Isolation and morphological characterization of cyanobacterial strains

5.1 Introduction

The large number of existing species of cyanobacteria constitutes a unique reservoir of biodiversity, which supports potential commercial exploitation of many novel products. Cyanobacteria have a worldwide distribution, and are well-adapted to survive under a different environmental stresses. Industrial wastewater rich with pollutants may exert stimulatory or inhibitory influences upon the metabolic activities of different

phytoplankton species, including filamentous cyanobacteria. However, many a times it is difficult to identify cyanobacteria in its natural population because of problems of distinguishing different species based on morphological, developmental and biochemical characteristics (Hiroki *et al*., 1998). Thus, isolation from their natural habitats and development of unialgal or axenic cultures facilitate the study and exploitation of the potential cyanobacteria. Information on cultivable cyanobacterial diversity in the industrially polluted sites and as well as unpolluted areas from the Panchgram is limited. Thus present chapter describes an account of isolation and morphological characterization of cultivable cyanobacteria from the soils of the selected sites of Panchgram in Hailakandi district, Assam, North East India.

5.2 Methodology

The detail methodology had already been discussed in **Chapter 3**. Dilution plate method (10^{-2}, 10^{-3}) was followed to quantify the cyanobacteria in the soil samples using BG-11 medium with or without combined nitrogen. Plates were incubated under the light intensity of 2000-3000 lux and made unialgal by repeated streaking on agar plates. The isolated strains were characterized in terms of their morphological and cultural behavior. Morphological characterization was done by analyzing cell dimensions (length and width of the vegetative cells, heterocysts and akinetes) of different filaments of each strain from late exponential phase fresh cultures. Behaviors of cyanobacteria in laboratory cultures were observed in liquid and agar-based medium. The taxonomic identification of the cyanobacteria was made based on the cell or colony morphology (Prescott, 1954 and Desikachary, 1959; Komárek & Komárková, 2004).

5.3 Results and discussion

The cyanobacterial strains were isolated from the soils of selected sites collected during the dry period. The genera wise distribution and abundance of cyanobacteria has been already discussed in **Chapter 4**. A total of 27 strains under 13 genera were made pure by repeated and frequent sub-culturing in liquid and solid medium. The name of the

Table 5.1 List of cyanobacterial strains along with the name of the sites

Sl. No.	**Name of the strain**	**location**
1	*Anabaena doliolum* Bharadw. (after Bharadwaja)	Solid wastes deposits
2	*Anabaena orientalis* Dixit (after Dixit)	Solid wastes deposits
3	*Aphanothece bullosa*	Paper mill wastewater
4	*Calothryx marchica* Lemm. (after Fremmy)	Paper mill wastewater
5	*Calothryx marchica* v. *crassa* Rao, C. B. (after Rao, C. B.)	Solid wastes deposits
6	*Calothryx marchica* v. *intermedia* Rao, C.B. (after Rao, C.B)	Solid wastes deposits
7	*Cylindrospermum majus* Kutz. (after Frémy)	Paper mill wastewater
8	*Cylindrospermum musicola* Kutzing ex. Born ex. Flah	Paper mill wastewater
9	*Cylindrospermum muscicola* var. longispora Dixit	Paper mill wastewater
10	*Fischerella muscicola* (Borzi) Gomont	Solid wastes deposits
11	*Lyngbya polysiphoniae* Frémy (after Frémy)	Paper mill wastewater
12	*Lyngbya limnetica* Lemmermann	Solid wastes deposits
13	*Nodularia spumigena* Mertens in Jürgens	Paper mill wastewater
14	*Nostoc carneum* Ag. (after Frémy)	Paper mill wastewater

15	*Nostoc commune* Vaucher ex Born. et Flah.	Paper mill wastewater
16	*Nostoc ellipsosporum v. violacea* Rao, C.B. (after Rao, C. B.)	Paper mill wastewater
17	*Nostoc linckia* v. (Roth) Born. et Flah. (after Frémy)	Paper mill wastewater
18	*Nostoc sphericum*	Solid wastes deposits
19	*Nostoc spongiforme v. tenue* Rao, C. B. (after Rao, C.B.)	Tree bark
20	*Oscillatoria formosa* Bory	Paper mill wastewater
21	*Phormidium autumnale (Ag.) Gom. (after Gomont)*	Solid wastes deposits
22	*Phormidium fragile (Menegh.) Gom.*	Tree bark
23	*Scytonema tolypothrichoides* Kutz.(after Kossinskaja)	Paper mill wastewater
24	*Tolypothryx byssoidea* (Berk.) Kirchn.	Tree bark
25	*Tolypothryx distorta* v. *penicillata* (Ag.) Lemm.	Tree bark
26	*Tolypothryx rechingeri* (Wille) Geitler (after Wille)	Paper mill wastewater
27	*Westiellopsis prolifica* Janet (after Janet)	Paper mill wastewater

cyanobacterial strains along with locations from where the cyanobacteria were isolated are given on the **Table 5.1**. Out of the 27 isolates, 15 strains from the industrially polluted river bank soil, 4 from tree barks and 8 from solid wastes were isolated. Cyanobacteria isolates included 21 heterocystous filamentous, 5 non-heterocystous filamentous and 1 unicellular form. Based on their morphological characteristics the strains were taxonomically assigned to 13 different genera and were identified as *Anabaena, Aphanothece, Calothryx, Cylindrospermum, Fischerella, Lyngbya, Nostoc, Nodularia, Oscillatoria, Phormidium, Scytonema, Tolypothryx* and *Westiellopsis*. Maximum number of strains was belonged to the genus *Nostoc* (5), followed by *Anabaena, Calothryx, Cylindrospermum, Phormidium* and *Tolypothryx*, each of which contained 3 strains. The

genus *Lyngbya* was represented by 2 species while the other 5 genera (*Aphanothece, Fischerella, Nodularia, Oscillatoria, Scytonema* and *Westiellopsis*) were represented by only one strain each.

Plate 5.1: *Anabaena doliolum* isolated from solid waste

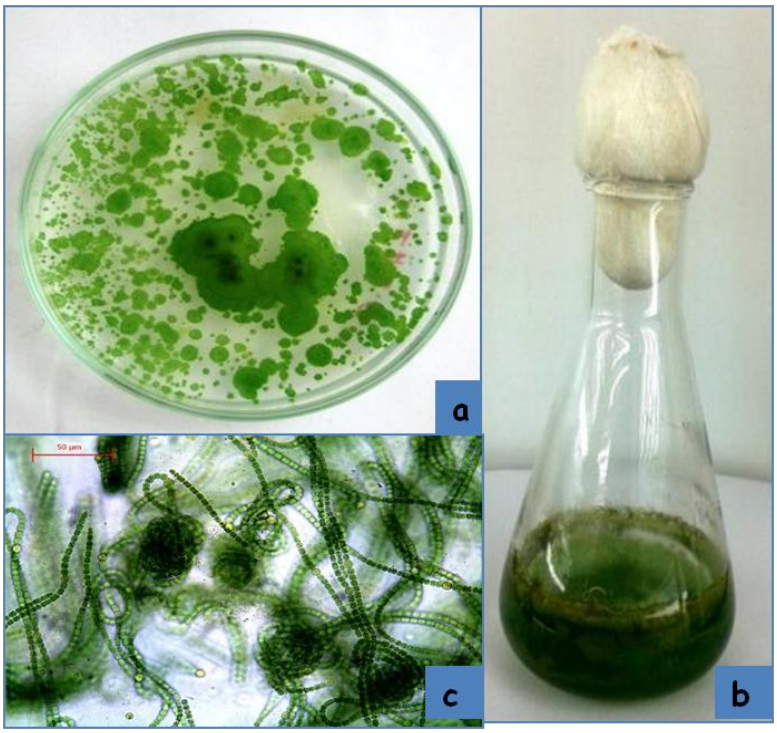

Plate 5.2: *Anabaena orientalis* isolated from solid waste

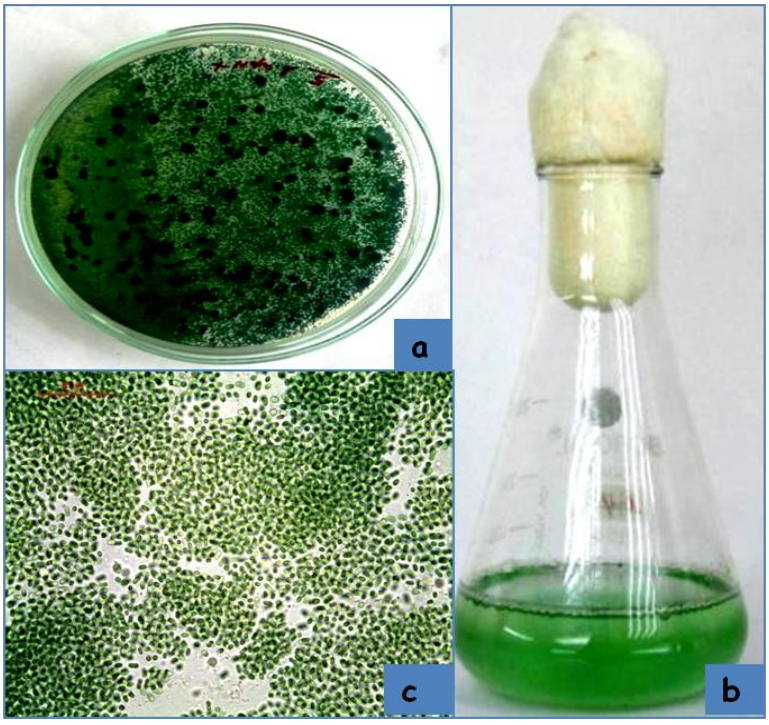

Plate 5.3: *Aphanothece bullosa* isolated from wastewater

Plate 5.4: *Calothryx marchica* isolated from wastewater

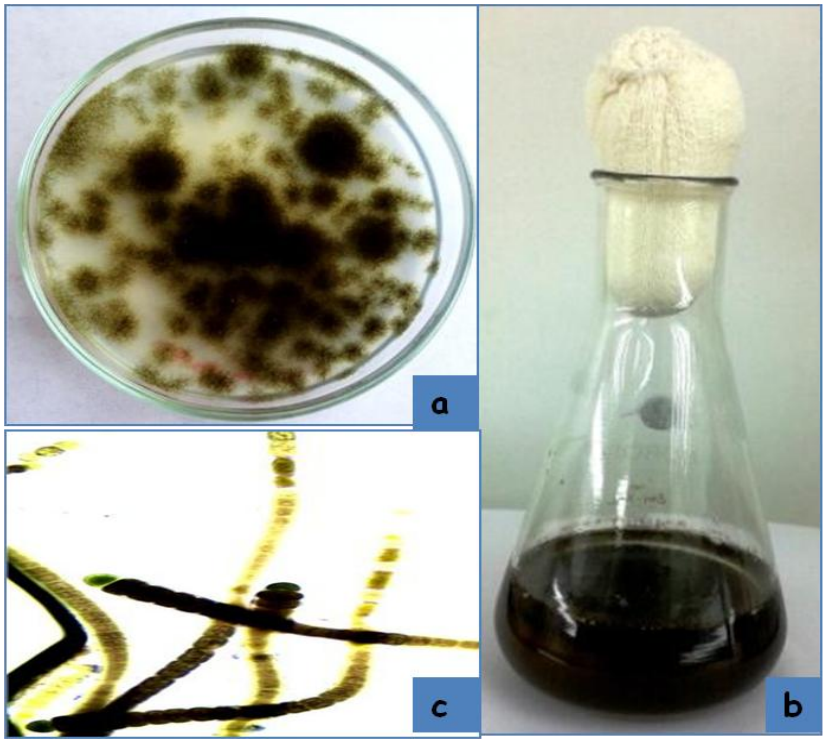

Plate 5.5: *Calothryx marchica* v. *crassa* isolated from solid waste

Plate 5.6: *Calothryx marchica* v. *intermedia* isolated from solid waste

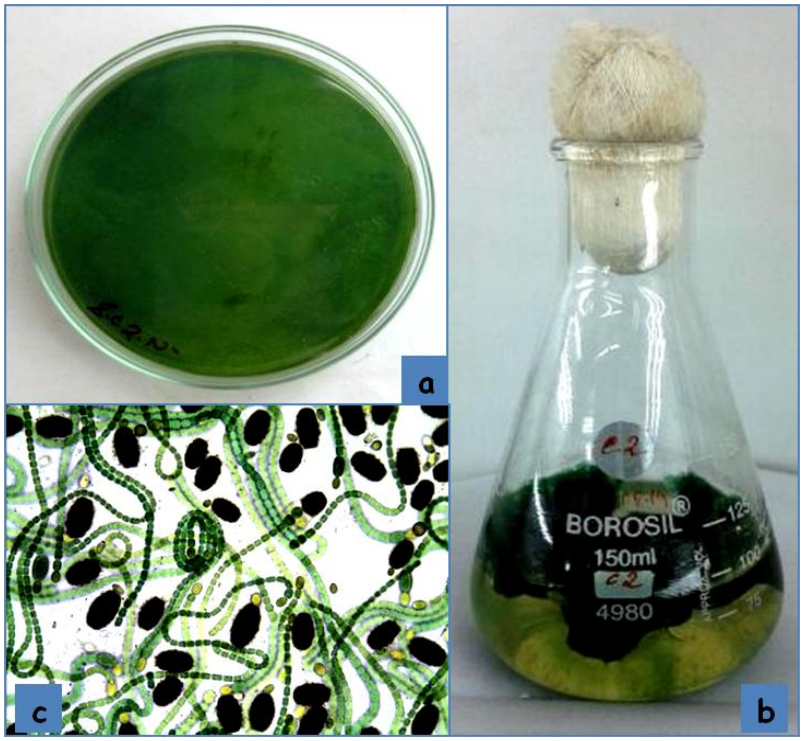

Plate 5.7: *Cylindrospermum majus* isolated from wastewater

Plate 5.8: *Cylindrospermum musicola* isolated from wastewater

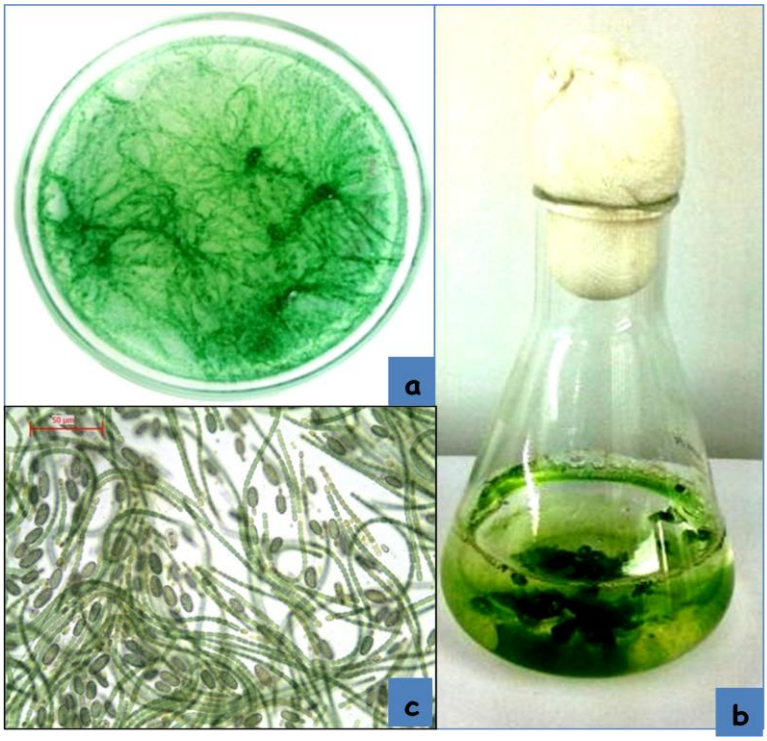

Plate 5.9: *Cylindrospermum muscicola* var. *longispora* isolated from wastewater

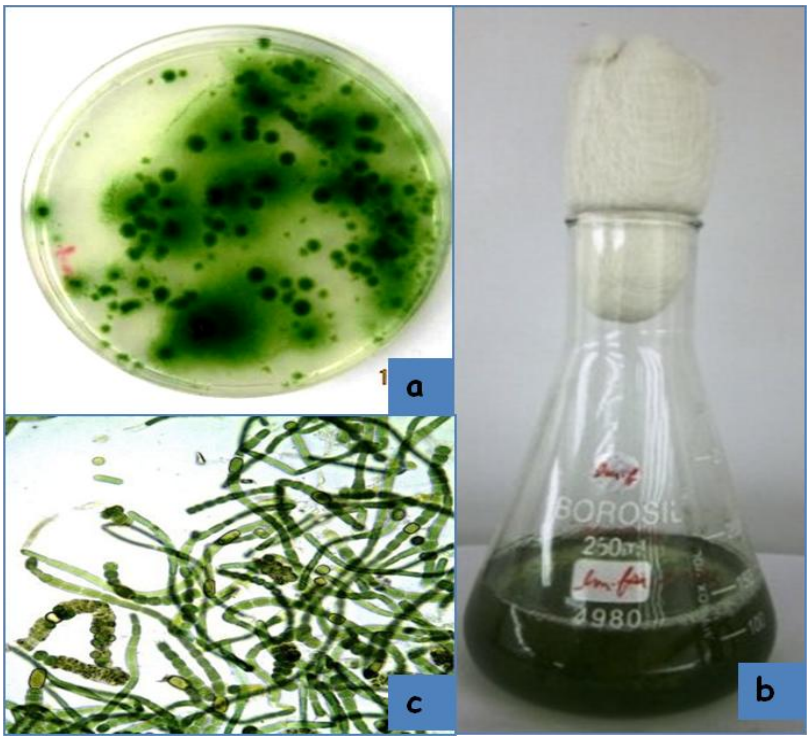

Plate 5.10: *Fischerella muscicola* isolated from solid waste

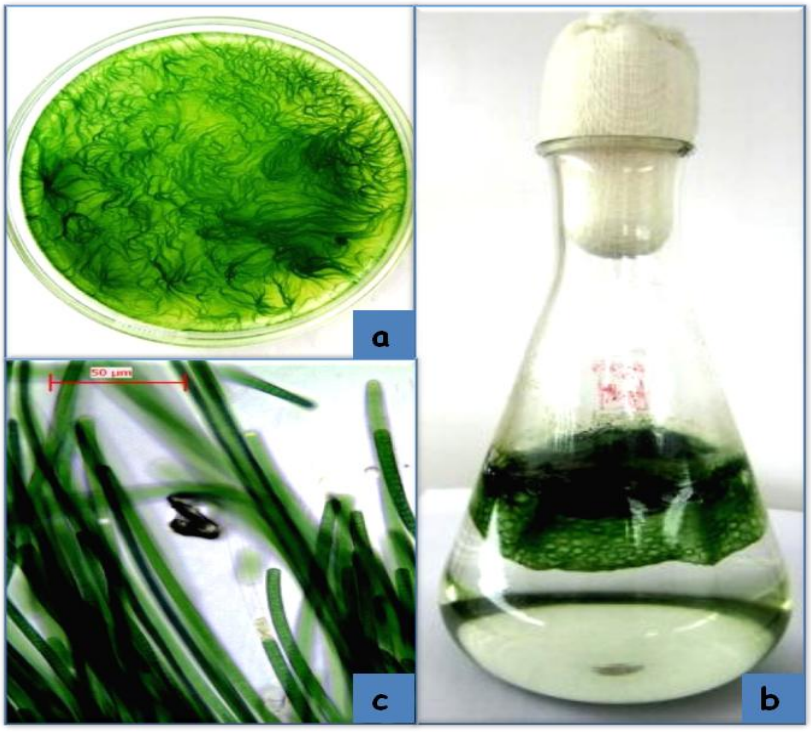

Plate 5.11: *Lyngbya polysiphoniae* isolated from wastewater

Plate 5.12: *Lyngbya limnetica* isolated from solid waste

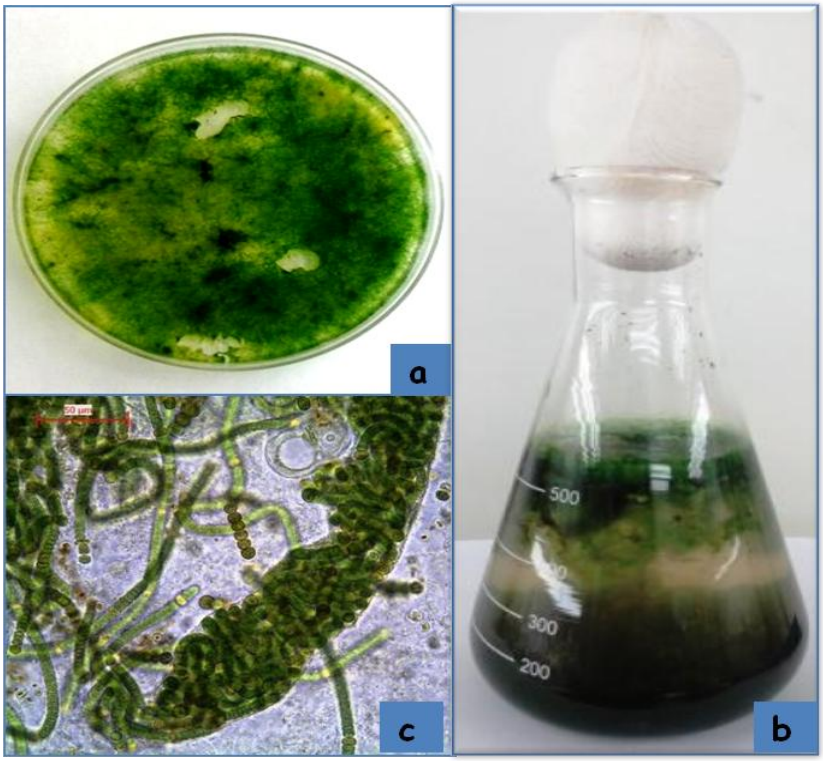

Plate 5.13: *Nodularia spumigena* isolated from wastewater

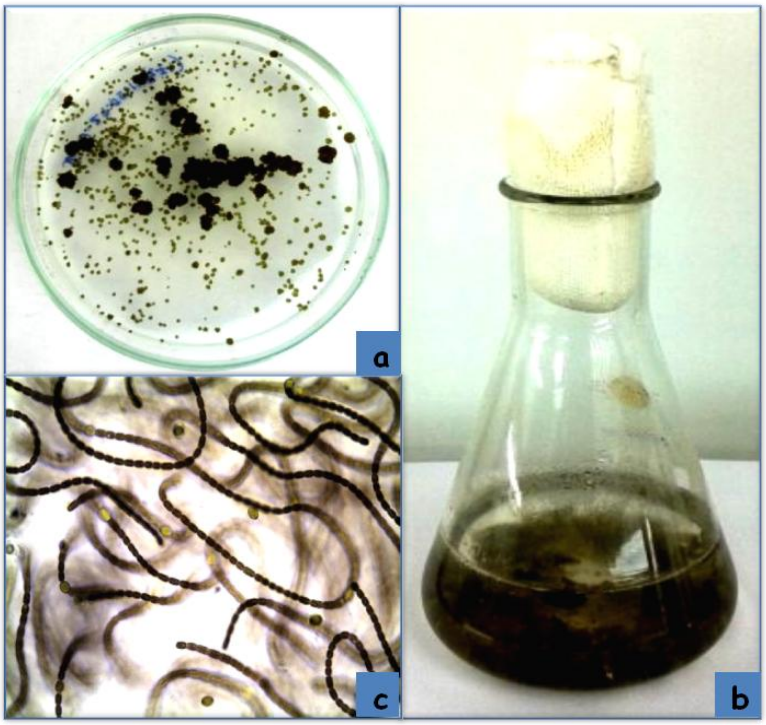

Plate 5.14: *Nostoc carneum* isolated from wastewater

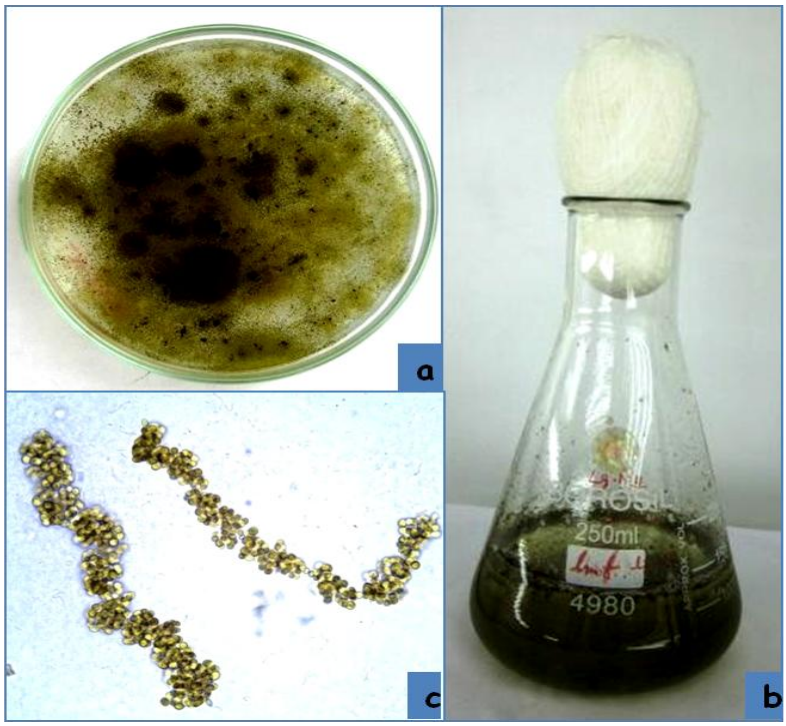

Plate 5.15: *Nostoc commune* isolated from wastewater

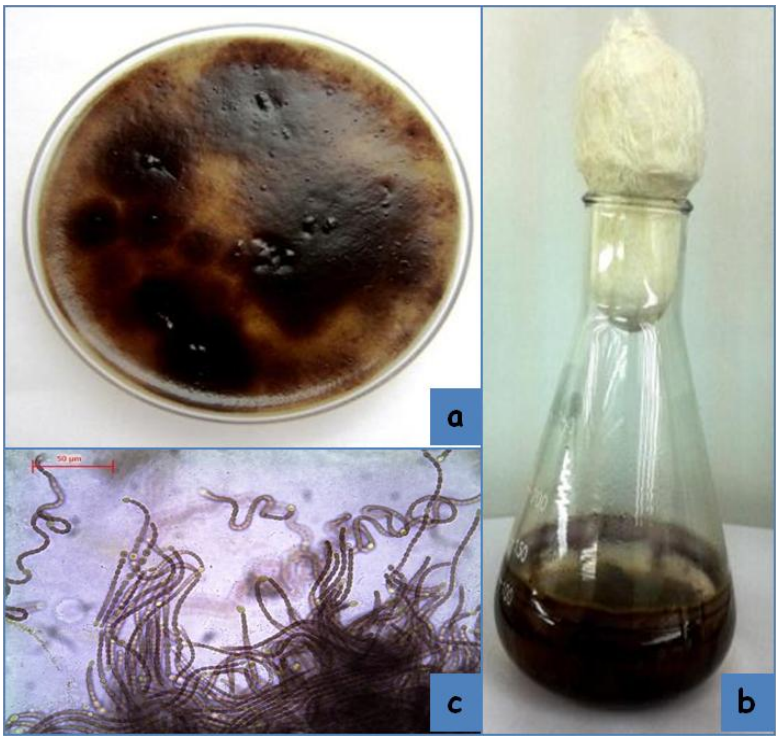

Plate 5.16: *Nostoc ellipsosporum* isolated from wastewater

Plate 5.17: *Nostoc linckia* isolated from wastewater

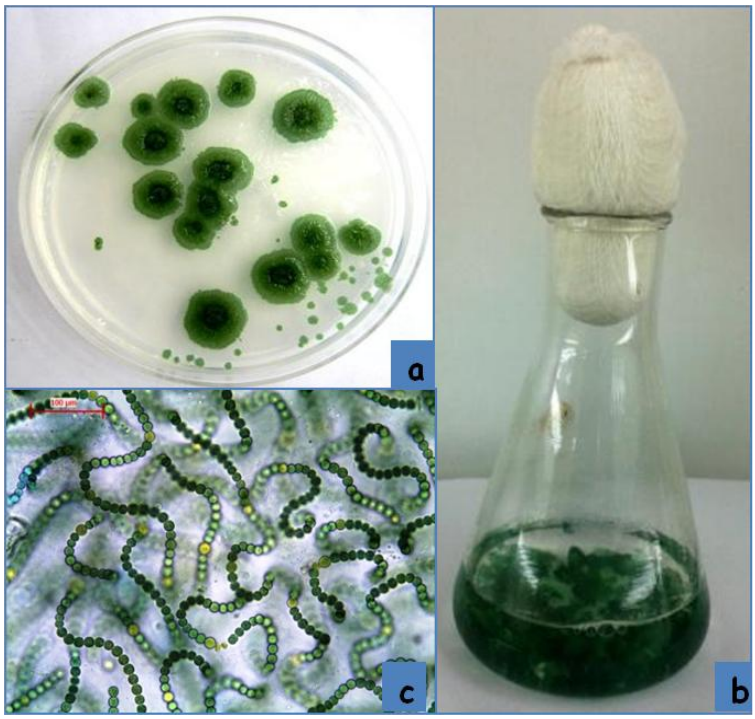

Plate 5.18: *Nostoc sphaericum* isolated from solid waste

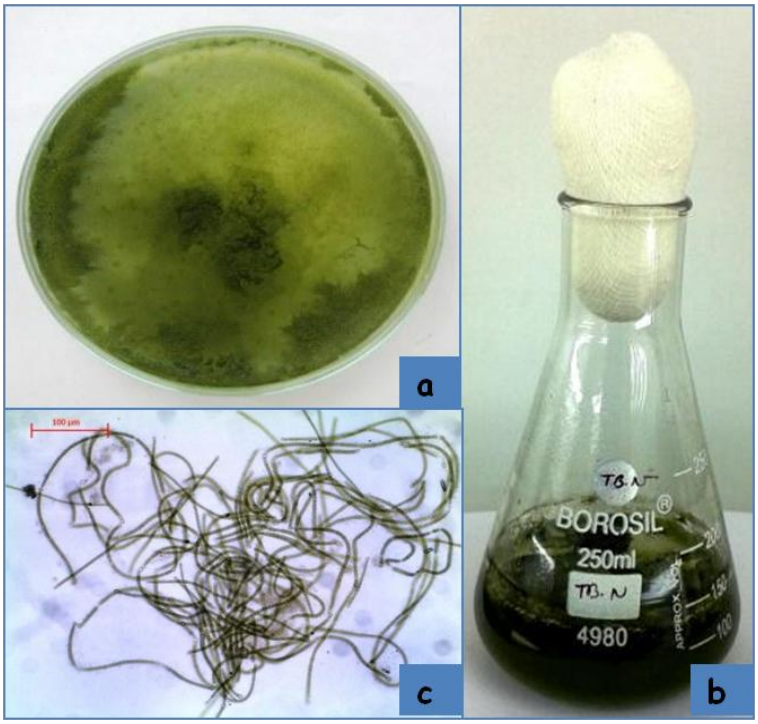

Plate 5.19: *Nostoc spongiforme* isolated from tree bark

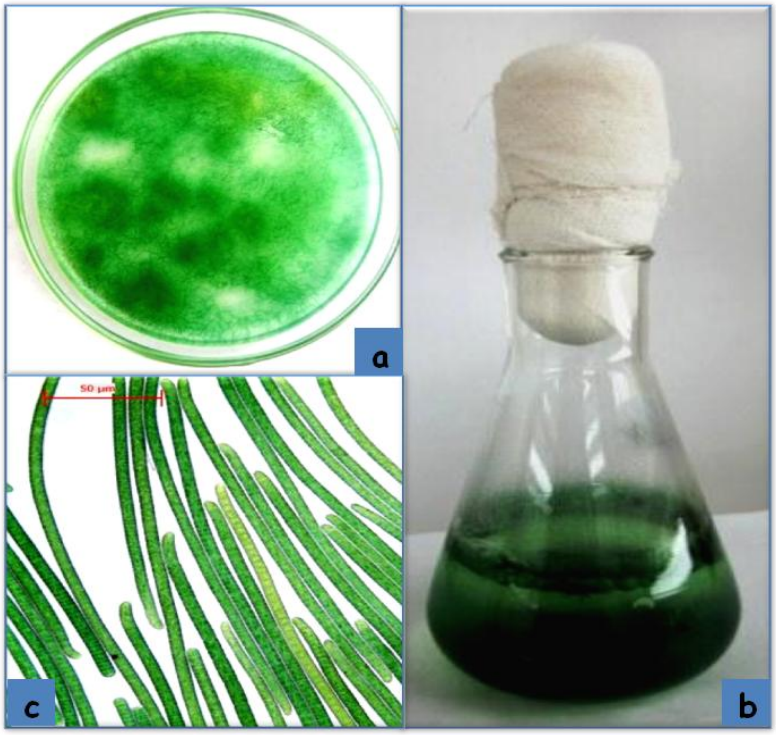

Plate 5.20: *Oscillatoria formosa* isolated from wastewater

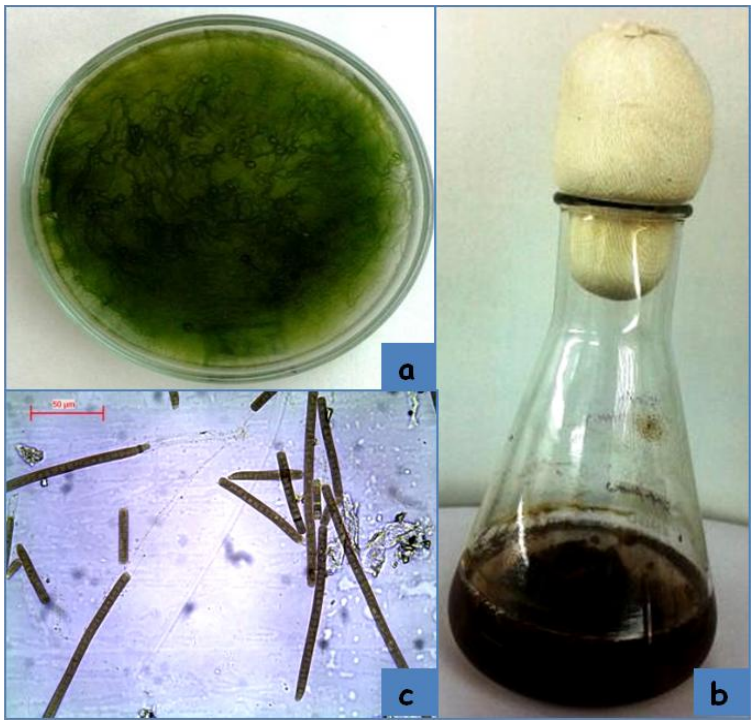

Plate 5.21: *Phormidium autumnale* isolated from wastewater

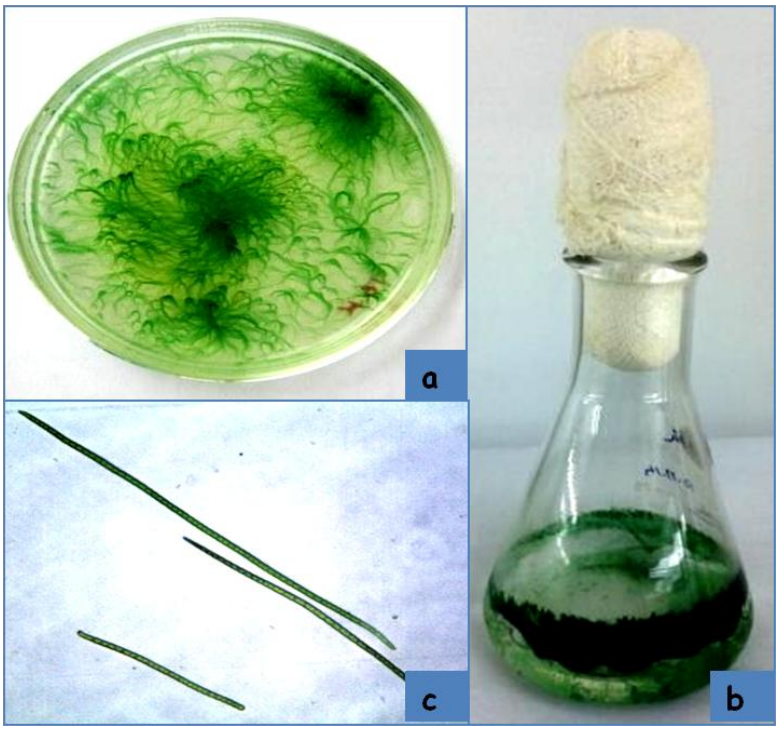

Plate 5.22: *Phormidium fragile* isolated from tree bark

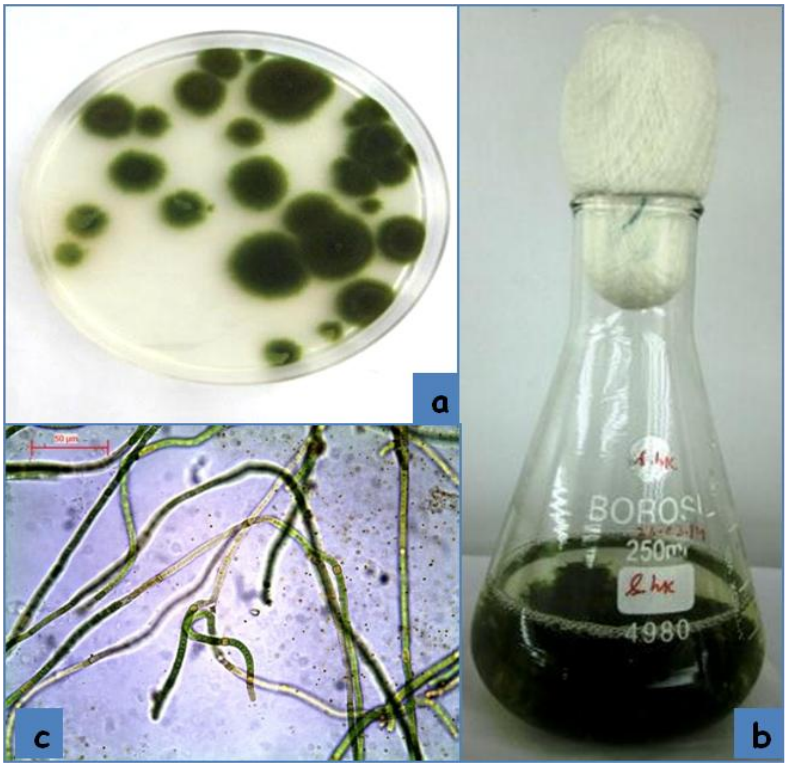

Plate 5.23: *Scytonema tolypothrichoides* isolated from wastewater

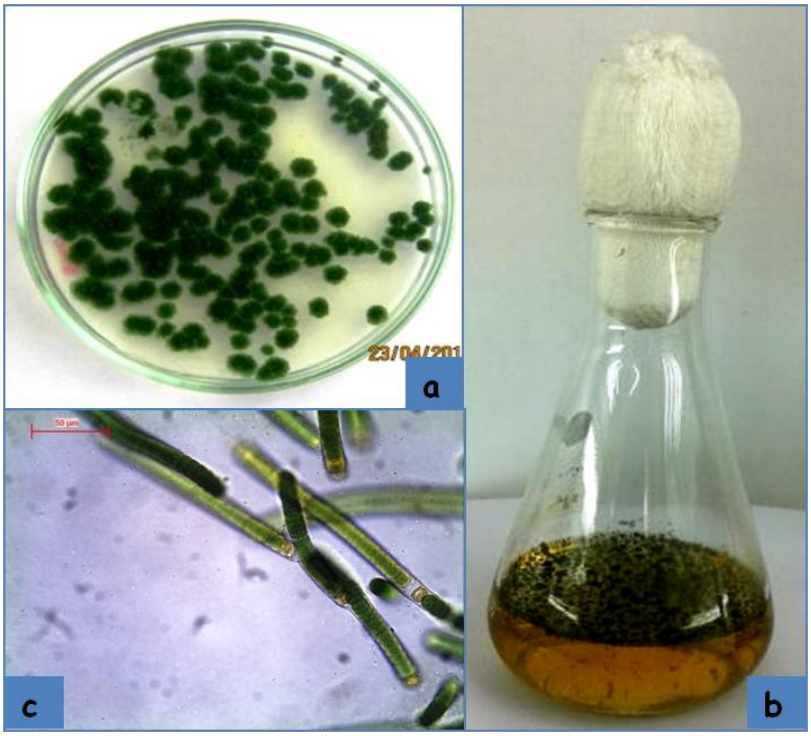

Plate 5.24: *Tolypothryx byssoidea* isolated from tree bark

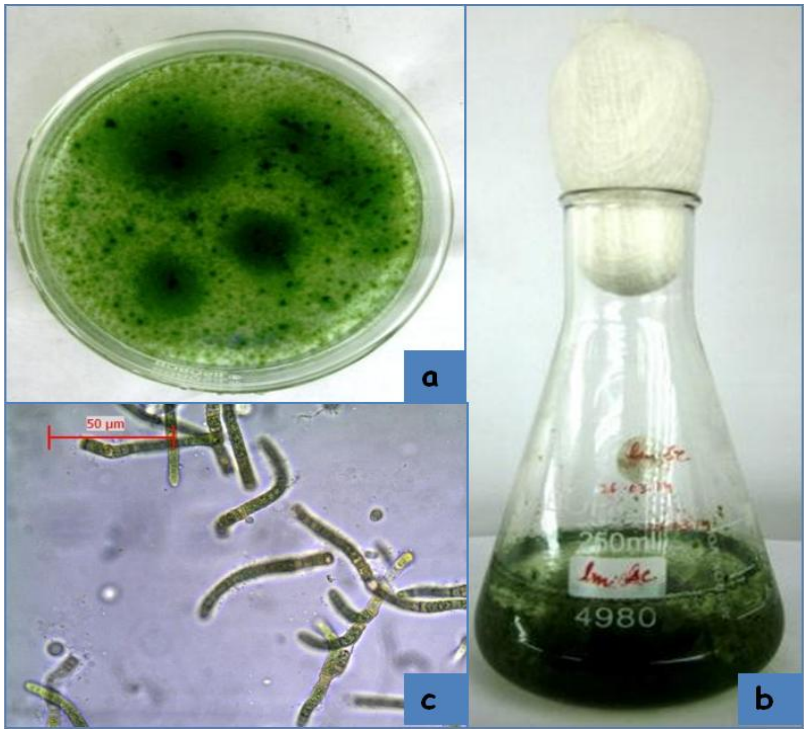

Plate 5.25: *Tolypothryx distorta* isolated from tree bark

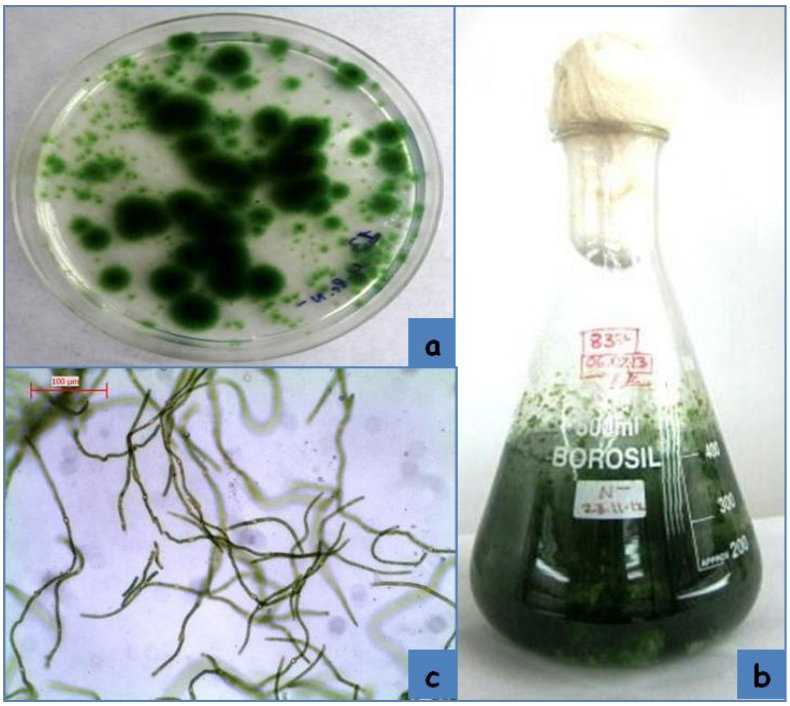

Plate 5.26: *Tolypothryx rechingeri* isolated from wastewater

Plate 5.27: *Westiellopsis prolifica* isolated from wastewater

5.3.2 Morphological characterization of the isolated pure strains

***Anabaena doliolum* Bharadw. (after Bharadwaja) (Plate 5.1)**

Thallus dark bluish green, single and expanded in the agarized medium. Compact biomass, globose, free-floating and submerged in the liquid medium. Filaments straight and sometimes bent. Cells barrel shaped, 5.5 µm long, heterocysts spherical or oval with 7 µm broad, end cell conical.

***Anabaena orientalis* Dixit (after Dixit) (Plate 5.2)**

The developed colonies in agarized medium were thick, blue green, broad, round and floating thin mats were formed in liquid culture, trichome single, straight or slightly curved, 2.8-4.2 µm broad, cells quadratic to cylindrical, longer than broad; end cell conical with rounded apex; Heterocysts single, intercalary, round, cylindrical and also ellipsoidal with rounded end walls, 5-5.7 µm broad and 7-8.6 µm long; spores not found.

***Aphanothece bullosa* (Plate 5.3)**

Thallus macroscopic, mucilaginous, round-shaped colony developed on agarized medium, dark blue green in color, homogenous sedimentary growth behavior in liquid medium; unicellular colonial algae, cells oval to cylindrical with rounded ends, 3.5-4.6 µm broad and 5.7-9 µm long.

***Calothryx marchica* Lemm. (after Fremmy) (Plate 5.4)**

Thallus filamentous, blakish-brown in color, colony spread out on agarized medium, planktonic in liquid culture forming free-floating fascicles, planktonic in cultural behavior having floccose type of growth pattern initially and later the filaments were generally found attached to the walls and the bottom of the flasks in liquid medium; filaments single,

slightly bent, with a close and thin colorless sheath at young stage, prominent sheath at old stage, without a terminal hair, end cell conical with rounded apex, vegetative cells quadrate or somewhat shorter than broad, heterocysts single, basal, spherical or subspherical, 4-4.3 µm broad and 5-5.7 µm long.

***Calothryx marchica* v. crassa Rao, C. B. (after Rao, C. B.) (Plate 5.5)**

Thallus filamentous, bright brown in color, planktonic in cultural behavior having floccose type of growth pattern initially and later the filaments were generally found attached to the walls and the bottom of the flasks in liquid medium; filaments in groups, irregularly bent and closely entangled; sheath somewhat thick, firm, hyaline; trichomes 5.6-7.3 µm broad, cell wall constricted, ends tapering but without a hair, end cell conical with a rounded apex, heterocysts single, basal, spherical or subspherical, 7 µm broad and 5.4-6 µm long.

***Calothryx marchica* v. *crassa* Var. Intermedia Rao (Plate 5.6)**

Thallus filamentous, dark brown in color. planktonic in cultural behavior having floccose type of growth pattern initially and later the filaments were generally found attached to the walls and the bottom of the flasks in liquid medium ie. bottom dweller. Thin membranous biomass, filaments elongate and straight, distinctly attenuated towards the apex, with a close thin colorless sheath at young stage, prominent sheath at old stage, without a terminal hair trichome, 5 µm broad, heterocyst spherical and sub-spherical, basal, 5 µm broad.

***Cylindrospermum majus* Kutz. (after Fremy) (Plate 5.7)**

Plant mass mucilaginous, expanded, blakish-green in colour on the agarized medium. Trichome 4.3 µ broad, constricted at the cross-walls, blue- green in colour; Heterocysts

oblong, broader than the trichome, upto 5-5.5 µ broad; spore ellipsoidal 15.7-16 µ broad and 27 µ long; epispore brownish with distinct papilliae.

***Cylindrospermum muscicola* Kutzing ex. Born ex. Flah (Plate 5.8)**

Individuals of *Cylindrospermum muscicola* are having thalli expanded, brownish green in color, short without sheath, cells cylindrical, constricted at the cross walls, heterocysts terminal occurring at both ends, spores single next to heterocysts, much bigger than the vegetative cells. Trichomes 2.5-4.3 µm broad, constricted at the cross walls, cells 4.3-5.8 µm long, cylindrical or nearly quadrate; heterocysts oblong, broad 4.3-5.7 µm, long 7.1-10 µm; spores oval 14.3 µm broad, 22.8 µm long, epispore smooth, yellowish brown.

***Cylindrospermum muscicola* var. *longispora* Dixit (Plate 5.9)**

Thallus filamentous, colony mucilaginous and expanded, bright blue- green in colour; filaments attached to the vertical glass surface in batch culture at young stage, later at the stage of spore development finger like protrude developed attached to the bottom of the conical flask, cells quadrate to cylindrical with slight constriction at the cross walls, 3-4 µm wide, 3.7-4.8 µm long; heterocysts 4-5 µm wide, 5-6.5 µm long; akinate single, ovoid, 9-11 µm wide, 11-18µm long, rounded at the ends, walls smooth.

***Fischerella muscicola* (Borzi) Gomont (Plate 5.10)**

Thallus bright green, filamentous, plant mass floccose; Main filaments creeping, torulose, flexous interwoven, initially submerged and later floating; thin membranous biomass, lateral branches almost erect with false branches, filaments elongated. Trichome uniseriate

and 5 µm broad, heterocyst elongate, spherical and sometimes compressed with 7 µm broad.

***Lyngbya limnetica* Lemmermann (Plate 5.11)**

Thallus bright green, round colonies developed on agarized medium. Thin membranous biomass, initially submerged and later floating in the liquid medium. Filament straight, thichome 1.4 µm broad, not attenuated at the end. Cells longer than broad, apical cells rounded, hyaline sheath present.

***Lyngbya polysiphoniae* Frémy (after Frémy) (Plate 5.12)**

Thallus dark blue green, spread over the agarized medium, loop-shaped structures were formed in the flat mat of the strain in the liquid medium, filaments both straight and curved, sheath thin; trichome blue-green, constricted at the cross walls, 5.7-6 µm broad and 1.4-2.9 µm long, cross-walls visible, not granulated; end cells convex.

***Nodularia spumigena* Mertens in Jürgens (Plate 5.13)**

Thallus yellowish green, spread over the agarized medium; thin membranous biomass, initially submerged and later floating in the liquid medium. Filaments usually entangled and clustered in a loose, gelatinious mass. Cells disc-shaped, very compressed, constricted at the cell walls; 3-4.3 µm broad. Gonidia intercalary, 10-11 µm broad.

***Nostoc carneum* Ag. (after Frémy) (Plate 5.14)**

Thallus at first globose , later bullose -tuberculate, leathery and irregularly expanded, gelatinous, flesh coloured in the agarized medium, planktonic in nature in the liquid

medium; sheath indistinct, colourless; trichome 4-4.6 µm broad; cells oblongo-cylindrical, up to twice as long as broad. Heterocyst oblong, 6-7.1 µm broad.

***Nostoc commune* Vaucher ex Born. et Flah. (Plate 5.15)**

Thallus firm, expanding, undulated, membranous, brownish on the agarized medium. homogenized powdery sedimentation was observed in liquid medium; filaments flexous, closely entangled forming globose membranous structure at young stage, sheath mostly distinct only at the periphery; at old stage, filaments more or less straight, loosely entangled, cells spherical, epispore smooth colorless, trichome 3.9-4.3 µm broad. Cells short barrel shaped or nearly spherical, mostly shorter or a little longer than broad, 5µm long, heterocysts nearly spherical, about 4.3 µm broad.

***Nostoc ellipsosporum v. violacea* Rao C.B (after Rao, C.B.) (Plate 5.16)**

Thallus gelatinous, irregularly expanded, dark violet in the agarized medium; filaments flexous, loosely entangled, sedimentary growth behavior in batch culture; trichome about 2.9-3.6 µ broad, constricted at the cross-walls; cells almost quadrate to cylindrical, cell length 3.2-5.7 µm; heterocysts spherical to ovate, length 4.2-7.1 µm, breath 4.3-7 µm; spores ellipsoidal, in long chains, 4.5-6.7 µ broad, length 5-7.2 µm.

***Nostoc linckia* v. (Roth) Born. et Flah. (after Frémy) (Plate 5.17)**

Thallus gelatinous, brownish green in color, varying in size, at first globose later irregularly expanding on agarized medium, sedimentation was seen in liquid medium; filaments densely entangled, flexuous or highly coiled; trichomes 4-5 µ broad, cells short barrel-shaped; heterocysts spherical.

***Nostoc sphericum* (Plate 5.18)**

Plant mass light blue-green, at first gelatinous and almost spherical and later, becoming expanding with age in the agarized medium; Trichomes spiral, Plant mass are planktonic, in the liquid medium. Cells spherical or compressed-spheroidal, 5.7-7.5µ broad. Heterocysts spherical, 7-8 µ broad. Gonidia spherical; 8-8.6 µ in diameter; adjacent to the heterocysts.

***Nostoc spongiforme* v. *tenue* Rao, C.B. (after Rao, C.B.) (Plate 5.19)**

Plant mass gelatinous, brownish green, at first globose, later expanding in the agarized medium. Sheath hyaline, thallus densely entangled, cells spherical, subspherical, ellipsoidal or barrel-shaped, cells adjoining to the heterocysts are slightly drawn out, 3.5-4.2 µ long, end cells usually with a pointed apex, heterocysts spherical, subspherical, ellipsoidal or barrel-shaped with rounded ends, 5.7-6 µm broad and 6.6-7 µm long; spores in chains, spherical, subspherical, ellipsoidal, 5.4-7µm broad and 5.7-7.1 µm long.

***Oscillatoria formosa* Bory (Plate 5.20)**

Thallus thin, light blue green at the young stage and yellowish green at the matured stage on the agarized medium, in liquid medium the mucilaginous filaments were entangled to form a slimy mat and by the group behavior of the filaments some big bubbles were formed; filamentous, trichomes showed oscillatory motions, straight, slightly constricted at the cross walls, 3.5-7 µm broad, end-cells nearly obtuse, attenuated at the ends and bent, 2.8-4.9 µm long, septa slightly granulated, cells nearly quadrate.

***Phormidium autumnale* (Ag.) Gom. (after Gomont) (Plate 5.21)**

Thallus brownish violet, filamentous, spread over the agarized medium, plant mass planktonic floccose; Filament straight, thichome 6-7.2 µm broad, variously entangled. Sheath firm and mucilaginous. Cells not constricted at the cross walls, 5.7-7.1 µm broad. End cells briefly attenuated, prominently capitate. Cells quadrate to half as long as broad, 2.9-4.2 µm long, septa granulated. End cell with a truncated conical calyptra.

***Phormidium fragile* (Menegh.) Gom. (Plate 5.22)**

Thallus green at young stage and yellowish blue green at mature stage on agarized medium, in liquid medium the mucilaginous filaments were entangled to form a slimy mat and by the group behavior of the filaments some big bubbles were formed; trichomes more or less flexuous, parallel, little constricted at the cross walls, attenuated at the ends, 2.8 µm broad, cells quadrate, 2.8-4.6 µm long, end cell acute conical.

***Scytonema tolypothrichoides* Kutz. (after Kossinskaja) (Plate 5.23)**

Thallus caespitose brownish green, filamentous on the agarized medium. Plant mass are planktonic, floccose. Filaments 8-9 µm broad, repeatedly false branched, false branches very similar to the main filament; sheath hyaline, later brown; trichome 6-8.7 µm broad, olivaceous- yellow; cells subquadrate or longer, densely granulated; heterocysts varied, sometimes short and some long.

***Tolypothryx byssoidea* (Berk.) Kirchn. (Plate 5.24)**

Stratum wooly, cushion like, dark green on agarized medium, planktonic thin layer formed on the surface of liquid media; filaments 13-14.4 µm broad, irregularly false branched;

false branches short, curved; sheath thin, light brownish, fragile, trichomes 11.4-13 µm broad, torulose; trichomes barrel- shaped, 1/2 – 1/3 as long as broad; heterocysts basal, single.

***Tolypothryx distorta* v. *penicillata* (Ag.) Lemm. (Plate 5.25)**

Thallus yellowish green, filamentous, bright green in color in the agarized medium. Plant mass are planktonic, floccose in the liquid medium. filaments 8-9 µm broad, false branched; flase branches erect; sheath thin, colouless, close to the trichomes, trichomes 6.5-7.7 µm broad; cells as long as broad or shorter or longer than broad; heterocysts single, yellowish, cylindrical, quadratic, spherical.

***Tolypothryx rechingeri* (Wille) Geitler (after Wille) (Plate 5.26)**

Thallus cushion-like, olivaceous-green, agarized medium. False branching in the basal parts of the thallus 9-10 µm broad; sheath thin colorless; trichome 6.7-8 µm broad, cells broader than long or up to 1/3 as long as broad, blue-green; heterocysts round, somewhat flattened, or nearly quadratic.

***Westiellopsis prolifica* Janet (after Janet) (Plate 5.27)**

Thallus filamentous, bright green in color in the agarized medium. Plant mass are planktonic, floccose in the liquid medium. Main filament torulose, with short barrel shaped cells, 7.7-11.4 µm broad as long as broad or slightly longer, branch filaments are thinner and elongate not constricted at the cross walls with elongate cylindrical cells, 4-6 µm broad, heterocysts oblong, cylindrical 6-7 µm broad and 10-18 µm long.

5.4 Conclusion

The industrially polluted soils were found to be rich in cultivable cyanobacterial diversity. The cultural studies of the potent isolates revealed unique morphologies and distinctive growth attributes on the both solid and liquid medium. Heterocystous forms were found to be dominant over the nonheterocystous forms. *Nostoc* was found to be the most dominant genera among all the isolated species in the selected sites. For solid wastes deposits which are otherwise very rare to be colonized with visible algal growth, the soils were found to be rich in cultivable cyanobacterial growth. In all the study sites relative contribution of the *Nostoc* species were found to be the highest. Reduced number of cyanobacterial growth was observed during the unfavorable period of the study sites. It is due to the dryness that is faced by the upland soil during winter and premonsoon period of the year while washes out of the soil during monsoon and post monsoon period results in less number of algae of the river bank soil.