J. Agrofor. Environ. 2(1): 1-6, 2008 ISSN 1995-6983

Ecology of cyanobacteria in some selected soils

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Abstract: Ecological studies of cyanobacteria in some selected soils were carried out at Soil Microbiology Laboratory, Department of Soil Science, Bangladesh Agricultural University, Mymensingh. The selected soils were BAU soil, Brahmaputra river bank rice soil, Brahmaputra river bank soil, Madhupur rice soil, Madhupur forest soil and Bhaluka rice soil. Indigenous cyanobacterial population in the selected soils was determined. Cyanobacterial population varied considerably from 139×10^4 to 542×10^4 g^{-1} soil of the studied soils. Maximum cyanobacterial population of 542×10^4 g^{-1} soil was observed in Brahmaputra river bank rice soil and minimum of 139×10^4 g^{-1} soil was recorded in Madhupur forest soil. Highly positive correlation was observed with the cyanobacterial population and soil pH, organic matter, available phosphorus and available sulphur which a weak positive correlation was observed with exchangeable K and total N content of soil.
Key words: Ecology, cyanobacteria.

Introduction

The modern concept of ecology is the interaction of organisms with their environment and with other organisms. Ecology is the study of the relationship between living organisms and the living (biotic) and non-living (abiotic) factors in the environments. Cyanobacteria, an exciting prokaryotic organism, have a wide range of ecology. They are common inhabitants of terrestrial and aquatic ecosystems. They grow abundantly in tropical and subtropical regions and are particularly common in rice fields. The occurrence of these organisms are ubiquitous and has been recorded in soils throughout the world, but no definite geographical locations of families, genera or species has yet been presented. As a group, cyanobacteria are moderately adaptable to environmental change, persisting in unfavorable circumstances, such as, in saline, alkaline, acid and desert soils. Cyanobacteria are more abundant in habitat in which moisture is adequate and light is accessible. Some cyanobacteria grow in hot springs at temperatures as high as 90ºC, although the optimum growth temperatures of these thermal cyanobacteria are between 50 to 54º C. Their presence can be demonstrated readily by the addition of a small amount of soil to a medium containing potassium phosphate, magnesium sulphate, calcium and iron salts, and trace of their inorganic nutrients. Studies in the field and laboratory indicated that cyanobactria prefer neutral to alkaline environments. Cyanobacteria have the capacity to fix nitrogen from atmosphere and improve the fertility of soils. Kaushik (2000) reported that application of cyanobacteria biofertilizers to rice crops also improves the soil structure (aggregation), increases availability of phosphorus and in saline alkali soils reduces soil pH, hence there is an overall improvement in physical-chemical and nutritional properties of soil. Bangladesh has thirty agro-ecological zones (AEZ) and seventeen general soil types, formed in different parent materials under varied topography, hydrologic, thermal and biological conditions. Soils are heterogeneous and their general fertility status as well as physical and chemical properties differs from place to place. Cyanobacteria are the component in different types of soils and they may have effect on soil properties. Thus, the present investigation has been designed with the following objectives to quantity of cyanobacteria in soil and its relationship with soil properties.

Materials and Methods

Soil samples were collected from the six different places of Bangladesh. i.e. BAU Soil, Madhupur rice soil, Madhupur forest soil, Bhaluka rice field soil, Brahmaputra river bank rice soil and Brahmaputra river bank soil upto at 0-15 cm depth. Care was taken to avoid exposing the samples to heat or drying during transportation. After collection, the samples were brought in the laboratory of the Department of Soil Science, Bangladesh Agricultural University. The collected soil samples were mixed thoroughly by hand on a thick paper sheet to make composite sample. The samples were then divided into two portions one for physico-chemical analysis and the other for microbiological study. Soil samples were air dried, ground and passed through a 2 mm sieve. After that the samples were analyzed for soil texture, soil pH, organic matter, total nitrogen, available phosphorus, available sulphur and exchangeable potassium, Particle size analysis of soil samples was done by hydrometer method (Piper, 1950) and the textural classes were determined following Marshall's "Triangular Coordinates" using USDA system. Soil pH was measured with the help of glass electrode pH meter, the soil water ratio being maintained 1:2.5 as described by Jackson (1962). Organic carbon in soil was estimated volumetrically by wet oxidation method with N K_2 Cr_2O_7 and conc. H_2SO_4 (96%) mixture, followed by rapid titration with freshly prepared N $FeSO_4$ solution (Walkley and Black, 1935). Organic matter content was then calculated by multiplying the percent organic carbon with the Van Bemmelen factor 1.73**.** Total nitrogen of soil was estimated by semimicro-Kjeldahl method. The samples were digested with 30% H_2O_2, Conc. H_2SO_4 and catalyst mixture (K_2SO_4: $CuSO_4$: $5H_2O$. Se in the ratio 100: 10: 1). Nitrogen in the digest was trapped by boric acid indicator solution following distillation with 35% Na_2OH and titration was made with 0.01 N H_2SO_4

(Page *et al.*, 1982). For the determination of available phosphorus, extraction was made with 0.5 M $NaHCO_3$ adjusted at pH 8.5 following the method of Olsen *et al.*, (1954). The phosphorus in the extract was then determined by developing blue colour using stannous chloride. The absorbance of the molybdophosphate blue was measured at 660 nm wave length and available P was calculated with the help of a standard curve. Available sulphur was extracted from the soil with $CaCl_2$ solution (0.15%). The extraction method was described by Page *et al.* (1982) and S content in the extract was determined turbidimetrically. The intensity of turbid was measured at 420 nm and values calibrated with the help of a standard curve. Exchangeable K was determined by flame photometer of the neutral ammonium acetate extract (Black 1965) Microbiological study conducted with the soil samples collected during the period of study covers.

Enumeration of cyanaobacteria was done according to the method as described by Prammer and Schmidt (1967). This part of the study includes:

a) Preparation of soil dilution series
b) Inoculation
c) Counting of tubes and enumeration of cyanobacteria
d) Preparation of media and tubing

Exactly 10 g soil from each sample was transferred to a 150ml Erlenmeyer flask containing 90ml of sterile distilled water. The flask was tightly covered with a rubber cork and shaken continuously for 10 minutes in a reciprocating shaker. Within few minutes after removing the flask from the shaker it was shaken vigorously by hand for a few seconds and immediately 10 ml aliquot from the centre of the suspension was transferred to 90ml sterile water blank with the help of a 10ml sterile pipette. This established to 10^{-2} dilution. Similarly 10ml aliquot from this second dilution was transferred to another 90ml water blank to obtain a 10^{-3} dilution. In this way a serial dilution upto 10^{-7} were prepared from each soil sample. For each set of dilution, 10 tubes containing growth media were inoculated with 1.0 ml portion of each soil dilution from 10^{-3} to 10^{-7}. The tubes were incubated in racks under constant light of 100-watt bulbs fixed at 20 cm apart and 50 cm from the test tubes in the laboratory for 30 days. The tubes were then observed occasionally for cyanobacterial growth as surface rings or pellicles on the surface of the culture fluid. After 30 days the tubes showing positive growth in each of the two successive lower dilution followed by negative growth in the next higher serial dilution were recorded. The readings were converted to most probable number (MPN) of cyanobacteria for determination of abundance of organisms in soil as described by Prammer and Schmidt (1976). A modified version of Chu 10D-N medium (Sinclair and Whitton, 1977) was selected to study the growth of cyanobacteria. The stock solution, which was used for media preparation, comprised of:

Reagents	g/ 250 ml
1. $MgSO_4.7H_2O$	6.25
2. $NaHCO_3$	3.96
3. $CaCl_2.2H_2O$	8.96
4. KH_2PO_4	1.95
5. a) $FeCl_3.H_2O$	0.61
b) Na_2-EDTA. $2H_2O$	0.80

$FeCl_3.6H_2O$ solution and Na_2 EDTA $2H_2O$ solution were prepared separately and were mixed.

6. Micronutrients	mg/250 ml
a) H_3BO_3	2.88
b) $MnCl_3.2H_2O$	0.18
c) $Na_2MoO_4.2H_2O$	0.03
d) $ZnSO_4.7H_2O$	0.25
e) $CuSO_4.5H_2O$	0.08
f) $CoSO_4.7H_2O$	0.04
g) $NiSO_4.7H_2O$	0.04

These components were weighed separately and taken in a 250ml measuring bottle. Distilled water was added to it and the materials were dissolved thoroughly and finally the volume was made to 250ml with distilled water. Thus the stock solutions were made ready for media preparation. Distilled water taken in a one liter volumetric flask which was maintained by 7.7 pH value. Every one ml (except micronutrient) stock solution and 0.25ml micronutrient was taken in this flask. The solutions were mixed up thoroughly and finally the volume was made to 1 liter with distilled water. Thus, the media was prepared for tubing. Fifty test tubes were taken and 10ml media was transferred in each test tube, plugged with cotton and were sterilized by autoclaving at 15 1bs of steam pressure for 15 minutes and cooled to room temperature.

Results and Discussion

Indigenous cyanobacterial population in the selected soils under study: Result of cyanobacterial population of selected six different soils are presented in these results showed that marked variation was observed in cyanobacterial population among the soils. The range of cyanobacterial population recorded in all the selected soils were from 139×10^4 to 542×10^4/g soil. The maximum cyanobacterial population of 542×10 /g soil was observed in Brahmaputra river bank rice soil and the minimum of 139×10^4/g soil was found in Madhupur forest soil. Results presented also showed that cyanobacterial population in rice soils of the selected areas is higher than any other selected areas like Madhupur forest and Bhaluka rice soil. The higher population value of cyanobacteria in rice soils may be due to favourable agro-ecology and year round production of rice. Cyanobacteria grow well in moist soils or in soils with standing water. They are phototropic organisms which require sufficient light, ample moisture and moderate temperature for their growth. More over, rice fields provide an ideal condition for the growth of cyanobacteria. Biswas (1994) reported that cyanobacterial population in soils from different agro-ecological zones of Bangladesh ranged from 0.42×10^4 to 28.0×10^4/ g soil. Roger *et.al* (1987) reported that cyanobacterial population in rice soils of Philippines, India, Malaysia and Portugal was

1.0×10^2 to 8.6×10^6/ cm^2 soil. In Thialand cyanobacterial population was 1.0×10^3 /g dry soil (Araragi and Tangcham, 1979) and in 10^3- 10^6 /g soil in mali (Traore *et.al*. 1978). From those data, it is clear although cyanobacteria are ubiquitous in nature but their population density shows a wide range of variation from soil to soil, location to location and country to country.

Soil pH and cyanobacterial population

Cyanobacterial population was positively correlated with pH of the studied soils (Figure 1). The pH range of the selected soils under study was 5.50 to 6.98. The highest soil pH was found in Brahmaputra river bank rice soil where the cyanobacterial population was maximum and the lowest pH was found in Madhupur forest soil where the cyanobacterial population was minimum thus the pH status of the studied soils showed highly positive correlation with the growth of cyanobacterial population. Among other chemical changes in soil under this situation increase in soil pH and well aerated soils led to the drop in soil pH by forming complex with Fe, Al, or Mn. The lower pH also creates environments where the uptake of metal cations by the plants are reduced due to availability of H^+ ions. This situation might be responsible for lower population of cyanobacteria in acid soils.

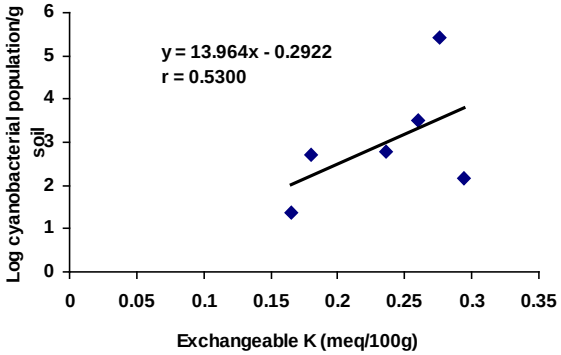

Fig. 1. Regression line showing the relationship between soil pH and cyanobacterial population

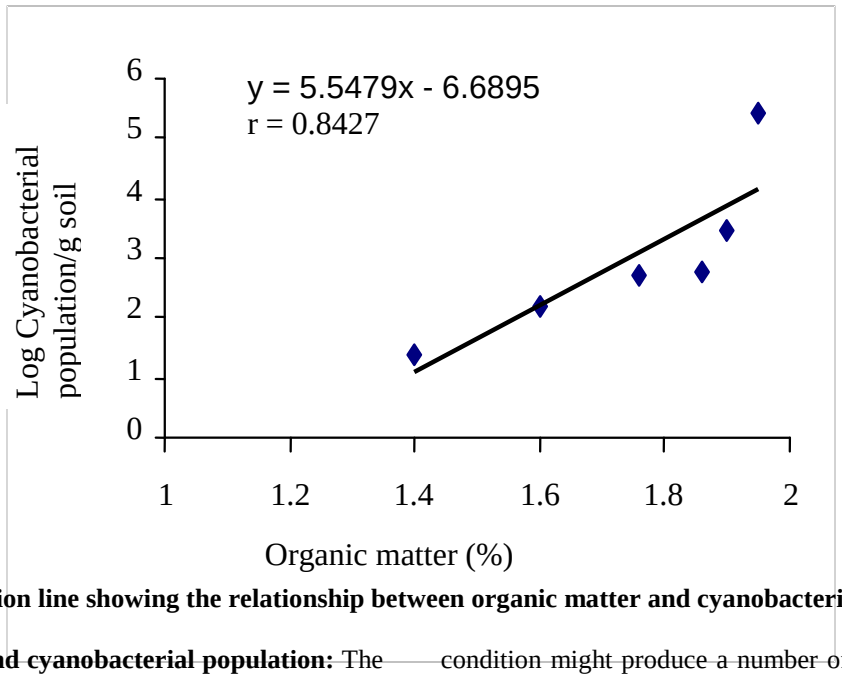

Fig. 2. Regression line showing the relationship between organic matter and cyanobacterial population

Organic matter and cyanobacterial population: The population of cyanobacteria was positively correlated with soil organic matter of the studied soils (Figure 2). The highest organic matter of 1.95% was found in Brahmaputra riverbank rice soil where cyanobacterial population was maximum and the lowest organic matter of 1.40% was observed in Madhupur forest soil where cyanobacterial population was minimum. So, a highly positive correlation was observed between soil organic matter and cyanobacterial population. The predominantly positive effect of soil organic matter on cyanobacterial population was noticed in all the soils. This may be due to the fact that organic matter is a store house of plant nutrients and mainly supplies N, P and S to the plants. It increases the water retention and available water holding capacity of soils (Tamhane *et al.* 1970). In the rice fields partial reduced soil condition might produce a number of organic acids or different sugars in the environment. Cyanobacteria have been reported to use of these low molecular organic acid and sugars for their growth (Khoja and Whitton, 1975). It might be possible that in soils with higher organic matter different types of carbohydrates might produce which could be utilized by the cyanobacteria. In rice fields, the penetration of light to the surface of water or soil surface greatly reduced with the increasing of plant canopy. During that period, growth of cyanobacterial population as well as N_2 – fixing capacity are greatly reduced. Under this situation cyanobacteria which are capable of taking organic substrates might grow well. Organic matter plays a key role in buffering the soil against rapid changes in soil acidification which might arise a result

of using acid forming fertilizers thus favouring the growth of cyanobacterial population.

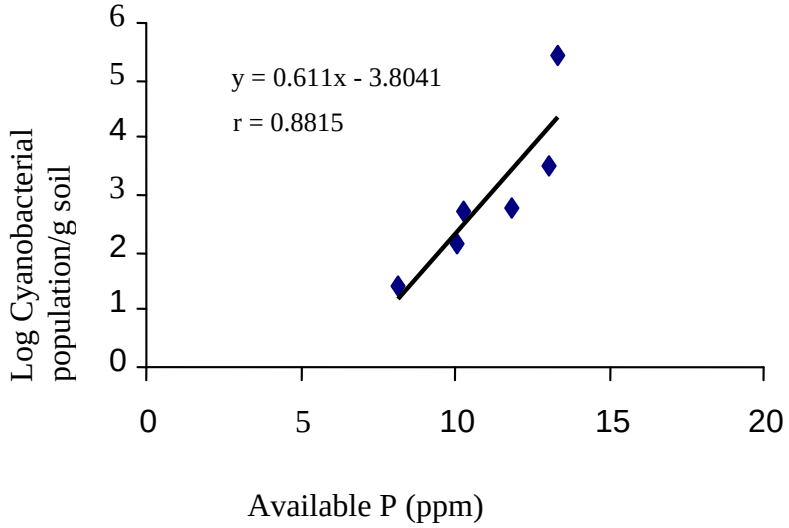

Fig. 3. Regression line showing the relationship between available P and cyanobacterial population

Available phosphorus and cyanobacterial population

Highly positive correlation was also found with cyanobacterial population and available phosphorus of the studied soils (Figure 3). The highest available P of 13.36 ppm was observed in Brahmaputra river bank rice soil and the indigenous cyanobacterial population was maximum in this soil. The lowest available P of 8.15 ppm was found in Madhupur forest soil and the minimum cyanibacterial population was observed in this soil. So, they are positively correlated with each other. Under this situation other chemical changes is increased and it in soilinhance the phosphorus availability in soil. Phosphorus has been reported for a number of physiological roles in plants as it is a component of many enzymes (Tisdale *et.al* 1985). In case of N_2-fixing cyanobacteria P has a special role in N_2-fixation process. To fix one molecule of nitrogen at least 147 K cal energy is needed. This energy has been supplied by ATP, where P in an integral component of the compound.

References

Araragi, M. and Tangcham, B. 1979. Microflora related to the nitrogen cycle in ten tropical paddy soils. Soil Sci. Plant Nutri. 25 (3): 297-309.

Banu, N.A., Hashem, M.A. and Islam, M.R. 1993. Growth and nitrogen fixation of blue-green algae (cyanobacteria) isolated from the saline rice soils of Bangladesh. Bangladesh J. Soil Sci. 24 (1& 2): 63-72.

Biswas, B.K., hashem, M.A. and Islam, M.R. 1994. Influence of soil properties on the population of blue-green algae in different agro-ecological zones of Bangladesh. Bangladesh J. Crop Sci. 4(1& 2): 11-18.

Black, C.A. 1965. Methods of soil Analysis. Part II . American Soc. Agron. Inc. Publisher, Madison, Wisconsin, USA.

Jackson, M.L. 1962. Soil Chemical Analysis. Constable and Co. Ltd. London. First Print.

Kaushik, B.D. 2000. Algal biotechnology in rice cultivation. Int. Conf. on Biotech and Biodiversity (Nov.14-16) Kathmandu, Nepal. pp. 65.

Khoja, T. and Whitton, 1975. Heterotrophic growth of filamentous blue green algae. Br. Phycol. J. 10: 139-148.

Olsen, S.R., Cale, C.V., Watanabe, F.S. and Dean, La.A. 1954. Estimation of available phosphorus in soils by extraction with sodium carbonate. U.S. Dept. Agril. Circ. p. 929.

Page, A.L., Miller, R.H. and Keeney, D.R. 1982. Methods of Soil Analysis. Part-2, 2nd ed. American Soc. Agron. Inc. Pub. Madison, Wisconsin, USA.

Piper, C.S. 1950. Soil and plant analysis. Adelaide Univ. Hassel Press, Australia

Prammer, D. and Schmidt, E.L. 1967. Algae. In: Experimental Soil Micrology. Burgess Publishing Co. Minneapolis 15, Minnesota, pp. 25-27.

Roger, P.A., Grant, I.F., Reddy, P.M. and Watanabe, I. 1987. The photosynthetic aquatic biomass in wetland rice fields and its effect on nitrogen dynamics. pp.43-68. In: Efficiency of nitrogen fertilizers for rice. IRRI, Manila, Philippines.

Sinclair, C. and Whitton, B.A. 1977. Influence of nitrogen sources on morphology of Rivulariaceae (cyanophyta). J. Phycol. 13: 335-340.

Tamhane, R.V. Motiramani, D.P. Bali., Y.P. and Donahue, R.L. 1970. Soils their chemistry and fertility in tropical Asia. Prentice Hall of India pvt. Ltd, New Delhi.

Tisdale, S. L., Nelson, W. L. and Beaton, J.D. 1985. Soil fertility and fertilizer. 4th Ed. The Macmillan Pub. Co. New Yprk.

Traore, T.M., Roger, P.A., Rreynaud, P.A and Season, A. 1978. N_2-fixation by blue green algae in a paddy soil in Mali. ORSTOM Ser. Biol. 13(2): 181-185.

Walkley, A. and Black, A.I. 1935. An examination of the Degtijareff method for determining soil organic matter and a proposal modification of the chromic acid titration method Soil Sci. 37:29-38.