



## Biochemical evaluation of freshwater cyanobacteria isolated from different freshwater habitats of Southern Karnataka

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### ABSTRACT

In the present study, efforts are made to study the freshwater cyanobacteria for the production of biomass and biochemical compounds. Eight species were isolated from different aquatic habitats of Southern India, mass-cultured in BG<sub>11</sub> media and evaluated for biochemical composition and also compared among themselves. They showed significant quantities of nutrients like total carbohydrate (up to 20%), reducing sugar (up to 15%), total protein (15-23%), total free amino acid (8-12%), and total lipids, up to 8- 15% dry weight of cells. Vitamin C (1.5 – 2.8 mgg<sup>-1</sup>) and vitamin E (2.0 – 4.5 mg g<sup>-1</sup>) was also present in smaller amounts. Sugar profiling using GC revealed the presence of ten neutral monosaccharides and one amino sugar (glucosamine) in the eight species of cyanobacteria of different locations/habitats. Fatty acid profile was carried out by Gas chromatography. The study reveals the presence of n-saturated, unsaturated and long chain fatty acids, majority of them belong to polyunsaturated fatty acids (PUFA). Essential fatty acids like  $\alpha$ -linolenic acid,  $\gamma$ -linolenic acid and arachidonic acid were also detected. Amino acid profile was done by HPLC method and the study noticed the presence of seven essential amino acids. Photosynthetic pigments such as chlorophyll a (6-18.5  $\mu$ g/ml) and carotenoids (2.5 - 10.5  $\mu$ g/ml) were also found in considerable quantities. Phycobillin pigments such as phycocyanin (3.5-8.8  $\mu$ g/ml), phycoerythrin (0.5-5.2  $\mu$ g/ml) and allo-phycoyanin (1.5-8.2  $\mu$ g/ml) were also found to be in better range. The results of atomic absorption spectroscopy reveal the presence of certain essential minerals and trace elements in cyanobacteria which are of high commercial value. The overall study suggests that biomass of such cyanobacterial species with implications in food and other pharmaceutical industry as a source of basic materials particularly in the preparation of nutrient supplement, products and other fine chemicals.

**Key Words:** Cyanobacteria, Biomass, Biochemical constituents, Pigments, Minerals, Gas Chromatography, Atomic absorption spectroscopy.

### Introduction

Cyanobacteria are gram negative, oxygenic, photosynthetic prokaryotes. They constitute the base of the food web in the aquatic ecosystems and play an important role in primary productivity (Klemer et al. 1990). They offer a greater opportunity for the production of biomass of a number of species which are useful to mankind as energy source, natural pigments, proteins, carbohydrates, lipids, important fatty acids, amino acids and vitamins. Some species are widely used in mariculture, fertilizer, food and pharmaceuticals and also in pollution abatement (Tajuddin et al. 2005). They contain considerable amount of proteins, carbohydrates, lipids, certain important fatty acids, amino acids and vitamins and hence are a good source of nutrients. Only a few cyanobacterial species (including *Spirulina*) have been well characterized and exploited commercially. The *Anabaena*, *Nostoc* and *Spirulina* species are consumed as food due to their high protein and fibre content (Anupama, 2000). Hence basic research is required to identify some new cyanobacterial strains of high value products and can able to withstand varied environmental conditions. The cyanobacteria contain pigments such as chlorophyll a, carotenes and phycobillins. The absorption of light energy is based on the occurrence of one or two forms of chlorophylls together with carotenes and phycobillins. In addition, minerals like zinc, magnesium and selenium are reported in some of the cyanobacterial species (Jensen and Ginsberg 2000). Due to their ability to accumulate heavy metals either by bioaccumulation through surface binding or biosorption, cyanobacteria are useful for heavy metal removal from the polluted waters (Karna et al. 1999). The species like, *Spirulina platensis* was shown to contain detectable levels of mercury and lead when grown under contaminated conditions In this regard, except the reports of Slotton et al. (1989), not much work has been carried out. In this view a study has been carried out to determine the biochemical composition, trace metal elements and pigment content in the biomass of the eight species of freshwater cyanobacteria isolated from different aquatic locations of Southern Karnataka, India.

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## Materials and methods

**Cyanobacterial identification:** The species were identified as per the approaches adopted by Anagnostidis and Komarek (1998) and Desikachary (1959).

**Cyanobacteria cultivation:** The species were cultured in the laboratory and grown aseptically on dry agar (Dor et al. 1987) and then in BG-11 growth medium with nitrate (Stainer et al. 1976) and were maintained at  $26 \pm 2^\circ\text{C}$  under constant illumination (14 h light: 10 h dark at 2000 lux). The cultures were harvested at their exponential phase by centrifugation (3000 rpm for 10 min), lyophilized and kept in a refrigerator. The species namely, *Oscillatoria perornata*, *Oscillatoria pseudogamiata*, *Lyngbya spiralis*, *Phormidium fragile*, *Phormidium ambiguum*, *Anabaena variabilis*, *Nostoc ellipsosporum* were isolated from the tanks and reservoirs and *Oscillatoria raciborskii* from a sulfur spring in the Western Ghats of Karnataka.

**Biochemical analysis:** The lyophilized cells were analysed with slight modification of Sadashivam and Manickam (2008) method for biochemical composition. Carbohydrates were determined by phenol-sulphuric acid, proteins by Lowry's method (Lowry et al. 1951) with Bovine Serum Albumin as standard; lipids by Soxhlet apparatus, total amino acids by Ninhydrin method; reducing sugars by di-nitro salicylic method. Sugar profile (monosaccharides) and fatty acids were determined by gas chromatography (GC-2010, Shimadzu, Japan) following Miller and Berger (1985). Amino acids profiling was done by HPLC method. Vitamin-C and vitamin-E was determined by colorimetric method. Trace mineral elements were analyzed by atomic absorption spectrophotometer (GBC Avanta).

Pigment extraction was done with 0.1 gm fresh weight of the sample for the estimation of chlorophyll a by following Jeffrey and Humphrey (1975) while carotenoids were estimated following Parsons and Strickland (1963) and both the pigments were extracted using 80% acetone by addition of pinch of magnesium carbonate ( $\text{MgCO}_3$ ). Estimation of phycobilin pigments like phycocyanin, allophycocyanin and phycoerythrin were done by spectrophotometric method (Abalde et al., 1998).

## Results

Measurable differences in biochemical composition of eight species of cyanobacterial biomass were noted (Table 1). The total carbohydrates were found to be up to 20 % of dry weight of cells, whereas in *Anabaena variabilis* it was high (20.53%) followed by *Nostoc ellipsosporum* (18.82%). It was least in *Phormidium ambiguum* (10.23%). Total proteins were found between 15 and 23% of dry weight of cells; *Nostoc ellipsosporum* showed maximum amount of protein (23.26%), whereas in *Lyngbya spiralis* it was least (7.5%). Similarly, total amino acids vary between 8 and 12  $\text{mg g}^{-1}$  and in *Anabaena variabilis* it was maximum (12.38  $\text{mg g}^{-1}$ ) and was low in *Phormidium fragile* (3.25%). Reducing sugars were found up to 15  $\text{mg g}^{-1}$  which was maximum in *Nostoc ellipsosporum* (14.56  $\text{mg g}^{-1}$ ) and minimum in *Phormidium ambiguum* (6.7  $\text{mg g}^{-1}$ ) and the total lipids varied between 8 and 15 % dry weight of cells, out of which it was high in *Oscillatoria pseudogamiata* (15.65%) and least in *Lyngbya spiralis* (4.6%). Our study shows the presence of low levels of Vitamin-C, (1.5 and 2.8  $\text{mg g}^{-1}$ ) and in *Oscillatoria pseudogamiata* (2.85  $\text{mg g}^{-1}$ ) and low in *Oscillatoria raciborskii* (1.5  $\text{mg g}^{-1}$ ). Vitamin E also found to be low (2.0 to 4.5  $\text{mg g}^{-1}$ ). In *Anabaena variabilis* it was high (4.5  $\text{mg g}^{-1}$ ) and low in *Nostoc ellipsosporum* (1.29  $\text{mg g}^{-1}$ ).

In the present study, a total of 11 monosaccharides including 1 amino sugar (Glucosamine) were detected in the eight species of cyanobacteria (Table 2). Among these sugars glucose and fucose were detected in all the eight species, whereas other sugars such as Rhamnose, galactose, mannose, glucuronic acid and fructose were detected at moderate levels in most of the species studied. Arabinose and xylose were detected in lower ranges in all the species studied.

In the present study reveals the presence of n-saturated, unsaturated and long chain fatty acids. The presence or absence of fatty acids in the eight species of cyanobacteria is shown in the Table.3. Fatty Acid composition of cyanobacteria isolated freshwater habitats of Southern Karnataka are listed in Table 4. In the present study most of the fatty acids were unsaturated (50-63%) although n-saturated fatty acids were also found out in most of the isolates (15-50%). In these major fatty acids were palmitic acid (C16:0) ranging from 5 to 40% of the total fatty acids, oleic acid (C18:1) and lenoleic acid (C18:2) were also present as higher components (5 to 35%). The predominant unsaturated fatty acids obtained were oleic acid (C18:1) and lenoleic acid (C18:2) up to 50%. Other monounsaturated fatty acids observed at palmitoleic acid (C16:1) as lower components (up to 12%). There were n-saturated FA such as caprylic acid (C8:0), capric

acid (C10:0), lauric acid (C12:0), tridecanoic acid (C13:0), myristic acid (C14:0) heptadecanoic acid (C17:0) and stearic acid (C18:0) were also determined in less amounts (up to 10%) in which myristic acid (C14:0) was found only in *Oscillatora resiborskii*. It was also found out that n-saturated FA palmitic acid (C16:0) which was present in all the eight isolated strains of cyanobacteria.

PUFA such as  $\alpha$ -linolenic acid,  $\gamma$ - linolenic acid and arachidonic acid were found only in *Anabaena variabilis* and *Nostoc ellipsosporum* (up to 12%). Similarly, Cis-11-eicosenoic acid (C20:1) was found only in *Anabaena variabilis* (up to 9%). By this investigation it was also observed that saturated long chain fatty acid lignoceric acid (C24:0) in some of the isolates (up to 4%) as lower components.

Amino acid profile of individual species eight of cyanobacteria is listed in Table 5. The study noticed the presence of seven essential amino acids viz., threonine, valine, methionine, phenylalanine, lysine, leucine and isoleucine.

Photosynthetic pigments such as chlorophyll a, and carotenoids content is shown in Fig.1. Photosynthetic pigments such as chlorophyll a (6-18.5  $\mu\text{g/ml}$ ) and carotenoids (2.5 - 10.5  $\mu\text{g/ml}$ ) were also found in considerable quantities. It was noticed that chlorophyll a was high in *Lyngbya spiralis* (18.05  $\mu\text{g/ml}$ ) and low in *Oscillatora raciborskii* (6.08  $\mu\text{g/ml}$ ); the carotenoid content was high in *Anabaena variabilis* (10.58  $\mu\text{g/ml}$ ) and low in *Phormidium fragile* (2.14  $\mu\text{g/ml}$ ). Phycobilin pigments such as phycocyanin (3.5-8.8 $\mu\text{g/ml}$ ), phycoerythrin (0.5-5.2 $\mu\text{g/ml}$ ) and allo-phycocyanin (1.5-8.2 $\mu\text{g/ml}$ ) were better (Fig.2). Phycocyanin was high in *Phormidium ambiguum* (8.91  $\mu\text{g/ml}$ ) and was least in *Oscillatora pseudogamiata* (3.17  $\mu\text{g/ml}$ ). In case of allophycocyanin it was maximum in *Anabaena variabilis* (8.32  $\mu\text{g/ml}$ ) and minimum in *Lyngbya spiralis* (1.19  $\mu\text{g/ml}$ ). Phycoerythrin was found to be rich in *Phormidium ambiguum* (5.24  $\mu\text{g/ml}$ ) and it was low in *Oscillatora perornata* (0.77  $\mu\text{g/ml}$ ).

In this study sixteen metal elements including certain heavy metals were detected by Atomic Absorption Spectroscopy (Table 6). It was noticed that Mg, Mn and Fe were found in higher levels followed Zn and Cu. Minerals like Na, K, and Ca were obtained in better range. Selenium was detected in lower levels in all the species studied, whereas heavy metals such as Pb, Ni, Co, Cr, As and Cd were found in low levels.

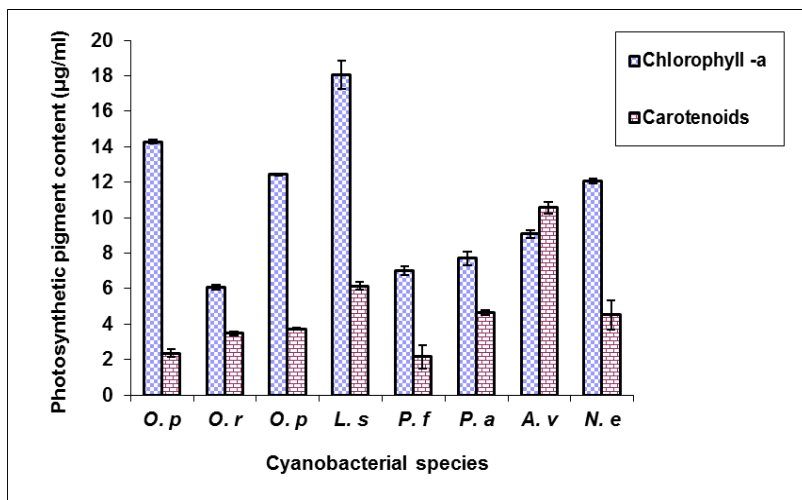


Fig. 1: Photosynthetic pigments in the eight species of cyanobacteria\*.

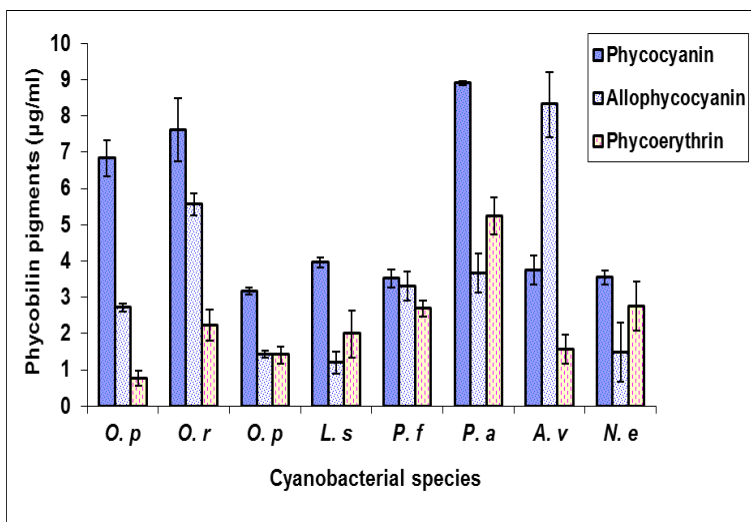


Fig. 2: Phycobilin pigments in the eight species of cyanobacteria\*.

### Discussion

The carbohydrate and protein contents were found in higher rates followed by reducing sugars and lipids. Amino acids and carbohydrates are considered as an important group of cell constituents in cyanobacteria. Cyanobacterial protein either as a supplement or as an alternative source has been received worldwide attention. The determination of free amino acid content is of great value from nutritional and biochemical point of view. Similarly, vitamin C and vitamin E which are of interest for its stress combating mechanism. *Spirulina* a well known cyanobacteria is being used as food supplements due to excellent nutrient compounds and digestibility. Besides higher content of protein (60–70 %) it has 20% carbohydrate, 5% lipids, 7% minerals and 6% moisture. It is also a rich source of beta-carotene, thiamine and riboflavin and is one of the richest sources of vitamin B12 (Thajuddin and Subramanian 2005). In the present study, *Nostoc* and *Anabaena* species showed also nutritional constituents in better range as compared with *Spirulina*.

Bertocchi et al. (1990) identified glucose, galactose, arabinose, xylose and rhamnose in the extracellular products of several species of cyanobacteria. During spring blooms of *Phaeocystis* in the North Sea, several monosaccharides (glucose, arabinose, xylose, galactose and mannose) were identified by Janse et al. (1996). It is of interest to note that although glucose has been referred to be the major monosaccharide by most investigations including the present data, other monosaccharides (rhamnose, fucose, galactose) were dominant in some mono specific diatom cultures indicating that their relative abundance might depend on species composition (Marchetti, 1992; Myklestad, 1995). Metaxatos et al. (2003) reported the quantitative composition of 8 monosaccharides such as glucose, galactose, xylose, fucose, mannose, rhamnose, arabinose and glucosamine from a mixture of four phytoplanktonic species and reported that glucose was the major monosaccharide among the species studied. This agrees with our data where, in the present study among the eight species studied glucose was found at higher range compared with other monosaccharides.

The fatty acid composition of cyanobacteria was studied by Holton et al. (1964) in *Anacystis nidulans* and by Lennarz (1966) in *Anabaena variabilis*. Major fatty acids so far studied in cyanobacteria are hexadecanoic (16:0), 9-hexadecenoic (16:1), hexadecadienoic (16:2), octadecanoic (18:0), and 9-octadecenoic (18:1). In the present study also fatty acid composition of *Anabaena variabilis* showed the presence of such fatty acids. Polyunsaturated fatty acids have an important role in human metabolic pathways, particularly as precursors of a particular type of prostaglandin E1 (Mendes et al. 2006). In our study most of the species were rich with polyunsaturated fatty acids and particularly *Nostoc* and *Anabaena* showed the presence of essential fatty acids like  $\alpha$ -linolenic acid,  $\gamma$ -linolenic acid and arachidonic acid. The lipids of some of the cyanobacterial species also rich in essential fatty acids such as the linoleic (C18:2) and linolenic (C18:3) acids and their C20 derivatives, eicosapentanoic acids (C20:5) and arachidonic acid (C20:4). These fatty acids are essential compounds of the diet of humans and animals and are also important feed additives in aquaculture (Borowitzka 1988).

The medicinal value of cyanobacteria especially strains of *Nostoc* were used to treat gout, fistula and several forms of cancer (Pietra 1990). Cyanobacteria may contain significant amounts of lipids with compositions similar to those of vegetable oils. The thylakoid membranes of cyanobacteria containing high level of PUFAs tend to decrease the phase transition temperature and increase the fluidity of membrane lipids. Besides, it has been found that PUFAs are important for the growth and the ability to tolerate photoinhibition of photosynthesis at low temperature (Wada et al. 1990). The present study also (PUFAs upto 60%), agrees with such findings.

Most of the species cultured under optimum conditions contained about 4% of total chlorophyll on dry weight basis. Chlorophyll provides a chelating agent activity which can be used in ointment, treatment for pharmaceutical benefits especially liver recovery and ulcer treatment. Besides that, it repairs cells, increases hemoglobin in blood and faster the cell growth (Puotinen 2009). Tiwari et al. (2005) have studied maximum chlorophyll content in *Phormidium* sp. (10.8µg/ml) and minimum in *Nostoc linckia* (0.7µg/ml). The present study agrees with these results. Some of the cyanobacteria are rich in vitamins and they can excrete them into the surrounding environment (Borowitzka et al. 1988). The carotenoides are characteristic of cyanobacteria have high commercial value. They are used as natural food colorants, as food additives to enhance the color of the flesh of Salmonid fish and to improve the health and fertility of cattle (Emodi et. al. 1978).

Bioaccumulation of metals by cyanobacteria is used for removal of metal ions from polluted waters. The potential of algal biomass culture for production of a variety of industrially important products and for water treatment has been studied (Vonshak 1997). Some cyanobacteria have remarkable affinity for heavy metals (Rai et al. 2000) and are grown frequently in metal contaminated locations. They are also used as a bioremediator of heavy metals in aquatic bodies (Nakanishi et al. 2004). Nickel is known to be accumulated by algae (Trollope and Evans 1976) and its deleterious effect on phytoplankton community has been well studied (Rai et al. 2000). The BGA and green algae are able to bind large amounts of metals due to the presence of mucilaginous sheath (Tien et al. 2002). Cyanobacteria growing in metal-polluted environments display the ability to tolerate high concentration of toxic metals, Cu, Cd, and Zn (Mallick et al. 1990).

Such findings show the possibility of manipulating or over expressing existing resistance mechanisms and the use of such organisms to remove harmful metals from the environment. The overall study suggests that biomass of such cyanobacterial species have with implications in food and other pharmaceutical industries. This study also focuses on application of cyanobacteria in aquaculture developments as fish feed and also in bioremediation in order to remove the toxic metal effluents from aquatic ecosystem.

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**Table 1: Biochemical constituents in eight species of cyanobacteria of Southern Karnataka, India.**

Species	Carbohydrate (% of dry wt.)	Protein (% of dry wt.)	Lipid (% of dry wt.)	Reducing sugar(mgg <sup>-1</sup> )	Free amino acid (mgg <sup>-1</sup> )	Vitamin –C (mgg <sup>-1</sup> )	Vitamin –E (mgg <sup>-1</sup> )
<i>Oscillatoria perornata</i>	17.125 ±0.025	15.45±0.02	8.12±0.40	9.13±0.10	11.38±0.03	1.60±0.05	1.6±0.12
<i>Oscillatoria raciborskii</i>	12.21±0.04	14.85±0.02	9.75±0.05	10.30±0.01	8.43±0.06	1.50±0.03	1.48±0.11
<i>Oscillatoria psuedogamiata</i>	12.55±0.05	10.425±0.13	15.65±0.15	9.43±0.06	7.37±0.10	2.85±0.05	2.15±0.05
<i>Lyngbya spiralis</i>	14.57±0.02	7.50±0.023	4.60±0.01	14.52±0.20	5.80±0.02	1.91±0.01	2.91±0.01
<i>Phormidium fragile</i>	12.13±0.13	12.175±0.03	8.25±0.03	7.23±0.50	3.25±0.05	1.55±0.03	2.33±0.23
<i>Phormidium ambiguum</i>	10.23±0.03	15.53±0.01	7.41±0.01	6.70±0.01	4.50±0.05	1.84±0.01	1.44±0.11
<i>Anabaena variabilis</i>	20.53±0.08	15.83±0.013	11.55±0.03	8.33±0.08	12.38±0.20	1.58±0.08	4.58±0.08
<i>Nostoc ellipsosporum</i>	18.82±0.05	23.26±0.08	6.45±0.08	14.56±0.14	8.48±0.22	1.79±0.01	1.29±0.01



**Table 2: Monosaccharide composition in the eight species of cyanobacteria isolated from various locations.**

S/N	Monosaccharides	Monosaccharide concentration ( $\mu\text{mole g}^{-1}$ dry wt.)							
		<i>O. p</i>	<i>O. r</i>	<i>O. p</i>	<i>L. s</i>	<i>P. f</i>	<i>P. a</i>	<i>A. v</i>	<i>N. e</i>
1	Glucose	120.5	140.2	88.4	65.0	160.7	55.5	170.8	78.2
2	Fucose	7.5	12.5	8.20	15.0	20.7	10.55	22.25	14.5
3	Rhamnose	ND	1.90	3.20	ND	5.20	2.15	7.70	6.55
4	Arabinose	0.80	ND	ND	1.30	2.50	ND	5.20	30.2
5	Galactose	20.5	35.5	26.25	18.0	42.35	15.0	55.30	ND
6	Mannose	9.5	2.55	ND	3.10	5.50	2.90	6.15	3.2
7	Xylose	0.75	ND	ND	1.55	8.5	ND	10.65	ND
8	Fructose	15.5	20.8	ND	10.5	ND	8.85	28.7	32.35
9	Ribose	5.2	3.20	8.2	0.95	5.8	ND	10.5	ND
10	Glucuronic acid	20.5	15.0	32.20	22.0	30.7	25.5	42.5	28.55
11	Glucosamine	ND	ND	ND	ND	ND	ND	8.55	ND

ND: Not detected

**Table 3: Presence or absence of fatty acids in the eight species of cyanobacteria.**

S/N	Fatty Acids	Cyanobacteria*							
		<i>O.p</i>	<i>O.r</i>	<i>O.p</i>	<i>L.s</i>	<i>P.f</i>	<i>p.a</i>	<i>A.v</i>	<i>N.e</i>
1.	Caprylic acid C8:0	+	+	-	+	-	+	+	+
2.	Capric acid C10:0	-	+	+	-	+	-	+	+
3.	Lauric Acid C12:0	+	+	-	-	-	-	-	-
4.	Tridecanoic Acid C13:0	-	+	-	+	-	+	-	+
5.	Myristic Acid C14:0	-	+	-	-	-	-	-	-
6.	Palmitic Acid C16:0	+	+	+	+	+	+	+	+
7.	Palmitoleic Acid 16:1	+	+	-	-	-	+	-	+
8.	Heptadecanoic Acid C17:0	+	+	-	-	-	-	+	-
9.	Stearic Acid C18:0	+	+	-	+	-	+	+	+
10.	Oleic Acid C18:1	+	+	+	-	+	-	+	+
11.	Linoleic Acid C18:2	+	+	+	+	+	-	+	+
12.	$\alpha$ -Linolenic Acid C18:3	-	-	-	-	-	-	+	+
13.	$\gamma$ -linolenic Acid C18:3	-	-	-	-	-	-	+	+
14.	Cis-11-eicosenoic Acid C20:1	-	-	-	-	-	-	+	-
15.	Arachidonic acid 20:4( $\omega$ -6)	-	-	-	-	-	-	+	+
16.	Lignoceric Acid C24:0	-	-	+	-	+	-	-	-

+ = present ; - = absent

Table 4: Fatty acid composition (%) of eight species of cyanobacteria isolated from Southern Karnataka.

Fatty Acids	Cyanobacteria*							
	<i>O.p</i>	<i>O.r</i>	<i>O.p</i>	<i>L.s</i>	<i>P.f</i>	<i>P.a</i>	<i>A.v</i>	<i>N.e</i>
n- Saturated								
Total (%)	30.8	43.26	36.4	15.1	45.2	52.05	56.05	57.4
Octanoic (8:0)	2.5	3.1	-	5.2	-	7.8	4.0	8.5
Decanoic (10:0)	-	2.15	3.0	-	4.7	-	6.2	5.4
Dodecanoic (12:0)	3.12	2.2	-	-	-	-	-	-
Tridecanoic (13:0)	-	2.16	-	1.5	-	3.0	-	6.3
Tetradecanoic (14:0)	-	1.60	-	-	-	-	-	-
Hexadecanoic (16:0)	15.6	20.3	33.4	5.8	40.5	35.0	28.2	24.8
Heptadecanoic (17:0)	3.48	8.35	-	-	-	-	12.4	-
Octadecanoic (18:0)	6.10	3.40	-	2.6	-	6.25	5.25	12.4
Long chain saturated								
Tetracosanoic acid (24:0)	-	-	2.8	-	3.6	-	-	-
Unsaturated								
Total (%)	40.54	45.10	57.62	9.2	51.84	12.2	63.35	63.55
9- Hexadecanoic (9-16:1)	4.72	8.10	-	-	-	12.2	-	10.5
9-octadecanoic (9-18:1)	23.82	16.80	24.42	-	28.44	-	22.5	16.3
9,12-Octadecadienoic (9,12-18:2)	12.1	20.2	33.2	9.2	23.4	-	14.8	10.2
<i>all-cis</i> -9,12,15-Octadecatrienoic (9,12,15-18:3)	-	-	-	-	-	-	5.35	7.85
<i>all-cis</i> -6,9,12-Octadecatrienoic (6,9,12-18:3)	-	-	-	-	-	-	8.2	10.30
5,8,11,14-20:4( $\omega$ -6)Eicosatetraenoic acid	-	-	-	-	-	-	12.5	8.4
Long chain Unsaturated								
Cis-11-Eicosenoic acid (20:1)	-	-	-	-	-	-	9.4	-

**Table 5: Amino acid profile (composition g/16gN) in the eight species of cyanobacteria of Southern Karnataka.**

S/N	Amino acids (g/16gN)	Species*							
		<i>O. p</i>	<i>O. r</i>	<i>O. p</i>	<i>L. s</i>	<i>P. f</i>	<i>P. a</i>	<i>A. v</i>	<i>N. e</i>
1.	Aspartic acid	6.95	8.24	6.67	6.51	5.97	8.29	4.42	8.39
2.	Glutamine	7.19	7.74	7.11	7.05	7.96	6.51	6.12	6.96
3.	Serine	4.88	4.86	2.39	3.97	5.15	4.11	5.54	4.67
4.	Glycine	11.93	11.21	10.84	11.87	11.75	12.31	15.12	11.05
5.	Histidine	1.49	1.03	0.93	0.92	1.15	0.79	1.11	1.31
6.	Arginine	5.29	4.82	3.82	4.82	5.56	4.60	6.35	5.88
7.	Threonine*	5.48	5.69	1.92	3.93	5.70	5.23	6.06	5.37
8.	Alanine	10.10	10.69	11.29	11.03	10.81	11.08	9.03	10.87
9.	Proline	6.19	5.68	8.48	7.70	5.63	7.07	9.54	5.69
10.	Tyrosine	3.75	3.09	0.70	2.45	3.62	3.03	5.16	2.88
11.	Valine*	7.22	7.61	9.31	8.07	7.49	7.02	6.27	7.50
12.	Methionine*	1.44	0.81	0.82	0.95	1.31	0.76	1.53	1.36
13.	Cysteine	0.29	0.15	0.11	0.12	0.06	0.05	0.07	0.14
14.	Isoleucine*	6.20	6.11	7.89	6.81	6.02	5.39	5.23	5.67
15.	Leucine*	9.06	9.63	11.00	10.04	9.00	8.07	7.81	8.69
16.	Phenylalanine*	5.33	5.20	3.94	4.72	4.80	4.40	6.44	4.55
17.	Lysine*	6.10	6.06	8.16	7.18	6.25	6.64	2.74	7.76
Total		98.89	98.62	95.38	98.14	98.23	95.35	98.54	98.74

\* Essential amino acids

**Table 6: Metal elements in the eight species of cyanobacteria isolated from different aquatic habitats.**

Cyanobacteria	Metal content (in ppm.)															
	Na	K	Ca	Zn	Mg	Mn	Fe	Ni	Cu	Cd	Pb	Co	Cr	Se	As	Hg
<i>Oscillatoria perornata</i>	28.40	50.30	36.37	65.6	7800	976	541.4	8.7	16.7	Nil	6.2	Nil	8.30	9.7	4.2	Nil
<i>Oscillatoria resiborskii</i>	24.57	68.23	25.79	125.2	7635	1300	5132.5	7.7	29.9	Nil	9.9	4.7	2.31	17.2	3.1	Nil
<i>Oscillatoria psuedogamiata</i>	25.58	50.49	24.06	55.3	8830	401	512.8	7.6	11.6	1.20	9.4	1.8	2.66	10.2	9.2	Nil
<i>Lyngbya spiralis</i>	44.30	95.64	35.89	62.6	5240	464	562.7	12.8	26.0	0.80	15.2	1.6	8.08	11.4	Nil	Nil
<i>Phormidium fragile</i>	22.24	62.37	25.92	122.9	5050	597	978.2	5.4	20.0	Nil	8.6	Nil	5.77	11.4	Nil	Nil
<i>Phormidium ambiguum</i>	40.81	89.48	49.69	56.9	2102	469	589.0	8.0	72.3	Nil	9.6	0.3	7.69	3.0	5.8	Nil
<i>Anabaena variabilis</i>	110.38	82.01	41.86	100.4	3790	595	777.5	10.3	17.9	2.50	7.8	1.9	3.49	12.3	4.2	Nil
<i>Nostoc ellipsosporum</i>	125.30	112.15	57.84	133.0	3780	533	1101.5	10.8	19.0	Nil	11.5	2.2	5.10	9.0	Nil	Nil

\*  
*O. p* : *Oscillatoria perornata*      *P. f* : *Phormidium fragile*  
*O. r* : *Oscillatoria raciborskii*      *P. a* : *Phormidium ambiguum*  
*O. p* : *Oscillatoria pseudogamiata*      *A. v* : *Anabaena variabilis*  
*L. s* : *Lyngbya spiralis*      *N. e* : *Nostoc ellipsosporum*