SOIL PH AND ITS ROLE IN CYANOBACTERIAL ABUNDANCE AND DIVERSITY IN RICE FIELD SOILS

S. NAYAK – R. PRASANNA*

Centre for Conservation and Utilization of Blue-Green Algae, Indian Agricultural Research Institute (IARI), New Delhi-110012, India
(phone: +91-011-25848431; fax : +91-011-25741648)

e-mail: *radhapr@gmail.com*

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Abstract. The influence of soil pH was evaluated on the abundance and generic diversity of cyanobacteria in soil samples collected from diverse rice soil ecologies of India. Qualitative and quantitative studies of the 52 soil samples collected from nine agroecologies was carried out using enrichment, MPN (Most Probable Number) techniques and diversity indices were measured. A total of 166 forms, including 130 heterocystous and 36 non-heterocystous isolates were isolated and the highest percentage of abundance of heterocystous forms was observed at pH of 8.1. Highest Shannon's diversity index was recorded at a pH of 6.9, followed by pH of 7.4, while indices of richness and evenness (J and E) were highest in soil samples of pH of 9.3. This study highlighted the successful colonization of cyanobacteria in rice field soils of diverse pH and the need for enrichment of the native flora as a means of exploiting the full potential of cyanobacterial biofertilizers in agriculture
Keywords: *abundance; cyanobacteria; diversity indices; rice ecologies; soil pH*

Introduction

Cyanobacteria represent cosmopolitan prokaryotes, which can be found in almost every conceivable habitat, including soil, on rocks, in fresh water, and in salt water [6,11]. In aquatic environments, cyanobacteria often form thick microbial mats, serving as crucial components in such ecosystems, as they are the primary producers at the base of the microbial food web. Cyanobacteria also increase the oxygen concentration and improve other physico-chemical parameters of the environment, in which they grow and flourish [13]. They are also found in the deserts, where they remain dormant for most of the time, taking advantage of the occasional rains, although this is not the most common [7]. Because they secrete polysaccharides that bind soil, cyanobacteria help to control stability, erosion, runoff, and site availability for germination by higher plants. In nature, cyanobacteria are abundant in places where there is a major nitrogen-deficiency.

Among soil properties, pH is a very important factor in growth, establishment and diversity of cyanobacteria, which have generally been reported to prefer neutral to slightly alkaline pH for optimum growth [23,10]. Acidic soils are therefore one of the stressed environments for these organisms and they are normally absent at pH values below 4 or 5; eukaryotic algae, however, flourish under these conditions. Soil pH is also known to have a selective effect on the indigenous algal flora, especially cyanobacteria and their succession and abundance in soil. Species of *Nostoc, Anabaena, Tolypothrix, Aulosira, Cylindrospermum, Scytonema, Westiellopsis* and several other genera are widespread in Indian rice field soils and are known to contribute significantly to their fertility [29,9'15]. There are very few reports on the existence of cyanobacteria at low pH (acidic range) as they are in general, intolerant to low pH conditions [1,8,5]

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Among the diverse habitats, rice fields constitute one of the favourable ecologies for the growth and proliferation of cyanobacteria [30,26,14]. In the 1970s, algalization or the enrichment of soil via inoculation of selected cyanobacterial strains led to the promotion of these biofertilisers among the farming community in South East Asia [26,28]. However, in recent years, an urgent need has felt to address inherent deficiencies, which have limited their extensive exploitation in diverse rice ecologies and soil types. In a country such as India, rice is grown under diverse ecologies with

Therefore, this investigation was aimed at quantification of the cyanobacterial diversity – in terms of population counts, relative generic abundance, and their correlation with pH of the various soil samples collected and isolation of dominant genera from diverse rice ecologies of India.

Materials and methods

Collection of soil samples and isolation and enumeration of cyanobacteria

Soil samples collected from diverse agroecological regions and soil types *(Fig. 1 and Table 1)* were measured for their EC and pH range and utilized for enrichment studies in BG –11 medium with/ without nitrogen supplementation. Enumeration of populations was carried out by MPN (Most Probable Number) technique and tabulated for each site under the various locations. The enrichment flasks and MPN tubes were regularly monitored for growth and observed microscopically. Standard plating / streaking techniques were used for isolation and purification of cyanobacterial strains [24].

Identification and purification of cyanobacteria

The growth pattern and morphological examination of the cyanobacterial strains was carried out at different stages of growth in nitrogen-free liquid and solid (agar) BG-11 medium. The strains were viewed under a Nikon (Microphot-FX) microscope and the nature of filaments and the shape and size of vegetative cells, heterocysts and akinetes, were analysed and assigned to different genera , using the keys of Desikachary [4].

Measurement of soil EC and pH

The soil samples (soil : water = 1: 2.5) were analysed with respect to their EC (Electrical Conductivity) and pH range following the methodology outlined by Black [2].

Measurement of acetylene reducing activity

Gas chromatographic quantification of ethylene formed (acetylene reduction activity, ARA) was utilized as an index of nitrogen fixation. The vials with log phase cultures (14d) were injected with acetylene (10% gas phase) after removing an equal amount of air, using airtight syringes and incubated for 90 minutes under optimal conditions of temperature and illumination [18]. The samples (1 ml) were injected into a Gas Chromatograph (Chemito, model GC 1000), fitted with an oven containing a 2m long column of stainless steel (2mm internal diameter) packed with Poropak N (80-100 mesh). Nitrogen gas flowing at the rate of 35ml min^{-1} was used as the carrier, while hydrogen and air were used to produce the flame in the Flame Ionisation Detector. The oven, injector and detector were maintained at 100-120°C to allow for ionization and detection of ethylene produced.

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Commercially available standard ethylene was utilized for quantification and vials with an equivalent volume of water served as controls [18]. The ARA values were expressed per mg chlorophyll. Spectrophotometric estimation of the chlorophyll content of cells was carried out following the method of Mackinney [12]. All values presented are means of triplicate measurements.

Statistical analyses

The diversity indices (Shannon's diversity index and Simpson's index of diversity) were calculated using the standard formulae.

Correlation coefficients were calculated using Microsoft Excel package and analysed for their significance using Pearson's tables

Results and discussion

The trophic independence from carbon and nitrogen, together with a great adaptability to environmental variations, enables cyanobacteria to be ubiquitous. Their structural-functional flexibility provides them with not only great versatility, but also makes them among the most successful in extreme environments including high temperatures, high levels of UV light, and high salinity and inhabit a wide range of environments and niches. Their role in the soil ecosystem is manifold, the most important consequences being the fixation of nitrogen and carbon, besides promoting release of nutrients and reducing the rate of loss of water and soil through erosion. In paddy fields, their relative occurrence varies within large limits, ranging from 0 to 76-85%. However, contrary to the general belief, nitrogen fixing forms are not invariably present in tropical rice soils. All India survey showed that out of 2,213 soil samples from rice fields, only about 33% harboured nitrogen-fixing forms [27], and limited systematic analyses on their limited distribution has been undertaken in relation to major environmental factors [19,21,22].

Among the soil properties, pH is certainly the most important factor determining the flora and fauna composition. In culture media, the optimal pH for the growth of cyanobacteria ranges from 7.5 – 10, with a lower limit of 6.5 – 7.0. However, in soil-culture experiments, soils having slightly alkaline reaction were more favourable, while in natural environments cyanobacteria prefer neutral to alkaline pH [3,20]. The development of soil acidity is generally believed to be associated with the base unsaturation caused by leaching out of bases and genesis from base-poor acidic rocks. The dissolved or free acidic substances, such as sulphuric acid and ferric and aluminium sulphate, accentuate acidity in acid sulphate soils [5].

In the present investigation, soil samples were collected from nine locations *(Fig.1),* differing in their EC and pH values and evaluated for cyanobacterial abundance and generic diversity. A total of 166 forms, including 130 heterocystous and 36 heterocystous isolates were recorded. A predominance of heterocystous forms (68 – 95%) was observed at all locations, while non-heterocystous forms exhibited 5-32% abundance in the various locations *(Table 1).* Highest % abundance of heterocystous forms was observed at pH of 8.1, followed by 7.9 (83 and 80% respectively). In terms of non-heterocystous forms, soil samples with pH of 7.4 and 9.3 recorded highest % abundance.

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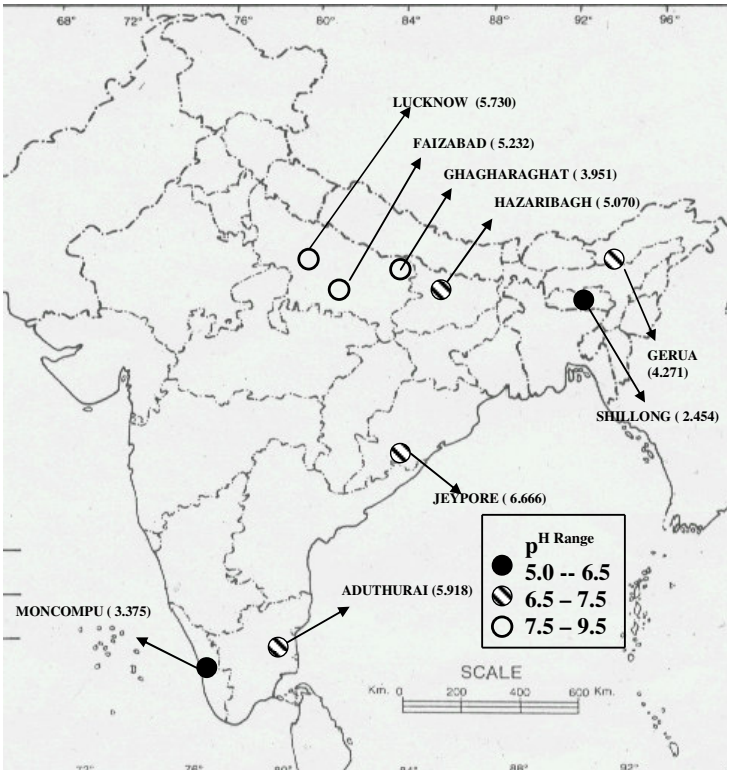

Figure 1. *Map of India depicting the various locations sampled, along with their pH range and Simpson's Reciprocal diversity indices (given in parentheses)*

Table 1. *Occurrence and distribution of cyanobacteria in various locations in India ,along with selected diversity indices*

Locations	EC (dS/m)	Total no of Genera	Non Heterocystous forms	Heterocystous forms	Shannon H	J	E
Aduthurai (Tamil Nadu)	3.4	19	6 (32%)	13 (68%)	1.909	0.918	0.843
Jeypore (Orissa)	2.7	20	5 (25%)	15 (75%)	2.038	0.927	0.853
Hazaribagh (Bihar)	2.9	17	4 (23%)	13 (77%)	1.785	0.903	0.828
Lucknow (Uttar Pradesh)	4.4	19	5 (26%)	14 (74%)	1.836	0.943	0.896
Faizabad (Uttar Pradesh)	3.5	15	3 (20%)	12 (80%)	1.859	0.894	0.802
Ghagraghat (Uttar Pradesh)	2.9	18	3 (17%)	15 (83%)	1.541	0.860	0.778
Gerua (Assam)	2.9	31	7 (23%)	24 (77%)	1.691	0.813	0.678
Shillong (Meghalaya)	2.5	09	1 (5%)	8 (95%)	0.964	0.878	0.874
Moncompu (Kerala)	4.2	18	2 (11%)	1 (89%)	1.442	0.804	0.704

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Simpson's Reciprocal indices *(Fig.1)* were highest in Jeypore soil samples, followed by those from Aduthurai. Shannon's diversity index was highest at a pH of 6.9, followed by pH of 7.4, indicative of the higher number of genera recorded in these soil samples. Indices of richness and evenness (J and E) were highest in soil samples of pH of 9.3 *(Table 2).* Shannon's indices are strongly biased towards richness, as it is calculated from proportional abundances of the species. On the other hand, Simpson's index is a measure of diversity, which takes into account both richness and evenness, although it gives more weight to the more abundant species in a sample.

Table 2. *Major genera and their relative abundance in the samples*

Genus	Total number of strains	Relative abundance
Anabaena	46	100 %
Nostoc	51	100 %
Calothrix	14	89 %
Scytonema	3	22 %
Westiellopsis	3	22 %
Hapalosiphon	8	89 %
Aulosira	1	11 %
Cylindrospermum	5	56 %
Oscillatoria	9	78 %
Phormidium	20	89 %
Lyngbya	1	11 %
Aphanocapsa	5	22 %

The extensive diversity in pH and EC led to a significant effect on the abundance of cyanobacterial species, depicted as log values of MPN *(Fig. 2 and 3).*

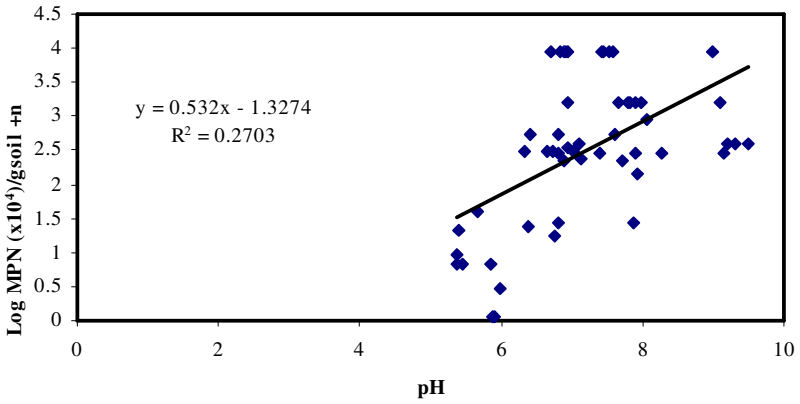

Figure 2. *Abundance of cyanobacterial populations (log MPN/g soil) as a function of pH (r = 0.52 ; P < 0.01)*

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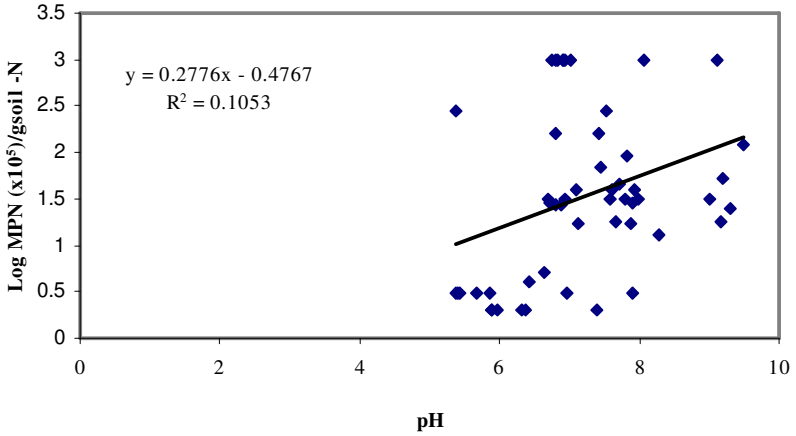

Figure 3. *Abundance of cyanobacterial populations (log MPN/g soil) as a function of EC (r =0.32; P < 0.01)*

The pH values and to a lesser extent EC of the various locations showed a significant positive correlation with MPN (expressed as log values), especially in relation to the enrichment studies in nitrogen supplemented media. The correlation between EC and pH was observed to be positive, but Pearson's coefficient was not significant *(Fig.4).*

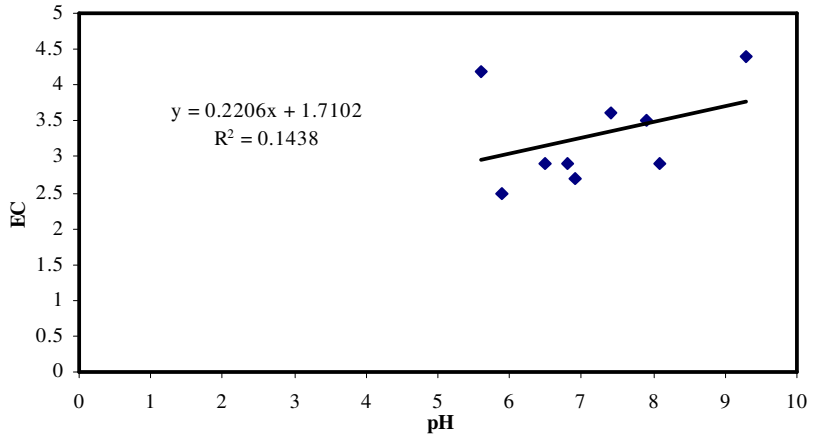

Figure 4. *Correlation between EC and pH*
(average over all locations; r=0.38, not significant)

Cyanobacteria belonging to 12 genera were isolated which included 8 heterocystous forms: Anabaena, Nostoc, Westiellopsis, Calothrix, Scytonema, Aulosira, Hapalosiphon, Cylindrospermum and 4 non-heterocystous forms: Phormidium, Oscillatoria, Lyngbya and Aphanocapsa. The genera-wise distribution of the five dominant cyanobacterial forms at various pH levels is illustrated in *Figure 5.*

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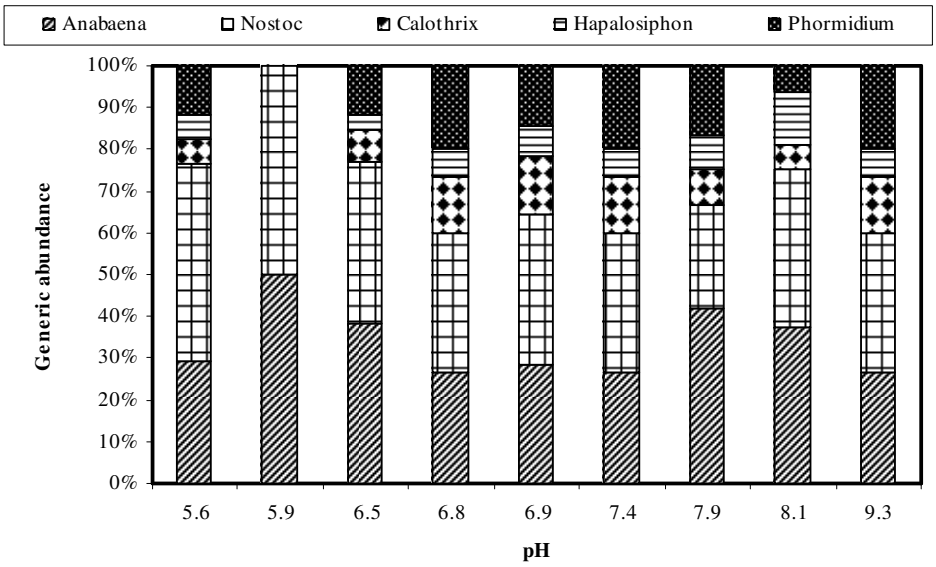

Figure 5. *Distribution of the selected dominant genera as a function of soil pH.*

In general, *Nostoc* and *Anabaena* recorded maximum number of isolates i.e. 10 at pH of 6.5. The number of isolates belonging to the genera *Anabaena* and *Nostoc* also showed the highest relative abundance of 100%, while lowest values were recorded for *Aulosira* and *Lyngbya,* as they were isolated only from one of the 9 locations. The relative abundance of cyanobacteria in rice soils and biofertilizer inocula from four countries revealed that significant correlation could be made with respect to pH and available P content of soils [21]. Algalization, when effective, is generally associated with increase in yield but the success of algalization is dependent on a number of factors that include flooding due to rains, simultaneous use of inorganic fertilizers, animal manures, pesticides and amount of light available to the cyanobacteria as the plant grows.

Earlier studies on the distribution pattern of cyanobacteria in soils of Andhra Pradesh, Haryana, Delhi, Rajasthan, Uttar Pradesh and Punjab revealed that although recurrent combination of forms were discernable, there appeared to be a localized distribution of cyanobacteria depending upon the soil pH, electrical conductivity and exchangeable sodium. Species of *Nostoc, Calothrix, Scytonema, Hapalosiphon* and *Wetiellopsis* were recorded in salt-affected soils of Maharashtra and Andhra Pradesh (where pH of the soil varied from 6.0 to 9.0), although the species of *Nostoc* and *Calothrix* were predominant. On analyzing the total cyanobacterial flora, it was observed that out of the total 37 species, 50 percent were nitrogen- fixing strains, including the non-heterocystous nitrogen fixers. Unicellular and colonial forms, with extensive mucilage like *Aphanocapsa, Aphanothece, Chroococcus, Gloeocapsa* and *Gloeothece* and filamentous species of *Scytonema, Lyngbya* and *Tolypothri* were also very common in Maharashtara soils. A significant reduction in soil salinity (12-35%) due to repeated cultivation of *Anabaena torulosa* in soils rendered saline owing to bad farm management has also been reported Cyanobacteria have been found not only to grow in highly saline-alkali soils, but also improve the physico-chemical properties of the soil by enriching them with carbon, nitrogen and available phosphorus [9,10]. Successive cultivation of BGA makes the environment more favourable and after a few

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years it may help to produce a reasonably good yield of crops, as observed by Singh [23] for sugarcane after 3 years of reclamation with BGA. Although infrequent at pH below 6.0, their ability to grow in diverse pH ranges and modify their environment makes them successful in any niche. Acidic soils, in general do not support their growth, although a few reports on their presence in soils with pH values between 5 and 6 are available [1,16,17].

Correlation between the number of isolates from each of the dominant genera and pH of the location sampled also showed a positive correlation for *Calothrix, Phormidium* and *Hapalosiphon (Fig. 6).* The numbers of isolates belonging to the genera *Nostoc* and *Anabaena*, however, exhibited a negative correlation, indicating that although these two genera show highest relative abundance quantitatively, their relative tolerance to pH is low. *Nostoc* and *Anabaena*, therefore, exhibited superior establishing and adaptive traits, although in terms of numbers, they showed an uneven distribution at different pH. However, the correlation was not statistically significant in any of these cases. Among the non-heterocystous cyanobacteria, *Phormidium* was the most cosmopolitan –20 isolates as against 9 and 5 belonging to *Oscillatoria/Lyngbya.* A similar trend was observed on correlating EC values and number of isolates belonging to the dominant genera (data not shown). Species of *Calothrix* and *Aulosira* have been reported to be ubiquitous in rice fields of Kerala where pH ranges from 3.5 – 6.5 [1]. Also, the number of spore producers is known to show a positive correlation with soil pH. Enrichment of such soils with the indigenous cyanobacterial isolates may help in ameliorating the land and making them suitable for obtaining higher yields as benefits, other than nitrogen fixation, include solubilisation of phosphorus, improved soil structure and synthesis of growth promoting substances are also known.

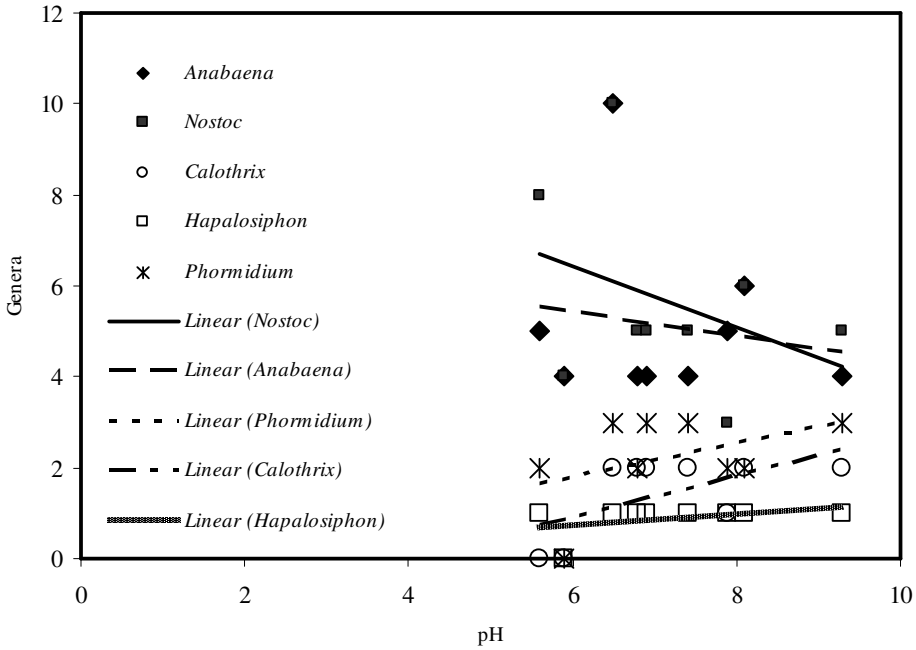

Figure 6. *Correlation of number of isolates of dominant genera with soil pH.*

The diversity within a genus was also further analysed by measuring the nitrogen fixing potential (using acetylene reducing activity as an index) of 46 isolates of

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Anabaena (Fig.7). Highest values were recorded in the isolates from Assam (A43 and 44), followed by the isolate from saline-alkali soil samples from Lucknow (A15).

The effect of pH on algal flora is generally difficult to evaluate as it is often correlated with other factors, for *e.g.* arid soils are almost universally alkaline and many continuously wet soils acidic. Among correlations between the relative abundance of the individual groups of heterocystous cyanobacteria and soil physico-chemical properties, only the correlation between pH and the relative abundance of *Nostoc* was found to be statistically significant, but a degree of bias was introduced when dry and wet samples were tested separately [21]. Contradictory reports regarding the occurrence in acid and very acid environments are available. However, one of the most acid lakes (pH 2.9) was observed to be inhabited by *Oscillatoria/Limnothrix* and *Spirulina* [25]. Despite the preference for neutral-highly alkaline environments, acidic soils do exhibit low diversity and abundance of cyanobacteria [11].

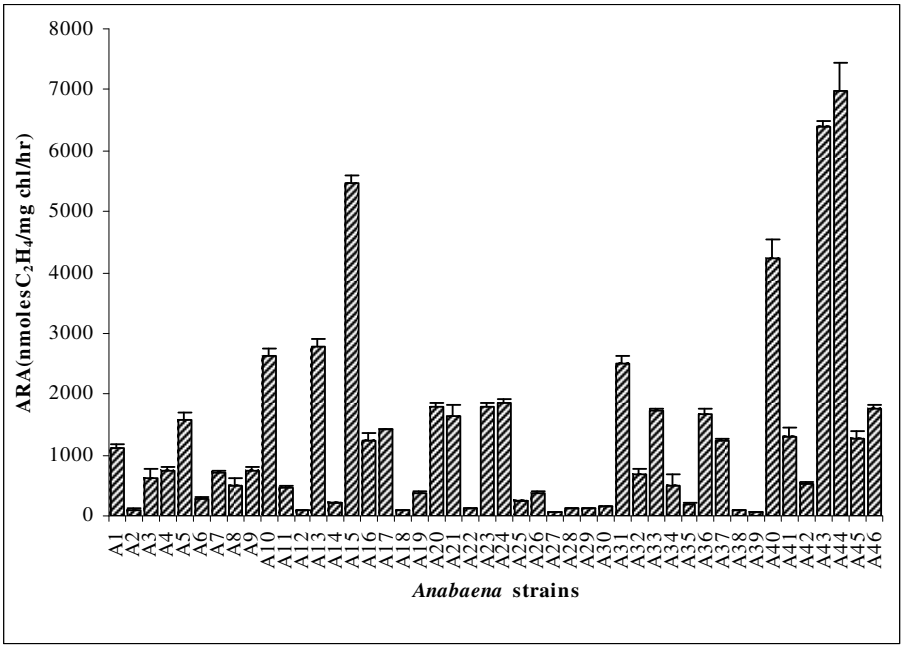

Figure 7. *Diversity in nitrogen fixing potential (measured as acetylene reducing activity, ηmoles C_2H_4 mg $chl^{-1}h^{-1}$) among the isolates of genus Anabaena*

Therefore, efforts need to be focused towards enrichment of indigenous cyanobacterial populations, which are better adapted to the specific niche, through development of multiple inocula preparations on a regional basis. Research programs should be oriented towards agricultural practices, including application of biofertilizers, which enhance the growth and proliferation of indigenous strains.

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