

## Taxonomic studies of Cyanobacteria and algae in soil crust of USTM Campus, Ri Bhoi District, Meghalaya

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### Abstract

Cyanobacteria and algae in soil crust of USTM Campus located in Ri Bhoi District, Meghalaya were investigated. A total of 22 species were observed from crust collected from various sites. Cyanobacteria was dominant (14 species) followed by Chlorophyceae (4 species) and Zygnematophyceae (2 species). Trebouxiophyceae and Ulvophyceae were observed with one species each. Cyanobacteria such as *Scytonema* and *Schizothrix* were dominant while filamentous green algae of the genus *Microspora* was also observed on the upper surface of the soil. Significant positive correlation ( $p < 0.05$ ) between the chlorophyll content in crust with soil parameters revealed that the growth of Cyanobacteria/algae positively influenced the moisture content and organic carbon in the soil.

**Key words :** Soil crust, Cyanobacteria, Moisture content, Organic carbon

**B**iological soil crusts (BSCs) are patch-like structures of different colours that occur on the upper surface of the soil. They represent an association of Cyanobacteria, bacteria, Chlorophycean algae, fungus and bryophytes forming a film tightly adhering to the soil particles. The upper millimetre of dry soil often appears crusty in nature due to growth of such microorganisms<sup>5,23</sup>. Biological soil crusts often occur in hostile environmental regimes that include extremes in temperature and light, and scarcity of water and are predominantly found in semi-arid and arid

regions of the world<sup>3</sup>. As a component of the soil crust, the microflora in the crust acts as reservoir of plant nutrients such as influencing the soil structure and also incorporation of carbon and nitrogen<sup>3,4,5</sup>.

Reports available from Indian subcontinent on the role of soil crust were from other parts of India<sup>7,9,15,16,19,21</sup>. Since study of soil crust from this region is scanty, therefore this study was undertaken to survey the soil crust in USTM campus.

*Study site :*

The study was carried out in the campus of University of Science & Technology, Meghalaya (USTM), located in Ri Bhoi, Meghalaya, with latitude and longitude of 26.1030°N and 91.8464°E respectively (Fig. 1).

*Collection of samples :*

Crust samples from the upper surface of the soil were from different sites of USTM campus, Ri Bhoi, Meghalaya. The samples were collected in a sterilized plastic bags and brought to the laboratory for analysis. The soil crust samples were wetted with distilled water and examined under the light microscope for immediate observation while some were culture in BG 11 and Bold's Basal medium for a period of 30 days at 25°C in  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

All algae observed were identified using standard books and monograph<sup>8,13,18</sup>.

*Physico-chemical parameters of soil :*

Soil pH was read using an electronic digital pH meter (pH tester 20). Soil moisture content was determined by oven dry basis, where 10 g of freshly collected soil was dried in hot air oven at 105°C for a period of 24 hours. Organic carbon was also estimated<sup>2</sup>.

*Chlorophyll content :*

Air dried samples of about 1 g was extracted with 5 ml of 90% methanol for 3 hours followed by incubation for 2 min at 60°C in a water bath. Absorption spectra was read at 663 nm measured in Cary 60 UV-Vis spectrophotometer. The amount of chlorophyll a was determined<sup>14</sup>.

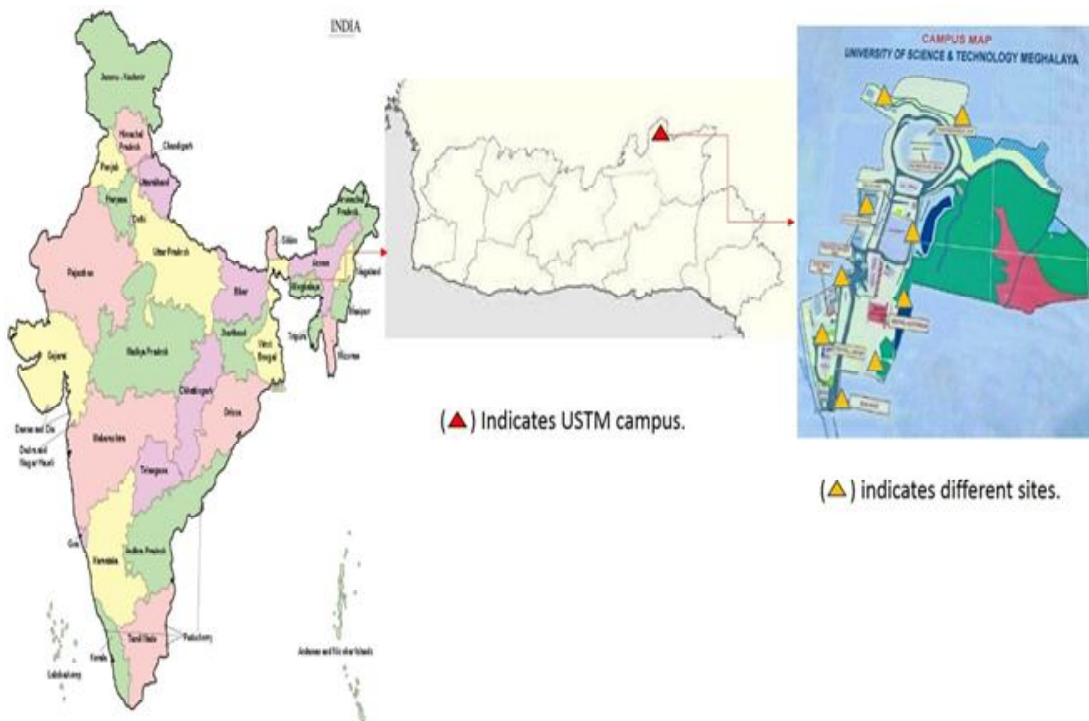


Fig. 1: Map of the study site.

Chlorophyll a ( $\mu\text{g g}^{-1}$ ) = O.D at 663 nm x 12.63.

*Data Analysis :*

Pearson's correlation was performed in order to determine the relationship of the Chlorophyll content in the crust on soil parameters and vice versa.

*Morphology of the crust :*

Soil crusts formed were mostly brownish or blackish mat, crust or patches. Some dark greenish patches were also observed with the onset of light shower in the month of May. Mat forms were 1 - 3 mm thick and sometimes were with or without moss association. Patchy forms were 0.5 - 1.0 mm thick and were strongly attached to soil surfaces. Since collection was conducted during the dry season mostly the dark coloured crust were observed (Fig 2).

A total of 22 species were observed from crust collected from various sites. Cyanobacteria was dominant with 14 species followed by Chlorophyceae with 4 species,

Zygnematophyceae with 2 species. Trebouxiophyceae and Ulvophyceae were observed with one species each. Cyanobacteria like *Scytonema* and *Schizothrix* were observed in crust which had thick sheath material and was seen winding through and among soil particles. Filamentous green algae of the genus *Microspora* formed thick multilayered mat like structure intertwined with the upper millimetres of which were dominant on the month of May. Unicellular algae like *Chlorococcum acidum*, *Chlorella vulgaris* and *Closterium navicula* were present in the crust and were protected by the filamentous mat (Table-1).

Species of *Scytonema*, *Stigonema*, *Oscillatoria*, *Schizothrix*, *Leptolyngbya*, *Anabaena*, *Asterocapsa*, and *Plectonema* mostly occurred as green coloured mat, brownish crust or green patches in soil crust of Khasi Hills<sup>9</sup>. Green algae forms occurred in crust only during the rainy seasons which could also be applied in our study since dominant species of *Microspora* were observed on May<sup>16</sup>.

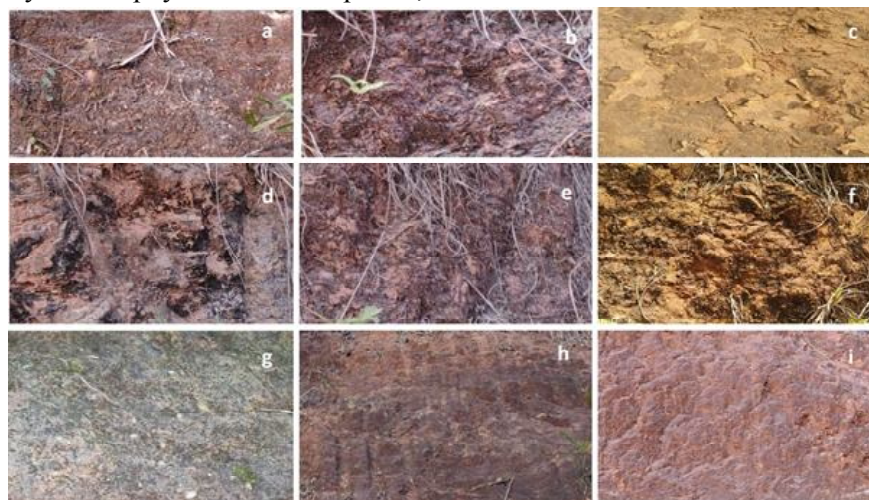


Fig. 2: Photographs of soil crust – a. Blackish brown crust, b. Black crust, c. Light brown crust, d. Black patch e. Brownish black patch f. Dark brown patch, g. Greenish black patch, h. Black mat, i. Brown mat.

The Systemic account of Cyanobacteria and other algal species recorded from the soil crust are described below (Fig. 3 & 4).

*Class – Cyanobacteria*

1. *Fischerella* sp.

Filaments with true branches, cells spherical to cylindrical, generally longer than broad (6.1 - 15.4 × 6.1 - 9.2 μm). Heterocysts spherical (8.5 × 6.9 μm) or even compressed (shorter than broad) and intercalary.

2. *Gloeotheca tepidariorum* (A. Braun) Lagerheim

Round to oblong colonies, 18.9–45.7 μm diameter. Sheath firm, hyaline, conspicuous, intensely lamellate, smooth to finely granulated (rare). Cells oblong to ellipsoid, 6.1 – 11.1 × 4.4 – 5.5 μm, 1.7 – 2.2 times longer than wide. Cell content finely granulated, greyish green to olive green.

3. *Hapalosiphon* 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, cell content granulated, pale green to pale blue green, heterocyst cylindrical to sub-quadrate.

4. *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; cells isodiametric or slightly longer than wide, cells end rounded conical, not capitate.

5. *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.

6. *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, 2.7 - 5.6 μm long, not constricted at the cross-walls, cross wall often granulated; sheath thick, yellow brown, lamellated cells.

7. *Nostoc commune* Vaucher ex Born. et. Flah:

Thallus brownish; filament coiled, densely entangled; cells 2.5-3.7 μm broad, 3.7–6.4 μm long; heterocyst at two different ends, sub-spherical to spherical, 5 -7 μm diameter.

8. *Nostoc* sp. :

Filaments straight or flexuous; mucilage hyaline, cells barrel-shaped to spherical (3.3 - 5.4 × 3 - 3.7 μm); heterocytes spherical (4.4 - 10.1 × 4.4 μm), distributed at the end of the trichome.

9. *Oscillatoria limosa* C. Agardh ex Gomont

Filament bluish green, unbranched, unsheathed, straight, 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.

10. *Schizothrix telephoroides* Gomont:

Filaments 1 - 2 in each sheath; sheath firm, reddish inside, outside colourless; constricted at cross walls, 4 - 9  $\mu\text{m}$  wide, 6 - 14  $\mu\text{m}$  long, cells 1 - 2 times as long as broad, end cell rounded.

11. *Scytonema ocellatum* (Dillwyn) Lyngb. ex Bornet & Flahault, Komárek :

Filament blackish to blackish green in colour, 24 - 25  $\mu\text{m}$  broad, short false branches present, cells are shorter than broad, 8 - 9  $\mu\text{m}$  long and 14 - 15  $\mu\text{m}$  broad, sheath firm, brownish, lamellated, heterocysts quadrate.

12. *Scytonema* sp. :

Filament brownish; false branched; sheath thick, gelatinous; 12 - 20  $\mu\text{m}$  broad; cell 1/2 - 1/3 shorter than broad, sub quadratic.

13. *Synechococcus aeruginosus* Nägeli :

Cells single or as a pair during division, short cylindrical with widely rounded ends. Cells 7-12  $\mu\text{m}$  wide, 10 - 45  $\mu\text{m}$  long.

14. *Westiellopsis prolifica* Janet :

Filament bluish green, heterocystous, branched, lateral filaments thinner than main branch, end with sporulated cells, 5.9 - 7.2  $\mu\text{m}$  broad, 9 - 12  $\mu\text{m}$  long; heterocyst intercalary.

Class - *Chlorophyceae* :

1. *Chlorococcum acidum* (Schrank) Meneghini :

Cells spherical rarely ovoid, green in

colour, solitary or in groups; cells 6.6 - 20.0  $\mu\text{m}$  wide; chloroplast a hollow sphere with a lateral notch.

2. *Microspora floccosa* (Vaucher) Thuret:

Cells are cylindrical in shape and rarely contracted at cross walls. The filaments were unbranched, length is 22 - 23  $\mu\text{m}$  and diameter 13 - 14  $\mu\text{m}$ . Light blue in colour and filaments are dissociating into H - pieces on fragmentation.

3. *Microspora pachyderma* (Wille) Lagerheim :

Cells cylindrical, 12 - 18  $\mu\text{m}$  long and 16 - 16.5  $\mu\text{m}$  broad, cell walls are separated by H- pieces, chloroplasts perforated plate like, covering more than half of the cell.

4. *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  $\mu\text{m}$  broad.

Class - *Zygnematophyceae* :

1. *Closterium navicula* (Breb.) Lutkem. Ettl and Gartner :

Cells are solitary, straight, ellipsoid, 140 - 143  $\mu\text{m}$  long, gradually narrowed towards both the ends, breadth 42 - 43  $\mu\text{m}$  at the centre and 18 - 20  $\mu\text{m}$  at the tip, chloroplasts 2, one on each side with 2 pyrenoids.

2. *Zygnema* sp. :

Filaments unbranched, 15 - 18  $\mu\text{m}$

wide chloroplast 2 per cell, star shaped, suspended in centre of cell, each with central pyrenoid.

*Class – Trebouxiophyceae :*

1. *Chlorella vulgaris* Beyerinck :

Cells spherical, 5 - 10 µm in diameter; chloroplast a parietal cup, sometimes without a pyrenoid.

*Class – Ulvophyceae :*

1. *Cladophora* sp. :

Filaments usually with profuse lateral branching, attached by well- defined rhizoidal cells or free- floating as entangled masses of indefinite shape; vegetative cells with thick stratified walls, multinucleate, much longer than broad; chromatophores single.

Table-1. Nature of the substratum and the species composition.

Crust Appearance	Moss association	Class	Species Composition
Blackish brown crust	Yes	Cyanobacteria Cyanobacteria	<i>Westiellopsis prolifica</i> <i>Fischerella</i> sp
Black crust	Yes	Cyanobacteria Cyanobacteria	<i>Schizothrix telephoroides</i> <i>Leptolyngbya scottii</i>
Light brown crust	No	Chlorophyceae Cyanobacteria Cyanobacteria	<i>Microspora floccosa</i> <i>Hepalosiphon</i> sp <i>Synechococcus aeruginosus</i>
Black patch	No	Cyanobacteria Chlorophyceae	<i>Nostoc</i> sp <i>Chlorococcum acidum</i>
Brownish black patch	Yes	Cyanobacteria Cyanobacteria	<i>Scytonema</i> sp <i>Lyngbya aestuarii</i>
Dark brown patch	Yes	Zygnemaceae Chlorophyceae Ulvophyceae	<i>Zygnema</i> sp <i>Chlorella vulgaris</i> <i>Cladophora</i> sp
Greenish black patch	No	Chlorophyceae Chlorophyceae Cyanobacteria Cyanobacteria	<i>Microspora willeana</i> <i>Microspora pachyderma</i> <i>Oscillatoria limosa</i> <i>Closterium navicula</i>
Black mat	No	Cyanobacteria Cyanobacteria	<i>Scytonema ocellatum</i> <i>Gloeotheca tepidariorum</i>
Brown mat	No	Cyanobacteria Cyanobacteria	<i>Leptolyngbya vincentii</i> <i>Nostoc commune</i>

*Physico-chemical parameters of soil :*

pH was acidic and ranged from 5.87 in black patches to 6.32 in brownish black patch. Accumulation of plants litter, mosses, lichens and activity of the microbiota on litter led to higher amount of organic matter in soil crust<sup>4</sup>. Thick crust with higher organic matter compared to thin crust with low organic matter content was recorded from few studies<sup>11,22</sup>. This was also true in our observation where organic carbon of 1.45% was observed in black patch and 0.67% in brown mat. Crust formation did not play any role on soil pH but greatly enhanced soil moisture and nitrogen content<sup>6,10</sup>.

Chlorophyll content was however observed to be higher in greenish black patch (132  $\mu\text{g g}^{-1}$ ) and lower in light brown crust (43  $\mu\text{g g}^{-1}$ ) (Table-2). Chlorophyll a content of soil from four different regions of India was recorded which ranged from 121  $\mu\text{g g}^{-1}$  to 284  $\mu\text{g g}^{-1}$ <sup>20</sup>.

Positive correlation between soil moisture content with organic carbon clearly indicated that moisture content in the crust greatly enhanced the amount organic carbon. Correlation between the chlorophyll content in crust with soil parameters revealed that the growth of Cyanobacteria/algae on the soil surface positively influenced the moisture content and organic carbon in soil (Table-3).

Table-2. Physico-chemical parameters and Chlorophyll content of the soil crust

<b>Crust appearance</b>	<b>pH</b>	<b>Soil moisture (%)</b>	<b>Soil organic carbon (%)</b>	<b>Chlorophyll content (<math>\mu\text{g g}^{-1}</math>)</b>
<b>Blackish brown crust</b>	6.13	11.23	1.32	87
<b>Black crust</b>	6.22	19.34	1.21	97
<b>Light brown crust</b>	5.87	12.65	0.76	43
<b>Black patch</b>	6.08	9.54	1.45	95
<b>Brownish black patch</b>	6.32	9.12	1.21	98
<b>Dark brown patch</b>	6.12	14.23	1.19	116
<b>Greenish black patch</b>	5.94	21.67	1.13	132
<b>Black mat</b>	6.10	19.54	0.87	87
<b>Brown mat</b>	6.09	19.54	0.67	89
<b>Average <math>\pm</math>SD</b>	<b>6.09<math>\pm</math>0.13</b>	<b>15.20<math>\pm</math>4.85</b>	<b>1.09<math>\pm</math>0.26</b>	<b>93.77<math>\pm</math>24.18</b>

Table-3. Pearson's correlation coefficient between soil parameters with Chlorophyll content in crust

<i>Parameters</i>	pH	Moisture content (%)	<i>Organic carbon (%)</i>
Moisture content (%)	-0.26	1	-
Organic carbon (%)	0.41	0.48*	1
Chlorophyll content ( $\mu\text{g g}^{-1}$ )	0.2762	0.63*	0.46

\* indicates significant at  $p < 0.05$



## Photoplates

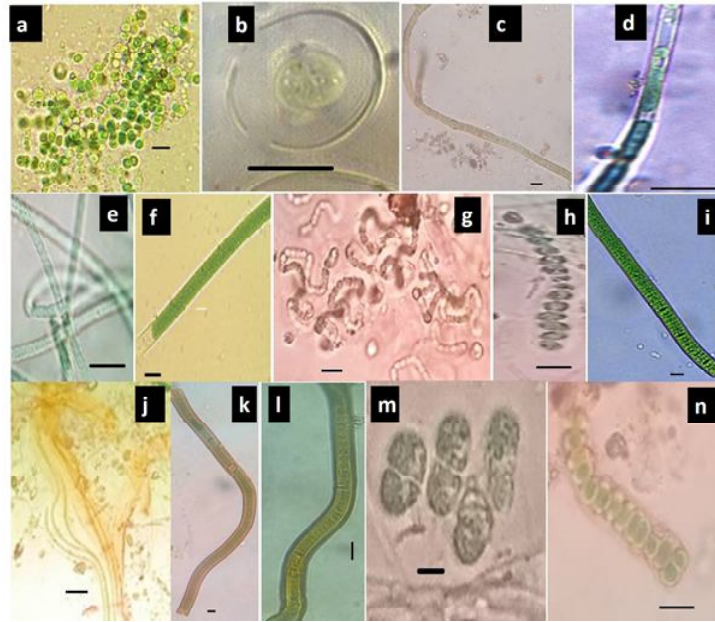


Fig. 3. Cyanobacteria- a. *Fischerella* sp, b. *Gloeothece tepidariorum*, c. *Hapalosiphon* sp, d. *Leptolyngbya scottii*, e. *Leptolyngbya vincentii*, f. *Lyngbya aestuarii*, g. *Nostoc commune*, h. *Nostoc* sp, i. *Oscillatoria limosa*, j. *Schizothrix telephoroides*, k. *Scytonema ocellatum*, l. *Scytonema* sp, m. *Synechococcus aeruginosus*, n. *Westiellopsis prolifica*.



Fig 4: Chlorophyceae - a. *Chlorococcum acidum*, b. *Microspora floccosa*, c. *Microspora pachyderma*, d. *Microspora willeana*; Zygnematophyceae - e. *Closterium navicula*, f. *Zygnema* sp; Trebouxiophyceae - g. *Chlorella vulgaris*; Ulvophyceae - *Cladophora* sp.



## References :

1. Adhikary, S. P. and J. K. Sahu (2000). *Indian J. Microbiol.*, 40: 53–56.
2. Anderson, J. M. and J.S.I. Ingram (1993). *Tropical soil biology and fertility: A Handbook of Methods* (2nd Edition) C.A.B. International Wallingford, UK.
3. Belnap, J.B. Büdel and O.L. Lange (2001) *Biological soil crust: Characteristics and distribution* In; *Biological soil crusts: Structure, Function and Management*, 3-30
4. Belnap, J. and K. T. Harper (1995). *Arid Soil Res. Rehabil.*, 9: 107-115.
5. Büdel, B. (2002) Diversity and ecology of biological soil crusts. *Progress in Botany, Springer*, Berlin, 63: 386-404.
6. Chamizo, S., Y. Cantón, I. Miralles-Mellado and F. Domingo (2012). *Soil Biol. Biochem.*, 49: 96-105.
7. Deb, S., B. Sarma, J. Rout, and M. Sengupta (2013) *Phykos.*, 43(1): 56-67.
8. Desikachary, T. (1959) *Cyanophyta*, I. C. A. R. Monograph on algae, New Delhi.
9. Dirborne, C.M. and P. Ramanujam (2020) *J. Algal Biomass Util.*, 11(2): 13-24.
10. Garibotti, I. A., M. G. Polo, and S. Tabeni, (2018) *Funct. Ecol.*, 32: 1065-1078.
11. Jafari, M., Tavili, A., Zargham, N. Heshmati, G. A. Zare Chahouki, M. A., Shirzadian, S., Azarnivand, H., Zehtabian, G. R. and M. Sohrabi (2004). *Pak. J. Nutr.*, 3: 273-277.
12. Johansen, J. R. (1993) *J. Phycol.*, 29 : 140–147.
13. John, D. M., B. A. Whitton, and A. J. Brook (2002) *The freshwater algal flora of the British Isles: An identification guide to freshwater and terrestrial algae*, Cambridge University Press, Cambridge.
14. Mackinney, G. (1941) *J. Biol. Chem.*, 140: 315-320.
15. Samad, L. K., M. Jena, and S. P. Adhikary (2008) *Bull. Bot. Sur. India*, 50(1-4): 51-56.
16. Sethi, S.K., L.K. Samad and S.P. Adhikary (2012) *Phykos.* 42(1): 1-9.
17. Smith, G.D., R.M. Lynch, G. Jacobson and C. J. Barnes (1990) *FEMS Microbiol. Ecol.*, 74: 79–90.
18. Tiffany, L.M. (1951) *Manual of Phycol. Chron. Bot.*, 293-311.
19. Tirkey, J. and S.P. Adhikary (2006) *Feddes Repert.*, 117(3–4): 280–306.
20. Tirkey, J. and S. P. Adhikary (2005) *Current Science*, 89(3): 515-521.
21. Vinoth, M., P. Muruganantham, G. Jeevanantham, M.J. Hussain, B. Balagura and A. K. Ahamed (2017) *RJLBPCS*, 3(3): 215-241.
22. Wang, J. and R. Wang (2019). *Sustainability*, 11: 1-10.
23. Zhao, J., Y. Zheng, B. Zhang, Y. Chen, and Y. Zhang (2009). *Front. Biol.*, 4(2): 143–150.