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Characterization and estimated diversity of cyanobacteria in biological soil crust in sacred grove forest of Tamil Nadu, India

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Abstract

Cyanobacteria constituted the main component of biological soil crusts (BSCs) that cover scarce area of sacred groves forest in the Ariyalur and Pudukottai district of Tamil Nadu, India. Cyanobacterial population numbers were estimated in three types of biological soil crust of sacred groves forest. Each type of crust exhibited different composition of cyanobacteria. The cyanobacteria count (CFU), pigment absorption spectrum, species abundance, evenness, dominance, diversity index values and Bray cluster analysis result showed large variations of BSCs at each sacred groves forest. Cyanobacterial species diversity was high in the studied grove forest which implied on increased soil fertility, ends with afforestation in the Ariyalur and Pudukottai district sacred groves forest. These results proved that diverse of cyanobacterial species increased the organic content in the soil of sacred groves forest.

Keywords: BSCs, cyanobacteria, diversity, sacred groves

Introduction

Sacred groves are small forests protected by local people. Sacred groves have been reported from parts of indigenous societies existed worldwide. These are one of the initiatives in conserving practices for native biodiversity [16, 10]. Many sacred groves constitute pristine vegetation and they are rich in vegetation and associate group of organisms such as rare or endangered flora and fauna. In India, sacred groves occur in a variety of ecological conditions. They have evolved under resource-rich condition as in Tamil Nadu [20, 21, 14].

The health of sacred groves vegetation is closely associated with biological soil crust (BSCs) diversity. Moreover the microbial communities of BSC [23, 22]. Flourished by numerous living things are important components in monsoon and the BSCs surface become wrinkled by withering in summer. These crust communities include bacteria, cyanobacteria, green algae, diatoms, lichen, mosses, liverworts and non-lichenized fungi. In sacred groves where vascular plants are scarce or absent [4], these crusts represent the primary biological growth within the sacred groves. Amongst the various microbial communities present in the crusts, cyanobacteria play an important eco-biological role in sacred groves environment by fixing the atmospheric nitrogen, segregating extra-cellular polymorphic substances (EPS) helping to soil stability, increasing soil water retention, contributing to nutrient cycling and facilitating seed germination of several plant species [3, 19, 8, 9, 17].

The biological soil crust communities and its diversity were protected and stabilized by the sacred groves forest vegetation. The biological soil crust occurs in different morphological forms in the sacred groves, namely mats, patches and crusts. Each form has varied in habitat, thickness, pigmentation and cyanobacterial composition [22].

The biological soil crust succeeds development of soil, nutrient cycling (C, N) and plant vegetation in sparse area. The diversity and abundance of biological soil crust cyanobacteria as a first colonizer of sacred groves forest may profoundly affect nutrient availability for pioneer vascular plants [18, 12, 5].

Study of these plant communities has increased considerably in recent years in different sacred groves of Tamil Nadu. In recent years, the sacred groves are subjected to disturbance by industrialization, urbanization and intensive human activities, but the biological soil crusts were left unaffected and reported to grow well by protecting sacred grove's vegetation against nutrient defect, wind and water erosion [22, 1, 11].

Biological soil crust and its population in arid and semi arid environment are reported worldwide.

The India level distribution of soil crust biota and communities are not available for many

regions. The present study reports the characterization of biological soil crust its cyanobacterial population and diversity of different sacred grove forests in Ariyalur and Pudukottai districts of Tamil Nadu, India.

Materials and methods

The study area comprising twelve sacred groves are located in Ariyalur and Pudukottai, district of TamilNadu, India. The field work was conducted in June 2018. Samples of BSC's were collected along twelve sacred groves belonging to the above said two districts.

Collection of biological soil crusts

Biological soil crusts were collected during early summer from different sacred groves sites in Ariyalur and Pudukottai. Samples were collected from the top soil along a transect line. Bio-crust communities within sacred groves were divided into three BSC types. The crusts were collected in 15 × 15 cm steel dishes, for each sample, the lower lid of the dishes was pressed approximately 2 - 4 cm deep into soil. We used a spatula for transportation sample to the polythene bags. The bags were properly labeled and noted (Dates, sites name, habitat, sample type). The collected samples were processed in the laboratory at Department of Botany, Jamal Mohamed College, Tiruchirappalli, India.

Pigment analysis

Pigment was extracted from known quantity of crust with 90 % methanol (v/v) and absorption spectra of all samples were measured in a double beam spectrophotometer in the wavelength range of 200-800 nm using quartz cuvettes. The data were analyzed with software provided by the manufacturer.

Enumeration of cyanobacterial population

The BSCs were wetted with sterile water and examined under light microscope. Serial dilution plate method was followed for the isolation of cyanobacterial population. The BSCs have been divided into three types. The samples were air dried, powdered and sieved. About 1g of samples was inoculated in 100ml of BG11 medium with or without nitrogen to give a 1:100 dilutions. Cyanobacteria were enumerated using 1%

agar-based plates. In each case 1ml of the BSCs suspension was spread onto plates containing 15ml of the solidified medium and incubated at 30° ± 2° C for 20-25 days in 23 K lux lights intensity. Enumeration of cyanobacterial number was carried out by Colony Forming Unit and it was calculated on the dry weight basis.

$$\text{CFU/g Dw} = \frac{\text{number of colony form} \times \text{dilution factor} \times \text{inoculum}}{\text{Dry - weight of soil (g)}}$$

Identification of cyanobacteria species were done by following the keys given by Desikachary (1959)^[7] based on their morphological structures. The relative abundance (%) was calculated using the following formula:

$$\text{Relative abundance (\%)} = \frac{\text{total number of the colonies of individual species}}{\text{Total number of colonies of all species}} \times 100$$

Statistical analysis

Simpson diversity index, Shannon index, dominance, evenness and relative abundance were calculated using standard formulae in "Microsoft Excel" package and "Biodiversity pro" packages^[15].

Results

Three different BSCs observed in the each field based on distribution, thickness, pigments and consist organism. Three types of BSCs namely BSCs1, BSCs2 and BSCs3 (Fig.1;Table 1). The first types BSCs1 were sited absent of vegetation places, directly affect by sun, dark green or black in colour. *Scytonema* and *Microcoleus* dominated. The second type BSC-2 is the only present in shade and moister places, light green in nature. This types of crust is the *Oscillatoria*, *Phormidium* species dominate. The third types is the BSCs3 were *Scytonema* species dominated, pink in nature and exposed to direct sun light. In all the BSCs filamentous forms cyanobacteria was abundance. BSCs species combination is varied with the sun light exposure, environment stress and physicochemical properties in the habitat.

Table 1: Cyanobacterial morphotypes distinguished from the biological soil crusts of sacred groves forest of Ariyalur and Pudukottai districts, Tamil Nadu, India

Types of BSCs	Nature of biological crusts	Dominant species
BSCs-1	Blackish green colour, Tightly associated with soil, > 2 mm thickness.	<i>Oscillatoria sancta</i> , <i>Nostoc punctiforme</i> and <i>Scytonema arcangeli</i> .
BSCs-2	Greenish colour >3 mm thickness, present in shade and wet places.	<i>Phormidium autumnale</i> and <i>Anabaena cylindrica</i> .
BSCs-3	Brownish colour, 2 to 3mm thickness, present in shade places.	<i>Calothrix desertica</i> , <i>Microcoleus acutissimus</i> , and <i>Scytonema aerugineo</i> .

Overall 46 species of cyanobacteria were reported from the three different biological soil crust in Ariyalur and Pudukottai sacred groves forest (Table 2). 16 different genera commonly associated with BSC were identified morphologically, of which *Anabena*, *Calothrix*, *Chroococcus*, *Gloeocapsa*, *Hapalosiphon*, *Lyngbya*, *Mastigocoleus*, *Microcoleus*, *Microsystis*, *Nostoc*, *Oscillatoria*, *Phormidium*, *Plectonema*, *Scytonema*, *Stigonema*, *Synechococcus*, *Spirulina* and *Synechocystis* were found from sacred groves BSC (Figs. 2-3). Although a few small lichen thalli and moss frond were also observed, these are not discussed here. *Phormidium*, *Lyngbya*,

Microcoleus, *Nostoc* and *Scytonema* taxa are main structural component in most of the sacred groves BSC.

Pigments profile of three biological soil crusts from all sacred groves in given in (Fig. 4) the absorption spectra showed absorption at 665 nm due to chlorophyll a, at 470 nm due to carotenoids, at 309-362 nm due to MAAs and at 386, 278 and 254 due to Scytonemin. In all the BSCs samples pigments content was found to be quite prominent. Indicating their vital role in survival of cyanobacteria in extreme environmental conditions in addition to photo protection.



Fig 1: Appearance of different types of biological soil crusts in Ariyalur and Pudukottai sacred groves forest. A and B, BSC1 (Blackish green) type crust; C and D, BSC2 (Greenish) type crust; E and F, BSC3 (Brownish) type crust

Table 2: Check list of BSC forming cyanobacteria of different biological soil crusts of Ariyalur and Pudukottai districts

S. No	Cyanobacterial species	Ariyalur			Pudukottai		
		BSC-1	BSC-2	BSC-3	BSC-1	BSC-2	BSC-3
1	<i>Microcystis aeruginosa</i> Kutz.,	+	+	-	+	-	-
2	<i>Microcystis</i> sp.	-	+	-	-	-	-
3	<i>Gloeocapsa rupestris</i> Kutz.,	-	-	-	+	-	-
4	<i>Synechococcus elongatus</i> Nag.	-	+	-	-	-	-
5	<i>Synechocystis pevalekii</i> Erceg.,	-	-	-	-	+	+
6	<i>Spirulina laxissima</i> West, G. S.,	-	-	+	-	-	-
7	<i>Oscillatoria obscura</i> Bruhl and Biswas.,	-	-	-	-	-	+
8	<i>Oscillatoria proteus</i> Skuja.,	-	-	-	-	+	-
9	<i>Oscillatoria sancta</i> (Kutz.,) Gom.,	+	-	-	-	-	-
10	<i>Oscillatoria subbrevis</i> Schmidle.,	-	-	-	-	+	-
11	<i>Phormidiumfoveolarum</i> Gom.,	-	-	-	+	-	-
12	<i>Phormidium abronema</i> Skuja.,	-	-	-	+	-	-
13	<i>Phormidium africanum</i> Lemm.,	-	-	-	-	+	-

14	<i>Phormidium angustissimum</i> West, W. and G.S.,	-	-	-	+	+	+
15	<i>Phormidium autumnale</i> (Ag.) Gom.,	-	+	-	-	-	-
16	<i>Phormidium bohneri</i> Sohmidle.,	+	-	-	-	-	-
17	<i>Phormidium foveolarum</i> Gom.,	+	-	-	-	+	+
18	<i>Phormidium fragile</i> (Menegh.) Gom.,	-	-	-	-	-	-
19	<i>Phormidium rubriterricola</i> Gardner.,	-	+	-	-	-	-
20	<i>Phormidium</i> sp.	-	-	-	+	-	-
21	<i>Phormidium tenue</i> (Menegh.) Gom.,	-	+	-	+	-	-
22	<i>Phormidium usterii</i> Schmidle.,	-	+	-	+	-	-
23	<i>Lyngbya kasyapii</i> Ghose.,	+	-	-	-	-	-
24	<i>Lyngbya lachneri</i> (Zimm.) Geitler.,	+	+	-	-	-	-
25	<i>Lyngbya palmarum</i> (Maetens) Bruhl et Biswas.,	-	-	+	-	-	-
26	<i>Lyngbya rivularianum</i> Gom.,	+	-	-	-	-	-
27	<i>Microcoleus acutissimus</i> Gardner.,	+	+	-	+	-	+
28	<i>Microcoleus orissia</i> West, W.,	-	-	+	-	-	-
29	<i>Microcoleus sociatus</i> West, W. and G.S.,	-	-	-	-	+	-
30	<i>Nostoc punctiforme</i>	-	-	-	+	-	-
31	<i>Anabaena cylindrica</i> Lemmermann	-	-	-	-	+	-
32	<i>Anabaena</i> sp.	-	-	-	+	-	-
33	<i>Plectonema nostocorum</i> Born.,	-	-	-	-	+	-
34	<i>Plectonema puteale</i> (Kirchn) Hansg.,	-	-	-	-	-	+
35	<i>Plectonema radiosum</i> (Schiederm.) Gom.	-	-	-	-	-	+
36	<i>Scytonema aerugineo - cinereum</i> Kutz.,	-	-	-	-	-	+
37	<i>Scytonema arcangeli</i>	+	-	-	-	-	-
38	<i>Scytonema javanicum</i> (Kutz.) Born.,	-	-	-	-	-	+
39	<i>Scytonema julianum</i> (Kutz.) Menegh.,	+	-	-	-	-	-
40	<i>Scytonema millei</i> Born.,	+	-	-	-	-	-
41	<i>Scytonema pseudopunctatum</i> Skuja.,	+	-	-	-	-	-
42	<i>Scytonema schmidtii</i> Gom.,	+	-	-	-	-	-
43	<i>Scytonema</i> sp.	+	-	-	-	-	-
44	<i>Calothrix desertica</i>	-	-	+	-	-	-
45	<i>Mastigocoleus testarum</i> Lagerh.,	-	-	+	-	-	-
46	<i>Hapalosiphon baroni</i> West, W and G, S.,	+	-	-	-	-	-

The colonies forming unit(CFU) of Ariyalur BSCs sample ranged from 46×10^3 cfu/g cells/1gm of soil, 29×10^3 cfu/g cells/1gm of soil and 18×10^3 cfu/g cells/1gm of soil of BSC1, BSC2 and BSC3 respectively. Pudukottai BSCs sample ranged from 35×10^3 cfu/g cells/1gm of soil, 23×10^3 cfu/g cells/1gm of soil and 31×10^3 cfu/g cells/1gm of soil of

BSC1, BSC2 and BSC3 respectively as shown (Fig.-5). Although there were differences in the average total cyanobacterial counts of the different locations, lowest total bacterial counts observed in Pudukottai BSCs, and highest count was observed Ariyalur sacred groves BSCs.

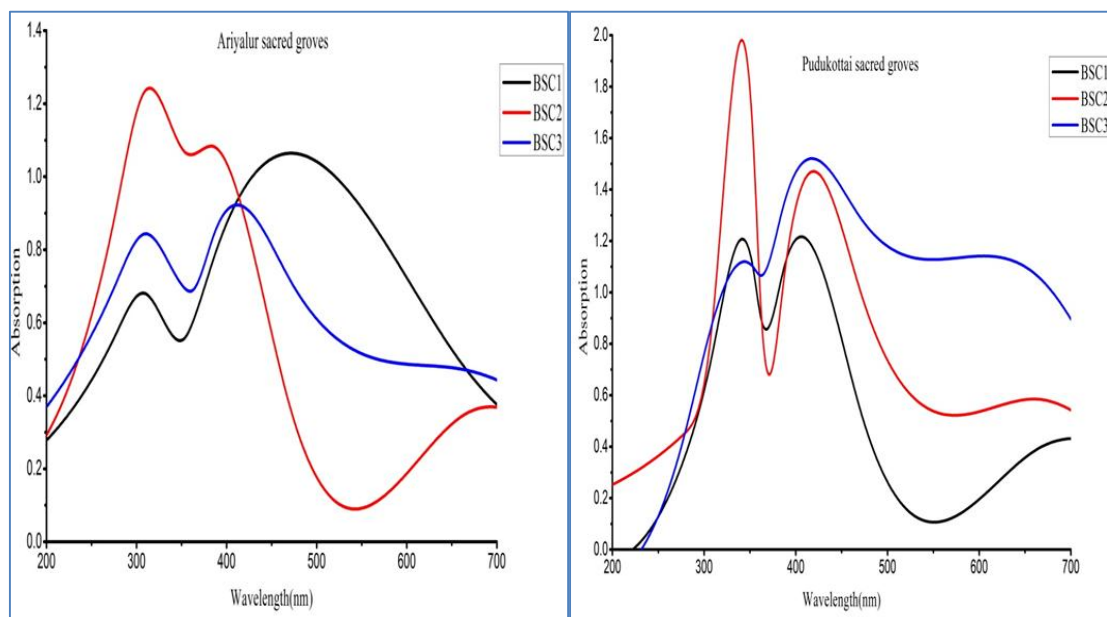


Fig 4: Absorption spectra of pigment of biological soil crusts from different sacred groves forest of Ariyalur and Pudukottai.

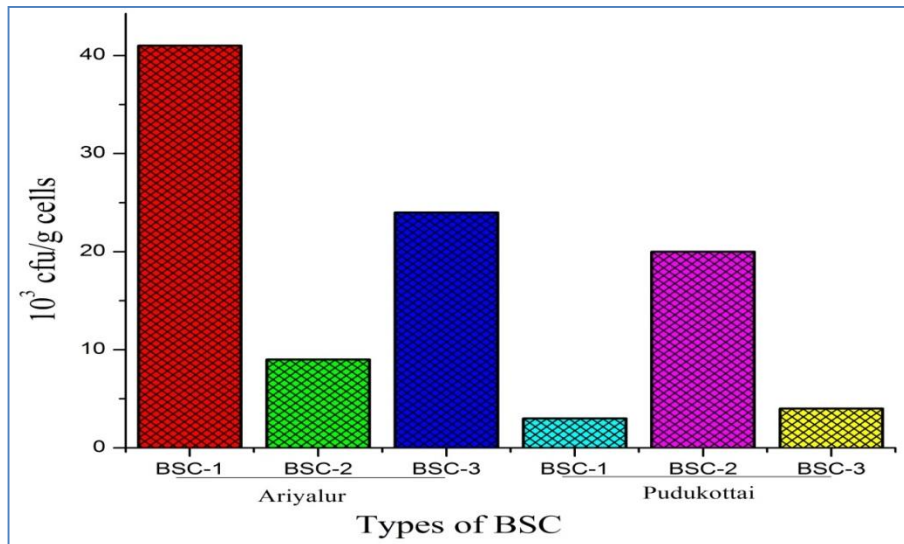


Fig 5: Colony forming unit (CFU) of cyanobacteria in biological soil crust of sacred groves forest

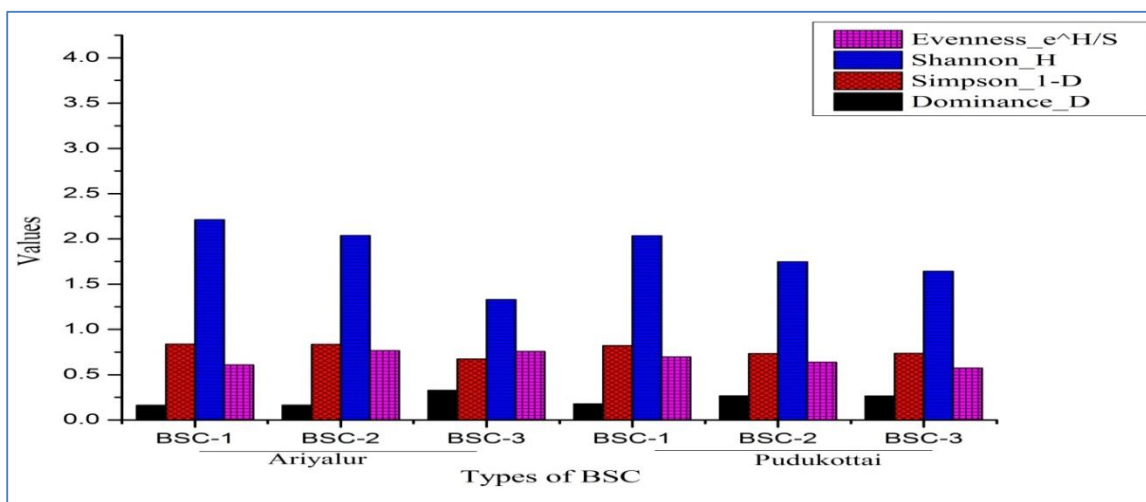


Fig 6: Diversity index, Dominance and Evenness of cyanobacteria in biological soil crust of sacred groves forest

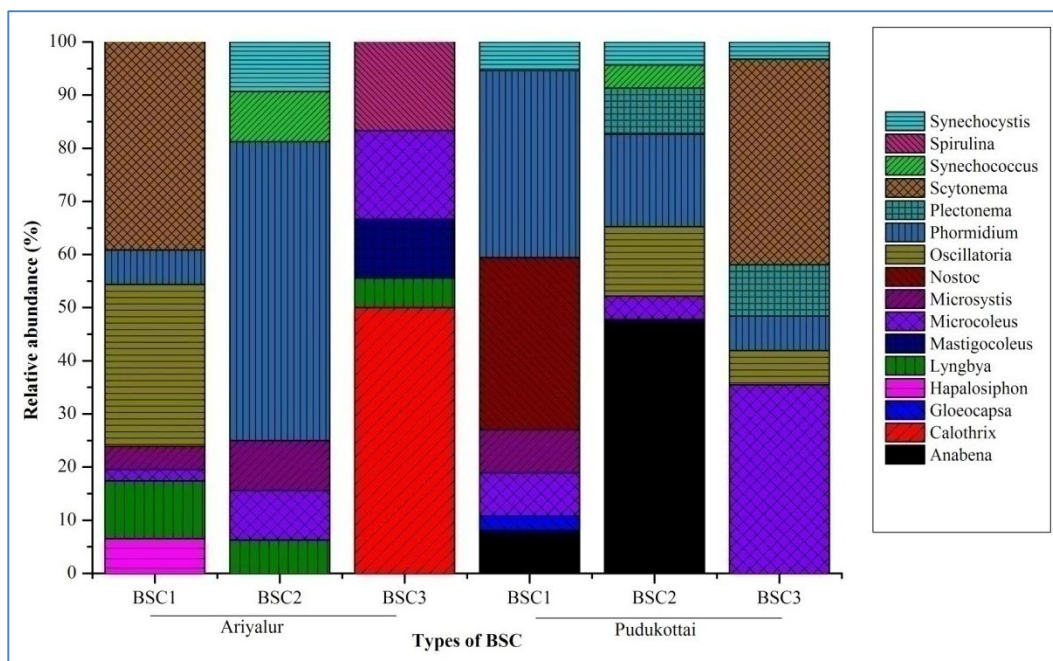


Fig 7: Major number of cyanobacteria genera and their relative abundance in the BSC samples

In diversity indices and relative abundance result indicate some taxa responsible particular types of BSC (Fig.6-7).

Scytonema(39.13%) and *Oscillatoria*(30.4%) showing highest percentage of relative abundance. The lowest abundant

genera were *Lyngbya*(10.8%), *Microcystis*(4.34), *Phormidium*(6.25) and *Hapalosiphon*(6.5). Including *Anabena*, *Calothrix*, *Gleocapsa*, *Mastigocoleus*, *Microcoleus*, *Nostoc*, *Plectonema*, *Spirulina*, *Synechocystis* and *Synechococcus* taxa that were undetected in ABSC-1 types.

Phormidium(56.25%) taxa showing highest percentage of relative abundance and *Microcoleus*(9.37%), *Microcystis*(9.37%), *Synechocystis*(9.37%) *Synechococcus*(9.37%) are lowest abundant. *Plectonema*, *Spirulina*, *Scytonema*, *Oscillatoria*, *Nostoc*, *Mastigocoleus*, *Hapalosiphon*, *Gleocapsa*, *Calothrix* and *Anabena* and *Calothrix* taxa that were undetected in ABSC-2 types.

Calothrix(50%) showing highest percentage of relative abundance. The lowest abundance genera *Mastigocoleus*(11.11%), *Microcoleus*(16.6%) *Spirulina*(16.6%) and *Lyngbya*(11.11%). *Synechocystis*, *Synechococcus*, *Plectonema*, *Scytonema*, *Oscillatoria*, *Phormidium*, *Nosto*, *Microcystis*, *Hapalosiphon*, *Gleocapsa* and *Anabena* taxa that were not detected in ABSC-3 types.

In PBSC-1 types *Phormidium*(35.1%) and *Nostoc*(32%) taxa having highest relative abundance. *Anabena*(8.1%), *Gleocapsa*(2.7%), *Microcoleus*(8.1%), *Microcystis*(8.1%), *Plectonema*(17.3%) and *Synechocystis*(5.4%) lowest

abundance. *Synechococcus*, *Spirulina*, *Scytonema*, *Mastigocoleus*, *Lyngbya*, *Hapalosiphon* and *Calothrix* taxa that were undetected.

Anabena(47.8%) taxa having highest relative abundance. *Plectonema*(17.3%), *Microcoleus*(4.3%), *Oscillatoria*(13%) *Synechocystis*(4.3%), *Synechococcus*(3.4%) are lowest abundance. *Calothrix*, *Gleocapsa*, *Lyngbya*, *Hapalosiphon*, *Mastigocoleus*, *Microcystis*, *Nostoc*, *Phormidium* and *Scytonema* taxa that were undetected.

Scytonema(38.70%) and *Microcoleus*(35.4%) showing having highest relative abundance. *Oscillatoria*, *Plectonema*(6.45%) and *Synechocystis*(3.2%) taxa that were lowest abundance. *Anabena*, *Calothrix*, *Gleocapsa*, *Lyngbya*, *Hapalosiphon*, *Mastigocoleus*, *Nostoc*, *Microcystis*, *Phormidium*, *Synechococcus* and *Spirulina* taxa that were undetected in PBSC-3 types.

Bray- curtis cluster analysis (single link) of cyanobacteria yields six types. PBSC-1, PBSC-2, PBSC-3, ABSC-1, ABSC-2 and ABSC-3 biological soil crusts groups having very different similarity percentages (Fig.8). PBSC-1 and ABSC-2 biological soil crust cyanobacteria species composition are little similar to other types of biological soil crust.

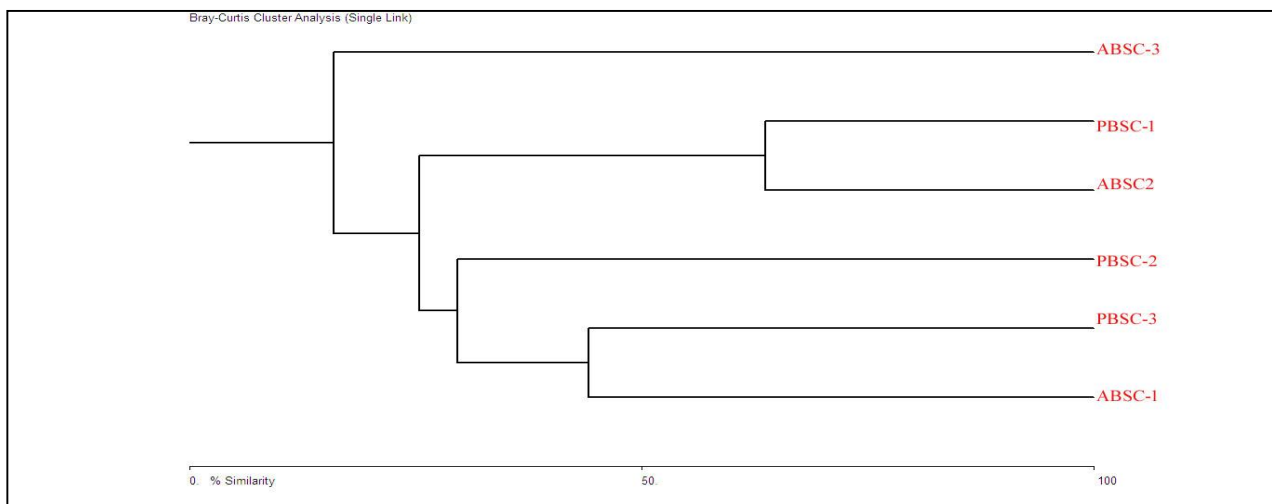


Fig 8: Bray- curtis cluster analysis of biological soil crust based on genera of cyanobacteria.

Discussion

Researchers report the Biological soil crust in worldwide in desert, arid and semi arid region analysis research in sacred groves forest [22]. They reported that biological soil crust cyanobacteria are dominance and diversity responsible for forest distribution and its stabilization. The three types of biological soil crust are widely distributed in Ariyalur and Pudukottai sacred groves forest, cyanobacteria species are major component in biological soil crust. This function is similar but species composition is very varied based on this habitat. The environmental factor and soil physic-chemical properties are affecting biological soil crust communities and its diversity.

Drought is the main factors for formation of different types of soil crust, The ABSC-1, ABSC-3, PBSC-1 and PBSC-3 biological soil crusts are present in plain places and away from vegetation cover area, It's affected by solar radiation, so the crust cyanobacteria was secreted more amount of photo protecting pigments. Which is dark and brown in colour due to UV absorbing compounds like Scytonemin and Mycosporine-like amino acids and is present in the pigment produce of several cyanobacteria [6]. This type soil crust

cyanobacteria had drought tolerate cyanobacteria such as *Microcoleus*, *Phormidium*, *Scytonema* etc. the ABSC-2 and PBSC-2 crust always present in vegetation cover and wet areas of sacred groves forest.

Relative abundance and diversity index result is demonstrated that filamentous cyanobacteria were greatly represented in sacred groves forests which were present majority of the all types of biological soil crust. The activities of various cyanobacteria enhance the fertility of the soil in sacred groves forest. So the biological soil crust used indirectly for the development of forest.

Soil crusts within the sacred groves forest are unique species assemblages of cyanobacteria. The cyanobacteria from sacred groves forest formed very different types of crust forming species reported from Ariyalur and Pudukottai sacred groves forest. Even though, the most common cyanobacteria species with in sacred groves forest are also recorded from different biological soil crust. The assemblage at sacred groves forest forms Bray- curtis cluster analysis. We expected the each crust types varied 40%. The biological

Conclusion

The results of the present study showed that biological soil crust had more number of cyanobacteria such as unicellular, heterocystous and non heterocystous forms. Their population varied between each type of biological soil crust. The species diversity, richness was evenly in all types of crust. These results proved that ecosystem rich with biological soil crusts showed an enhanced population of cyanobacterial

species which in return increased the growth of floral species in their places.

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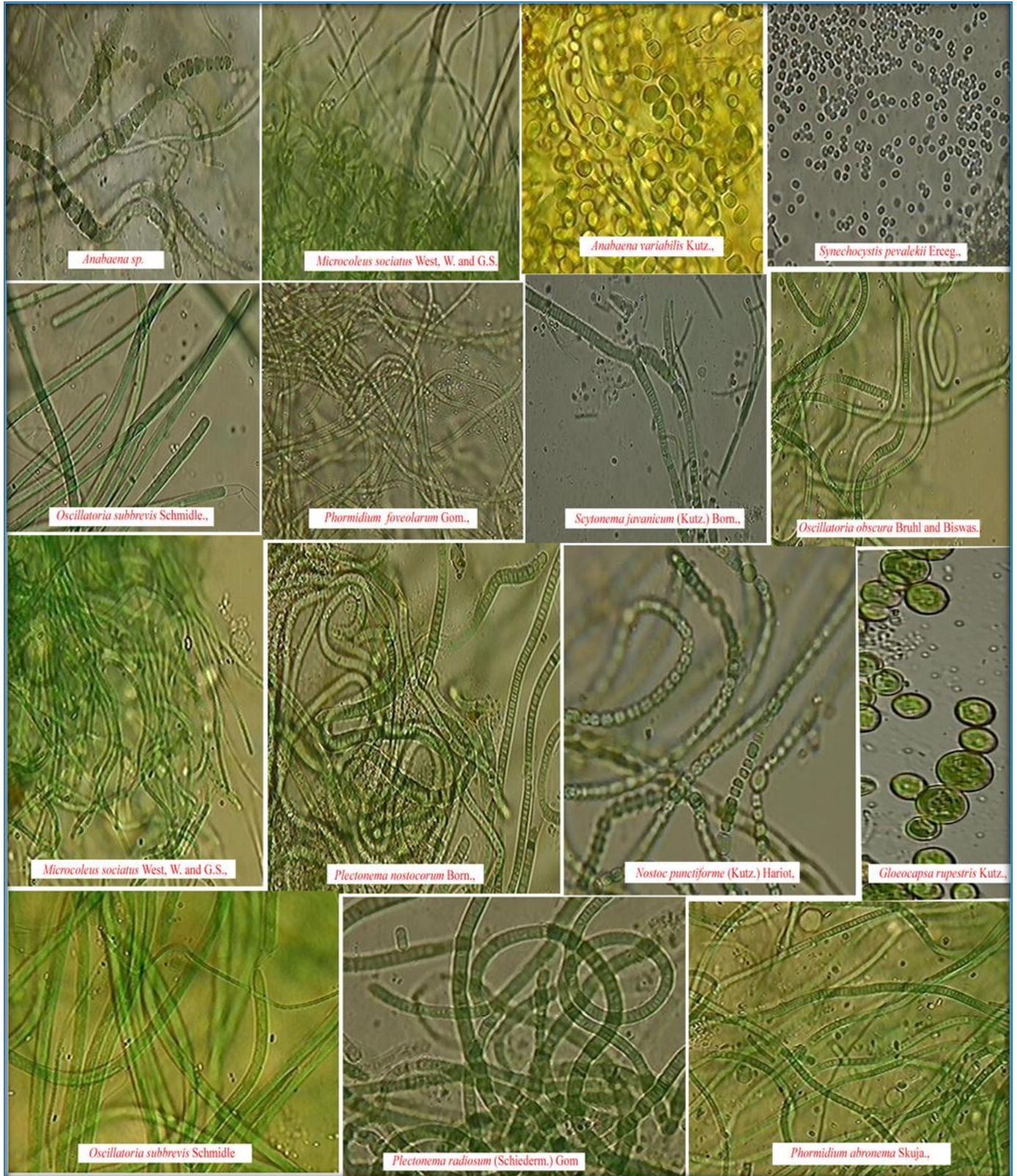


Fig 2: Common cyanobacteria from Ariyalur sacred groves biological soil crust

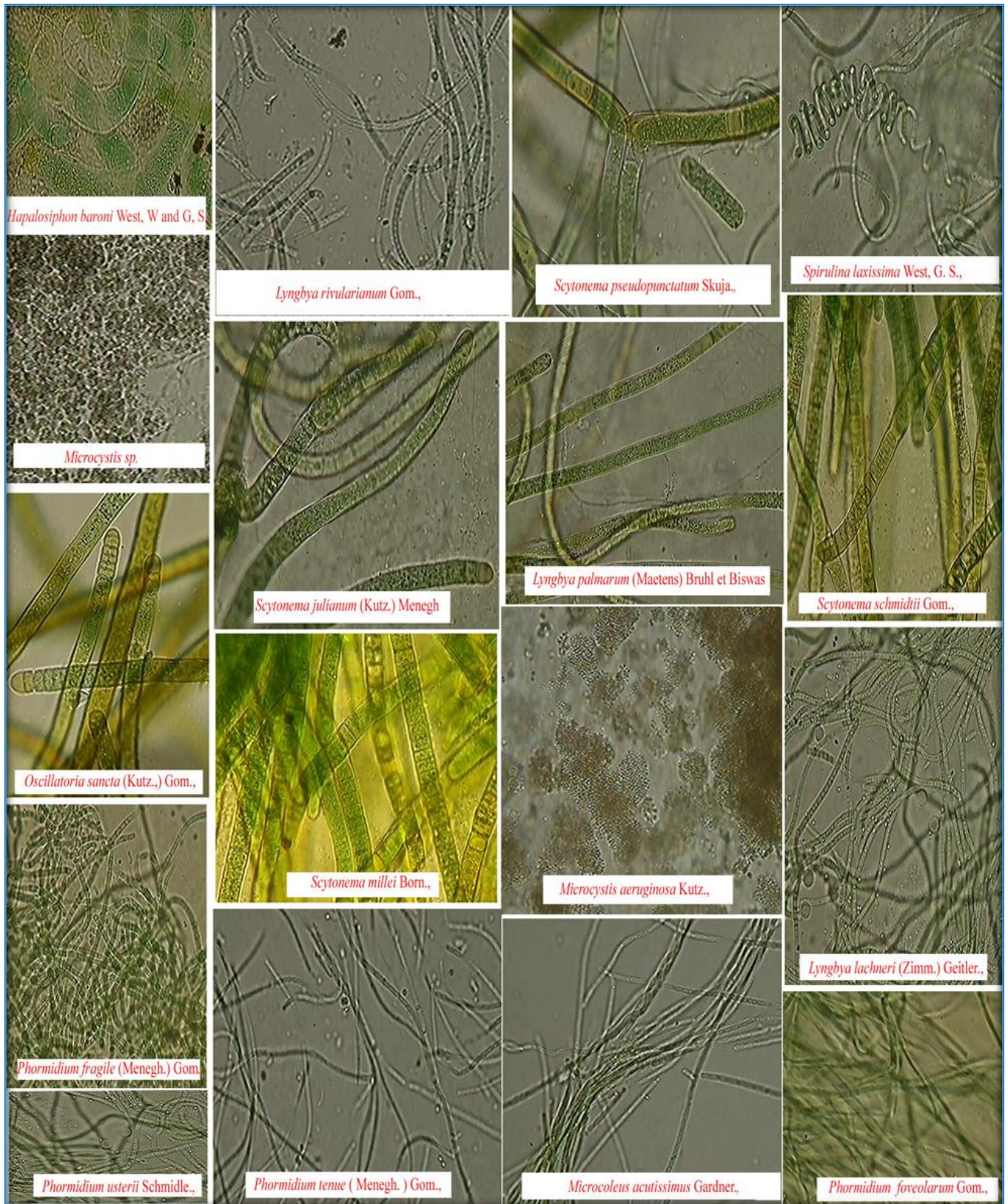


Fig 3: Common cyanobacteria from Pudukottai sacred groves biological soil crust.

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