported a profuse growth of N_2-fixing BGA in sugarcane fields in India from beginning of the rainy season. Among the strains *Cylindrospermum licheniforme* was assumed to add about 90 kg N ha^{-1} in about 75 days.

The same species was reported to very successfully colonize the clean soil surface of the weed-free maize fields. In grasslands of the Banaras Hindu University a thick and sometimes continuous BGA mat developed during the rainy season. The thicker the grass cover, the better the growth of BGA was. In protected and enclosed grasslands the total N content of the soil increased 88% in 5 months, an increase attributed mainly to the growth of *C. licheniforme*. From the above results it appears that BGA may contribute to maintain the N level of natural and cultivated ecosystems, but generally the extent of this contribution is poorly documented.

5. Relations between BGA and higher plants

5.1. Availability of fixed N_2 to higher plants

N_2 fixed by BGA is released either through exudation or through microbial decomposition after the death of the cells, but the process involved in the transfer of fixed N_2 to the plants is largely a mystery [30]. It has been shown in the laboratory, that BGA liberate large portions of their assimilated nitrogenous substances [17, 70]. However, the large amounts of nitrogenous substances recorded may result from methodological artifacts such as the osmotic shock occurring when resuspending the cells or any other physical damage of the algal material. No information on the exudation of fixed N_2 by BGA under field conditions is available, but it is clear that only part of it is available to rice, other parts being reincorporated by the microflora or volatilized. Nutrients released through microbial decomposition after the death of the algae appear to be the principal manner in which N is made available to the crop. Field experiments with *Tolypothrix tenuis* for 4 consecutive years indicated that only 1/3 of the field algae was decomposed and absorbed by rice plants in the first year: the rest remained as residual soil N, which could have been responsible for continued yield increases in the succeeding years [80]. The transfer of N from algae to higher plants has been investigated using ^{15}N-tracer techniques [34, 64]. Wilson *et al.* (personal communication) recently recovered from a rice crop 39% and 51% of the N from ^{15}N-labeled *Aulosira* spp. spread on or incorporated into the soil, thus showing that BGA N is readily available to rice. A similar experiment is underway at the International Rice Research Institute (Philippines). Results of the analysis of the first crop show a recovery of 13% and 38% of the N from ^{15}N-labeled *Nostoc* sp. spread on or incorporated into the soil. This indicates that the availability of algal N for the plant varies either with the strain or with the physiological state of the algal material; i.e. N from an algal material rich in akinetes, not so easily decomposable, will be less available than that from vegetative cells which are more susceptible to decomposition.

5.2. Growth-promoting effect

Besides increasing N fertility, BGA have been assumed to benefit higher plants by producing growth-promoting substances. This hypothesis is based on the additive effects of BGA inoculations in the presence of nitrogenous fertilizers. Most of these results have been obtained with rice but similar results were observed also with vegetables such as radishes and tomatoes [51].

More direct evidence of hormonal effects has come primarily from treatments of rice seedlings with algal cultures or their extracts. Presoaking rice seeds with BGA cultures or extracts enhances germination, promotes the growth of roots and shoots, and increases the weight and protein content of the grain [19, 23]. It has also been established that algal growth-promoting substances are beneficial to other crops besides rice [11, 26, 39] and that the production of such substances is not confined to BGA. Whether these substances are hormones [19], vitamins [73], aminoacids [7] or any other components is still unknown.

5.3. Harmful effects

Blooms caused by filamentous algae can be harmful to rice, mainly due to a mechanical effect on the young plants [6]. However detrimental, the effects of BGA are incidental [12, 58]. Even when BGA produced a bloom at the beginning of the growing cycle, their effect on grain yield was rarely negative [2].

5.4. Epiphytism

Epiphytic BGA have been observed on wetland rice [57] deepwater rice [28] and on weeds growing in rice fields [27]. In wetland rice fields, epiphytic BGA on rice and weeds make a limited contribution to the N input but are important in providing an inoculum for the regeneration of the algal blooms that are periodically affected by adverse conditions. In deepwater rice, which offers a much greater biomass for colonization, the addition of N by epiphytic BGA is of agronomical significance [28]. BGA were found to grow preferentially on submerged decaying tissues. An endophytic growth inside the leaf sheath was also observed in deepwater rice. The results obtained did not confirm the existance or absence of biotic relationships between the algae and their hosts, but indicated that a mechanical effect in relation to the roughness of the support was involved in algal epiphytism and endophytism.

6. Role of BGA in soil colonization

Despite a worldwide distribution of BGA in terrestrial habitats their ecology has been less studied than that of heterotrophic microorganisms such as soil fungi and bacteria. This has sometimes given rise to the fallacious impression that they are unimportant soil microorganisms [17]. Terrestrial algae in fact play a major role in soil genesis and soil conservation. They constitute the initial successional stage on substrata which are poor in plant nutrients such as recent volcanic deposits, sand, and barren infertile soils denuded of macrovegetation. The interwoven algal growth consolidates the surface, leading to the formation of a soil crust which improves infiltration, may limit sheet erosion and affords a substratum upon which seeds of higher plants germinate. Algae produce a surface humus after death and dissolve certain soil minerals maintaining a reserve supply of elements in a semi-available form for higher plants [61]. Experiments on algal crusts from the Botanical Garden at the University of India indicated that these crusts (i) do not slow the rate of water infiltration [7], (ii) have a very efficient protective effect on erosion by buffeting rain, (iii) increased the moisture content of the soil underneath the algal stratum 10–15% [63]. The resistance of algal crusts to erosion is apparently the result of binding the soil surface particles into a non-erosible layer, which is also effective in breaking the force of falling water. The favourable effect of BGA on aggregation of the soil was demonstrated by Roychoudhury *et al.*, [60] who observed a 50–70% increase in the water-stable aggregates after algal inoculation. This was attributed to the action of polysaccharides released by the algae and the pressure of filamentous BGA growing in the soil. In sugarcane fields in India, a profuse growth of *Porphyrosiphon notarisii* was reported to check the erosion of the soil and to help the crop stand erect on the intact ridges during the monsoon period [63]. The same author in a study of grasslands subjected to close and heavy grazing by sheep and cattle concluded that in such deteriorated grassland, where soils are light and sandy, a thick growth of BGA during the monsoon is perhaps the only check for erosion. *Porphyrosiphon notarisii* and *Microcoleus chthonoplastes* [8] were reported to be the most efficient colonizing strains growing as a mat that provides a suitable substratum for the germination of grass seeds. Booth [7] conducted a detailed investigation on the importance of BGA in badly eroded soils in south-central US. He concluded that (i) soil losses from plots with an algal stratum were greatly reduced as compared with the losses from bare areas, (ii) BGA constituted and initial stage in plant succession, and (iii) BGA colonization lasted for several years until higher perennial plants were able to form abundant ground cover. Singh [63] described the characteristic and common algal cover caused by *Aphanothece pallida*. Although the fresh thalli were extremely mulicaginous and fragile, they formed a compact dark grey stratum firmly adherent to the soil when dried; 0.3 g of algae were sufficient to bind 34.6 g of soil.

The floristic composition of algal crusts developing on cultivated sandy soils in Senegal was recently studied by Reynaud. The L.P.P. (*Lyngbia, Phormidium, Plectonema*) group [50] was dominant in most of the soils studied. A similar observation was made by Marathe and Anantany [31] in Indian arid soils. The dominance of the L.P.P. group in arid soils can be related to its ability to develop in drastic conditions a gel-like protoplasm and a thick mucilaginous sheath able to readily adsorb water and retain it. Among N_2-fixing genera, *Nostoc* was the most frequently observed, confirming the observation that it is one of the most consistently present N_2-fixing forms in arid soils [61]. Examples of the strains isolated from algal crusts on sandy Senegalese soils are given in Figs. 2 and 3.

7. Agronomical use of BGA

Most information on the effects of agronomical practices on BGA is related to paddies. Land preparation and management seem to have only incidental effects [58] and were reported not to interfere with the establishment and activity of inoculated BGA [2]. Pesticides depending on their nature and their concentration, could have inhibitory, selective or stimulatory effects on BGA. Experiments mainly with flask cultures suggest that BGA are generally more resistant to pesticides than other algae and are capable of tolerating pesticide levels recommended for field application [77]. Insecticides are generally less toxic to BGA than other pesticides [42] and have a secondary beneficial effect by controlling the grazers.

Organic manure may favour or depress BGA growth depending on its nature and mode of application. Incorporation of plant residues has frequently been reported to temporarily depress the algal populations and to have a negative effect on inoculated BGA (refer to Roger and Kulasooriya [58]). On the contrary the surface application of straw very significantly enhanced BGA growth and ARA [33].

The nature and the quantity of inorganic fertilizers as well as application techniques have a considerable influence on the algal flora. The surface application of NPK generally results in a profuse green algae growth. To prevent such a growth the incorporation of fertilizers into the soil is recommended. The deep placement

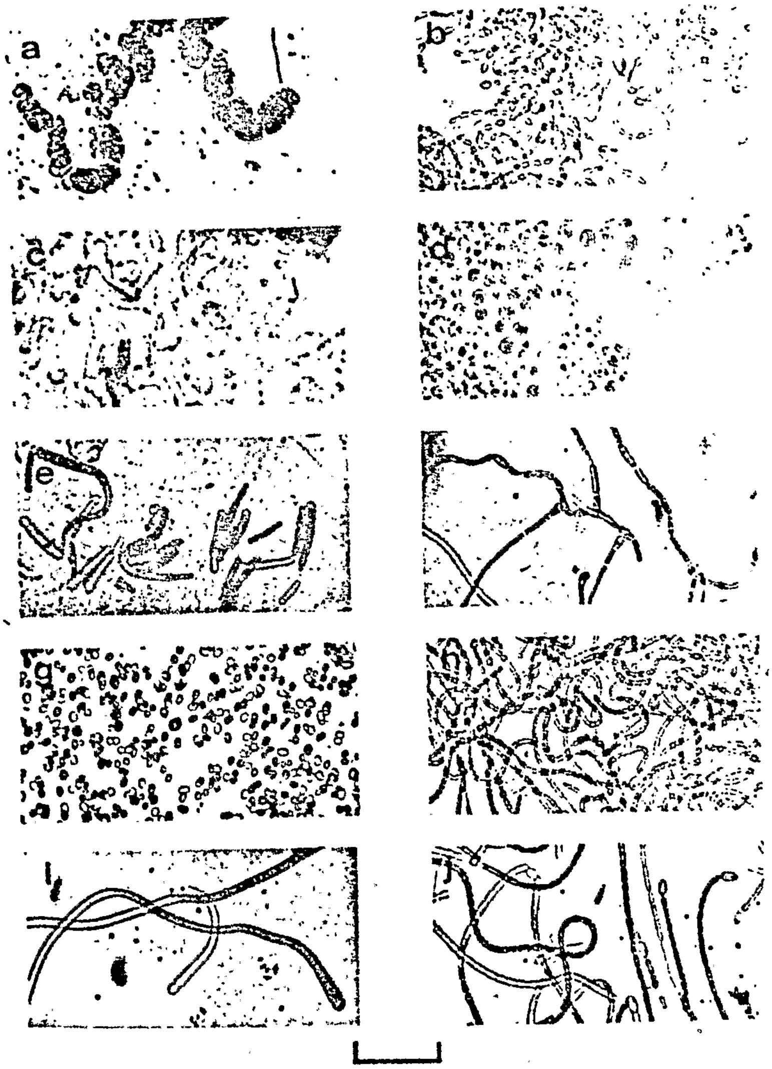

Fig. 2. BGA isolated from algal crusts in Senegal, in a medium without N. (a) *Nostoc* sp.; (b, c, h) *Anabaena* spp.; (d) *Dermocarpa*; (i, e) *Calothrix* sp.; (f) *Hapalosiphon* sp.; (g) *Gloeocapsa* sp.; (j) *Anabaenopsis* sp. (the scale represents 50 μm).

of urea supergranules prevents the dense growth of green algae that occurs when urea is surface broadcast; moreover it does not inhibit the growth and N_2 fixation in BGA [59]. Since the growth of N_2-fixing BGA in paddy fields is generally limited by P deficiency, P application alone or together with lime should be recommended (refer to Roger and Kulasooriya [58]).

In N-deficient conditions, N_2-fixing BGA are not hindered by competition from the other algae and they can develop profusely if the other environmental factors are not limiting. When nitrogenous fertilizers are applied their ARA is inhibited or

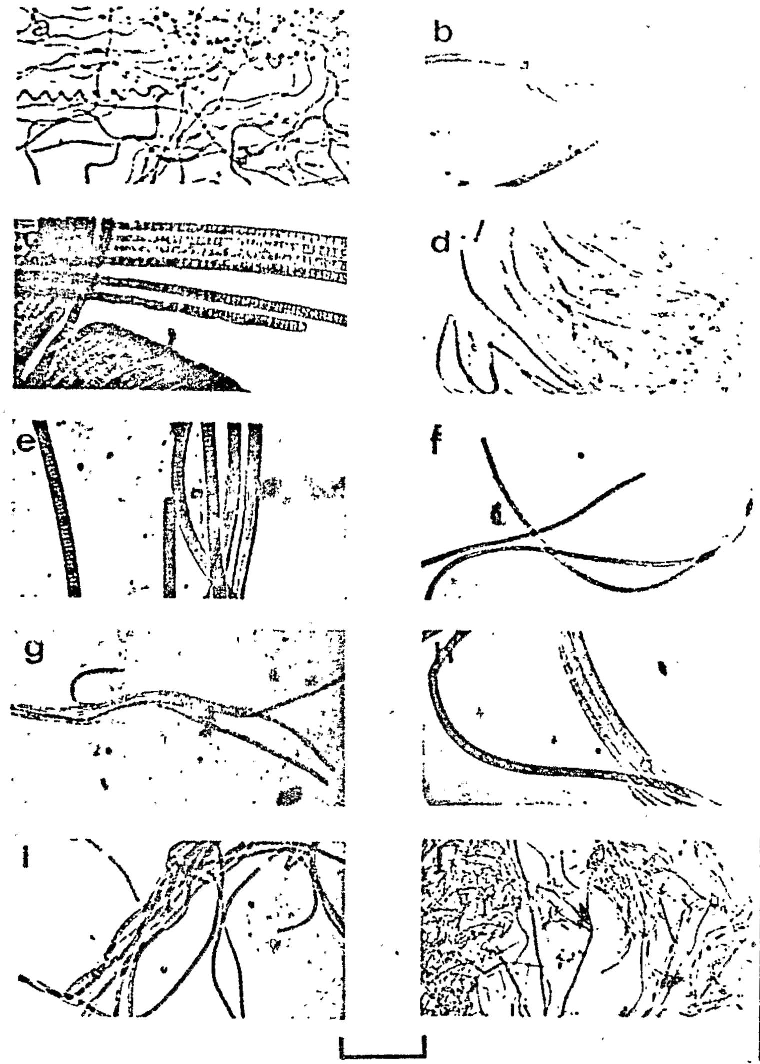

Fig. 3. BGA isolated from algal crusts in Senegal, on a medium with N. (a) *Pseudanabaena* sp.; (h, d) *Oscillatoria* spp.; (b, c, e, f, g, i, j) LPP (*Lyngbia, Phormidium, Plectonema*) group (the scale represents 50 μm).

at least affected. Moreover, N fertilizers appear to enhance the growth of non-N_2-fixing algae thus increasing the competitive pressure against N_2-fixing forms [59]. However, this effect seems to be transient as suggested by pot experiments [49] and preliminary field data. Simultaneously the effect of N fertilizers on ARA is only partial. Venkataraman [79] reported that ARA was not depressed in a soil–rice–BGA system with less than 40 ppm ammonium-N in stagnant paddy water. Within mixed algal masses combined N may diffuse at a slower rate than the readily available dissolved N_2, and a local depletion in combined N may favour N_2-fixing algae. Thus in the fields, the lack of competition with N_2-fixing algae in the presence of mineral nitrogen may not be as clear-cut as was first thought.

Molybdenum, because of its function in nitrogenase, is required by N_2-fixing BGA. Subrahmanyan *et al.* [68], suggested the addition of sodium molybdate (0.25 kg ha^{-1}) to the soil to improve N_2-fixing algal growth. This addition has been shown to be beneficial in some situations.

Other nutrients (Fe, Mg, K, etc.) are required for optimal growth of BGA but their ecological implications as limiting factors or as factors affecting the composition of the algal community in paddies have not been documented.

7.1. Reclamation of Usar lands [62]

Usar soils are saline (solonchak) and alkaline (solonetz) unproductive soils, found extensively throughout India. They are characterized by impermeablity, extreme hardness and the occasional presence of undesirable salts on the surface, all of which adversely affect the plant growth. The pH value is usually high throughout the profile. The subsoil water table is generally found between 3.0 and 4.5 meters below the surface. These soils are usually very deep. The method of reclamation arose from the observation [62] that in the alkaline Usar soils of northern India, while other plants fail to grow blue-green algae form a thick stratum on the soil surface during the rainy season (July–September) and the retreating monsoon (December–January). These soils, characterized by an alkalization process, can be reclaimed by replacing sodium with calcium through the addition of chemical correctives such as gypsum, a rather expensive method. Another possibility is to help the reaction of calcium carbonate with the sodium clay by waterlogging the soil and by adding organic matter and N. The abundant growth of N_2-fixing BGA on waterlogged soils fulfills these requirements.

The following reclamation process was tested. During May and June, before the rains started, the land was divided into plots of less than 0.4 ha. The plots were enclosed by an earth embankment. After the first showers, N_2-fixing BGA formed a thick and compact stratum. Later, when the soil was waterlogged, N_2-fixing forms characteristic of rice fields developed and continued their active growth as long as the soil was waterlogged. In field trials, after a year of reclamation, a transplanted paddy crop produced a yield of 1,600–2,200 kg grain ha^{-1} and allowed the successful growth of sugarcane after 3 years. Laboratory experiments over a 3-year period indicated tremendous changes in the soil characteristics after algal growth compared with the control covered with a black cloth: pH decreased from 0.2 to 7.5. Large increase in organic matter content (69%), total N (46%), water-holding capacity (35%), and exchange calcium (31%), were observed. The most significant changes concerned the different forms of P. CO_2-soluble P was 0.45 in the control and 8.15 ppm in the treated plot with BGA. The role of BGA in converting the sodium clay into a type of calcium clay was not explained but a possible effect of oxalic acid excreted by the algae was suggested.

Subsequent improvements of the reclamation technique were proposed: doing the process several times in a year where irrigation water is available, contour farming (field operations such as plowing, etc. are done at right angles to the slope of the land) to retard water flow and increase water penetration; and introducing *Nostoc commune* where it does not occur naturally.

7.2. Algalization

BGA were among the first N_2-fixing agents recognized to be active in flooded rice soils. Since De [14] attributed the natural fertility of the topical paddy fields to these organisms, many trials have been conducted to increase rice yield by inoculating the soil with BGA. This practice, also called algalization, a terminology introduced by Venkataraman [74], has been reported to have a beneficial effect on grain yield in different agroclimatic conditions. However, some reports also indicate failure of algalization. The conclusions of the review of Roger and Kulasooriya [58] on algalization are summarized thereafter.

7.2.1. Methodology. Most experiments on algalization were on a 'black box' basis, where only the last indirect effect (grain yield) of algalization was observed and the intermediate effects were not studied. There is little information on the qualitative and quantitative variations of the N_2-fixing algal flora and the N balance in inoculated paddy soils. Pot and field experiments have been conducted, usually on a single crop. The relative increase in grain yield over the control was an average 28% in pot experiments and 15% in field experiments. The better growth of BGA in pot experiments is probably attributable to the reduction of climatic disturbance and to the mechanical effect of the pot walls, where BGA frequently seem to grow preferentially and profusely. Pot experiments may therefore only be suitable for qualitative studies, since they overestimate the effects of BGA inoculation. Most of the field experiments on the other hand, were conducted in only one growing season and may underestimate the effects of algalization since the advantages of a slow N release from dead BGA may not be apparent in the first algalized crop.

7.2.2. Effects of algalization on rice. Algalization may affect plant size, its nitrogen content, and the number of tillers, ears, spikelets, and filled grains per panicle. The most frequently used criterion for assessing the effects of algalization has been better grain yield. Results of field experiments conducted mainly in India report an average yield increase of about 14% over the control, corresponding to about 450 kg grain ha^{-1} per crop where algal inoculation was effective. A higher grain yield increase was observed when algalization was in combination with lime, P and sometimes molybdenum application. Unfortunately it is not possible to separate the direct effect of PK fertilizers on rice from its indirect effect upon the growth of indigenous or introduced algae. The effects of algalization used with N fertilizers are controversial. Since biological N_2 fixation is known to be inhibited by inorganic N the beneficial effect of algalization in the presence of N fertilizers was most frequently interpreted as resulting from growth-promoting substances produced by algae or also by a temporarily immobilization of added N followed by a slow release through subsequent algal decomposition permitting a more efficient utilization of N by the crop (see Section 5.2.).

7.2.3. Effects of algalization on soil properties and microflora. Grain-yield measurements suggest that algalization produces both a cumulative and residual effect. This was attributed to a build up of both the organic N content and the number of BGA propagules in the soil, facilitating the reestablishment of the BGA biomass. Several reports indicate an increase in organic matter and organic N; algalization was also reported to increase: aggregation status of the soil [61], water-holding capacity [63], and available P, total microflora, *Azotobacter, Clostridia*, and nitrofiers [21].

7.2.4. Limiting factors for algalization. Among the limiting factors responsible for the failure of algalization only pH and available P content of the soil have been studied. Low pH is an important limiting factor for algalization as demonstrated by unsuccessful trials in acidic soils of India, Sri Lanka and Japan. In Sri Lanka and Japan algalization was effective only when the soil was supplemented with calcium carbonate, the effect of inoculation being related to the amount of lime added. Since in some soils, algalization is inefficient in spite of the addition of lime and phosphate [36], available P content should probably not be the only factor limiting the effect of algalization; texture, organic matter content, CEC of saturated extracts, total N are probably not important limiting factors [36, 68]. Amont the biotic factors that can possibly limit BGA growth inoculum, grazing by the zooplankton has been already mentioned. Other possible mechanisms involved such as antagonism, competition, etc. have been cited, but their role is not clear. Low temperatures, heavy rains, and cloudy weather have also been reported to limit the establishment of the inoculum.

7.2.5. Algalization technology

7.2.5.1. Inoculum production and conservation. The methodology of BGA production has been reviewed by Watanabe and Yamamoto [83] and Venkataraman [76, 77]. Methods of inoculum production in artificially controlled conditions have been developed mainly in Japan. Different types of tank cultures and outdoor closed circulating systems have been described [82]. Algae are grown either in liquid culture, mixed with an inert material (support) and dried, or grown directly on the support and dried. Various supports such as sand, pumise stone, volcanic earth, and blocks of synthetic sponge have been tested to conserve and facilitate transportation of the algae. Such inocula reportedly maintained their capacity for growth unimpaired for at least two years.

Producing the inoculum in artificially controlled conditions is well defined but relatively expensive. On the contrary, the open air soil culture, used in India, is more simple, less expensive and easily adoptable by the farmers. It is based on the use of a starter culture that is a multistrain inoculum of *Aulosira, Tolypothrix, Scytonema, Nostoc, Anabaena* and *Plectonema*, provided by the 'All India Co-ordinated Project on Algae' [2]. This inoculum is multiplied by the farmer in shallow trays or tanks with 5–15 cm water, about 4 kg soil m^{-2}, 100 g triple superphosphate m^{-2} and insecticide. If necessary, lime is added to correct the soil pH to about 7.0–7.5. In 1 to 3 weeks, a thick mat develops on the soil surface and sometimes floats. Watering is stopped and water in the trays is allowed to dry up in the sun. Algal flakes are then scraped off and stored in bags for use in the fields. With such a method, the ultimate proportion of individual strains in the algal flakes is unpredictable. It is assumed that, because the inoculum is produced in soil and climatic conditions similar to those in the field, dominant strains will be the most adapted to the local conditions. The recorded rates of production of algal flakes in the open air soil culture range from 0.4 to 1.0 kg m^{-2} in 15 days, indicating that a $2m^2$ tray can produce in 2–3 months enough algal material to inoculate 1 ha of rice field.

7.2.5.2. Inoculation of the rice field. The methods of field application have been reviewed by Venkataraman [77]. For transplanted rice the algal inoculum is generally applied 1 week after the transplanting. When rice is sown, seeds can be coated by mixing the algal suspension and 2–3 kg calcium carbonate per 10–20 kg seed and air-dried in the shade.

Recommendations for field application of dried algal inoculum (algal flakes) given by the 'All India Coordinated Project on Algae' [2] indicate that 8–10 kg of dry algal flakes applied 1 week after transplanting is sufficient to inoculate 1 ha; a large amount will accelerate multiplication and establishment in the field. Algalization can be used with high levels of commercial nitrogen fertilizer, but reduction of the N dose by one third is recommended. To benefit from the cumulative effect of algalization the algae should be applied for at least three consecutive seasons. Recommended pest-control measures and other management practices do not interfere with the establishment and activity of these algae in the fields.

7.2.5.3. Pay-off of algal technology. Trials in Indian station (in 1978), showed that adding 10 kg of algal culture ha^{-1} which costs about 4 US $, increases paddy yield; the average yield increase is worth 60 to 90 US $ [43]. The pay-off of algal technology based on the results of a large number of field experiments was discussed by the 'All India Coordinated Project on Algae' [2] and it was inferred that if algal technology was introduced into even 50% of the Indian rice area, it would supposedly result in a saving of about 3.8×10^5 t of N. In a large number of trials where the recommended level of N fertilizer (100 kg ha^{-1}) was complemented with algal application (10 kg ha^{-1}) an average increase of about 300 kg rice grains ha^{-1} was reported.

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