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Nitrogen Fixation Potential of Soil Blue-green Algae: A Quantitative Assessment

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ABSTRACT
The present study emphasizedthe nitrogen fixation potential of soil blue-green algae in quantitative terms. A total of 24 blue-green algal species belonging to eight different genera were analysed for their total nitrogen content by conventional Micro- Kjeldahl method. Tremendous variation was observed in the total nitrogen content not only between different genera but also within different species of the same genera. The lowest as well as highest amount of total nitrogen content was obtained in the members of family Nostocaceae. The obtained values of total nitrogen content among the studied blue-green algae were ranging from a minimum of 1.52% in Nostoccalcicola to a maximum of 5.28% in Anabaena circinalis. Simultaneously, from the obtained range of total nitrogen content, it was revealed that about 45.83 percent studied blue-green algal species were fit well in the higher range of total nitrogen content, while 37.5 percent species possessed medium range and 16.67 percent species were found in the lower range of total nitrogen content over the studied genera. The highest mean amount of total nitrogen content was recorded in the genus Calothrix (4.69%). The consequences of the results showed that the mean amount of total nitrogen content varied within different genera which can be arranged in such that Calothrix (4.69%) >Anabaena (4.08%) >Hapalosiphon(3.34%) >Nostoc(3.21%). However, the mean amount of total nitrogen content overall in twenty- four unialgal strains was recorded to be 3.64%. Calothrix, Anabaena, Hapalosiphon and Nostoc were found to be the dominant nitrogen fixing blue-green algae encountered in the soils of study area. These forms capable of growing on moist and shaded soil surface hold promise for crops like sugarcane, tomato, mungbeans and maize to improve nitrogenous soil fertility. The obtained results were discussed in detail with the previous reports.
Keywords: *Anabaena, Blue-green algae, Calothrix, Micro-* Kjeldahl method*, Total Nitrogen.*

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INTRODUCTION

The present day global interest in biological nitrogen fixation is a direct consequence of biofertilizer particularly blue-green algae. It has been proved as the most efficient source of organic nitrogen. The direct application of blue-green algae in fields is a practice that extends over centuries and is more successful than chemical fertilizers. The beneficial effect of blue-green algae is probably due to the fixing of atmospheric nitrogen and addition of some enzymes and growth promoting hormones to the soil [1].

Blue-green algae are unique in reducing the atmospheric nitrogen by the process "Biological nitrogen fixation" [2]. The cyanobacteria contain nitrogenase and fix atmospheric nitrogen for which these are used as biofertilizer to maintain and improve soil status [3]. They have tremendous potential in environmental management as soil conditioner, biomonitors of soil fertility, water quality, feed for animals and protein supplements [4].

Most of the heterocystous and non-heterocystous blue-green algae are known to fix nitrogen. The observation in this regard showed that *Nostoc, Calothrix, Hapalosiphon, Aulosira,* and *Anabaena* were dominant nitrogen fixing cyanobacteria encountered in various agro-practices areas of Maharashtra state. It has been reported that the part of nitrogen requirements for the crops (25- 35 %) could be met by algalisation under different agroclimatic conditions [5]. Reduction of chemical fertilizers input upto 30% by supplementing with blue-green algae as a significant finding when conservation of energy is contemplated. In India, the agro- ecological conditions are favourable for the growth of blue-green algae. Particularly, in Maharashtra state, there is great scope for its adoption to marginal farmers. However, the agronomic potential of blue-green algae is currently little exploited.

MATERIAL AND METHODS

To isolate and assess the nitrogen fixing potential of blue-green algae, 42 sampling sites were established in the study area during study period. Soil Samples were collected as per the procedure given by Singh [6]. A total 86 soil samples were collected from the study areas.

For isolation of blue-green algal species in the collected soil samples, the BG- 11 [7] and Fogg's [8, 9, 10] culture media were used. 5 gm of soil samples were inoculated in sterilized flasks having 100 ml of BG-11 and Fogg's media separately. Inoculation was done in laminar airflow unit. These inoculated flasks were incubated at 28 ± 2^0C under 2.5 K lux fluorescent light for 2- 3 weeks under 16/ 8 hours light / dark cycles. After 20- 25 days of incubation period, the visible growth of algae appeared in the enrichment cultures.

The taxonomic identification of blue-green algal species was done by following the monographs and keys given by Prescott [11, 12, 13]. On completion of experiments, the unialgal isolates from each replication were used for mass cultivation in laboratory using nitrogen free BG-11 medium. Total nitrogen content was estimated by conventional Micro- kjeldahl distillation method [14]. A blank was run without blue-green algal extract and readings were noted.

RESULTS

A total of 24 blue-green algal species belonging to eight different genera were analysed for their total nitrogen content in the laboratory cultures. The values of total nitrogen estimation are expressed as percentage (%) of fresh weight basis (Table- 1). The raised unialgal culture in nitrogen free media gave the evidence of nitrogen fixing potential.

Wonderful distinctions were observed in the total nitrogen not only between different genera but also within different species of the genus. The lowest as well as highest amount of total nitrogen content was pragamtic in the members of the family Nostocaceae. The highest total nitrogen content was observed in *Anabaena circinalis*(5.28%) followed by *Calothrixjavanica*(5.15%), *Nostoc punctiforme* (4.94%), *Nostoc muscorum* (4.89%), *Nostoccarneum* (4.84%), *Anabaena orientalis*(4.80%), *Westiellopsis prolifica*(4.70%) and *Cylindrospermum musicola* (4.64%). The least amount of total nitrogen content was recorded in *Nostoccalcicola* (1.52%).

Table-1: Total nitrogen content of blue-green algal speciesisolated from soil of the study area.

Sr. No.	Blue-green algal species	Total nitrogen content(%)	Range within genus	Range Overgenera
1.	*Cylindrospermum musicola*	4.64	--	H
2.	*Nostocpunctiforme*	4.94	H	H
3.	*N. entophytum*	2.67	M	M
4.	**N. paludosum**	4.09	H	H
5.	*N. linckia*	2.68	M	M
6.	*N. sporangiaeforme*	2.75	M	M
7.	*N. ellipsosporum*	2.29	M	L
8.	*N. carneum*	4.84	H	H
9.	*N. calcicola*	1.52	L	L
10.	*N. muscorum*	4.89	H	H
11.	*N. microscopicum*	2.31	M	L
12.	*N. verrucosum*	2.41	M	L
13.	*Anabaena spiroides*	3.78	H	H
14.	*A. oryzae*	2.89	H	M
15.	*A. fertilissima*	3.65	H	M
16.	*A. orientalis*	4.80	H	H
17.	**A. circinalis**	5.28	H	H
18.	*Aulosiraaenigmatica*	3.65	--	M
19.	*Scytonemasubtile*	2.52	--	M
20.	**Calothrixjavanica**	5.15	H	H
21.	*C. marchica*	4.24	H	H
22.	*Hapalosiphonwelwitschii*	3.21	H	M
23.	**H. hibernicus**	3.48	H	H
24.	*Westiellopsisprolifica*	4.70	--	H

The values represented are mean of three observations and expressed as percentage (%) of fresh weight basis.

Where, L= Lower; M= Medium; H= Higher

The highest total nitrogen content obtained in the species of eight different genera was *Nostoc* (*N. punctiforme*, 4.94%), *Anabaena* (*A. circinalis,* 5.28%), *Calothrix*(*C. javanica*, 5.15%) and *Hapalosiphon* (*H. hibernicus*, 3.48%). In addition to this, in the single species of the following genera, the total nitrogen content found was *Cylindrospermum* (*C. musicola*, 4.64%), *Aulosira*(*A. aenigmatica*, 3.65%), *Scytonema* (*S. subtile*, 2.52%) and *Westiellopsis* (*W. prolifica*, 4.70%). Likewise, the minimum amount of total nitrogen content recorded in the studied species of different genera was *Anabaena* (*A. oryzae*, 2.89%), *Nostoc* (*N. calcicola*, 1.52%) *Calothrix* (*C. marchica*, 4.24%) and *Hapalosiphon* (*H. welwitschii*, 3.21%).

In the present investigation wide variation was observed in the total nitrogen content of the analysed species of blue-green algae. This was also observed at intrageneric level of the blue-green algae. The calculated range of total nitrogen content within each studied genus was discussed here with great details (Table- 1 a). The four species of the genus *Nostoc* viz. *N. punctiforme, N. paludosum, N. carneum* and *N. muscorum* exhibited higher range of total nitrogen content within the genus. Whereas, six studied taxa in the genus *Nostoc* showed medium range and only *Nostoc calcicola* exhibited lower range. On the other hand, higher range of total nitrogen contents was obtained in all the studied species of the genus *Anabaena, Calothrix* and *Hapalosiphon* (Table- 1). The mean amount of total nitrogen content obtained in the studied genera was *Nostoc* (3.21%), *Anabaena* (4.08%), *Calothrix* (4.69%) and *Hapalosiphon* (3.34%).

Table- 1a: Range of total nitrogen content (%) within the studied genus.

Nostoc	**L= 1.14**	**M= 2.28**	**H= 3.42**
Anabaena	**L= 0.79**	**M= 1.58**	**H= 2.37**
Calothrix	**L= 0.30**	**M= 0.60**	**H= 0.90**
Hapalosiphon	**L= 0.09**	**M= 0.18**	**H= 0.27**

Table- 1b: Range of total nitrogen content (%) over the studied genera.

L	=	1.25
M	=	2.50
H	=	3.75

Note: The lower, medium and higher range was formed by subtracting the lowest amount from the highest and then divided by number of ranges. The value thus obtained is of range 'L', which is multiplied by 2 and 3 to get range 'M' and 'H' respectively.

Similarly, the calculated range of total nitrogen content over the studied genera was discussed here in detail (Table- 1 b). In the genus *Nostoc,* four species viz. *N. punctiforme, N. paludosum, N. carneum* and *N. muscorum* revealed higher range of total nitrogen content over the studied eight different genera embracing twenty- four species. While, the three species in the genus *Nostoc* such as *N. entophytum, N. linckia* and *N. sporangiaeforme* showed medium range and the rest four viz. *N. ellipsporum, N. calcicola, N. microscopicum* and *N. verrucosum* exhibited lower range. Out of the five tested species of the genus *Anabaena*, three showed signs of higher range of total nitrogen which are *A. spiroides, A. orientalis* and *A. circinalis* whereas, the remaining two species viz. *A. oryzae*and *A. fertilissima* exhibited medium range. Higher range of total nitrogen content over the eight genera were obtained in both the studied species of *Calothrix* while, medium range was obtained in both the tested species of genus *Hapalosiphon.* Single species of the following genera *Cylindrospermum*(*C. musicola*) and *Westiellopsis* (*W. prolifica*) showed higher range of total nitrogen content over the eight studied genera whereas medium range found in *Aulosira* (*A. aenigmatica*) and *Scytonema* (*S. subtile*) (Table- 1).

From the above results it was revealed that about 45.83 percent studied blue-green algal species were fit well in the higher range of total nitrogen content, while 37.5 percent species possessed medium range and 16.67 percent species were found in the lower range of total nitrogen content over the eight different studied genera.

The consequences of the present investigation showed that not much variation was observed in the total nitrogen content amongst the analysed species of *Calothrix* (*C. marchica,* 4.24% and *C. javanica,* 5.15%) and *Hapalosiphon* (*H. welwitschii,* 3.21% and *H. hibernicus,* 3.48%). While, the species of *Nostoc* and *Anabaena* exhibited wide variation with an array of 1.52% to 4.94% and 2.89% to 5.28% total nitrogen content, respectively. The highest mean amount of total nitrogen content was recorded in the genus *Calothrix* (4.69%). The results showed that the mean amount of total nitrogen content varied within different genera which can be arranged in such that *Calothrix* (4.69%) >*Anabaena* (4.08%) >*Hapalosiphon*(3.34%) >*Nostoc*(3.21%).

However, the mean amount of total nitrogen content overall in 24 unialgal strains was recorded to be 3.64%. (Table- 2)

Table- 2: Maximum and minimum amount of total nitrogen content of blue-green algal genera, number of species and mean amount of total nitrogen over the genera and within the genus.

Sr. No.	Genus	No. of species of each genus	Total nitrogen content (%) Max.	Min.	Mean
1.	**Cylindrospermum**	01	4.64	--	--
2.	*Nostoc*	11	4.94	1.52	3.21
3.	*Anabaena*	05	5.28	2.89	4.08
4.	*Aulosira*	01	3.65	--	--
5.	*Scytonema*	01	2.52	--	--
6.	*Calothrix*	02	5.15	4.24	4.69
7.	*Hapalosiphon*	02	3.48	3.21	3.34
8.	*Westiellopsis*	01	4.70	--	--
9.	**Overall**	**24**	**5.28**	**1.52**	**3.64**

The results recorded during the present investigation revealed that *Calothrix, Anabaena, Hapalosiphon* and *Nostoc* were found to be the dominant nitrogen fixers encountered from the soils of the study area along with *Cylindrospermum musicol a*(4.64%), *Aulosiraaenigmatica* (3.65%), *Scytonemasubtile* (2.52%) and *Westiellopsis prolifica*(4.70%).

DISCUSSION

Better performance of *Calothrix*spp. over the species of *Nostoc, Anabaena* and *Hapalosiphon* with respect to nitrogen fixation isolated from paddy field soils was reported by [15, 16]. Similarly, higher amount of nitrogen content was also recorded in *Calothrix, Anabaena, Nostoc, Aulosira* and *Tolypothrix* in rice fields by earlier workers [17, 18, 19].

The estimated value of total nitrogen in *Nostocmuscorum*(4.89%) appears close to the value of 5.4% and 5.09% for the same alga as reported by [20] and [21] respectively.The obtained total nitrogen content in *Anabaena fertilissima* (3.65%) and *Anabaena spiroides* (3.78%) is very close to the value for *Anabaena* variabilis (3.60%). Similarly, the estimated value of total nitrogen in the *Nostocsporangiaeforme*(2.75%) is in agreement with that of the reported value 3.38% for the same taxa by [22].

The anticipated total nitrogen content in *Anabaena circinalis* (5.28%) fit right in the range (5- 7%) reported by [23] for *Anabaena cylindrica* whereas in *Anabaena circinalis* (5.28%), total nitrogen content recorded is very close to the reported value for the *Anabaena cylindrica* (5.63%) by [24]. In the present study, the amount of total nitrogen fixed by *Nostoclinckia* was 2.68% that is lower than the value reported by [25] as (6.0%); [26] as (4.69%).

However, in *Scytonemasubtile* (2.52%) the value of total nitrogen content estimated is found to be very close to the value for *Scytonemamirabile* (2.69%). The estimated value for *Nostoc ellipsosporum* (2.29%) and *Anabaena oryzae* (2.89%) is found to be less than the reported value 4.7% and 4.30% respectively. Concomitantly in *Hapalosiphon welwitschii*, the reported value (3.10%) of nitrogen content is in agreement with the obtained value (3.21%). On the other hand, the obtained total nitrogen content in *Calothrixmarchica* (4.24%) is appears more than the stated value (2.68%) for the same alga as reported by [27]. While in *Westiellopsis prolifica*(4.70%) the obtained value of total nitrogen content is much closer to the report of [28] and less than the reported value of [29] for the same alga. These reports of earlier workers are appeared in close conformity with the findings of the present investigation.

CONCLUSION

The studied blue-green algae exhibited a wide range of variation in the total nitrogen content not only between different genera but also within different species of same genus. In the laboratory cultures, blue-green algal species, *Calothrix*and *Anabaena* showed better performance in terms of total nitrogen fixed over the species of *Nostoc, Scytonema, Westiellopsis*and *Hapalosiphon*. These forms capable of growing on moist and shaded soil surface hold promise for crops like sugarcane, tomato, mungbeans and maize to improve nitrogenous soil fertility.

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