

Distribution of algal diversity in the rice field

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ABSTRACT

A good understanding of the distribution of algal communities in paddy fields in the wetland ecosystem is essential. Moderate temperature, high light nutrient and water availability appears to be better conditions for algal growth in the rice field habitat. Diverse chlorophycean taxa with wide range of thallus structure were collected and identified of the algal communities using image processing techniques from microscopic images. It's belonging to various orders that is, Oedogoniales, Zygnematales, Cladophorales, Chlorococcales, Ulotrichales etc. The collected algal samples of dry matter, nitrogen, carbon and sugar content are analysed. *Oedogonium australe* showed that higher percentage of dry matter averaged 6.25% whereas, *Navicula* (2.70%) showed very low amount of dry matter compared to other algae communities. Similarly, Nitrogen content of the collected algal biomasses was equivalent to values ranging from 1.20 to 3.95. *Oedogonium australe* showed highest percentage of sugar (22.5) and carbon content (23.7), whereas, *Tribonema regulare* revealed very low percentage of carbon (10.3) and sugar (10.1). At the same time, experimental paddy field soil nutrients were changed because of the algal growth. This study reveals that the varietal numbers of algal diversity will help to increase the nutrient status in the soil.

Key words: Rice field, Algae and soil

Introduction

Rice (*Oryza sativa* L.) is one of the first leading ancient (3,000B.C.) cultivated crops of the world. This is of the genus *Oryza* and the Poaceae family, has 22 known species and has great economic significance (Bajaj and Mohanty, 2005). Worldwide, rice species commonly cultivated are *Oryza glaberrima* and *Oryza sativa* (L.) (Lu, 1999). This is one of the most common staple food crops, serving more than half a billion people worldwide, primarily in Asian countries, as a source of food and calories. These are adapted to various climatic conditions, and can be grown at high and low altitudes in both dry and wetland environments. Rice can be cultivated in Bhutan and Nepal at altitudes above 300 m from sea

level and in India (Kerala) 3 m below sea level (Khush, 1984). Overall it covers an area of almost 158 million hectares (M ha), producing 470 million tons (Mts) of milled rice in 2009 (IRRI, Africa Rice and CIAT, 2010). Nearly 90 percent of the world's rice is obtained from Asia (around 640 million tons), China and India being the main contributors. The world population in 2050 is projected to be 9 billion, entailing an incessant increase in food production to ensure food security. However, rice productivity remains poor in several parts of the world owing to numerous constraints, including biotic and abiotic stresses.

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 good 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 chemical nitrogen. Although nitrogen occurs in the atmosphere in the form of molecular nitrogen in a concentration of 77 volume percent (about 77,000 t/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 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. The study of the rice field algal flora in tropics has attracted considerable attention. Rice fields are a peculiar character in artificial biotopes. The biocenosis in them is the synthesis of two micro organisations, that of the water and soil, although they are not mutually exclusive. However, biocenotic life in rice field water is of a short duration which extends in total over three to six months. Most aquatic micro-organisms either die or permanently removed after the harvest. Throughout India, algal flora from the rice field displays a distinct periodicity before and just after the monsoon (Gupta, 1964).

Singh (1961) observed that a general mixture of nitrogen-fixing species initially appears in Uttar Pradesh soils, which is soon followed by a 'massive

and pure growth of *Aulosira fertilissima* thick brownish gelatinous mass. *Cylindrospermum* species are dominant as the soil dries out. Pandey (1965) found blue-green algae to be around 70 per cent of the algal group. Potential nitrogen-fixing species such as *Aulosira*, *Anabaena*, *Anabaenopsis*, *Calothrix*, *Camptylonema*, *Cylindrospermum*, *Fischerella*, *Hapalosiphon*, *Microchaete*, *Nostoc*, *Westiella*, *Westiellopsis* and *Tolypothrix* have all been recorded in the rice fields of many localities in India. In the present investigation was undertaken to study the succession of algae in paddy fields, algae samples collected from these fields and to correlate the growth of algae in nature with the growth found in soil.

Materials and Methods

The location of the sample collection

In this study algal and soil samples were collected from rice fields in the wet land and surrounding area (Farm Management Department, Tamil Nadu Agricultural University, Coimbatore). The experimental location is situated in western agro-climatic zone of Tamil Nadu at 11°N and 77°E with an altitude of 426.7 m above MSL. Weather parameters have been recorded for the normal weeks. Total rainfall of 530 mm occurred in 26 rainy days. The maximum and minimum temperatures ranged from 29.0 to 32.7 °C and from 19.2 to 23.7 °C, respectively. With respect to relative humidity, there was a fluctuation from 77.5 to 94 per cent (0722 hours) and from 49.6 to 77.3 per cent (1422 hours). The evaporation and bright sunshine hours of first day ranged from 2.8 to 6.6 mm and 3.1 to 12.4 hr respectively. In the wetland farm and surrounding area, floating algal mass colonies were collected using a net, 20 cm in diameter, 1 mm mesh. Stuff was rinsed with floodwater from rice field and cleaned out organic waste. Shortly after compilation an experiment was performed.

Identification algae microscope

Sample preparation

The collected specimens were brought to the, University department of Agricultural Microbiology, Coimbatore. Samples were carefully washed and preserved in 4% formaldehyde solution. A permanent slide was prepared by aniline blue (1% aqueous solution with 4% HCl, Toluidine blue 0.05%

aqueous solution) and to visualizing the macro algae stains prepared the 2% Potassium permanganate solution. Additionally, taken the Indian ink to highlight the mucilage and some flagella like structures. After staining for 30 seconds to five minutes, rinse in water, then add a drop or two of glycerine solution (75% glycerine and 25% water) to a small piece of the algae placed on a microscope slide then carefully lower the coverslip. Once set the sides of the coverslip can be readily sealed using nail polish. Temporary Mounts of algal specimen were observed under compound microscope (ALCO AM-35) and taken the photography.

Methods of analysis

Dry algae weight was determined from the algal suspension pellets centrifuged for 15 min at 10 000 rpm. Dry weight was measured in the oven after 24 h of heating at 80 °C. Mineral contents were measured using analytical methods for plants in the TNAU analytical laboratory. Nitrogen, Protein, sugar and carbon have been measured using algal suspensions sonicated for 5 min. Nitrogen in algae was measured using the standard micro-Kjeldahl method. Sugars were measured using the phenol-sulfuric acid method (Dubois *et al.*, 1956). Carbon was measured using the Walkley and Black method (Black, 1965).

Soil nutrient analysis

For the assessment of soil nutrient status, the soil sample was randomly gathered, air-dried, and analyzed for various physico-chemical characteristics using standard procedures. The soil pH was determined at 1:2.5 soil/water ratio. Available P determined using a spectrophotometer (Mehlich, 1984), organic carbon by Walkley and black sulphuric acid-dichromate digestion method (Walkley and Black, 1934), available N using Kjeldahl method (Bremner *et al.*, 1982), whereas micronutrients were determined using atomic absorption spectrophotometer (Lindsay and Norvell, 1978).

Statistical analysis

One-way ANOVA was conducted to compare the effect of algae and soil properties. Similarly, Mean values of the treatments were compared by Duncan's multiple range tests at $p \leq 0.05$.

Results

During this present investigation, different taxa of chlorophyceae belonging to different orders such as, *Chlorococcales*, *Ulotrichales*, *Cladophorales*, *Oedogoniales* and *Zygnematales* have been found and the algal microscopic images were represented in

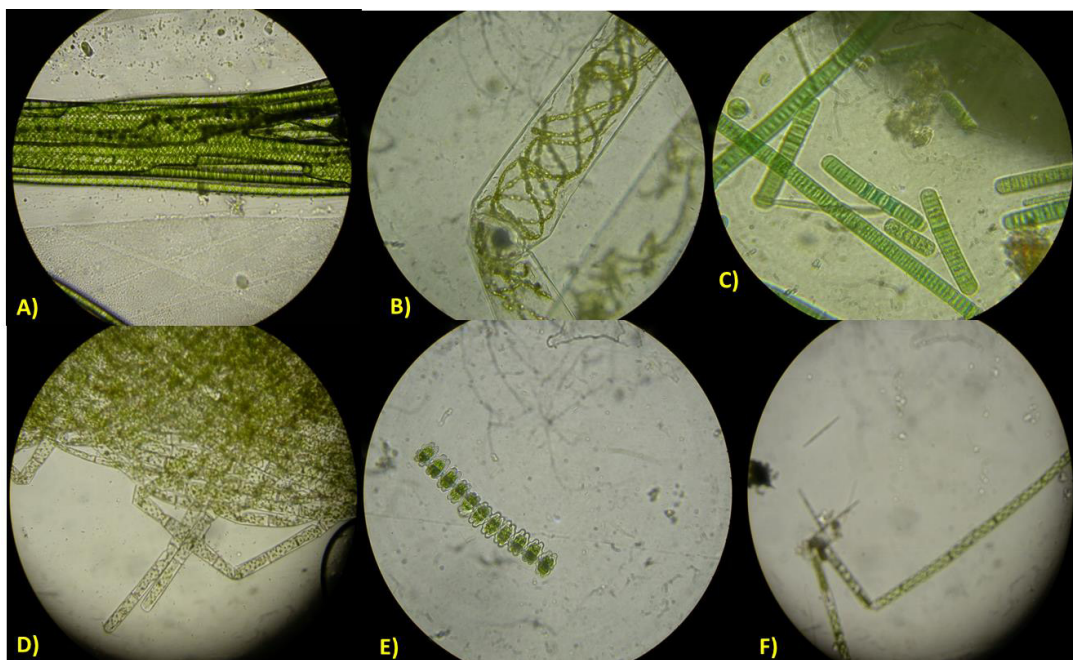


Fig. 1. Microphotograph of some taxa observed during study period: A) *Rhizoclonium* B) *Spirogyra dubia* C) *Oscillatoria tenuis* D) *Oedogonium australe* E) *Chroococcus* F) *Aphanizome*

Fig. 1 & 2. The collected algal samples of dry matter, nitrogen, carbon and sugar content are analysed and the results were described in Fig. 3. *Oedogonium australe* showed that higher percentage of dry matter averaged 6.25% whereas, *Navicula* (2.70%) showed very low amount of dry matter compared to other algae communities. Similarly, Nitrogen content of the collected algal biomasses was equivalent to values ranging from 1.20 to 3.95. *Oedogonium australe* showed highest percentage of sugar (22.5) and carbon content (23.7), followed by *Navicula*, shows (21.3% of sugar) and carbon about (20.5%) whereas, *Tribonema regulare* revealed very lowest

percentage of carbon (10.3) and sugar (10.1). At the same time, experimental paddy field soil characteristics were analysed and the results described below. The soil texture is clay loam in nature. The initial analyses of the experimental sites revealed that the soil was alkaline in pH (8.10 - 8.24), medium in organic carbon content (0.62 - 0.68 %), low in available nitrogen (210.4 kg ha⁻¹ to 223.1 kg ha⁻¹), medium in available phosphorus (15.4 kg ha⁻¹ to 18.8 kg ha⁻¹) and high in available potassium (ranged from 416.1 to 428.5 kg ha⁻¹). The details of physico-chemical properties of the soil of the experimental field are given in Table 1.

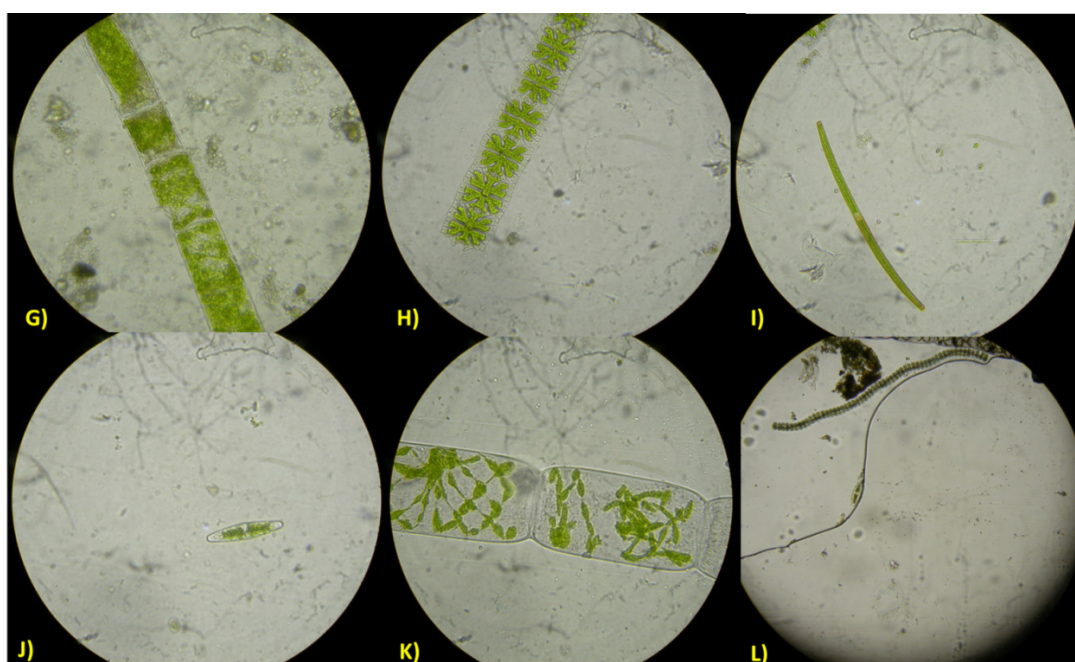


Fig. 2. Microphotograph of some taxa observed during study period: G) *Tribonema regulare* H) *Zygnema* sp. I) *Closterium acutum* J) *Navicula* K) *Spirogyra* L) *Spirogyra lagerheim*

Table 1. Analysis of paddy field samples of algae

Diversity of Algae	Dry matter	Nitrogen	Sugar	Carbon
<i>Rhizoclonium</i>	4.00	2.29	12	17.2
<i>Spirogyra</i>	4.12	2.32	20	20.1
<i>Spirogyra dubia</i>	6.21	3.81	18.2	18.9
<i>Oscillatoria tenuis</i>	5.43	3.23	18.4	13.5
<i>Oedogonium australe</i>	6.25	3.95	22.5	23.7
<i>Chroococcus</i>	5.43	2.14	12.1	14.0
<i>Aphanizome</i>	5.81	3.83	15.3	18.2
<i>Tribonema regulare</i>	3.47	2.20	10.3	10.1
<i>Zygnema</i> sp.	3.89	1.20	19.5	12.5
<i>Closterium acutum</i>	4.52	2.72	19.1	13.2
<i>Navicula</i>	2.70	2.35	21.3	20.5
<i>Spirogyra lagerheimini</i>	2.85	2.89	20.1	9.25

Table 2. Soil characteristics of the experimental paddy field

Mechanical analysis	Values	Chemical analysis	Values	Water characteristics	Values
Clay (%)	44.2	pH	8.3	pH	7.6
Silt (%)	19.5	Electrical conductivity (dS m ⁻¹)	0.42	Electrical conductivity (dS m ⁻¹)	1.18
Course sand (%)	18.2	Organic carbon (%)	0.58		
Fine sand (%)	18.1	Available nitrogen (kg ha ⁻¹)	216		
Textural class	Clay loam	Available phosphorus (kg ha ⁻¹)	16.2		
		Available potassium (kg ha ⁻¹)	426		

Nitrogen, carbon, sugars are expressed in percentage of dry weight. Dry matter content is in percentage of fresh weight.

Discussion

The occurrence of rich algal flora results generally at the place where there are high levels of nutrients present, together with the occurrence of favorable environmental conditions. The quantity of N fixed by BGA to support the growth and development of the rice plants. BGA increase in grain production was due to the nitrogen fixation of the algae which cause the increment in the plant height and grain quality of rice plant. The application of nitrogen fertilizer could increase the yield of rice, chemical fertilizer resulted the poor performance than combined with BGA. Likewise, in our study revealed that much diversity of algae was present in paddy fields and it was more effective in the growth attributes of rice plants. Blue green algae are reported to contribute to the higher nitrogen fertility of rice fields. They grow on the surface of paddy soil and water enriching with good source of nitrogen. Like our results suggesting that, *Rhizoclonium*, *Spirogyra dubia*, *Oscillatoria tenuis*, *Oedogonium austral*, *Chroococcus*, *Aphanizome*, *Tribonema regulare*, *Zygnema sp.*, *Closterium acutum*, *Navicula*, *Spirogyra* and *Spirogyra lagerheim* were present in the rice fields. In the present situation of world the algae are beneficial in various ways and its potential uses for benefit of soil health and crop improvement by nutrients enrichment as well as for other organisms. So, it is necessary to conserve algal genetic resources of local habitat and to do more systematic work on it which is possible only after understanding the ecology and habitats of various algal forms and to find out the soil nutrient enhancement by algal diversity. The results suggest that application of cyanobacteria with lower level of applied urea-N was effective in enhancing the growth attributes of rice plant. This indicates the better efficiency of cyanobacteria in promoting the growth of rice plants in soil low in

nitrogen fertility. Likewise our results revealed that, algal biomasses were equivalent to values ranging from 1.20 to 3.95. *Oedogonium australe* showed highest percentage of sugar (22.5) and carbon content (23.7), followed by *Navicula*, shows (21.3% of sugar) and carbon about (20.5%) whereas, *Tribonema regulare* revealed very lowest percentage of carbon (10.3) and sugar level is 10.1. So, these algal diversity increased the rice yield percentage approximately 10 to 25 per cent. We conclude, these findings will be of great use to scientific works in future to explore more and more about fresh water chlorophyceae of rice fields and other habitat of the area.

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