

**Research article**

First report of diversity of Cyanoprokaryotes and Algae from aero-terrestrial biofilms on Roxburgh building of Acharya Jagadish Chandra Bose Indian Botanic Garden, Howrah, West Bengal, India

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Abstract: Roxburgh Building in Acharya Jagadish Chandra Bose Indian Botanic Garden (AJCBIBG), Howrah is more than 200 years old. Dr. William Roxburgh, an important botanist, is a significant figure in the natural history of both Britain and India. Dr. William Roxburgh was appointed as the first salaried superintendent of the Garden in 1793. He laid the foundation of modern plant taxonomy (post Linnean) in India by establishing a large Herbarium (the present-day Central National Herbarium - CAL) during his tenure (1793–1814). Cyanoprokaryotes and algae develop on the building surfaces which may form more complex consortia i.e. biofilms. The investigations of the microorganisms on the surface of Roxburgh Building have shown that microorganisms thrive, particularly Cyanoprokaryotes, Algae, etc. that form biofilms and crusts on the surfaces. During study altogether 16 species of different classes of Cyanoprokaryotes and algae viz. Cyanoprokaryota/Cyanobacteria, Ulvophyceae, Conjugatophyceae, Phaeothamniophyceae and Bacillariophyceae have been recorded. Out of sixteen species of Cyanoprokaryotes and algae, 02 species of Cyanoprokaryotes have been reported as new to the science of India. This is the first study report on the diversity of Cyanoprokaryotes and algae from Roxburgh Building of AJCBIBG.

Keywords: Biofilm - Algae - Cyanoprokaryotes - Bbuilding surface - Roxburgh Building.

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INTRODUCTION

Indian Botanic Garden, Howrah established in 1787 by Col. Robert Kyd under East India Complaný's patronage, the Company Bagan presently known as Acharya Jagadish Chandra Bose Indian Botanic Garden, Howrah which spread in 273 acres on the west bank of the river Ganga (Hooghly) depicted in Figure 1. Geographically it is located at 22° 35' N latitude and 88° 21' E longitude at an elevation of 4.6 m above the sea level.

India, scientifically knowing about the plants with developing concept of taxonomy started in 1793 by Dr. William Roxburgh while working in the 'Company Garden', Shibpur, Howrah. Roxburgh Building is a building of 18th century, >200 years old situated in Acharya Jagadish Chandra Bose Indian Botanic Garden, Howrah where the "Father of Indian Botany", William Roxburgh, lived. On right side adjacent to the Roxburgh House is the herbarium established by Scottish surgeon-cum-botanist for his collection of plant specimens, and made beautiful coloured drawings of these collected specimens by Indians. Many of the advances to the original gardens were brought about during botanist William Roxburgh's tenure as its Superintendent. He brought plants from all over the country and created a vast herbarium on the premises and worked extensively on a variety of plants and published multiple works on Indian Botany. He has not only done the distinction of naming numerous species, but also number of species was named in his honour. William Roxburgh left behind a large

collection of coloured Icons, more than 2500 original colour paintings of Indian plants using vegetable dyes, a large number of manuscripts, which are preserved in the archives of the Central National Herbarium, Botanical Survey of India, Howrah.



Figure 1. Base Map of Acharya Jagadish Chandra Bose Indian Botanic Garden, Howrah.

Aero-terrestrial biofilms are microbial mats with a wide range of temperature and along with atmospheric moisture act as an ambience for Cyanoprokaryotes and algal growth. The Extracellular Polymeric Substances (EPS) secreted by Cyanoprokaryotes and algae also help in the adherence of these biofilms to the substratum (Rosenberg, 1989). The climate is the regulating factor in the composition of microbes in these biofilms. In different climatic regions, a different group of algae dominates in biota (Samda & Adhikary 2008, Adhikary *et al.* 2015). Studies on the diversity of Cyanoprokaryotes and algae from aero-terrestrial biofilms from Roxburgh building has remain untouched. Keeping this in view, an attempt has been made to study and present the factual diversity of Cyanoprokaryotes and algal profiles from the biofilm on Roxburgh Building.

MATERIALS AND METHODS

Cyanoprokaryotes and algal samples were collected from the surface of Roxburgh Building (Fig. 2) of Acharya Jagadish Chandra Bose Indian Botanic Garden, Howrah. Samples from different levels of the cemented wall like upper, middle and lower portion, etc. were collected depending upon the availability of Cyanoprokaryotes and algae in different parts of the Roxburgh Building by gently scraping the green, bluish-greenish, yellowish-green patches with sterilized scalpel and forceps in screw cap sampling vials of Tarsons (size 25×50 mm & 25×75 mm) and brought to the laboratory. Then added double distilled water to make the volume at least 10 ml in each vial and preserved the specimens by adding 2–4 drops of 4% Formalin for studies.

All specimens collected were observed under Leica DM 2500 Microscope (4x, 10x, 20x, 40x, 63x and 100x magnification) using Leica Qwin V 3.2 Image Processing and Analysis Software and Leica Application Suit V4 Software. The specimens of varying morphological and structural characteristic, shape and size of cell(s), colonies, filaments, trichomes, heterocysts, sheath, protoplasm and striae, etc. were studied in detail. Photomicrographs were taken by using Leica DFC 500 digital camera with annotation for documentation.

The specimens were identified consulting standard monographs, books, proceedings like Geitler (1932), Tiffany & Britton (1952), Desikachary (1959), Prescott (1982), Starmach (1985), Kant & Gupta (1998), Komárek & Anagnostidis (1998, 2005), Bertalot & Genkal (1999), Guiry (2022), Kristiansen & Preisig (2007), Karmmer & Bertalot (2008a, b).

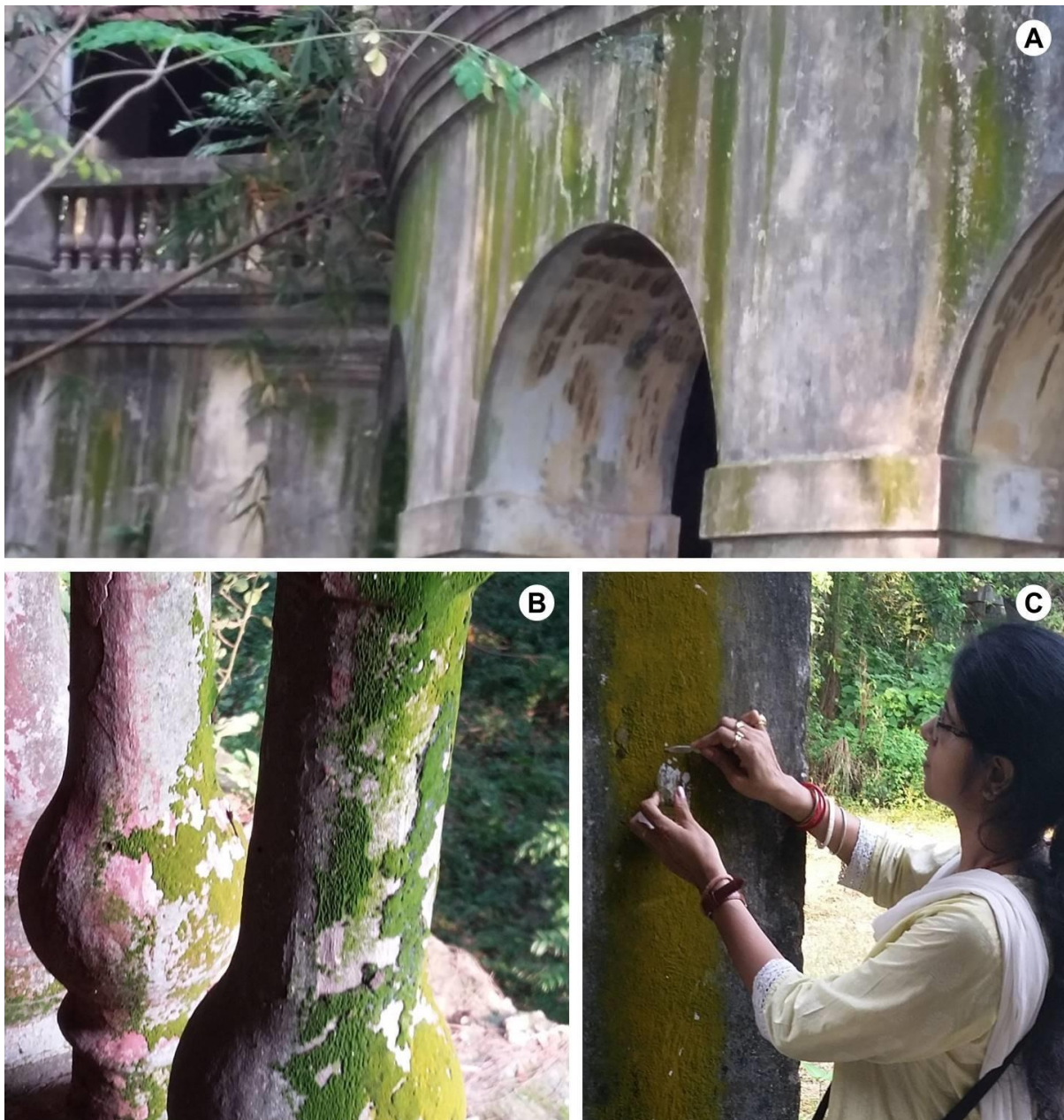


Figure 2. A, Algal patches on Roxburgh Building; B, Close-up of algal patches; C, collection of different groups of algal patches.

All specimens in screw cap sampling vials with proper labelling are properly maintained in the Central Botanical Laboratory, Botanical Survey of India, Ministry of Environment Forest and Climate Change, Government of India, Botanic Garden, Howrah.

In the text, authority name are cited as described in Authors of Plant Names by Brummitt & Powell (1992), books in accordance with Stafleu & Cowan (1976, 1979, 1981, 1983, 1985, 1986, 1988) and supplements as described by Stafleu & Mennege (1992, 1993, 1995, 1997, 1998, 2000), Journals, Periodicals with Botanical content as described by Bridson (2004a, b).

RESULTS AND DISCUSSION

Systematic Enumeration

Systematic studies provide basic information about cyanoprokaryotes and algal diversity observed from aero-terrestrial biofilms on the surface of Roxburgh Building of Acharya Jagadish Chandra Bose Indian Botanic Garden (AJCBIBG). Group-wise quantitative details of cyanoprokaryotes and algae depicted in figure 3.

Taxonomic Enumeration of Cyanoprokaryotes and Algae

Taxonomic enumeration of identified Cyanoprokaryotes and algae are described here along with their details including nomenclature.

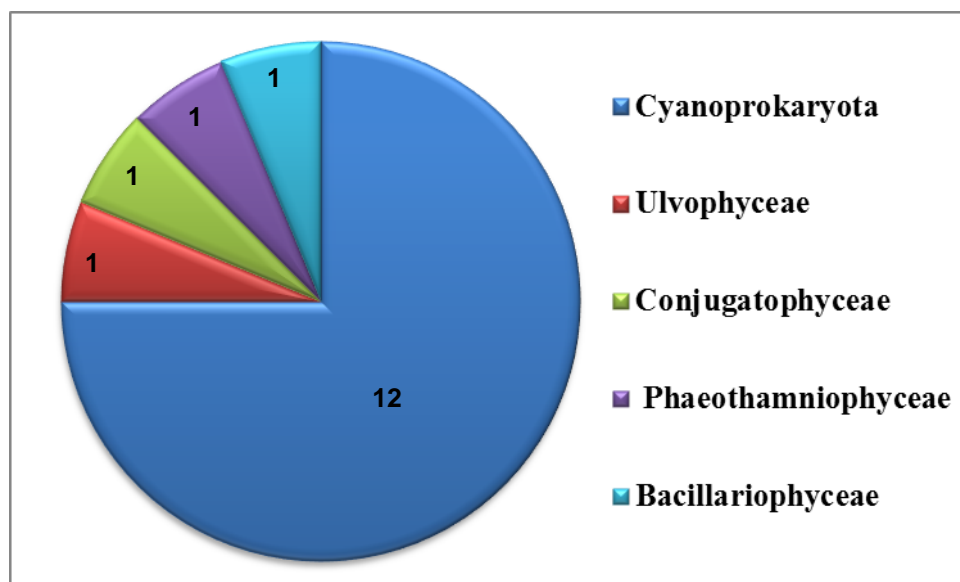


Figure 3. Cyanoprokaryotes and Algae recorded from Roxburgh Building.

Cyanophyceae
 Chroococcales
 Chroococcaceae
Chroococcus Nägeli

Chroococcus minor (Kütz.) Nägeli, Neue Denkschr. Allg. Schweiz. Ges. Gesamten Naturwiss. 10[7]: 46. 1849; Desikachary, Cyanophyta 105, t. 24, f. 1. 1959. **[Fig. 4A]**

Protococcus minor Kützing, Phycol. Germ.: 144. 1845.

Thallus slimy gelatinous, dirty blue-green or olive green in colour; cells more or less spherical, single or in pairs, sometimes 4 or 8; sheath colourless, very thin, hardly visible.

Dimension: Cells 3.0–4.5 µm in diameter.

Environment: Freshwater and Marine species.

Habitat: Generally found in wide range of freshwater environments, but especially shallow ponds and slow-flowing streams with submerged macrophytes.

Chroococcus minutus (Kütz.) Nägeli, Neue Denkschr. Allg. Schweiz. Ges. Gesamten Naturwiss, 10(7): 46, 1849. **[Fig. 4B]**

Protococcus minutus Kütz., Phycol. General. 168, 1843.

Microscopic colonies gelatinous or slimy, colourless, dirty olive-green or brownish; cells solitary or in 2–4 celled groups or sometimes 8 cells, rarely in multicellular, irregular slimy clusters; colonial slime wide, indistinctly lamellate or homogeneous, refractive, colourless or yellowish to orange-yellowish; cells spherical or hemispherical, cells and their small group (2–4 celled) surrounded by individual colourless, indistinctly and concentrically lamellate envelopes, cells green, olive-green, rarely yellowish content; cell division usually irregular in different oblique planes.

Dimension: Cells 4.47–5.59 µm in diameter; with sheath 7.6–12.75 µm in diameter.

Environment: Aerophytic, Epilithic and Freshwater species.

Cyanosarcina L.Kováčik

Cyanosarcina parthenonensis Anagn., in Anagnostidis & Pantazidou, Hydrobiol. Suppl. 92 (Algol. Stud. 64): 144, f. 24. 1991; Komárek & Anagn., Cyanoprokaryota Part 1: Chroococcales 19(1): 312, f. 415. 1998. **[Fig. 4C]**

Thallus olive-green, consisting of 2–16 or more celled colonies, later compound and multicellular in more or less cubic, dense, packet-like aggregates, with thin colourless and structure less, mucilaginous envelope; cells spherical after division sub globose or sub spherical, olive-green, yellow-green rarely olive-brown, finely granulate protoplasts.

Dimension: Colony 35.68 µm in diameter and cells 4.61–5.9 µm in diameter.

Environment: Terrestrial species.

Gloeocapsopsis Geitler ex Komárek

Gloeocapsopsis crepidinum (Thuret) Geitler ex Komárek, Bull. Natl. Sci. Mus. Tokyo, ser. B (Bot.), 19: 24, 1993; Komárek & Anagn., Cyanoprokaryota Part 1: Chroococcales 19(1): 275, f. 361. 1998. [Fig. 4D]
Protococcus crepidinum Thuret, Mém. Soc. Imp. Sci. Nat. Cherbourg 2: 388, 1854.

Colonies large, gelatinous, irregular, dirty greenish; cells irregular spherical with thin colourless and sometimes diffuent envelope; envelope yellowish green or yellowish brown near the colonial surface, not lamellate; outer envelope hyaline; cell content pale blue-green usually homogeneous.

Dimension: Cells 3.05–4.01 µm in diameter.

Environment: Freshwater, Halophilic, Epilithic and Terrestrial species.

Gloeocapsopsis pleurocapsoides (Nováček) Komárek & Anagn. ex Komárek, Bull. Natl. Sci. Mus. Tokyo, ser. B (Bot.) 19: 24, 1993; Komárek & Anagn., Cyanoprokaryota Part 1: Chroococcales 19(1): 277, f. 362. 1998. [Fig. 4E–G]

Gloeocapsa pleurocapsoides Nováček, Mohelno, Arch. Svazu Ochr. Přír. A Domov. Zemi Moravskoslezské 3a: 93, 133, t.1: figs. 2, 6, 7; t. 3(lower half): figs. 1-12; t. 5, f. 7. 1934.

Colonies microscopic, irregular, composed of aggregations of solitary, ensheathed cells or their groups, crustaceous, dark olive-green, brownish or blackish-brown, gelatinous or flat, squamulose; cells sub-spherical, more or less oval, elliptical, irregularly rounded or of irregular outline, usually composed of sub-colonies, with greyish blue-green, pale olive-green or blue-green, homogeneous or finely granular content, with differentiated sominate and centropiasm, sometimes with small granules; envelop narrow, copying the cells, usually more or less lamellated, firm, not diffuent, yellowish inside more intensely, outer lightly coloured; resting cells more or less spherical, irregular, yellow-brownish or brown.

Dimension: Cells 2.24–7.51–12.0 µm in diameter.

Environment: Aerophytic species.

Entophysalidaceae

Entophysalis Kütz.

Entophysalis deusta (Meneghi.) F.E. Drouet & W.A. Daily, Lloydia 11: 79. 1948. [Fig. 4H]
Coccochloris deusta Meneghi., Atti Riun. Sci. Ital. 2: 173, 1841.

Colonies flat, granular, dirty yellow-brown composed of many small, irregular rounded sub-colonies; these sub-colonies with 1–2–4 more cells with lamellate, not very wide, firm, delimited, yellow-brown; cells more or less spherical or slightly elongate, pale blue-green; mucilaginous envelop colourless to brown, lamellate, with distinct outer brown layer.

Dimension: Cells 4.2–4.52 µm in diameter.

Environment: Marine and subaerophytic, Epilithic species.

Microcystaceae

Gloeocapsa Kütz.

Gloeocapsa aeruginosa Kütz., Phycol. General. 174, 1843; Komárek & Anagn., Cyanoprokaryota Part 1: Chroococcales 19(1): 239, f. 310. 1998. [Fig. 4I]

Colonies granular or gelatinous, sometimes forming amorphous mass, blue-green or greyish olive-green, composed of almost spherical, many celled sub-colonies; mucilaginous envelopes colourless, delimited, more or less inflated; cells small, spherical, blue-green.

Dimension: Cells 2.0–3.1 µm in diameter and 4.1–7.9 µm in diameter with sheath.

Environment: Aerophytic and Freshwater species.

Gloeocapsa novacekii Komárek & Anagn., Presilia 67: 19, figs. 1 - 7. 1995; Komárek & Anagn., Cyanoprokaryota Part 1: Chroococcales 19(1): 252, f. 328. 1998. [Fig. 4J]

Colonies gelatinous, granular, dirty or blackish brown, composed of subcolonies; mucilaginous envelops wide, finely granular; cells spherical or wide oval, pale olive-green, blue-green or yellowish.

Dimension: Cells 3.5–5.51 µm in diameter.

Environment: Aerophytic species.

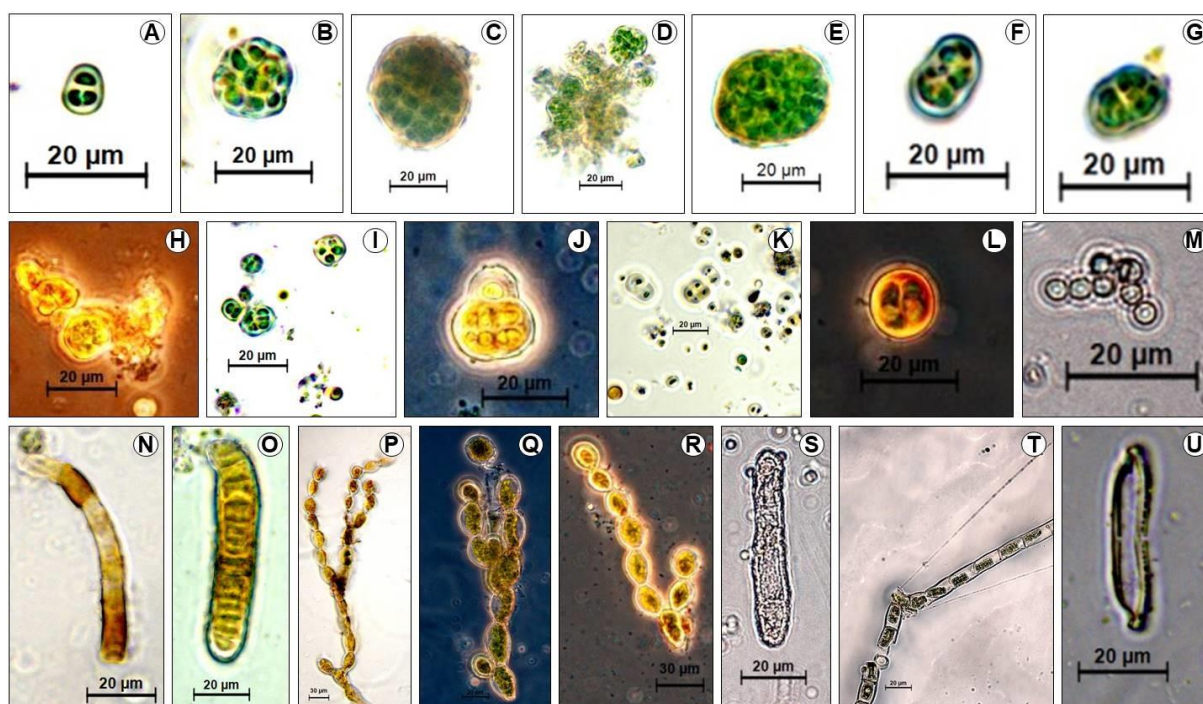


Figure 4. A, *Chroococcus minor* (Kütz.) Nägeli; B, *Chroococcus minutus* (Kütz.) Nägeli; C, *Cyanosarcina parthenonensis* Anagn.; D, *Gloeocapsopsis crepidinum* (Thuret) Geitler ex Komárek; E–G, *Gloeocapsopsis pleurocapsoides* (Nováček) Komárek & Anagn. ex Komárek; H, *Entophysalis deusta* (Meneghi.) F.E. Drouet & W.A. Daily; I, *Gloeocapsa aeruginosa* Kütz.; J, *Gloeocapsa novacekii* Komárek & Anagn.; K & L, *Gloeocapsa violacea* Kütz.; M, *Aphanocapsa thermalis* Brügger; N, *Calothrix brevissima* G.S. West; O, *Scytonema pseudopunctatum* Skuja; P–R, *Trentepohlia rigidula* (J.Müller) Hariot; S, *Penium margaritaceum* Bréb.; T, *Phaeothamnion confervicola* Lagerheim.; U, *Hantzschia amphioxys* var. *capitata* Zimmermann.

Gloeocapsa violacea Kütz., Tab. Phyc. 1: 25, t. 36, f. 9. 1847.

[Fig. 4K & L]

Colonies granular, blue-green, blackish; sub colonies usually 2–4 celled, rarely with more cells jointed together with wider outer colourless or bluish to very intensely coloured, usually not or slightly striated sheath layers; cells blue-green.

Dimension: Cells 4.5–5.71 µm in diameter.

Environment: Aerophytic species.

Synechococcales
Merismopediaceae
Aphanocapsa Nägeli

Aphanocapsa thermalis Brügger, Jahresber. Naturf. Ges. Graubündens, ser. 2, 8: 244, 1863; Komárek & Anagn., Cyanoprokaryota Part 1: Chroococcales 19(1): 156, f. 187. 1998. [Fig. 4M]

Colonies mucilaginous, irregular, formless with homogeneous colourless mucilage, usually with densely packed cells, they touch one to another, blue-green; cells spherical or slightly oval, bright blue-green, sometimes with individual fine, diffluent mucilaginous envelope especially in peripheral parts of colonies.

Dimension: Cells 3.56–4.39 µm in diameter.

Environment: Freshwater and sub-aerophytic species.

Nostocales
Rivulariaceae
Calothrix C.Agardh ex Bornet & Flahault

Calothrix brevissima G.S.West, J. Linn. Soc., London Botany 38: 180, t. 10, f. 8. 1907; Desikachary, Cyanophyta 533, t. 114, f. 1. 1959. [Fig. 4N]

Filament epiphytic, short, not or very little, very little attenuated, sheath firm, very close to the trichome, thin, nearly cylindrical, colourless; trichome short slightly attenuated with rounded end cells, not constricted at the cross-walls, olive green; cells at the base nearly as long as broad; heterocysts basal, single, more or less rounded, hemispherical or sub-spherical.

Dimension: Filament 4.03–7.51 µm in diameter.

Environment: Epiphytic and Freshwater species.

Scytonemataceae

Scytonema C.Agardh ex Bornet & Flahault*Scytonema pseudopunctatum* Skuja, Nova Acta R. Soc. Sci. Upsal, Ser. 414(5): 38, t. 4, figs. 1–9. 1949; Desikachary, Cyanophyta 469, t. 96, f. 3. 1959. [Fig. 4O]

Thallus pulvinate, attached to the substratum, not attenuated, olivaceous to slightly blackish; filament partly prostrate, mostly erect, more or less densely intertwined, false branched, rarely solitary or geminate, generally short; sheath moderately thick, with parallel lamellation, close, colourless or yellow or yellowish brown; trichome mostly constricted at the cross-walls, slightly constricted at meristematic region; cells isodiametric or in parts, cell content pale olivaceous; homogeneous or sparsely granulated; heterocysts cylindrical with rounded ends, frequently lightly constricted in the middle region or discoid or rounded quadrate, yellowish membrane; propagation by hormogones and hormocysts formed from the apices.

Dimension: Filament 14.02–17.0 µm in diameter; trichome 10.5–16.04 µm in diameter; heterocysts 15.0–16.01 µm in diameter.

Environment: Terrestrial species.

Ulvophyceae

Trentepohliales

Trentepohliaceae

Trentepohlia C.Martius*Trentepohlia rigidula* (J.Müller) Hariot, J. Bot. 3: 403, 1889. [Fig. 4P–R]*Coenogonium igidulum* J.Müller, Flora 65: 490, 1882.

Thallus forms compact crusts of filaments on tree bark and varied from orange to yellowish-red in colour; uniseriate filaments showed prostrate and an erect portion; vegetative cells are arranged in uniseriate chain and sporangia borne on the stalk or superimposed on the vegetative cells, vegetative cells are elliptical, barrel shaped, two cells divided by a strong constriction or septum; cell wall thick and sometimes rough; sporangium varied from globular, orbicular or dome shaped, with 15–30 µm diameter, sporangia globose, sometimes oval and larger than the vegetative cells, generally formed on the filament in apical, intercalary or in lateral position.

Dimension: Vegetative cells 6.0–12.0 µm in diameter and 12.86–15.0 µm in length.

Environment: Aerophytic species.

Habitat: Polymorphic species widely distributed in tropical and subtropical regions, usually found on tree bark but also on rocks, cement walls and artificial substrata.

Charophyta

Conjugatophyceae (Zygnematophyceae)

Desmidiales

Peniaceae

Penium Bréb ex Ralfs*Penium margaritaceum* Bréb., Brit. Desmid 149, t. 25, f. 1; t.33, f. 3. 1848; Tiffany & Britton, The Algae of Illinois 176, t. 51, f. 540. 1952. [Fig. 4S]

Cells cylindric or subfusiform, very slightly attached to the truncately rounded apices; cell-wall brown with longitudinal rows of minute granules; chromatophores axial, with about 10 longitudinal ridges; pyrenoids numerous, in a median series.

Dimension: Cells 8.90–12.8 µm in diameter.

Environment: Freshwater species.

Ochrophyta

Phaeothamniophyceae

Phaeothamniales

Phaeothamniaceae

Phaeothamnion Lagerheim.*Phaeothamnion confervicola* Lagerheim., in Wittrock & Nordstedt, Bot. Not. 124, 1884 (as '*conferviculum*'); S. Kant and P. Gupta, Algal Flora of Ladakh 141 & 263, t. 68, f. 7. 1998. [Fig. 4T]

Thallus forms branched filaments; Cells cylindrical barrel-shaped to sub-ovoid jointed end to end in branched filament with a conspicuous central axis and sub-erect lateral branches prominent hemispherical cell without chromatophores attaches the plant to the substratum, mostly grows epiphytic.

Dimension: Cells 10.4–13.52 µm in diameter and 18.8–19.2 µm long.

Environment: Epiphytic and Freshwater species.

Bacillariophyceae

Bacillariales

Bacillariaceae

Hantzschia Grunow

Hantzschia amphioxys var. *capitata* Zimmermann, Brotéria Bot. 16: 88, t. 2, f. 8. 1918; B.N. Prasad & M.N. Srivastava, Algal Flora of Andaman and Nicobar Island Vol. 1: 313. [Fig. 4U]

Valves narrowly linear-lanceolate, dorsal side convex, ventral side slightly concave with distinct deep median depression; ends slightly attenuated, constricted rounded-capitate; keel punctae distinct, thick, slightly elongated median two set apart, central nodule prominent; striae fine, lineate, parallel, throughout the valve.

Dimension: Length 41.0–42.34 µm and 7.0–8.74 µm in diameter; keel punctae 7–8 µm; striae 22.0–24.0 in 10 µm.

Environment: Freshwater and Terrestrial species.

DISCUSSION AND CONCLUSION

Altogether sixteen species of Cyanoprokaryotes and algae were observed from Roxburgh Building of AJCBIBG. Maximum 12 species of Cyanoprokaryotes of 08 genera followed by single species of class Ulvophyceae, Conjugatophyceae, Phaeothamniophyceae and Bacillariophyceae were observed during the study. In Cyanoprokaryotes maximum number of 03 species of genera *Gloeocapsa* Kütz. recorded namely *Gloeocapsa aeruginosa* Kütz., *Gloeocapsa novacekii* Komárek & Anagn. and *Gloeocapsa violacea* Kütz. followed by 02 species of *Chroococcus* Nägeli viz. *Chroococcus minor* (Kütz.) Nägeli, *Chroococcus minutus* (Kütz.) Nägeli and 02 species of *Gloeocapsopsis* Geitler ex Komárek viz. *Gloeocapsopsis crepidinum* (Thuret) Geitler ex Komárek and *Gloeocapsopsis pleurocapsoides* (Nováček) Komárek & Anagn. ex Komárek. However, only single species of Cyanosarcina L.Kováčik - *Cyanosarcina parthenonensis* Anagn., *Endophysalis* Kütz. - *Entophysalis deusta* (Meneghi.) F.E. Drouet & W.A. Daily, *Aphanocapsa* Nägeli - *Aphanocapsa thermalis* Brügger, *Calothrix* C.Agardh ex Bornet & Flahault - *Calothrix brevissima* G.S. West and *Scytonema* C.Agardh ex Bornet & Flahault - *Scytonema pseudopunctatum* Skuja. recorded. Besides this, single species of rest of the 04 classes were observed namely Ulvophyceae - *Tentepohlia rigidula* (J.Müller) Hariot, Conjugatophyceae - *Penium margaritaceum* Bréb., Phaeothamniophyceae - *Phaeothamnion confervicola* Lagerheim. and Bacillariophyceae - *Hantzschia amphioxys* var. *capitata* Zimmermann from aero-terrestrial biofilms of Roxburgh Building of AJCBIBG. *Trentepohlia rigidula* (J.Müller) Hariot, *Chroococcus minor* (Kütz.) Nägeli, *Gloeocapsa aeruginosa* Kütz. and *Gloeocapsa violacea* Kütz. were commonly observed species in the samples. Two species of Cyanoprokaryotes viz. *Gloeocapsa novacekii* Komárek & Anagn. and *Entophysalis deusta* (Meneghi.) F.E.Drouet & W.A.Daily reported for the first time from India. Few studies of Cyanoprokaryotes and algae has been reported from historic buildings, limestone walls, monuments, etc. (Chadha & Pandey 1982, Danin & Caneva 1990, Adhikary 2000, Crispim *et al.* 2003, Deepa *et al.* 2011, Nikola *et al.* 2017). Cyanobacteria and algae develop on the building surfaces which may form more complex consortia (biofilms). Cyanoprokaryotes and algae as organisms that combine water cause physical corrosion of such materials via penetrating into the porous systems of building materials and contribute to the formation of micro-fissures. Due to higher moisture contents Cyanoprokaryotes and algae increased quantitatively and erode the surface and surrounding. Along with the impurities from the atmosphere (dust particles, microorganisms, etc.) they form a bio-layer of the material surface that further supports the retention of water. In addition, Cyanoprokaryotes and algae may also participate in the formation of a crust as they produce the so-called Extracellular Polymer Substances (EPS). These substances significantly affect the physicochemical properties of materials like building walls, stones, etc. and support the Cyanoprokaryotes and algal growth and activity leading to the release of inorganic substances useful for organisms in the same environment.

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