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Studies on nitrogen fixing ability of blue green algae (BGA) from trimbakeshwar tehsil of Nashik district

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Abstract
The present study deals with the estimation of potential of cyanobacteria for nitrogen fixation in paddy field of Trimbakeshwar area. Water and soil sample were collected from different site and were used for identification and isolation of cyanobacterial species. Ocular micrometry method was used for identification of species classified under cyanobacteria. Thus *Calothrix*, *Nostoc*, *Anabeana*, And *Oscillotoria Sp.* Was identified and used for study purpose. These species were isolated in BG-11 media for growth. Further the nitrogen content was estimated by using the famous Kjeldahl method. According to the result obtained *Anabeana* have the highest capability of Nitrogen fixation (71.05 mg/l) followed by *Oscillotoria* (50.51 mg/lit), then Nostoc (68.80 mg/lit) and the minimum Nitrogen fixation was observed for the *Calothix* species (47.00 mg/lit). For agricultural growth, the *Anabeana* can be used as a biofertilizer. Moreover, Nostoc Sp., *Oscillotoria* Sp., and Calothrix Sp. have demonstrated the ability to fix nitrogen and can take the place of *Anabeana* sp. As a result, the study might draw the conclusion that harmful chemical fertiliser can be replaced with nitrogen-fixing cyanobacterial species.

Keywords: cyanobacterial, nitrogen fixation, biofertilizer

Introduction
Our atmosphere contains around 79% nitrogen, but because agricultural plants cannot use nitrogen in its elemental form, crops suffer in the midst of abundance. Across the tropical, subtropical, and temperate regions, BGA is extensively spread. An algal community of BGA made up primarily of *Aulosira fertilissima* is growing in abundance in the rice fields of U.P. and Bihar. According to a survey of the CRRI, species of *Aulosira, Cylindrospermum, Nostoc, Anabaena, Aphanothece*, and *Gloeotrichia* predominate (Singh, 1978) [8].

The abundance of cyanobacteria in rice fields is crucial for maintaining the fertility of the rice fields by fixing N2. Around 50% of the cyanobacterial taxa present in rice paddy fields are heterocystous filamentous cyanobacteria, and the soil of many rice fields has a high concentration of cyanobacteria. (Kim, Dong & Lee, 2006) [5]. The well-known fact that the N-fixing BGA greatly improve the N status of the soil's aquatic habitats has drawn a lot of attention to them. Almost 40% of the entire area under cereals in India—40 million hectares—is used for the cultivation of rice. The fixation of atmospheric nitrogen by BGA is what allows rice to continue to grow year after year even in the absence of manure.

Cyanobacteria, sometimes referred to as blue-green algae, are a kind of photosynthetic bacteria made up of either single cells or colonies. Only one form of chlorophyll, Chlorophyll a, a green pigment, is found in cyanobacteria. They also include pigments like phycobilin and carotenoids. In both freshwater and marine environments, these bacteria spontaneously multiply. In hot springs as well as in dams, rivers, lakes, and reservoirs, they flourish.

These bacteria are typically green but can occasionally become blue when scum is dying. These bacteria almost all float on the water's surface and create floating mats because they are buoyant. There are lots of blue-green algae everywhere. Certain blue-green algae fix nitrogen from the atmosphere when they repair carbon from carbon dioxide. These include symbiotic and free-living types and are referred to as nitrogen-fixing blue-green algae.

The region under inquiry is Trimbakeshwar, which is located on the Western Ghats' eastern spur. Studies of bryophytes are said to take place in a "green paradise" known as the Western Ghat. Road travel from Nasik, Maharashtra, is 28 km. Trimbakeshwar is located at an altitude of around 600 metres above sea level. It is supported by lateritic black soil and dark basalt rock. The usual maximum and minimum temperatures in the region range from 30 to 34 °C and 18 to 22 °C, respectively, with around 2500 mm of annual rainfall. (Aruna K.B. and Krishnappa M 2014) [10]. As the Trimbakeshwar region is well known for its paddy fields, the present study investigates the Nitrogen fixing ability of BGA from the fields of the study area and these BGA is can be a useful replacement for chemical fertilizer as a nitrogen source.

Material and Method
The research was divided into four stages of methodology that contain water and soil sample collection, Identification of species, isolation of selected BGA and finally Nitrogen estimation was performed by using the Kjeldahl method.

Water and soil sample collection
As the paddy crop grows in waterlogged conditions, the Trimbakeshwar region with the highest rainfall has a variety of paddy species grown in the area. So the water sample from the crop field is collected. Also, the moist soil from standing crop fields is collected for the study of cyanobacteria.

Identification of species
The species were identified with the help of their size. The determination of size was done by the Ocular micrometry method. An ocular micrometre, also known as an eyepiece

micrometre, is a glass disc with a ruled scale etched on it that fits in a microscope's eyepiece and is used to measure the size of tiny objects through magnification.

Using an ocular micrometre is a quick and accurate way to measure things under a microscope. This device is essentially a ruler that has been carved into a microscope's ocular. It is made up of several ruled markings. The distance between each mark must be calculated for each microscope objective. If you've already calibrated for a certain purpose, you don't need to do it again until you change the target. The size of an item is measured in ocular micrometre units. The calibration factor is then multiplied by these units to get real units. Usually, a stage micrometre is used to calibrate ocular micrometres. This glass slide is uniform and has a scale that is properly marked.

Isolation of blue-green algae

A universal method for growing and caring for blue-green algae is BG11 broth (cyanobacteria). *Synechococcus* elongates is one of the chosen cyanobacteria that BG-11 Media is tailored for growing and maintaining. They need light as an energy source. This medium is made up of synthetic nitrogen and carbon sources as well as various inorganic salts.

Estimation of nitrogen

The estimation of Nitrogen was conducted by using the Kjeldahl method. This method was specifically developed for determining the nitrogen contents in organic and inorganic substances. The main objective or purpose of this procedure is the oxidation of organic compounds using concentrated sulphuric acid. Overall, the Kjeldahl method is divided into three main steps. The method has to be carried out in proper sequence. The steps include digestion, distillation, and titration

$$\text{Formula For Calculation Of Nitrogen Content -Sample (X)} = \frac{[\ (V3 - V4) * C * 14 * 2 * 1000\ ]}{V}$$

Result and discussion

The methodology used showed a successful result. After the use of Ocular micrometry, major four types of cynobacteria were identified those are *Calothrix*, *Nostoc*, *Anabeana*, and *Oscillotoria*

Fig 1: *Calothix*

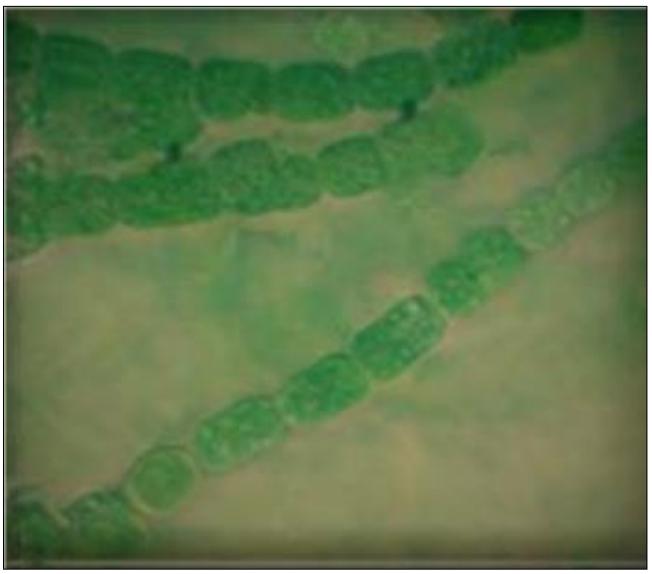

Fig 2: *Anabeana*

Fig 3: *Oscillotoria*

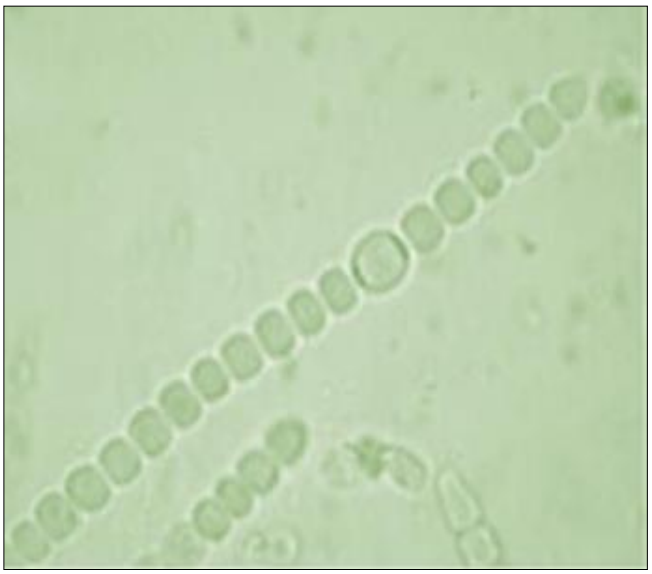

Fig 4: *Nostoc*

Calothix filaments are not radially oriented; instead, they frequently occur alone or in small groups. They are not poisonous. *Alothrix* filaments feature a spherical basal heterocyst and a tapered, hairlike structure at the apical end. They are adhered to a substrate either alone or in tiny clusters. They are non-planktonic. Anabaena is a genus of nitrogen-fixing blue-green algae that is found as plankton in shallow water and on moist soil. Its cells resemble beads or barrels and are interspersed with larger spores (heterocysts). There are both solitary and colony forms, with the latter resembling the genus *Nostoc*, which is closely related. The body of Oscillatoria is a single, unbranched thread known as a thallus. It consists of a single row of cells that grow into unbranched filaments covered with gelatin. Oscillatoria can be linked or free-floating and are seldom encountered on their own. Unbranched, filamentous nostoc. Oval, spherical, or cylindrical cells can exist. Heterocysts are differentiated cells that are present in the filament. A protective covering called a mucilaginous sheath surrounds each filament. Colonies come in a variety of forms, dimensions, and hues.
These species were grown on BG-11 media for further Nitrogen estimation. The nitrogen estimation was done by Kjeldahl method twice for confirmation. The following results were obtained after analysis.

Table 1: Nitrogen Content from different species

Sr. no.	Cyanobacteria	Estimated Nitrogen (mg/l)
1	*Calothrix*	47.00 mg/l
2	*Anabeana*	71.05 mg/l
3	*Oscillotoria*	50.51 mg/l
4	*Nostoc*	68.80 mg/l

After the analysis of nitrogen content from each species, it was observed that among the observed Cyanobacteria, *Anabeana* have the highest capability of Nitrogen fixation (71.05 mg/l) followed by *Oscillotoria* (50.51 mg/lit), then Nostoc (68.80 mg/lit) and the minimum Nitrogen fixation was observed for the *Calothix* species (47.00 mg/lit).

Conclusion

The Trimbakeshwar area have great importance due to origin of Godavari-river. So, it is important to maintain the ecosystem in the particular area. Continuous use of Chemical fertilizer in the field may cause contamination of water and soil and lead to pollution. Thus, use of such Alternative fertilizer for crop growth could help the environment and economic balance. As observed in the study the *Anabeana Sp.* Have highest capability to fix atmospheric nitrogen and provide to the plant. The *Anabeana* can be used as biofertilizer for the crop growth. Also *Nostoc Sp*. *Oscillotoria Sp.*, *Calothrix Sp.* Has shown the nitrogen fixing quality, it can also replace *Anabeana sp.* Thus, the study can conclude that Nitrogen Fixing Cyanobacterial Species Are Alternative to Harmful Chemical Fertilizer.

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