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A systematic account of algae and cyanobacteria from different soil crusts in the Khasi Hills of Meghalaya

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Abstract
In the present investigation, a total of 40 species were recorded from coloured soil crust located in different landuse type in Khasi Hills, Meghalaya. Cyanobacteria was recorded as the most dominant group represented by 31 species followed by Chlorophyceae, Trebouxiophyceae and Zygnematophyceae represented by 4 species, 3 species and 2 species respectively. Cyanobacteria were the major group in these crusts during the dry seasons while filamentous green algae mostly prevalent during wet seasons. Cyanobacterial genera of *Scytonema* and *Stigonema* were mostly dominant during the dry seasons while those of *Oscillatoria*, *Schizothrix* and *Leptolyngbya* were prevalent during the wet seasons. Green filamentous algae like *Microspora* and *Zygnema* were also observed during the wet seasons. Soil crust formed during wet seasons were greenish or blue green mats, crusts or patches while during the dry seasons, brown or blackish mats or crusts were observed.

Keywords: algae, cyanobacteria, soil crust, Khasi hills

Introduction

Biological soil crusts (BSCs) are association between microorganisms such as cyanobacteria, algae, fungi, lichens and sometimes bryophytes which are closely associated with the substratum (Belnap and Lange, 2001) [1]. They are also referred to as cryptogamic, crytobiotic, microphytic, microbiotic, or microfloral soil crust by many authors (Harper and Marbel, 1988; West, 1990) [2] [3]. Soil crust occurred in variety of substrata ranging from exposed rock to desert, arid areas, forest and agricultural soils (Sethi *et al*. 2012) [4]. Soil crust, a crusty layer on the upper soil surface thus is the result of growth and activity of the microorganisms intertwined by EPS (exopolysaccharides) compounds or hyphae. Soil crust have been reported on rocks, hot deserts, arid areas, forest soils and rice fields (Belnap, 2002; Zhang *et al*., 2010) [5, 6]. Although filamentous Cyanobacteria have been reported as the key components of soil crust, however in alpine habitat, deserts and in the arctic, green algae were responsible for formation of the pioneer community as they were more resistant to unfavourable conditions (Büdel *et al*., 2016; Karsten and Holzinger, 2014) [7, 8].

Since, biological soil crusts are in close association with the soil surface they play an important role in stabilization of soil surfaces, reduction in erosion and retaining moisture, thus providing suitable habitat for plant growth. Information on the composition of Cyanobacteria and green algae in Indian subcontinent is less (Venkataraman *et al*., 1974; Marathe and Kushaldas, 1997; Adhikary and Sahu., 2000; Tirkey and Adhikary, 2005, 2006; Sethi *et al*., 2012; Vinoth *et al*., 2017) [9][10][11][12][13][4][14] and very limited in the northeastern India. Thus the present study was undertaken to provide an insight on the organisms occurring in crust of this region.

Material and Methods

Different coloured crust samples were collected from different landuse type of Khasi Hills, Meghalaya, during dry and wet seasons, as visible differences were observed during these two seasons. The samples were then kept in sterilised bottles and brought to the laboratory for analysis. Some samples were wetted for immediate observation while some were incubated in BG 11 medium (cyanobacteria) and Bold's basal medium (green algae) at 25ºC in light intensity of 40 µmol photons m^{-2} s^{-1} for a period of 30 days. The organisms were morphometrically analysed under Olympus BX41. All algae observed were identified using standard books and monograph such as Fritsch (1935), Desikachary (1959), Philipose (1967), Prescott (1982), Gandhi (1998) and John *et al*. (2002) [15][16][17][18][19][20]. Classification of cyanobacteria and algae was carried out in accordance with Komárek *et al*. (2014), Lewis and McCourt (2004) and Guiry and Guiry (2018) [21] [22][23].

Results and Discussions

Species composition in crust varied according to the nature of substratum and season. Soil crust formed during wet seasons were greenish or blue green mat, crust or patches while during the dry seasons, brown or blackish mat or crust were observed (Plate 1). These crusts consist of cyanobacteria and unicellular green algae in the form of spores, cysts or vegetative cells.

During the dry seasons, cyanobacteria was dominant in brownish and blackish crust and represented by *Gloeothece samoensis*, *Lyngbya aestuarii*, *Microcoleus vaginatus*, *Nostoc muscorum*, *Schizothrix tinctoria*, *Scytonema hyalinum*, *Scytonema*

papilicapitatum, Scytonema mirabile, Stigonema mammilosum and *Stigonema ocellatum*.

During the wet seasons, green algal forms were also represented as dominant genera. Filamentous green algae observed were *Microspora willeana* and *Zygnema* sp while unicellular alga was represented by *Chlorococcum acidum.* Cyanobacteria observed were *Leptolyngbya scotia, Leptolynbya vincentii, Microcoleus chthonoplastes, Oscillatoria curviceps, Oscillatoria rubescens, Phormidium retzii Schizothrix telephorioides and Oscillatoria limosa*. The associated components in those crusts and mats were represented by Cyanobacteria genera like *Geitlerinema* and *Plectonema*, while green algal form *Trebouxia erici* and *Chlorella vulgaris* were also observed**.**

Study on soil crust from different parts of India recorded the occurrence of same forms like *Scytonema*, *Plectonema* or *Lyngbya* as the dominant components along with other species like *Oscillatoria*, *Phormidium*, *Microcoleus*, *Aulosira*, *Fishcherella*, *Nostoc*, *Westiellopsis* and *Hepalosiphon* (Tirkey and Adhikary, 2005) [12]. According to Sethi *et al*. (2012) [4] species of *Scytonema stuposum*, *Lyngbya anomala*, *Calothrix clavata*, *Leptolyngbya tenuis*, *Gloeocapsa aeruginosa* and *Aphanocapsa biformis* were observed from the brownish crust or mat on all soil during summer and winter. Further they also reported green algae like *Microspora willeana, Chlorococcum infusionum*, *Chlorella vulgaris*, *Pandora morum*, *Volvox carteri* and *Closterium lunulain* in the crust of rice field during rainy seasons. Species of *Phormidium*, *Aphanocapsa*, *Lyngbya*, *Microcoleus*, *Anabaena*, *Nostoc*, *Plectonema*, *Scytonema* mostly occurred as green coloured mat, brownish crust or green patches in soil crust of Tamil Nadu (Vinoth *et al*., 2017) [14].

The Systemic account of cyanobacteria and other algal species recorded from the soil crust are described below.

Phylum - Cyanobacteria

Class - Cyanophyceae

1. Order - Synechococcales

***Leptolyngbya scottii* (Fritsch) Anagnostidis & Komárek**

Filament solitary or small clusters, straight to slightly coiled, 2.5 - 3.2 µm wide, pale blue-green; sheaths thin, colourless, later thickened; cells isodiametric or slightly longer than wide, cells end rounded conical, not capitate.

Collection site - Dark green patches from shifting cultivated land in Mawtawar during wet seasons (Plate 2, Figure a).

***Leptolyngbya vincentii* Komárek**

Filament thin, cylindrical, 0.6 - 1.8 µm, ends not narrowed, pale greyish blue-green, sheath very thin; cells isodiametric, longer than wide, cells end rounded.

Collection site - Thin dark blue green patches from potato/rice field in Umshing during wet seasons (Plate 2, Figure b).

***Schizothrix borealis* Komárek & Kovacik**

Filaments relatively thin, cylindrical, with slightly distant sheath, rarely constricted at cross walls, 1 - 6 arranged in parallel, solitary or in fascicles, 3 - 4 µm wide; sheath colourless, not or indistinctly lamellated.

Collection site - Dark blue green mat from sacred grove in Mawphlang during wet seasons (Plate 2, Figure c).

***Schizothrix telephoroides* Gomont**

Filaments 1 - 2 in each sheath; sheath firm, reddish inside, outside colourless; constricted at cross walls, 4 - 9 µm wide, 6 - 14 µm long, cells 1 - 2 times as long as broad; blue green in colour; end cell not attenuated, rounded.

Collection site - Thick dark blue green patch from pine forest in Umshing during wet seasons (Plate 2, Figure d).

***Schizothrix tinctoria* Gomont ex Gomont**

Filaments 1 - 5 in firm sheath; sheath cylindrical, thin firm colourless and hyaline, pale grey-green; cells cylindrical to barrel-shaped, isodiametrical or slightly longer or shorter than wide upto 2.3 µm wide.

Collection site - Brownish black crust from pine forest in Umshing during dry seasons (Plate 2, Figure e).

2. Order - Chroococcales

***Aphanothece castagnei* (Kützing) Rabenhorst**

Colonies irregular, round to elongated; sheath gelatinous, hyaline to brownish, inconspicuous to conspicuous; cells disposed sparsely, oblong to ellipsoidal, 3.8 - 5.9 x 2.6 - 3.8 µm, 1.4 - 2.0 times longer than wide; cells greyish green, slightly granulated.

Collection site - Thin dark blue green patches from potato/rice field in Umshing during wet season (Plate 2, Figure f).

***Asterocapsa* sp**

Colonies irregular, elongated and round, 5.5 - 79.7 µm, isolated or in groups; sheath firm, hyaline, conspicuous, smooth to granulate and rarely lamellate; cells ellipsoid to irregular, 3.8 - 5.8 x 2.5 - 3.9 µm, cell content green to blue green, granulated.

Collection site - Light brown crust from potato/rice field in Umshing during dry seasons (Plate 2, Figure g).

***Chlorogloea* sp**

Colonies elongated, 27 - 83 µm length; sheath firm to diffluent, conspicuous, non-lamellate, hyaline; cells elliptical or spherical, 1.7 - 3.6 µm, arranged in rows without individual envelopes.

Collection site - Light green patches from citrus plantation in Umshing during wet seasons (Plate 2, Figure h).

***Gloeocapsa atrata* Kützing**

Colonies variable in sizes, cells spherical, olive green; mother cells 22.5 - 33.8 x 19.2 - 20.8 µm, daughter cells are 6.6 - 8.9 x 5.7 - 7.3 µm; cells in periphery arranged in 2 - 4 celled sub colonies, granulated envelopes.

Collection site - Dark green patches from shifting cultivated land in Mawtawar during dry seasons (Plate 2, Figure i).

***Gloeothece rhodochlamys* Skuja**

Colonies irregular, round to ellipsoidal, sheath firm, conspicuous, concentric lamellate, finely granulated; cells oblong to ellipsoid, 4.5 - 6.0 x 2.6 - 5.0 µm, cells content homogenous to finely granulated, olive green in colour.

Collection site - Thin dark blue green patches from potato/rice field in Umshing during wet seasons (Plate 2, Figure j).

***Gloeothece samoensis* Wille**

Colonies round, oblong to elliptical with 9.7 - 21.3 µm diameter, 2 to 8 cells; cells oblong to ellipsoid, 5.4 - 8.4 x 3.3 - 5.8 µm, 1.3 - 1.7 times longer than wide; blue-green to olive green, cell content granulated.

Collection site - Blackish crust from potato/maize field in Sohiong during dry seasons (Plate 2, Figure k).

***Lyngbya aestuarii* Liebman ex Gomont**
Filament single or form a thallus of brown or dull green in colour, nearly straight or coiled. 10 - 16 µm wide. 1/3 - 1/6 times as long as broad, 2.7 - 5.6 µm long, not constricted at the cross-walls, cross wall often granulated; sheath thick, yellow brown, lamellated cells.
Collection site - Light brown crust potato/rice field in Umshing during dry seasons (Plate 2, Figure l).

3. Order - Oscillatoriales
***Geitlerinema splendidum* (Greville ex Gomont) Anagnostidis**
Filaments bright green to blue green or olive colour, not mucilaginous; filament without sheath, slightly bent and constricted at cross walls, 2 - 2.3 µm wide; cells longer than wide, end cells attenuated and elongated, narrowed, bent and spherically capitate up to 15 µm long.
Collection site - Light brown crust from potato/rice field in Umshing during dry seasons (Plate 2, Figure m).

***Microcoleus chthonoplastes* (Thuret) Gomont**
Filaments dark green, broad sheath with filaments arranged tightly towards the centre; 2.5 - 6.0 µm wide, 1 - 2 times longer than wide, cross walls narrowed; end cells not capitate.
Collection site - Dark blue green mat from sacred grove in Mawphlang during wet seasons (Plate 2, Figure n).

***Microcoleus vaginatus* (Vaucher) Gomont**
Filaments dark green, single or tight bundle with broad sheath and almost fill the sheath; cells 3.5 - 7.0 wide, 0.5 times longer than wide, cross walls narrowed with granules, end cells capitate, sheath diffluent.
Collection site - Brownish crust from citrus plantation in Umshing during dry seasons (Plate 2, Figure o).

***Oscillatoria curviceps* Agardh ex Gomont**
Filament blue-green, straight for a portion of its length then twisted and then entangled; apex not tapering, cell end rounded, not capitate, without a calypyta. Cells 10 - 17 µm in diameter, 3 - 5 µm long, granulate and not constricted at cross wall.
Collection site - Dark green patches from shifting cultivated land in Mawtawar during wet seasons (Plate 2, Figure p).

***Oscillatoria limosa* C. Agardh ex Gomont**
Filament more or less straight, brown or olive green; not constricted or slightly constricted at cross walls, 13 - 16 µm broad; cells about 2 - 5 µm long, end cells flatly rounded with slightly thickened membrane.
Collection site - Dark blue green mat from sacred grove in Mawphlang during wet seasons (Plate 2, Figure q).

***Oscillatoria rubescens* De Candolle ex Gomont**
Filament straight, gradually attenuated at the ends, 6 - 8 µm broad, cross walls not constricted; crimson or violet; 2 - 4 µm; end cell capitate, with convex calyptra.
Collection site - Light green patches from citrus plantation in Umshing during wet seasons (Plate 2, Figure r).

***Phormidium retzii* Kützing ex Gomont**
Filaments more or less straight, mostly constricted at cross walls, ends cells round, not attenuated or capitate, 11 - 13 µm broad, 6 - 9 µm long; sheath thin.
Collection site - Thick dark blue green patches from pine forest in Umshing during wet seasons (Plate 2, Figure s).

***Plectonema* sp**
Filament flexous, richly branched and narrower than the main branched; cells 1.3 - 2.0 µm wide, in the main axis slightly shorter or as long as wide while on branches longer than wide, cross walls narrowed, without granules.
Collection site - Brownish crust from citrus plantation in Umshing during dry seasons (Plate 2, Figure t).

4. Order - Nostocales
***Anabaena spiroides* Klebahn**
Filament single, helical, embedded in mucilage; cells almost spherical, 6.5 - 8.0 µm wide and 5.0 - 8.5 µm long. Heterocyst 6.5 - 8 µm wide.
Collection site - Light green patches from citrus plantation in Umshing during the wet season (Plate 2, Figure u).

***Hepalosiphon* sp**
Filament uniseriate, 6.0 - 10.7 µm wide; branches long; sheath thin to moderately thick, hyaline to yellowish brown; cells 3.5 - 10.5 µm long, 0.7 -2.3 times longer than wide, cell content granulated, pale green to pale blue green, heterocyst cylindrical to sub-quadrate.
Collection site - Blackish mat from sacred grove in Mawphlang during dry seasons (Plate 2, Figure v).

***Nostoc linckia* Bornet ex Bornet & Flahault**
Colony irregular, gelatinous; blue-green, brown or violet; filaments densely entangled, highly coiled, flexuous, 3 - 4 µm wide 6 - 7 µm broad, barrelled shaped, heterocysts subspherical; sheath colourless inside and distinct only in the periphery.
Collection site - Thick dark blue green patches from pine forest in Umshing during wet seasons (Plate 2, Figure w).

***Nostoc muscorum* C. Agardh**
Colony olive to yellow brown; filaments thickly entangled, cells short barrel-shaped or cylindrical, 3.5 - 5.0 µm wide; usually as long as wide or twice; heterocyst subspherical, 6 - 7 µm wide.
Collection site - Blackish crust from potato/maize field in Sohiong during dry seasons (Plate 2, Figure x).

***Scytonema hyalinum* Gardner**
Filaments cylindrical, 10 - 15 µm wide, disintegrating and divided in isolated segments; sheath firm, thin, slightly lamellate; heterocyst cylindrical, rounded ends, twice longer than wide, 5 - 18 µm x 6 - 12 µm.
Collection site - Brownish crust from citrus plantation in Umshing during dry seasons (Plate 2, Figure y).

***Scytonema mirabile* Bornet**
Filaments entangled 15 - 21 µm wide, 2 - 12 mm long mostly false branched; cells 6 - 12 µm wide, cylindrical at the end of trichome disc- shaped or more or less barrelled shaped, yellowish to blue or olive green; sheath yellow-brown, with slightly divergent layers.
Collection site - Blackish mat from sacred grove in Mawphlang during dry seasons (Plate 2, Figure z).

***Scytonema papillicapitatum* Sant'Anna & Komárek**
Filaments cylindrical, flexous and widened toward the end, 13 - 16 µm wide; false branching rare usually with two branches; sheath thick, yellow upto brownish; filaments 5 - 8 µm wide; heterocytes intercalary, solitary slightly wider than filaments, cylindrical, 10 - 15 x 6.0 - 6.5 µm.
Collection site - Light brown crust from potato/rice field in Umshing during dry seasons (Plate 2, Figure z1).

***Scytonema schmiditii* Gomont**
Filament 7 - 18 µm wide, Sheaths irregularly lengthwise structured; cells quadratic; constricted or rarely unconstricted at cross-walls.
Collection site - Brownsh crust from citrus plantation in Umshing during dry seasons (Plate 2, Figure z2).

***Scytonema* sp**
Filament brownish; false branched; sheath thick, gelatinous; 12 - 20 µm broad; cell 1/2 - 1/3 shorter than broad, sub quadratic; heterocyst 9.9 - 11.6 µm broad, 11.8 - 13.2 µm long.
Collection site - Dark blue green mat from sacred grove in Mawphlang during wet seasons (Plate 2, Figure z3).

***Stigonema mamillosum* Agarh ex Bornet & Flahault**
Filaments usually maybe up to about 70 µm or occasionally wider; base give out branches which are slightly narrower than the main filaments, each branch typically 15 - 24 µm wide; sheath initially colourless, usually turning yellow brown.
Collection site - Brownish black crust from pine forest in Umshing during dry seasons (Plate 2, Figure z4).

***Stigonema ocellatum* Lyngbye ex Bornet & Flahault**
Filaments 10 - 40 µm wide, entangled; false branches short; cells 6 - 14 µm wide, shorter than wide; sheath firm, lamellate, brown; heterocyst almost quadrate to cylindrical.
Collection site - Blackish mat from sacred grove in Mawphlang during dry seasons (Plate 2, Figure z5).

Phylum - Chlorophyta
Class - Chlorophyceae
1. Order - Chlamydomonadales
***Chlorococcum acidum* (Schrank) Meneghini**
Cells spherical rarely ovoid, green in colour, solitary or in groups; cells 6.6 – 20.0 µm wide; chloroplast a hollow sphere with a lateral notch.
Collection site - Thin dark blue green patches from potato/rice field in Umshing during wet seasons (Plate 3, Figure a).

2. Order - Sphaeropleales
***Gloeocystis vesiculosa* Nägeli**
Colony of ovoid or almost spherical groups 1 - 16 cells surrounded by concentrically striated mucilage; cells 8 - 12 µm broadly ova to almost spherical, sometimes slightly asymmetrical, chloroplast cupshaped.
Collection site - Brownish black crust from pine forest in Umshing during dry seasons (Plate 3, Figure b).

***Microspora willeana* Lagerheim**
Filament cylindrical slightly constricted at cross wall, cell wall thin, H piece scarcely visible in vegetative cell; chloroplast variable, cell 12.5 - 15.0 µm broad.
Collection site - Light green patches from citrus plantation in Umshing during wet seasons (Plate 3, Figure c).

3. Order – Oedogoniales
***Oedogonium anomalum* Hirn**
Filaments long, vegetative cells cylindrical, 35 - 45 µm in diameter and 85 - 120 µm long, oogonium solitary, ovoid to cylindrical in shaped, 50 - 60 µm in diameter and 60 - 80 µm long.
Collection site - Light green patches from citrus plantation in Umshing during wet seasons (Plate 3, Figure d).

Class - Trebouxiophyceae
1. Order - Chlorellales
***Chlorella vulgaris* Beyerinck (Beijerinck)**
Cells spherical; single parietal and cup-shaped chloroplast with one pyrenoid; cells 5.5 - 10.0 µm in diameter.
Collection site - Blackish crust from potato/maize field during dry seasons and thick dark blue green patches from pine forest in Umshing during wet seasons (Plate 3, Figure e).

2. Order - Trebouxiales
***Myrmecia biatorellae* J. B. Peterson**
Cells clustered in groups, spherical when young, becoming elongated and egg-shaped as they mature, 3 - 13 µm wide; chloroplast parietal, filling the cell but developing splits, without apyrenoid.
Collection site - Thick dark blue green patches from pine forest in Umshing during wet seasons (Plate 3, Figure f).

***Trebouxia erici* Ahmadjian**
Cells solitary or in groups of 2, 4 or 8 or more cells, 2 - 25 µm in diameter, spherical, ellipsoides or pear shaped, wall thin; chloroplast axile (center), massive.
Collection site - Blackish mat from sacred grove in Mawphlang during dry seasons (Plate 3, Figure g).

Phylum - Charophyta
Class - Zygnematophyceae
1. Order - Desmidiales
Closterium closterioides* var *intermedium
Cells broadly spindle shaped, 19 - 30 µm wide, 90 - 100 µm long, apices rounded 6.5 - 10 µm wide; girdle band absent, often with numerous sutures, chloroplast with 4 or 5 longitudinal ridges, each with 2 or 3 spherical pyrenoids; wall smooth, usually colourless.
Collection site - Dark green patches from shifting cultivated land in Mawtawar during dry seasons (Plate 3, Figure h).

***Zygnema* sp**
Filaments unbranched, 15 - 18 µm wide chloroplast 2 per cell, star shaped, suspended in centre of cell, each with central pyrenoid.
Collection site - Dark blue green mat from sacred grove in Mawphlang during wet seasons (Plate 3, Figure i).

Plate 1: Photographs of soil crust – a. Light brown crust, b. Brownish crust, c. Blackish mat, d. Blackish crust, e. Brownish black crust, f. Light green patches, g. Dark green patches, h. Dark blue green patch (thick), i. Dark blue green mat, j. Dark blue green patches (thin). (Dirborne and Ramanujam, 2020) [24].

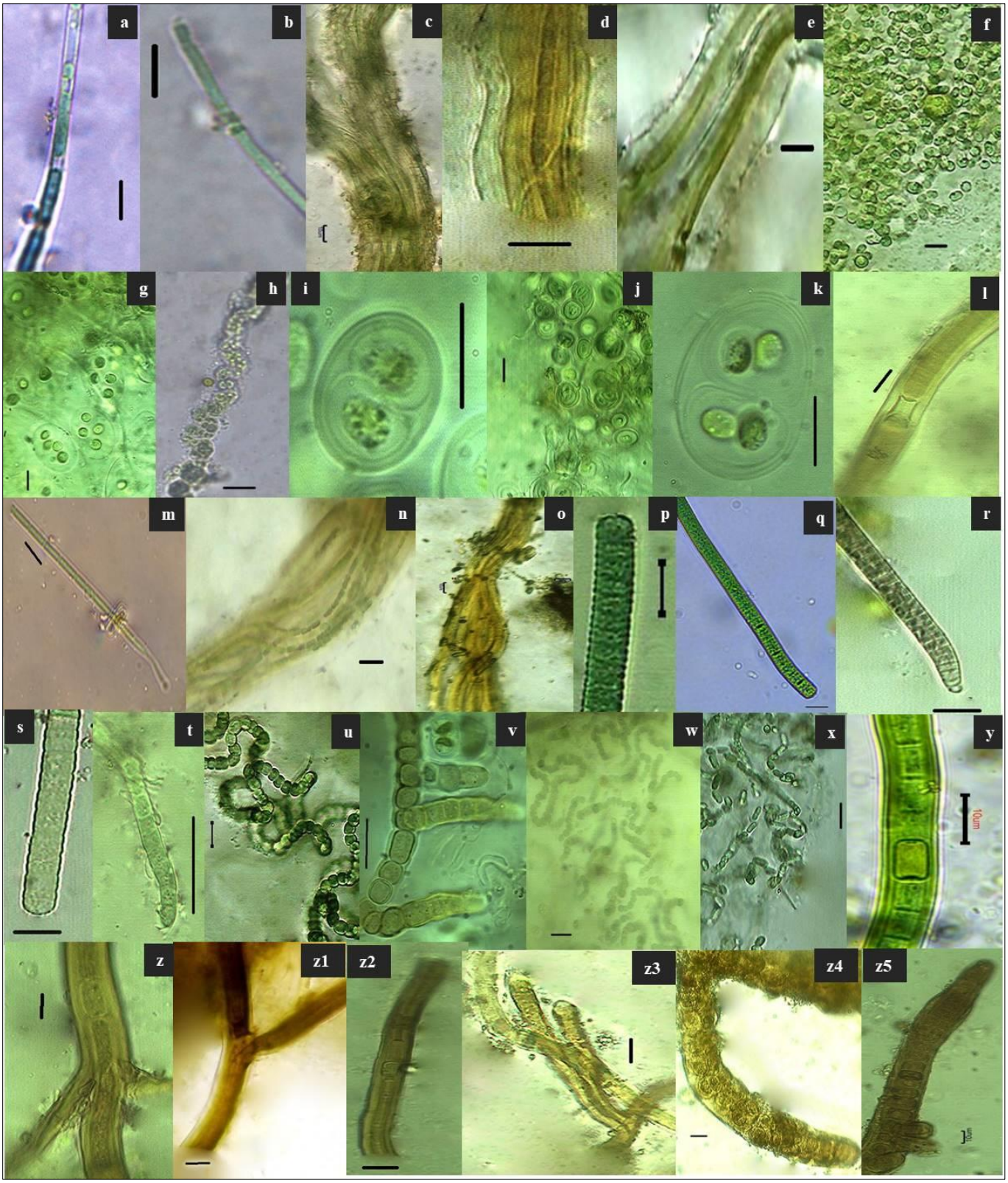

Plate 2: Cyanobacteria - Synechoccocales: a. *Leptolyngbya scottii*, b. *Leptolyngbya vincentii*, c. *Schizothrix borealis*, d. *Schizothrix telephoroides*, e. *Schizothrix tinctoria.* Chroococcales: f. *Aphanothece castagnei*, g. *Asterocapsa* sp, h. *Chlorogloea* sp, i. *Gloeocapsa atrata*, j. *Gloeothece rhodochlamys*, k. *Gloeothece samoensis* l. *Lyngbya aestuarii.* Oscillatoriales: m. *Geitlerinema splendidum*, n. *Microcoleus chthonoplastes*, o. *Microcoleus vaginatus*, p. *Oscillatoria curviceps*, q. *Oscillatoria limosa*, r. *Oscillatoria rubescens*, s. *Phormidium retzii*, t. *Plectonema* sp. Nostocales: *u. Anabaena spirioides*, *v. Hepalosiphon sp*, w. *Nostoc linckia*, x. *Nostoc muscorum*, y. *Scytonema hyalinum*, z. *Scytonema mirabile*, z1. *Scytonema papillicapitatum*, z2. *Scytonema schmiditii*, z3. *Scytonema* sp, z4. *Stigonema mamillosum*, z5. *Stigonema ocellatum.* Scale bar - 10 µm

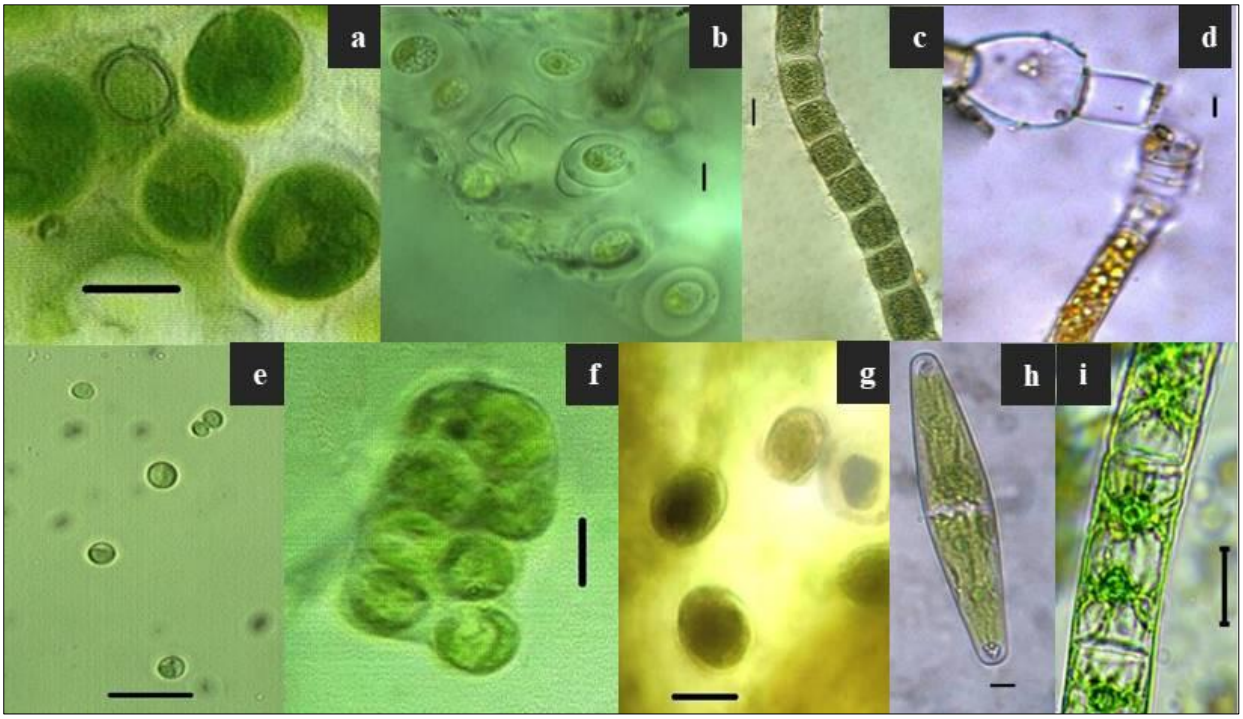

Plate 3: Green algae in soil crust – Chlorophyceae: a. *Chlorococcum acidum*, b. *Gloeocystis vesiculosa*, c. *Microspora willeana*, d. *Oedogonium anomalum*; Trebouxiophyceae: e. *Chlorella vulgaris*, f. *Myrmecia biatorellae* g. *Trebouxia erici*; Zygnematophyceae: h. *Closterium closterioides*, i. *Zygnema* sp. Scale bar - 10 µm

Conclusion

From the present study, it is evident that the composition of soil crust varied not only in different coloured crust but also depends on availability of moisture. During the dry seasons, cyanobacteria were dominant in brownish and blackish crust, while green algal forms were also represented as dominant genera during the wet seasons. Such intimate association of the organisms in crusts with soil has also been observed to improved soil quality and soil fertility by carbon and nitrogen fixation.

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