Review of literature

2.1 Paper production process

The papermaking process requires four major inputs: a source of fiber, chemicals, energy and water. Pulp and paper are manufactured from raw materials containing cellulose fibers, generally wood, recycled paper, and agricultural residues. In developing countries, about 60% of cellulose fibers originate from nonwood raw materials such as bagasse (sugar cane fibers), cereal straw, bamboo, reeds, esparto grass, jute, flax, and sisal (Nanda *et al.*, 2010). The manufacture of pulp, for paper production, involves mechanical (including thermo-mechanical), chemi-mechanical and chemical methods (Nanda *et al.*, 2010). Paper is made by pulping wood, bleaching this pulp and then spreading it out into sheets to make it into paper. At various stages of the process, chemicals are used to give the paper particular properties. However, paper industries in India are not as modernized as in Western Countries. The pulp and paper mills in India utilizes various cellulosic-based materials for paper production accounting about 43% from forest wood, 28% from agro-based product, and 29% from recycling of waste paper (Balakrishanan, 1999).

2.1. a Pulp and paper mill wastes discharges

Pulp & paper industries are complex in nature consisting of emissions from several processes determined by the quality and type of paper required and raw material used and the prevailing management practices. The sector is highly intensive in terms of consumption of raw material, chemicals, energy and water and consequently releasing huge amount of effluents. Every day about two million tons of sewage, industrial wastes and agricultural wastes are discharged in to world's water (UN WWAP, 2003). It is estimated that industry alone is responsible for dumping of 300-400 million tons of heavy metals, solvents, toxic sludge and other waste into waters each year (UNEP, 2010). A recent survey indicates that about 70% industrial waste in developing countries are dumped untreated into waters where they contaminate the existing water quantities (UN-Water, 2009). The l pollutants from a pulp and paper mill can be classified into four categories–liquid effluents, air pollutants, solid wastes and noise pollution (Mohanty and Srivastav, 1998).

Pulp and paper manufacturing process generates highly polluting waste water during various processing stages such as raw material preparation, pulping, pulp washing, screening, bleaching and coating operation etc. (Tripathy *et al*., 2013). The significant environmental impacts of the paper manufacture result mainly from the pulping and bleaching processes (Nanda *et al*., 2010). The wet processes used during the pulp making are responsible for removing large amounts of organic compounds from the processing wood (Vepsäläinen *et al*., 2011). Isopimaric, sandacopimaric, levopimaric, abietic, dehydroabietic and palustric acids are resin acids which can be generally found in pulping wastewaters (Vepsäläinen *et al*., 2011). The residual lignin present in the pulp is further removed by bleaching process. The process consists of sequential chemical operations of treatment with chlorine dioxide and washing, generating acidic effluents, which is followed by alkaline extraction and washing, generating alkaline effluents (Garg *et al*., 2004). The use of chlorine or chlorine dioxide during bleaching process, release chlorinated and nonchlorinated compounds from lignin and wood extractives and the resultant effluents contain high concentrations of chlorophenolic compounds, chloroacetones and chloroform, which are colored and recalcitrant (Mvula and Sonntag, 2003; Savant *et al*., 2006). The generated effluent is characterized by dark color, foul odour, high organic content and extreme quantities of chemical oxygen demand (COD), biochemical oxygen demand (BOD) and pH (Pokhrel and Viraraghavan, 2004). The organic matter present in these effluents favours the growth of various microorganisms as ideal substrates and depletes oxygen by rapid respiration and oxidation. As a result, a major oxygen deficiency is encountered by the aquatic ecosystem which is deleterious for the native flora and fauna (Nanda *et al*., 2010). Other ecologically important physical parameters such as conductivity, temperature, high suspended particulate matter, dissolved particulate matter sometimes impair the oxygen balance of the water body and sediments. The excess of nitrogen (N) and phosphorus (P) in the effluents may support eutrophication process which might lead to anoxic condition (Poole *et al.*, 1977). Paper mills, as already mentioned, release huge amount of effluent in the form of black liquor loaded with many pollutants (Ingle, 2000; Yedla *et al*., 2002). These units generate 150-200 m^3 effluent with a high pollution loading of 90-240 kg suspended solids, 85-370 kg biochemical oxygen demand (BOD) and 500-1100 kg chemical oxygen demand (COD) per ton paper (Mathur *et al*., 2004). It has been reported that production of one ton of paper contributes 100 kg of color imparting substances and 2-4 kg of organochlorines to the bleach plant effluents (Kansal *et al*., 2008). The high chemical diversity of these pollutants causes a variety of clastogenic, carcinogenic and mutagenic effects on aquatic communities in recipient water bodies (Ali & Sreekrishnan, 2001; Karrasch *et al*., 2006).

Kortekaas *et al*., (1998) reported the value of COD ranges from 1000 to 6000 mg/L during the thermo-mechanical pulping (TMP). Fazeli *et al*. (1998) studied accumulation of heavy metals in the paddy crop field irrigated with paper mill effluent and found increased level of heavy metals accumulated in plant tissue. Copper seems to be main contributor among other heavy metals of acute toxicity of the Kraft mill effluent (Reyes *et al*. 2009). Dubey *et al*. (2011) monitored the physicochemical characteristics of paper mill and pharmaceutical industries waste water and cyanobacterial diversity. High levels of organic matters, calcium, nitrate, phosphate and low levels of oxygen have been reported to favour the luxurious growth of the cyanobacteria. The physicochemical parameters of a paper mill effluent at Magahar (U.P.) were analyzed and the acute toxicity (LC50 evaluation) to a freshwater fish, *Mystus vittatus* was conducted (Mishra *et al*., 2011). The physico-chemical characteristics of another paper mill industry at

Nagaon, Assam were reported and its effect on different crops was analyzed (Medhi *et al.*, 2011). The result revealed some of the wastewater parameters were above the permissible limits as per Indian irrigation water standard. Kuzhali *et al*. (2012) investigated the physico chemical quality of effluent from the paper mills of Nilakotai Dindigul, TamilNadu and reported all the parameters to be higher than the WHO prescribed discharge limits for effluent. Maheswari *et al*. (2012) analyzed the physicochemical characteristics of different recycled paper mills of Northern districts of UP viz. Saharanpur, Muzaffarnagar and Meerut and inferred the selected parameters have high concentration values than the prescribed value of Indian Standard (BSI). Tripathi *et al*. (2013) compared the wastewater parameters of the small and large-scale paper industry and reported significant differences at all levels of both the pulp and paper mills and found Agro based small scale pulp and paper mill to be more contributor of pollution than wood based large scale. Yadav and Yadav (2014) analyzed the physicochemical parameters of the effluent water of the Taragram paper mill and reported the value of conductivity, TDS and acidity beyond the standards set by Central pollution control board, India. Bhatnagar (2015) analysed the paper mill effluents from both the inlet and outlet of the effluent treatment plant of paper mill and compared with the Indian standards of effluent discharge. Values showed that COD, BOD, SS are beyond the permissible standards even after treatment. Thus, the paper mill does meet the Standards set by Central Pollution Control Board, India.

Proportionate amount of solid wastes from paper mills is of additional concern. The solid wastes produced from the paper industry includes wastewater treatment plant (WWTP) sludges, lime mud, lime slaker grits, green liquor dregs, boiler and furnace ash, scrubber sludges and wood yard debris (Likon and Trebše, 2012). In general, solid wastes from pulp production and paper mill operations are humid and contain some organic compounds in the form of wood or recycled paper fibres, chlorinated organic compounds and pathogens, significant amounts of ash and trace quantities of heavy metals (Monte *et al.*, 2009). In 2005, the production of recycled paper in Europe was 47.3 million tons, generating 7.7 million tons of solid waste which represented 16 % of the total production from this raw material (Monte *et al.*, 2009). On average, the majority of waste generated from paper production and recycling is paper mill sludge, which is a by-product of up to 23.4 % per unit of produced paper, the quantity depending on paper production process (Miner, 1991). The paper mill generates large quantities of lime sludge every day (940 tons/day). Analysis and alternative utilization of lime sludge wastes from a paper mill at Nagaon (Assam) has been documented (Deka and Yasmin, 2006). Disposal of this solid waste has become a problem, as it causes abnormal increase in soil alkalinity and damage to vegetation in the vicinity of the dumping ground (Deka and Yamin 2006).

Fly ash being light gets airborne very fast and pollutes the atmosphere (Swamy, 2000) and water system. (H. Hazard) In the kraft pulping process, highly malodorous emissions of reduced sulfur compounds, measured as total reduced sulfur (TRS) and including hydrogen sulfide, methyl mercaptan, dimethyl sulfide, and dimethyl disulfide, are emitted, typically at a rate of 0.3–3 kilograms per metric ton (kg/t) of air-dried pulp (ADP ie. 90% bone-dry fiber and 10% water). Other typical generation rates are: particulate matter, 75–150 kg/t; sulfur oxides, 0.5–30 kg/t; nitrogen oxides, 1–3 kg/t; and volatile organic compounds (VOCs), 15 kg/t from black liquor oxidation. In the sulfite pulping process, sulfur oxides are emitted at rates ranging from 15 kg/t to over 30 kg/t. Other pulping processes, such as the mechanical and thermomechanical methods, generate significantly lower quantities of air emissions. Steam- and electricity-generating units using coal or fuel oil emit fly ash, sulfur oxides, and nitrogen oxides. Coal burning can emit fly ash at the rate of 100 kg/t of ADP (Pollution Prevention and Abatement Handbook, 1998).

2.2 Diversity of algae with special reference to cyanobacteria

Cyanobacteria represent cosmopolitan prokaryotes, occurring in almost every aquatic and terrestrial environment (Castenholz, and Waterbury, 1989) including soil, fresh water, salt water and as well as in several surface types such as rocks, urban walls, metals, tree barks, leaves and animal hairs (López-Bautista *et al.*, 2007). Algae represent the first community to colonise bare soil and newly exposed substrata of both natural and technogenic origin. As photoautotrophs, they generate organic matter from the inorganic substances and subsequently help in establishment of higher plant communities (Starks *et al.*, 1981). Moreover, the inherent capability of cyanobacteria to fix molecular nitrogen together with their desiccation tolerance enables them to be the most successful colonizers in low-fertility, high-pH areas (Englund, 1978; Metting, 1981). However, they are known to grow abundantly in submerged soils than the upland soils (Watanabe and Yamamoto, 1971) due to high temperature and limitation of water in the upland areas (Roger and Reynaud, 1982).

Algae are primarily known from aquatic habitats but they are also important contributors to the soil microflora (Metting, 1981; Starks *et al*, 1981). Studies on soil algae particularly the blue greens have been well documented (Arvik and Wilsson, 1974; Roger and Reynaud, 1982; Hahn & Kusserow, 1998; Mataloni *et al*, 2000; Singh, 2000; Zancan *et al*, 2006; Kalinowska *et al*, 2008 etc). Tirkey and Adhikary (2005) explored cyanobacteria in biological soil crusts of different states of India. They also measured photosynthetic activity of the crusts. Cyanobacterial population in the different sites of Odhisa was extensively studied by Ghadai *et al*. (2010). Sethi *et al*. (2012) reported 24 species of cyanobacteria and 6 species of micro-algae in biological crusts from different sub-aerial habitats. Chonudomkul *et al.* (1998) carried out study on diversity of blue-green algae and green algae in deciduous dipterocarp forest at Huai Kha Khang wildlife sanctuary of Thailand. They reported 18 genera of blue-green algae and 31 genera of green algae. Predominant blue-green algae were *Oscillatoria* and *Lyngbya*, while *Chlorella* and *Ulothrix* dominated the green algae. Khaybullina *et al.* (2010) examined the forest soil of Great Smoky Mountains National Park, USA for soil algae from soil, rocks and tree barks. They documented a total of 42 species with twenty new taxa records for the park was established. In Barak valley intensive study regarding seasonal algal diversity in both rice field as well as soil of Assam University campus were done by Rout and her group (Rout and Dey, 1999; Roy *et al*., 2008, Deb *et al*., 2013; Deb *et al*., 2015). Prevailing physicochemical parameters were analyzed and their influence on algal diversity was documented.

Most of the studies regarding soil algae were restricted generally to natural and disturbed soil and corresponding information on diversity of algae on industrial solid wastes are extremely scarce. Lukesˇova (2001) investigated the algal communities in two contrasting chronosequences established on reclaimed spoils in Czech Republic and in Germany. The Sokolov chronosequence was characterized by tertiary cypric clay substrate and high pH, while in Germany, chronosequence by pyritic sand of extremely low pH. A total of 122 species of algae was found in both areas with different proportion of individual algal groups and species composition. Green algae prevailed in both areas, but in Sokolov, cyanobacteria and diatoms were also quite diverse, and in younger sites they were abundant.

Role of cyanobacteria in reclamation of highly sodic and alkaline soil has been well documented by Kaushik (Kaushik, 1985; Kaushik, 1989; Kaushik, 1994). Subhashini and Kaushik (1981) and Goel *et al*. (1997) conducted laboratory and field level investigation and reported amelioration of sodic or alkaline soil by cyanobacteria through the accumulation of inorganic ions, organic compounds (sugars, polyols, quarternary amines) and osmoregulators.

Neustupa and Škaloud (2010) investigated species composition of subaerial epixylic algae from two Singaporean rainforest localities. In total, 57 species were identified. Green algae with 40 species were dominant and cyanobacteria (12 species) were the second most frequent group while heterokontophyceae showed least algal diversity with 5 algal species. Lemes-Da-Silva *et al.* (2012) conducted a floristic survey of the corticolous cyanobacteria from tropical forest remnants in northwestern São Paulo State, Brazil. Eighteen species of Cyanobacteria were found belonging to the genera *Aphanothece*, *Chroococcus*, *Lyngbya*, *Phormidium*, *Porphyrosiphon*, *Hapalosiphon*, *Hassalia*, *Nostoc*, *Scytonema*, and *Stigonema*. Bhakta *et al*. (2014) documented diverse algal forms collected from tree bark surfaces of different collection sites of the Similipal Biosphere Reserve of the Mayurbhanj district of Odisha. Of the total 19 species recorded, 18 were cyanobacteria and only one belonging to green algae. Kharkongor and

Ramanujam (2014) conducted the tree bark algal diversity in the three different study sites of in and around Shillong, the capital of Meghalaya. A total of 85 taxa had been recorded, 30 cyanobacteria and 55 algal species belonging to six classes of algae. Undisturbed area harboured the highest subaerial algal diversity compared to those of disturbed forest areas. Soni and Shukla (2006) investigated the algal flora from the three different tree species growing at Rono Hills in Arunachal Pradesh. The algal flora on bark of the three plants was dominated by cyanophycean algae followed by a few members of bacillariophyta and chlorophyta.

Phytoplankton, the most important biological phenomenon in nature on which the entire array of life depends is also the integral component of riverine ecosystem which determines the primary productivity of the system. The structure of algal assemblages which are the primary producers in lotic systems mainly rivers and streams depend on variations in ambient environmental factors (physical and nutrient concentration) prevailing in that area (Buzzi, 2002; Celekli and Kulkoyluoglu, 2007; Pilkaitite and Razinkovas, 2007). Diversity and abundance of algae have since long been considered as indicator of water quality as they are known to reflect the ecological conditions of aquatic systems (Palmer, 1969; Cascallar *et al.*, 2003, Denicola *et al.*, 2004). Kolkwitz and Marrson (1908) formulated the relation of aquatic organisms to the degree of water pollution. As stated by Venneaux (1976) the pattern of phytoplankton community and water quality are interdependent.

Studies on the river ecosystems indicate that the major Indian rivers are grossly polluted, especially beside the cities (Upadhyaya *et al.*, 1982; Srivastava, 1992). Major water pollutants comprise microbes, nutrients, organic chemicals, oil, heavy metals and sediments (Sunday *et al*., 2013). Understanding the contaminant source and quantification of pollutant inputs (natural or anthropogenic) and their nature is crucial to planning, mitigation and cleanup process of rivers (William, 1998). Worldwide, substantial studies have been made on hydrobiological studies regarding rivers but their relations with algae are few and moreover studies concerning small rivers in developing countries are rather scanty (Dassenakis *et al.*, 1998).

Marsh (1907) observed the effects of pollution of river water on fish population. Greenfield (1925) made a comparative study of chemicals and bacteriological aspects of Illinois River. Walling and Webb (1975) studied the water quality of river Exe and reported that water quality was affected by lithology and land use. Razumov and Tyutyunova (1999) investigated the physico-chemical characteristics of river Moskva, the main waterway of Moscow megapolis, and stated that the self purification capacity was severely damaged by inflow of heavily polluted wastewater. Jafari and Alavi (2010) reported *Oscillatoria, Anabaena, Nostoc, Spirogyra, Pediastrum, Navicula and Nitzschia* as the dominant phytoplankton species from the polluted water of river Talar. Li *et al*. (2013) investigated the dynamics of phytoplankton size structure in terms of concentrations of size-fractionated chlorophyll *a* (Chl *a*) in the Pearl River estuary.

In Indian sub-continent, studies on river hydrobiology and pollutional effect due to domestic and industrial wastes have been done by many workers for many rivers from time to time. Bhimachar and David (1946) studied the effect of paper mill effluents on the Bhadra river fisheries at Bhadravati. Chako and Ganapati (1951) determined the effect of paper mill effluents on physico- chemical aspects of Godavari River in India. Among the various inorganic contaminants of the river water, heavy metals are getting importance for their non-degradable nature and often accumulate through tropic level of plant and fish to man causing a deleterious biological effect (Jain, 1978). Effect of industrial effluents on phytoplankton communities of the river Ganga was studied by Bilgrami and Siddiqui (1980). Sudhakar *et al*. (1991) studied the impact of paper mill effluents on the distribution of cyanobacteria. Cyanobacteria found to constitute 30% and 67.8% by numerical abundance in unpolluted and highly polluted stations respectively. Fazeli *et al*. (1991) investigated the accumulation of heavy metals in different parts of coconut trees growing in the area irrigated directly by the wastewaters of a paper mill near Nanjangud, Karnataka. The concentrations of heavy metals like Cu, Pb, Zn, Ni, Co, and Cd in coconut water, root, and leaf are higher than the limits suggested by World Health Organization. A study on water quality of river Buriganga receiving discharge of tannery effluents revealed that the river was totally devoid of oxygen and upto 500m downstream no fish or other aquatic organisms were recorded during dry season of the year (Huq, 1998). Discharge of industrial effluents constitutes about 62% of total source of heavy metals responsible for degrading the water quality of a river (Garba and Abubakar 2006). Chandra *et al*. (2006) reported that the BOD, COD, TDS, TSS, chloride and nitrate contents of water of Gola increased by 20-30 times after confluence of the paper mill waste with the river. Shekhar *et al*. (2008) studied the water quality status of river Bhadra receiving Mysore paper mill and Iron and steel mill effluent. They reported that the water quality deteriorated along the length of river from upstream to downstream due to direct discharge of industrial effluents and domestic sewage. Rout and Sarma (2010) studied the algal colonization and distribution pattern in river Barak receiving effluents from a Paper mill in Panchgram. The presence of *Spirogyra crassa, Oscillatoria* *tenuis*, *Nitzschia* sp. in the effluent receiving point suggests the capability of these species to do anoxygenic photosynthesis. Sharma *et al*. (2011) monitored the water quality of Narmada river and reported that the water of the river is highly contaminated due to heavy mixing of effluent waste from Security Paper Mill (SPM) and domestic sewage and not safe for human use. Ferdous *et al*. (2012) reported 27 genera of phytoplankton with *Merismopedia* as the dominant species from the polluted water of river Buriganga, contaminated by the tannery effluent sewage water containing high concentration of heavy metals, viz. Cr, Zn, Pb, Ni, Cu, Cd and As. Saikia and Lohar (2012) conducted a study on the physico-chemical parameters of water and seasonal algal abundance of Elenga beel, a wetland receiving paper mill effluents. The results revealed that the wetland is highly polluted and is dominated by the resistant varieties of nuisance algal flora. Das and Dutta (2012) studied the effects of industrial effluents on ecology of a wetland of Nalbari district, Assam with special reference to Ichthyofauna. The results showed that industrial effluents affected the integument, gills and several organs of such fishes especially liver. Negi and Rajput (2013) conducted a study on impact of pulp and paper mill effluents on the qualitative and quantitative aspect of phytoplankton on river Ganga in Bijnor (UP). *Synedra, Cocconeis* and *Spirulina* were significantly abundant at the discharge point and downstream indicating high tolerance of these species to the various paper mill effluents. Mukherjee and Saha (2015) conducted a study on the effects of pulp and paper mill effluents on the water quality and fish diversity of the river Hooghly and the lowest diversity of fishes were observed in the effluent receiving station compared to other stations irrespective of seasons.

Numerous researches have been conducted on the floristic and ecology of lentic and lotic algae from both polluted and unpolluted water, but the algal flora of waste water system have not been investigated much (Vijaykumar *et al*., 2012). In comparison to freshwater system, algae in waste waters are exposed to different environmental stress and a study on the biological parameters of such water bodies paves the way for future waste treatment programmes using the indicator species. Kumar *et al*. (1974) made physico-chemical and biological analyses of the Indian Oil Refinery in Barauni, the Sindri Fertilizer Factory in Sindri and the Mohan Meakin Brewery in Ghaziabad. The studies indicate that algae can tolerate and grow in highly polluted waters and the abundance of blue-green algae, flagellates and euglenoids are mostly associated with organically rich effluents and low in dissolved oxygen. Ramaswamy *et al*. (1982) conducted an ecological study on algae in waste water from a rubber tyre factory near Mysore, Karnataka. He reported the occurrence of blue green alga *Microcoleus* for the first time in the waste water from this type of industry and in spite of the absence of nitrates and phosphates, diatoms were abundantly present in the effluent stream. Sahai *et al*. (1985) studied the physico-chemical characteristics and algal flora of effluents from waste releases of fertilizer factory, sugar factory, distillery and township sewage. In all the polluted habitats Cyanophycean members as *Oscillatoria, Microcystis, Chlorella, Closterium* dominated. Fifteen different strains of blue green algae (including one green alga) were collected by Sunita and Rao (2003) from the waste waters of fruit processing industrial areas. Vijayakumar *et al*. (2007) conducted a survey on cyanobacterial diversity of dye industry effluent. They isolated 24 species of cyanobacteria distributed in 9 genera falling under 5 different families. Among cyanobacteria, *Oscillatoria* with nine species was found to be the dominant genus in that habitat. Kirkwood *et al*. (2001) studied the occurrence of cyanobacteria in pulp and paper waste-treatment systems in Brazil, Canada, New Zealand, and the U.S.A. All the studied area contained dynamic cyanobacterial communities. No other viable photoautotrophic populations were detected in the ponds. Regardless of geographical location, Oscillatoriales including *Phormidium*, *Geitlerinema*, and *Pseudanabaena* were the dominant taxa. Biodiversity of cyanobacteria in industrial effluents such as dye, paper mill, pharmaceutical and sugar were surveyed by Vijayakumar *et al*. (2007). The physico-chemical characteristics of all the effluents studied were found to be more or less similar. Totally 59 species of cyanobacteria distributed in four different effluents were recorded. Among the effluents, sugar mill recorded the maximum number of species (55) followed by dye (54), paper mill (45) and pharmaceutical (30). In total 26 species of cyanobacteria were recorded in common to all the effluents analyzed. Of them, *Oscillatoria* with 13 species was the dominant genus which was followed by *Phormidium* (8), *Lyngbya* (2), *Microcystis* (2) and *Synechococcus* with single species each. An investigation was carried out by Boominathan *et al*. (2005) to evaluate the impact of dairy effluent on the microbial diversity viz., bacteria, fungi and cyanobacteria. Together 9 species of bacteria, 11 species of fungi and 20 species of cyanobacteria were isolated from the effluent stream. Among bacteria, *Pseudomonas* with two species was dominant. *Aspergillus* was dominant group of fungi. Inhabitant of all kinds of water (effluents) recorded 20 species of Cyanobacteria. *Oscillatoria* with 11 species was the dominant genus followed by *Phormidium* (5), *Plectonema* (2), *Aphanocapsa* and *Chlorogloea* with single species each. Cyanobacterial populations from three different industrial effluents such as chemical, distillery and oil refinary have been isolated and identified by Vijayakumar *et al*. (2012). Their diversity has been correlated with physicochemical characteristics of the effluents. Altogether 63 species of cyanobacteria were recorded from these effluents. Among the effluents, distilleries contained the maximum number of species (63) followed by chemical (52) and oil refinery (43). Except oil refinary effluent, others recorded heterocystous cyanobacteria. Totally 34 common species were observed in all the effluents. Of them, Oscillatoria with 14 species was the dominant genus followed by *Lyngbya* (7), *Phormidium* (6), *Chroococcus, Aphanocapsa, Aphanotheca, Synechocystis* and *Plectonema* with single species each. Paranthaman and Karthikeyan (2013) monitored the impact of paper effluent on the microbial diversity viz., bacteria, fungi and cyanobacteria for one year. Altogether 10 species of bacteria, 5 species of fungi and 42 species of cyanobacteria were isolated from the effluent stream. Among the bacteria, *Pseudomonas* with two species and others with single species each were recorded. *Aspergillus* was dominant among fungi with 2 species followed by *Penicillium* with three. Cyanobacteria, one of the dominant groups of algae, inhabiting all kinds of water (effluents), recorded 42 species. *Oscillatoria* with 14 species was the dominant genus followed by *Lyngbya* (8), *Phormidium* (4), *Chroococcus* and *Microcystis* with two species each. Higher amounts of phosphates and nitrates, with sufficient amount of oxidizable organic matter, limited dissolved oxygen content and slightly alkaline pH were probably the factors favoring the growth of microbes especially cyanobacteria.

2.3 Biochemical response of cyanobacteria to toxicity

The influence of environmental stress on the physiology of an organism also results in profound changes in the biochemical composition of the organism. Metal-tolerant cyanobacterium (*Nostoc linckia*) and metal-sensitive (*Nostoc rivularis*) were grown at three levels of sewage water (25, 50 and 75%). Low levels of sewage water increased chl *a* content, photosynthetic O2-evolution, respiration and protein content. About 65–60% of Cd or Zn were found in pellets (sediment) as insoluble form in the two species (El-Enany and Issa, 2000). Kumar *et al*. (2008) studied metabolic response and nutrient removal by *Tolypothrix tenuis* (kutz.) from fertilizer industrial effluent. The test concentrations caused a concentration dependent decrease in growth, pigment content and carbohydrate values. Removal of nutrients from the medium was found to increase in all the concentrations. The growth stimulation and inhibition of algae treated with pulp and paper mill effluent was investigated by Saikia *et al*. (2011) in laboratory culture condition. *Oscillatoria chlorina* tolerated higher concentration where as *Scenedesmus quadricauda* tolerated lower concentration of effluent. At 100% effluent concentration growth was retarded and inhibition was the result. Kiran and Thanasekaran (2011) investigated the response of an indigenous cyanobacterial strain *Lyngbya putealis* isolated from contaminated site to increasing levels of copper and cobalt. Metal treatments positively affected the chlorophyll and phycobiliproteins and biomass production at 0.5 mg/L as compared to that at control. Exopolymer production also increased significantly in response to both copper and cobalt in *L. putealis* and found to be maximum at metal concentration of 2.0 mg/L. This species also showed increased accumulation of starch and carbohydrates in presence copper or cobalt at 0.1 mg/L. But the overall response was better for copper as compared to cobalt in single metal systems for almost all the studied parameters. Rana *et al*. (2013) isolated *Oscillatoria tenius* and *Phormidium corium* from the sewage water irrigated soil of Rohtak city of Haryana. The isolates were exposed to elevated concentrations, 0.5mg/l, 1mg/l, 2mg/l, 5mg/l, 10mg/l of heavy metals using $CuSO_4$, $ZnSO_4$, $NiCl_2$, $CdCl_2$ and $PbNO_3$ respectively, supplemented with BG-11 medium. Low concentrations of heavy metals stimulated the growth of these species. These species were most sensitive to nickel metal and least sensitive to lead. The native cyanobacterial isolates *Lyngbya contorta* and *Phormidium foveolarum* were isolated, characterized and changes in their biochemical composition in response to different concentrations of sewage waste water were studied. The overall response of biochemical parameters (biomass, chlorophyll, carotenoid, soluble protein and total carbohydrate) was batter for 50% sewage wastewater concentration (Rana *et al*., 2013). Brahmbhatt *et al*. (2013) exposed *Oscillatoria* sp. to various concentrations (2, 5, 10, 20 & 30ppm) of Cr and Pb for 21 days. Algae accumulated appreciable amount of both metals from solution, however, accumulation of Cr was lower compared to Pb. Chlorophyll content in the algae was found to increase at all doses of the metals in comparison to unstressed algae which could be used as biomarker of metal toxicity. Sivasubramanian *et al*. (2012) reported preliminary result on the tolerance and growth of micro algae (*Chlorococcum humicola* and *Chroococcus turgidus*) in the sludge from hypochlorite manufacturing industry. The micro algal growth in the sludge was studied by diluting it with culture medium in two different concentrations (2.5% and 5%). *Chlorococcum humicola* and *Chroococcus turgidus* showed a significant growth at 2.5% concentration compared to 5% concentration. Swain *et al*. (2014) isolated *Anabaena iyengarii* and *Lyngbya* sp. from paddy field soils near the vicinity of J. K.

Paper Mill, Rayagada. The isolates were grown in varying concentrations of industrial effluent in defined culture media. Growth, chlorophyll *a*, nitrogen content and photosynthetic rate of the isolates were studied. *Anabaena iyengarii* exhibited good growth in J. K. Paper Mill effluents at lower concentrations compared to *Lyngbya* sp.

2.3 Bioremediation potential of cyanobacteria

2.3.a Cyanobacteria as removal agent of heavy metal

In both the terrestrial and aquatic habitats algae play the predominant role in biogeochemical cycling of heavy metals (Rajamani *et al*., 2007). In metal contaminated bodies, algae as a primary producer, accumulate and pass the metals to the subsequent higher tropic level (Murugesan *et al*., 2008). Thus, being an important basic component of the food chain and natural oxygenator of the freshwater ecosystem, it is obligatory to study the toxic effects of metal pollutant on algae. Major metal pollutants which are regularly found in industrial wastes are Cu, Zn, Ni, Co, Pb, Cr, Cd (Venkateswarlu *et al*., 1994; Kaushik *et al*., 1999). Slotton *et al*., (1989) observed that the cyanobacterium *Spirullina* sp. contains detectable level of mercury and lead when grown under the contaminated condition proving its capacity to uptake metals from the polluted environment. De Carvalho *et al*. (1995) evaluated the bioabsorption of Cd, Cu and Zn in two-metal systems using brown marine alga *Ascophyllum nodosum*. They observed that each of the metal inhibited the sorption of others. Rai *et al*. (1998) reported the bisorption potential of *Microcystis* sp. in removal of Ni and Cd and also the potential of alginated immobilized cells of the cyanobacterium that can be used for removal of Cu. De Philippis (2003) reported that the biomass of *Cyanospira* and *Nostoc* possesses high specific uptake for copper, suggesting the possibility to use these two cyanobacteria for the bioremoval of heavy metals from contaminated water bodies. During an experiment to assess the potential of metal removal of *Synechococcus* sp., showed the binding to metal ion as Cu (11.3 mg/g biomass), Pb (30.4 mg/g biomass), Ni (3.2 mg/g biomass), Cd ((7.2 mg/g biomass) (Yee *et al*., 2004). Lengke *et al*. (2006) investigated the gold bioaccumulation by cyanobacterium *Plectonema boryanum* from gold (III)-chloride solutions. They concluded that the reduction mechanism of gold (III) to metallic gold by this organism involves the formation of an intermediate gold (I)-sulfide due to a chelation process via some thiol compounds. Ruangsomboon *et al*. (2006) studied the Pb2+ removal ability of the living-freshwater cyanobacterium *Calothryx marchica*. At lower biomass concentration cyanobacterium showed higher absorption than that at high biomass concentration. *C. marchica* had Pb^{2+} binding capacity of 74.04 mg g-1 and indicators of adsorption capacity of 18.01. Anjana *et al* (2007) investigated the bioabsorption of chromium from metal contaminated soil in the premises of textile mill by using the cyanobacteria, *Nostoc calcicola*, and *Chroococus* sp. The biosorption of Cr was found to be optimum at initial pH 3-4 and at 30 minutes contact time. Kiran *et al*. (2007) reported biosorption of Cr(VI) by native isolate of *Lyngbya putealis* HH-15 in batch system under varying range of pH (2.0–10.0), initial metal ion concentration (10–100 mg/l) and salt concentration (0–0.2%). Maximum metal removal (94.8%) took place at pH 3.0 with initial Cr concentration of 50 mg/l. Kaushik *et al* (2008) reports on chromium (VI) tolerance of two cyanobacterial strains *Nostoc linckia* and *Nostoc spongiaeforme* isolated from salt affected soils using uni-algal and bi-algal systems. Prado *et al*. (2010) observed the rate of biosorption of cadmium and copper ions by nonliving biomass of the brown macroalga *Sargassum sinicola* under saline conditions. *Anabaena variabilis, Aulosira sp., Nostoc muscorum, Oscillatoria* sp. and *Westiellopsis* sp. were grown in 100% effluent supplemented with and without basal nutrients and in Siruvani river water as control. Results of the growth response of the BGA in wastewater showed that the algae can grow very well in polluted water. Among the tested isolates *Anabaena variabilis* performed well for biosorbing the heavy metals. The mean available Cr, Cd, Ni and Pb were reduced to 0.67, 0.57, 1.72 and 1.32 ppm respectively by *Anabaena variabilis* in 28 days. The algae were also able to completely remove the offensive odour of the effluent (Parameswari *et al*. 2010). Kumaran *et al*. (2011) studied the metal uptake capacity of the native strains of *Anabaena* sp., *Trichodesmium* sp., *Oscillatoria* sp., *Cylindrospermopsis* sp. and *Nostoc* sp. isolated from the contaminated water of Uppanar Estuarine Water, Southeast Coast of India. *Nostoc* sp. showed high resistant than the others. During this experiment 95.4% of Cd, 97.7% of Fe, 99.6% of Pb, 88.23% of Ni and 75.3% of Zn was removed by the *Nostoc* sp. Cecal *et al* (2012) deals with a study of the biosorption of UO2 2+ ions on two green algae: *Chlorella vulgaris* and *Dunaliella salina*. By kinetic investigations it was found that the biosorption process was higher for *Chlorella vulgaris* than for *Dunaliella salina*. Miranda *et al* (2012) observed two species of cyanobacteria, *Oscillatoria laete-virens* (Crouan & Crouan) Gomont and *Oscillatoria trichoides* Szafer isolated from a polluted environment was studied for their Cr6+ removal efficiency from aqueous solutions. Das (2012) studied the biosorption of chromium and nickel by dried biomass of cyanobacterium *Oscillatoria laete-virens*. Cr (VI) and Ni (II) adsorption was fast at initial stage of the contact period and maximum adsorption took place within the first 60-75 min. Initial metal ion concentration plays an important role in determining the adsorption capacity. The maximum adsorption capacity (q_{max}) of Cr (VI) and Ni (II) by the cyanobacterial biomass was 103.09 and 84.75 mg g-1 respectively. Karuppaiah *et al*. (2013) treated the tannery effluent using marine cyanobacterium *Lyngbya* sp. with coir pith. Removal of heavy metals such as Mercury, Cadmium, Iron, Copper and Lead contents was monitored on 30th day of incubation. All heavy metals were found to be reduced with all the treatments notably with the combined treatment of *Lyngbya* sp. with coir pith in tannery effluent due to absorption of heavy metal particles on the surface of *Lyngbya* sp. and coir pith. Sivakami *et al*. (2015) studied the removal of three heavy metals (Cd, Hg and Pb) by biosorption using Cyanobacteria *Oscillatoria limosa*. Results indicate that the order of uptake of Cd, Hg and Pb was found to be the order of 82, 78 and 72% respectively. The study also indicated that the metal uptake appeared to be a concentration independent phenomenon where an increase in metal concentration resulted in an increased uptake of metal.

2.3.b Cyanobacteria as removal agent of industrial effluents

Intensive studies have been done regarding the success of cyanobacteria to detoxify the pollutants rich wastewater. Uma and Subramanian (1990) used the marine cyanobacteria *Oscillatoria* sp. BDU 10742, *Aphanocapsa* sp. BDU 16 and a halophilic bacterium *Halobacterium* US 101, to treat ossein factory effluent which resulted in reduced calcium and chloride levels and enabled 100% survival and multiplication of Tilapia fish with only cyanobacteria as feed source. Over the last few decades, algae have been widely applied to perform the biological tertiary treatment of secondary effluents (de la Noüe *et al*., 1992). Manoharan and Subramanian (1992a, b and 1993a) investigated the physico-chemical parameters of domestic sewage, paper mill and ossein effluents under laboratory conditions by inoculating the *Oscillatoria pseudogeminata* var. *unigranulata*. Te result showed a significant reduction of BOD and COD and the removal of various nutrients such as nitrates, ammonia and phosphorus from the effluents. The cyanobacterium *Phormidium bohneri* was used by Lessard *et al*. (1994) to remove nutrients from municipal wastewater. Field experiments suggested that the use of cyanobacteria was a viable alternative for small communities. Satisfactory reductions in ammonium, nitrate, nitrite and phosphates were achieved. Shashireka *et al*. (1997) found that *Phormidium valderianum* BDU 30501 was able to tolerate and grow at a phenol concentration of 50 mg/l and removed 38 mg/l within a retention period of seven days. Kalavathi *et al*. (2001) explored the possibility of using a marine cyanobacterium *Oscillatoria* boryana BDU 92181 for decolorization of distillery spent wash and its ability to use melanoidins as carbon and nitrogen source. A mixed culture of cyanobacteria was used by Sharma *et al*. (2002) to study the decolourization and COD reduction of digested distillery spent wash. On supplementing the diluted effluent with 1 per cent single super phosphate, about 63 per cent decolourization and 72 per cent COD reduction were achieved after 20d of incubation at 30-35^{o} C. Vijayakumar *et al*. (2005) investigated the role of cyanobacterium, *Oscillatoria brevis* in the treatment of effluent from dye industry. The result showed that within 30 days, more than 60 per cent of colour has been reduced. Nutrients such as nitrates and phosphates have been completely removed. An increase in DO content and reduction in BOD and COD upto 90 per cent have also been reported. The potential of hypersaline *Phormidium tenue* in remediation of paper mill effluent water was investigated by Nagasathya and Thajuddin (2008).

Cyanobacteria have been used intensively in lagoons (Neel *et al*., 1961) and stabilization pond (Gloyns, 1971) for removing pollutants and surfeit nutrients from the wastewater (El-Bestawy, 2008). Therefore, culture and application of indigenous cyanobacteria in the paper mill wastewater remediation may serve as a potential and sustainable approach toward the effluent treatment. The investigation conducted by El-Bestawy (2008) for treatment of mixed-domestic industrial water using three indigenous species of *Anabaena oryzae, Anabaena variabilis* and *Tolypothrix ceytonica* proved to be highly efficient in removing organic matter, solids, FOG and heavy metal respectively. Kumar *et al*. (2008) studied metabolic response and nutrient removal by *Tolypothrix tenuis* (kutz.) from fertilizer industrial effluent. The removal of sulphate, phosphate and nitrate contents of fertilizer industry by the *T. tenuis* was more in 60 percent applied dose than other two (80 and 90 percent). Nitrate removal was higher than phosphate and sulphate removal by the organism used. Also, the fertilizer effluent seems to be non-toxic to *T. tenuis* and enhanced major metabolic activities like pigments, photosynthesis, protein, and amino acid synthesis below 80 and 90 percent treatments. Rajasulochana *et al*. (2009) studied bioremediation of oil refinery effluent by using *Scenedesmus obliquus*. Various physicochemical parameters such as pH, alkalinity, hardness, iron content, COD, BOD and free ammonia were estimated. It is observed that *Scenedesmus obliquus* proved to be an efficient removal of most of the parameters including COD and BOD. Nanda *et al*. (2010) employed *Nostoc* sp. for the bioremediation of paper mill effluents and as well as of tannery effluents. The results revealed a considerable decrease of 53.1% in colouration, 49.6% in BOD, 39.7% in COD and 53.0% in TDS of paper mill effluents after 4 weeks of treatment with *Nostoc*. Similarly, the results revealed a 57.5%, 37.8%, 48.6% and 66.1% decrease in BOD, COD, TDS and colour of the tannery effluents after 4 weeks of treatment with *Nostoc*. Dubey *et al*. (2011) employed native cyanobacterial species viz. *Oscillatoria* sp., *Synechococcus* sp., *Nodularia* sp., *Nostoc* sp. and *Cyanothece* sp. as single and mixed culture for biodegradation of pharmaceutical and textile industrial effluents of Mandideep, Bhopal. The contaminants removal efficiency (RE) percentage of cyanobacterial species ranged between 69.5 and 99.6% with a maximum of 97.0 to 99.6% at 5 ppm, 83.9% and 99.7% at 10 ppm and maximum between 95.5 and 99.7%. Mixed culture RE percentages ranged between 91.6 and 100% at 5 ppm with a maximum range of 99.3 to 100%, while at 10 ppm, the RE percentage ranged between 90.4 and 100%, with a maximum range of 96.0 to 100%. Vijayakumar and Monoharan (2012) stated 75% removal of colour, complete removal of nutrients and 95% reduction of organic matter of the dye industry effluent when treated with *Oscillatoria brevis* and *Westiellopsis prolifica*. Increase in DO content and reduction of BOD, COD upto 95% have been reported. It is concluded that *Oscillatoria* had a little edge over than *Westiellopsis* can successfully be used not only to reduce pollution load but also for colour removal purposes. Henciya *et al*. (2013) used *Lyngbya* sp. BDU 9001 with coir pith and also cyanobacterium culture with effluent alone to decolorize the textile dye effluent. The accuracy of 73% decolorization efficiency was performed at the 15^{th} day of incubation. The chlorophyll *a* and protein content were increased and decolorized activity was further confirmed by the standard techniques of Dissolved Oxygen (DO) and, biological oxygen demand (BOD). Similarly, David and Rajan (2014) recorded significant reduction in the BOD and COD concentration in textile industry effluent when treated with *Anabaena variabilis, Oscillatoria salina, Nostoc muscorum* and *Lyngbya majuscula*. Nandhini *et al*. (2014) applied marine cyanobacterium *Lyngbya* sp. to check its bioremediation ability in textile dye effluent. Ground nut shell was used as an adsorbent in the current study. The biochemical parameters like Nitrate, Nitrite, EC, TDS, Ammonia, Alkalinity, Calcium, and Magnesium were studied. 77.55% decolourisation was attained in the treatment of textile effluent with *Lyngbya* sp.

2.3. c Estimation of toxicity analysis to Cyanobacteria

Panigrahi *et al*. (2003) studied the toxicity of parathion-methyl to cells, akinetes and heterocysts of the cyanobacterium *Cylindrospermum* sp. and the probit analysis of toxicity. Arunakumara and Xuecheng (2009) studied the effects of heavy metals (Pb2+ and Cd2+) on the *Synechocystis* sp. PCC 6803. Higher doses resulted in reduction in growth and pigment contents of the unicellular cyanobacterium. Lethal concentration (96 h LC50) values (3.47 mg/L Cd2+ and 12.11 mg/L Pb2+) indicated that *Synechocystis* sp. PCC 6803 is more vulnerable to Cd2+ than Pb2+. Koksoy and Aslim (2013) determined the herbicide resistance in twelve aquatic Cyanobacterial species by probit analysis. Ozturk *et al*. (2009) studied the chromium (VI) removal behaviour by two isolates of *Synechocystis* sp. in terms of exopolysaccharide (EPS) production and monomer composition. The EC50 of the Cr (VI) for *Synechocystis* sp. BASO670 and *Synechocystis* sp. BASO672 were determined as 11.5 mg L^{-1}, and 2.0 mg L^{-1}, respectively. Rangsayatorn *et al*. (2002) exposed the *Spirulina* (*Arthrospira*) *platensis* TISTR 8217 to six different cadmium concentrations for 96 h. The IC50 at 24, 48, 72, and 96 h were 13.15, 16.68, 17.28, and 18.35 mg/l Cd, respectively. Mishra *et al*. (2011) studied the acute toxicity (LC50 evaluation) of paper mill effluent to a freshwater fish, *Mystus vittatus*. A well marked variation in the LC50 values in different exposure periods as well as in various months of the spawning phases of the test fish were observed. Hong and Shan-shan (2005) studied the bioremediation potential of *Spirulina* to remove aqueous lead of low concentration (below 50 mg/L) from wastewater. The 72 h-EC50 (72 h medium effective concentration) was estimated to be 11.46 mg/L (lead). The maximum biosorption capacity of live *Spirulina* was estimated to be 0.62 mg lead per 105 alga cells.

2.4 Cyanobacterial investigation using polyphasic approach

Kato *et al*. (1991) evaluated the allozyme divergence within and between three *Microcystis* species on the basis of genotype and indicated the relevance of these studies in solving taxonomic problems of cyanobacteria, especially at the species genus levels. *Synechococcus* diversity on the basis of RNA polymerase gene sequences of isolated strains was determined by Toledo and Palenik (1997). Teaumroong *et al.*, (2002) aimed to identify indigenous nitrogen-fixing cyanobacteria based on morphology, N2-fixing efficiency, and molecular genetic methods on the basis of DNA fingerprinting using the PCR method. The diversity among 853 isolates of nitrogen-fixing cyanobacteria obtained from soil samples collected from different ecosystems in the central, northern and northeastern regions of Thailand was examined. Most isolates showed slow growth rate and had filamentous, heterocystous cells. *Anabaena* and *Nostoc* were the dominant genera among these isolates. Phylogenetic analysis clearly distinguished all cyanobacterial strains even at intraspecies level. This showed a wide range of diversity among the N2-fixing cyanobacteria in Thai soil. Bérard *et al*. (2003) described a polymerase chain reaction (PCR) approach targeting algal 18S rDNA sequences of desoxyribonucleic acid (DNA) samples extracted either from unialgal eukaryotic microalgae culture, complex assemblages of microalgae populations or natural soil communities. The results showed that microalgae rDNA could be amplified by PCR from soil DNA samples. They also indicated difficulties extracting DNA from diatoms directly from soils, probably because of the presence of robust silicate valves. An 18S rDNA library has been established and preliminary phylogenetic analysis showed the feasibility of applying molecular methods to study edaphic algae community structure. This is promising for soil algae ecology and for developing soil biological indicators. M13 PCR fingerprinting, ERIC PCR fingerprinting and amplification of the internal transcribed spacer (ITS) region were used by Vale´rio *et al*. (2005) to characterize nine cultured strains of *Cylindrospermopsis raciborskii*, sourced from several freshwater lakes and rivers in Portugal, and two other Australian. Strains belonging to other taxa including *Microcystis aeruginosa*, *Aphanizomenon* sp., *Planktothrix agardhii* and *Oscillatoria neglecta* were also analysed to evaluate the taxonomical potential of the fingerprinting methods. Data obtained from genomic fingerprinting were used to perform hierarchical cluster analysis and demonstrated ability to differentiate strains at intra-specific level. Prasanna *et al*., (2006) studied 30 *Anabaena* strains, isolated from diverse geographical regions of India, were characterized using morphological and physiochemical attributes as well as molecular marker profiles. Significant differences were observed among the *Anabaena* strains with regard to the shape and size of trichomes and individual cells within a filament, besides qualitative and quantitative aspects of phycobiliprotein accumulation and activities of enzymes involved in nitrogen metabolism. Analyses of molecular polymorphisms in a selected set of 13 *Anabaena* strains, using primers based on repetitive sequences in the genome, led to unambiguous differentiation of the strains as well as understanding of their genetic relationships. Informative morphological, physio-chemical and molecular characters have been identified that could aid in differentiation and utilization of *Anabaena* strains as bioinoculants or as sources of pigments. Marquardt and Palinska (2007) studied phenotypic and genotypic diversity of cyanobacteria of the genus *Phormidium* from different habitat and geographical sites and their results confirms that *Phormidium* group is not phylogenetically coherent. The presence of repetitive DNA sequences viz., short tandemly repeated repetitive (STRR) and highly iterated palindrome (HIP), in the cyanobacterial genome were used by Selvakumar and Gopalaswamy (2008) to generate a PCR-based fingerprint pattern of nine cyanobacterial cultures (both stress tolerant and non-tolerant), belonging to the genus *Westiellopsis*. By this method it was possible to generate distinguishing fingerprint patterns for all the isolates and cluster isolates with similar stress tolerance properties. This study reveals the utility of repetitive DNA sequences in the cyanobacterial genome, for differentiation of *Westiellopsis* cultures and clustering strains that posses similar stress tolerance properties. Tuji and Niiyama (2010) conducted molecular analyses of nineteen species of planktonic *Anabaena* strains collected from eutrophic lakes and ponds in Japan were conducted and the phylogenetic relationships compared. Using cultured strains with clearly identified morphological characters, there was no discrepancy between the identification results from the morphological study and the cluster classification results from the DNA analyses. The cultured strains were divided into four clusters based on the information from the 16S rDNA and *rbcLX* (*rbcL* and *rbcX*) analyses. The forms and dimensions of vegetative cells and akinetes as well as the relative location of akinetes to heterocytes were found to be important for distinguishing planktonic *Anabaena* species. Ten isolates of freshwater filamentous heterocystous *Anabaena* spp. has been studied by Ezhilarasi and Anand (2010) amplified 16S rRNA gene restriction analysis and compared with morphological characters. They were morphologically discriminated two groups, each containing five *Anabaena* species based on the proximity of the akinetes to heterocyst, adjacent to or away from the spore in the trichome. The amplicons were digested with three restriction enzymes (*Alu*I, *Hhe*III, *Taq* I) and the banding patterns obtained were analyzed. Cluster analysis showed the separation of all the strains into two main clusters. The clusters for three different enzymes yielded heterogenous groupings of the morphotypes and resulted in unclear delineation of tested organisms. Thirty cyanobacterial strains of *Calothrix* (family *Rivulariaceae*) isolated by Singh *et al*. (2011) from diverse geographical regions of India were analyzed using morphological and molecular approaches. Most of the isolates were planktonic while some grew benthically. Significant differences were observed with regard to the shape and size of the vegetative cells, heterocysts, and akinetes. Analyses of molecular polymorphisms using Restriction Fragment Length Polymorphisms (RPLP) of 16S rRNA genes with the reference strain led to unambiguous differentiation of the isolates as well as understanding of their genetic relationships. Hašler *et al.* (2012) isolated filamentous epipelic cyanobacteria from ponds and lakes in the Czech Republic, Austria and Italy. Morphological and genetic variation of 20 isolated strains within the genera *Geitlerinema*, *Microcoleus* and *Phormidium* were studied. Partial sequences of the 16S rRNA gene were used for phylogenetic analyses, and secondary structure of the

16S–23S ITS region was used to additionally define clades. The *M. vaginatus* clade is well defined by an 11 bp insert in 16S rRNA gene (bp 423–433) and populations from different ecological conditions differ in secondary structure in the 16S–23S ITS regions, particularly in Box–B helices. *Ph. autumnale* and the genus *Geitlerinema* appear to be polyphyletic as presently defined. Hrouzek *et al*. (2013) described the cyanobacterial genus *Desmonostoc* gen. nov. including *D. muscorum* comb. nov. as a distinct, phylogenetically coherent taxon related to the genus *Nostoc*. The new genus includes the traditional species *Nostoc muscorum* Agardh ex Bornet et Flahault 1888, and several other strains previously assigned to the genus *Nostoc*, which present similar morphology and phylogenetic placement within the Desmonostoc lineage. The Desmonostoc clade is phylogenetically coherent according to 16S rRNA gene sequence analysis performed with four distinct approaches. In all phylogenetic trees, Desmonostoc formed a supported group separated from strains belonging to the related taxa *Nostoc, Trichormus*, and *Mojavia*. Amer *et al*. (2013) isolated and characterized the cyanobacterial community including a Microcystin-producing *Nostoc* sp. strain in the Nile River, Egypt. The combined use of morphological identification and phylogenetic characterization employing primers that target the 16S rDNA region led to the identification of ten isolates belonging to eight cyanobacterial genera in the Nile river. 16S-23S ITS region was amplified to confirm two isolates to be affiliated to genus *Nostoc*. Using MALDI-TOF/MS, the production of the hepatotoxic demethylated MC-LR was detected by one isolate that clustered together with the genus *Nostoc*. Protein phosphatase inhibition assay has confirmed toxicity. Results added to the rising importance of *Nostoc* as a hepatotoxin-producing cyanobacterium. Furthermore, the results stressed that water municipalities in the studied region need to be assessed the potential threat of toxic cyanobacteria that may pose to human health and economy. Keshari and Adhikary (2014) studied the colonization of cyanobacterial biofilms on various archaeologically important stone temples, caves, mortar monuments as well as building facades of India. Generally the genera belonging to *Hassallia*, *Tolypothrix*, *Scytonema*, *Lyngbya* and *Calothrix* soon appeared after wetting the biofilms. Molecular phylogenetic analysis based on 16SrRNA partial gene sequencing of the cyanobacteria isolated was compared with those species from tropical regions. The genetic diversity and the potential toxicity of bloom-forming cyanobacteria were studied in four lagoons located in the state of São Paulo and were evaluated using DGGE fingerprinting and 16S rDNA clone library (Elias *et al.*, 2015).