Growth kinetics, photosynthetic pigment analysis and biochemical characterization of some selected cyanobacterial strains

5.1 Introduction

The first use of edible blue-green algae by humans dates back 2000 years to the Chinese, who used *Nostoc* to survive during famine (Spolaore *et al.*, 2006) W.H. Harvey (1811-1866) was the first who divided the algae into four divisions, based on their pigmentation. This was the first use of a biochemical criterion in plant systematics.

However, algal biotechnology began to develop only a few decades ago (Horincar *et al*., 2011). Over the years, cyanobacterial biocomponents has received worldwide attention for either as food supplement or as a natural source of a variety of drugs for pharmaceutical and cosmetic applications. Pigments are of fundamental importance in algal cell physiology. The presence of photosynthetic pigments- the chlorophylls, carotenoids and biliproteins in algae is a specific feature of each species. Measurement of pigment evaluation is essential as a measure of cell growth, as well as a parameter to check the trophic level of waters. However, pigments - chlorophyll *a*, carotenoids and phycobiliproteins are adversely affected by the stress (Sundaram and Soumya, 2011).

Chlorophyll *a* is usually the parameter used as the trophic indicator because of the direct relationship between the content of this pigment and the amount of algal biomass. Chlorophyll *a* is the primary photosynthetic pigment of all oxygen-evolving photosynthetic organisms and is present in all cyanobacteria. Chlorophyll is used in treatment for pharmaceutical benefits. It repairs cells, increases haemoglobin in blood and faster the cell growth (Puotinen, 2009). Chlorophyll *a* has two in vitro absorption bands; in the red-light region at 660-665 nm and at lower wave lengths near 430nm. Primarily chlorophyll *a*, has been utilized to determine the in vitro growth kinetics of the cyanobacteria. Their interest lies in their convenient use as a biotechnological biomass, as they can easily be grown in controlled conditions and handled as conventional lab microorganisms. Under these conditions, the cells reproduce rapidly and the dynamics of the microbial growth can be charted by means of a population growth curve, which is constructed by plotting the increase in cell numbers versus time of incubation and can be used to delineate stages of the growth cycle. It also facilitates measurement of cell

numbers and the rate of growth of a particular organism under standardized conditions as expressed by its generation time, the time required for a microbial population to double. In the growth of a cyanobacterial culture, a succession of phases, characterized by variations of the growth rate, may be conveniently distinguished. The growth cycle in the system passes through four phases (lag, log, stationary and death phase) which together constitute a typical growth curve.

Apart from chlorophyll *a*, accessory pigment compounds (chlorophyll *b* and *c*, carotenoids and phycobilliproteins) also play a significant role in photosynthesis and as well as photoprotection against environmental stress. Carotenoids constitute a class of terpenoid pigments, derived from a 40-carbon polyene chain, which can be envisaged as their molecular backbone (Campo *et al*., 2007). The recognized therapeutic value of some carotenoids (especially lutein) in prevention and treatment of degenerative diseases and the rising market demand for pigments from natural sources has promoted large-scale cultivation of algae for synthesis of carotenoids (Guedes *et al*, 2011).

Phycobiliproteins are water-soluble naturally occurring light harvesting pigments commonly present in cyanobacteria and some eukaryotic algae (rhodophytes, cryptomonads, and glaucophytes). Phycobiliproteins are covalently attached linear tetrapyrrole chromophoric group called bilins or phycobilins because of their close structural relationship to the well-known bile pigments of human- bilirubin and biliverdins (Lemberge and Legge, 1949). As natural colorants phycobiliproteins are gaining importance over synthetic ones due to their nontoxic and non-carcinogenic nature. They are widely used in cosmetic industry and clinical laboratories as label for antibodies and receptors. Antioxidants, anti-inflammatory, neuroprotective and

hepatoprotective properties are also exhibited by phycobiliproteins (Spolaore *et al*., 2006). Cyanobacterial pigment composition isolated from paper mill effluents and around papermill of Panchgram has not been studied so far. Although, identification of the organisms are most often carried out purely on the morphological basis, physiological and biochemical data are the integral parts of polyphasic microbial identification. Thus, the present chapter deals with the study of growth kinetics, photosynthetic pigment analysis and biochemical characterization of some of the selected cyanobacterial strains isolated from the areas around Panchgram of Hailakandi district of Assam, North-East India.

6.2 Methodology

In detail methodology for estimation of the growth curves and biochemical analysis had been discussed in **Chapter 3**. The abbreviations used for different strains of cyanobacteria are given in **Table 6.1**. All the experiments were replicated three times. Cyanobacterial biomass was estimated in terms of chlorophyll *a* concentration in each alternate day. Growth rate and generation time were determined according to the standard procedures mentioned in **chapter 3.7**. Other photosynthetic pigments (Total carotenoid content-TCC and phycobiliproteins) and biochemical properties (soluble protein and total carbohydrate) were estimated in stationary period of growth following standard procedure as mentioned in **Chapter 3**. The data obtained were subjected to one way ANOVA by Tukey multiple comparison of the means using SPSS V-19. Significant differences were indicated at $p< 0.05$.

6.3 Results and Discussion

Table 6.1: List of abbreviations of the cyanobacterial strains

Sl. no.	Name of the strain	Abbreviation used
1	*Oscillatoria formosa* Bory	D1
2	*Lyngbya polysiphoniae* Frémy (after Frémy)	D2
3	*Nostoc linkia* v. (Roth) Born. et Flah. (after Frémy)	D3
4	*Nostoc commune* Vaucher ex Born. et Flah.	D4
5	*Nostoc carneum* Ag. (after Frémy)	D5
6	*Cylindrospermum muscicola* var. longispora Dixit	D6
7	*Cylindrospermum majus* Kutz. (after Frémy)	D7
8	*Cylindrospermum musicola* Kutzing ex. Born ex. Flah	D8
9	*Scytonema tolypothrichoides* Kutz.(after Kossinskaja)	D9
10	*Tolypothryx rechingeri* (Wille) Geitler (after Wille)	D10
11	*Westiellopsis prolifica* Janet (after Janet)	D11
12	*Calothryx marchica* Lemm. (after Fremmy)	D12
13	*Nostoc ellipsosporum v. violacea* Rao C.B (after Rao, C.B.)	D13
14	*Lyngbya limnetica* Lemmermann	D14
15	*Anabaena doliolum* Bharadw. (after Bharadwaja)	D15
16	*Nostoc sphaericum* Vaucher	D16
17	*Anabaena orientalis* Dixit (after Dixit)	D17
18	*Fischerella muscicola* (Borzi) Gomont	D18
19	*Calothrix marchica* v. intermedia Rao, C.B (after Rao, C.B)	D19
20	*Calothryx marchica* v. crassa Rao. C.B (after Rao, C.B)	D20
21	*Phormidium fragile* (Menegh.) Gom.	D21
22	*Nostoc spongIforme v. tenue* Rao, C. B. (after Rao, C. B.)	D22
23	*Tolypothryx byssoidea* (Berk.) Kirchn.	D23
24	*Tolypothryx distorta* v. *penicillata* (Ag.) Lemm.	D24
25	*Nodularia spumigena* Mertens in Jürgens	D25
26	*Aphanothece bullosa*	D26
27	*Phormidium autumnale* (Ag.) Gom. (after Gomont)	D27

6.3.1 Growth kinetics of the isolated strains

Fig. 6.1- 6.7 depicts the growth curve obtained for 27 (**D1-D27**) purified cyanobacterial isolates. Growth curves were estimated on the basis of chlorophyll *a* analysis in each alternate day including the day of inoculation. Culture of cyanobacteria in the laboratory with suitable culture conditions depends on the eco-physiological requirements of a particular species. The physico-chemical factors such as temperature, acidity and light (Lobban and Herrison, 1994), aeration (Chen and Johns, 1991) or nutrient concentration (Bjornsater and Wheeler, 1990) influence the growth rate, physiological status and biochemical composition of cyanobacteria in culture condition (Lee and Kim, 2002). One of the most striking features of algal physiology is the marked phenotypic variation in chemical composition and rates of physiological processes (Geider, 1987). Microbial growth is an autocatalytic process. As obligate photoautotrophs, cyanobacteria utilize organic carbon compounds for their growth (Droop, 1974; Smith, 1982; Tuchman, 1996), and the potential of growth depends on the genetic background of the specific organism, the nature of the substrate as well as environmental factors. In the present study, four phases of growth viz. lag, log, stationary and death have been observed. In the study a variation between each of the growth phases among the isolates was noticed. Most of the isolates were having a long lag phase which might be attributed to the adjusting time required by the organisms to the new environment (Madigan *et al.*, 2000) and synthesizing the enzymes needed to exploit the new medium. All the non-heterocystous forms were observed to have comparatively short lag period than heterocystous forms. However the lag phase is least when inoculated strain is in exponential phase of growth (Gopinathan, 1984) or if organisms have been transferred from an identical medium, at

the same temperature, the lag phase may be very short. The length of exponential phase depends on the concentration of nutrition sources however the growth rate though the exponential phase does not change. Microbial population enters the stationary phase from several reasons. Population growth may be limited by the nutritional limits, oxygen shortage or increased concentration of toxic metabolites. Most of the isolates were having long growth period and gradually entered the declining phase after 3rd week. The growth kinetics of the isolates expressed in terms of specific growth rate (K) and as well as generation time (G) showed wide differences in the values of the isolates. In case of the filamentous isolates, exponential growth commenced very rapidly, with almost no log phase apparently evident. Growth rate **(Table 6.2)** was found highest in *Oscillatoria formosa* ($0.46d^{-1}$) isolated from the wastewater with lowest generation time ($51.71h^{-1}$) followed by *Nostoc linkia* v with growth rate $0.40d^{-1}$ and generation time $59.52h^{-1}$. *Nodularia spumigena* was found to be the slowest grower (growth rate, $0.09d^{-1}$; generation time, 241 h^{-1}) among the isolates.

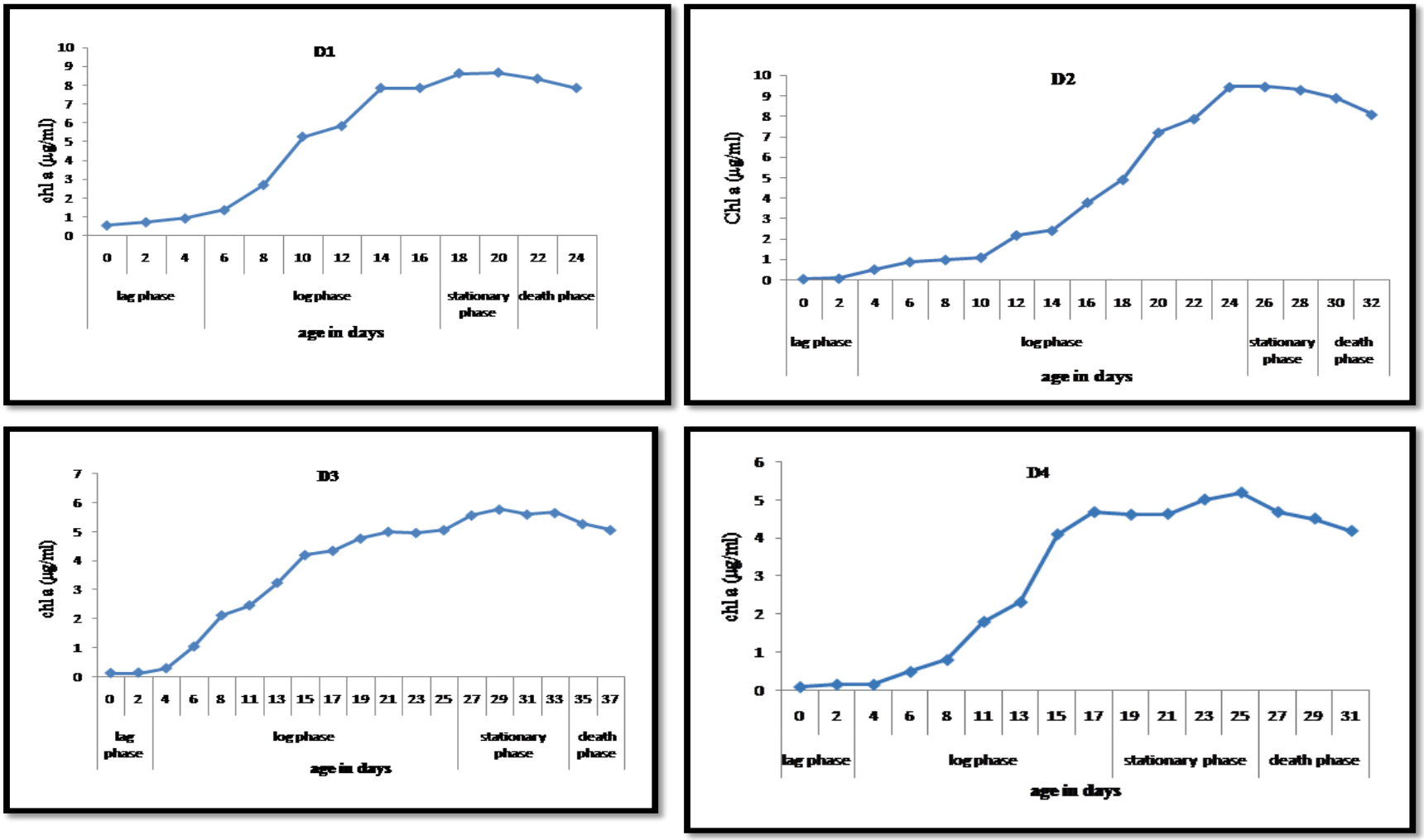


Fig. 6.1 Growth curves of the isolated cyanobacteria (D1-D4)

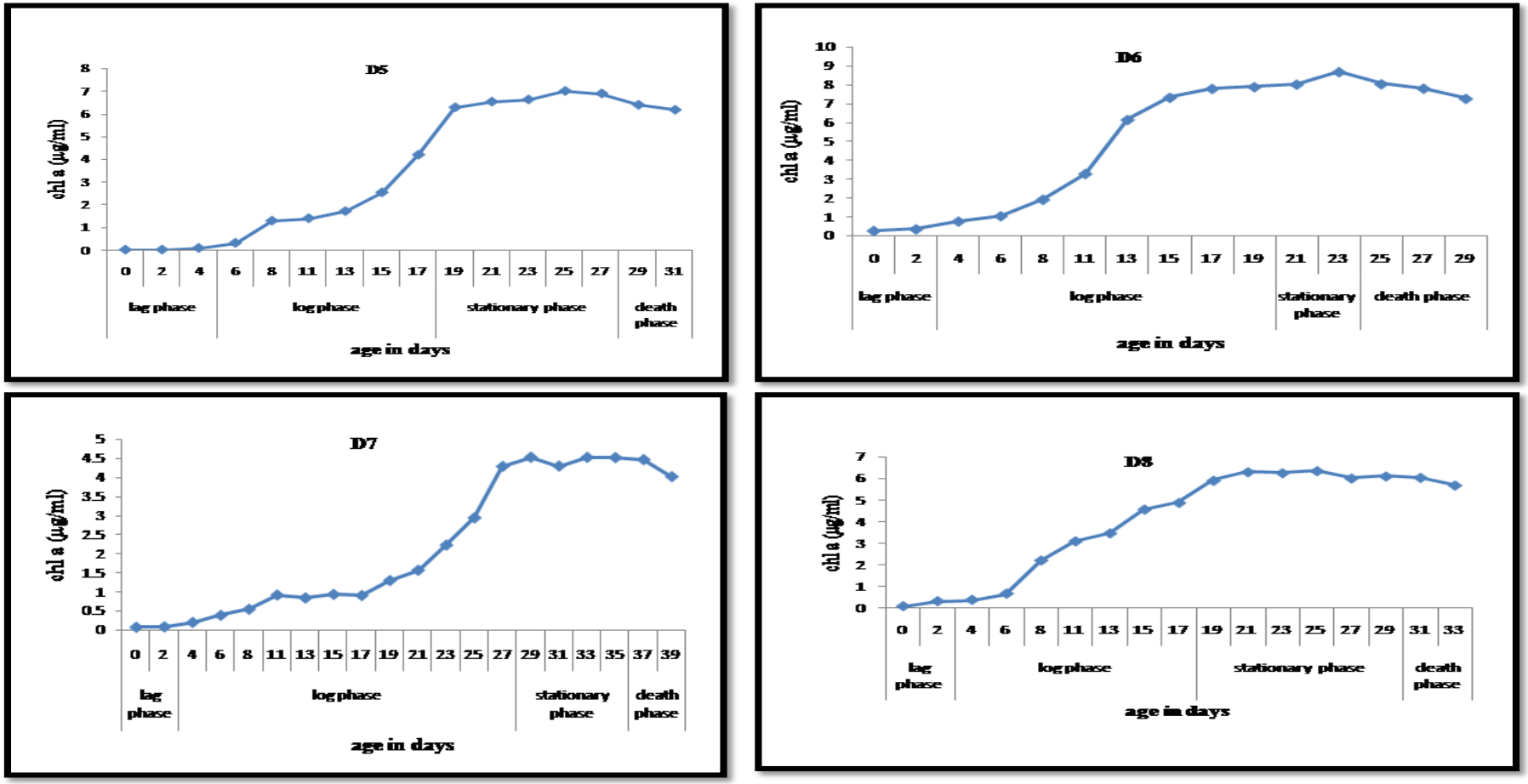


Fig. 6.2 Growth curves of the isolated cyanobacteria (D5-D8)

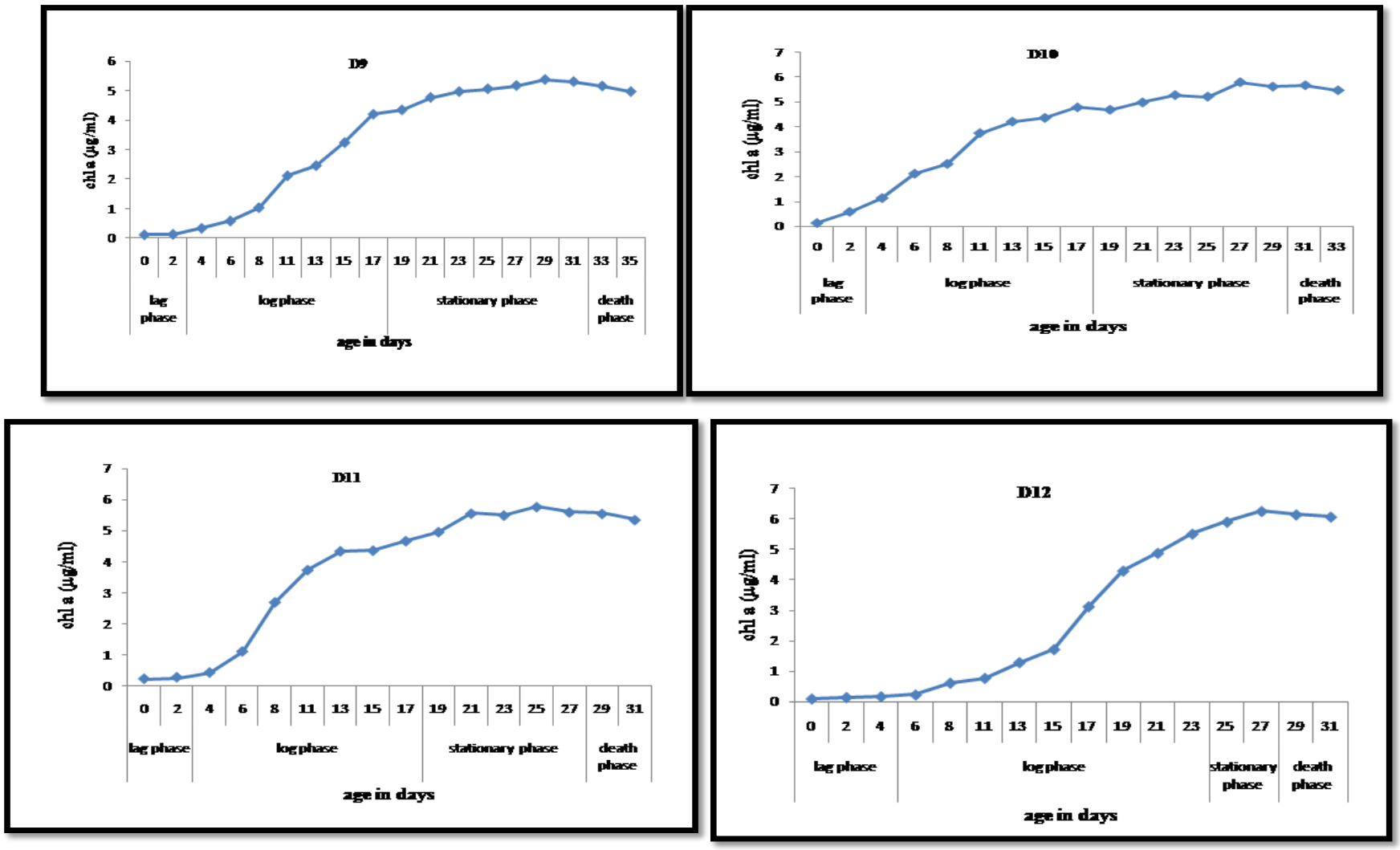


Fig. 6.3 Growth curves of the isolated cyanobacteria (D9-D12)

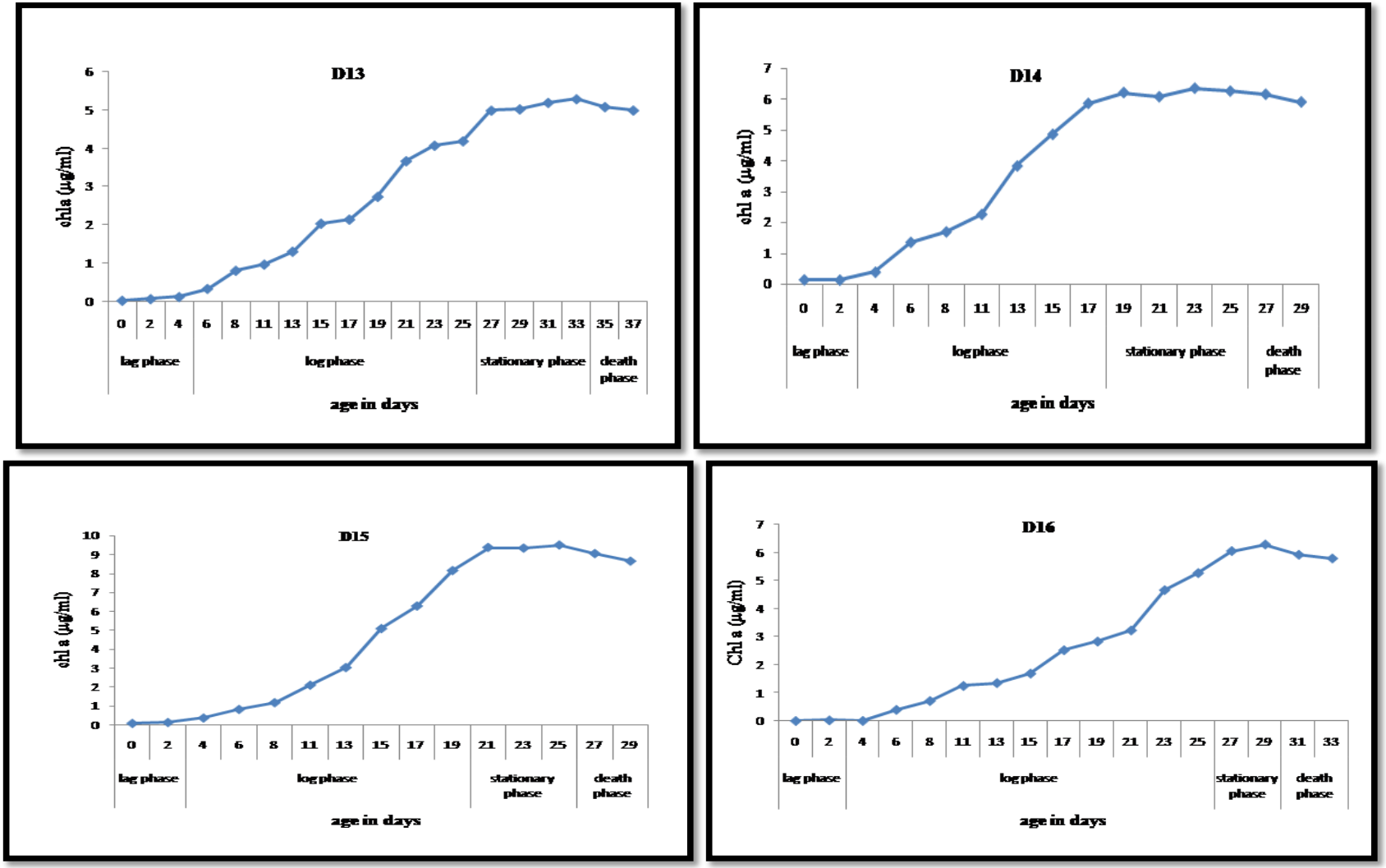


Fig. 6.4 Growth curves of the isolated cyanobacteria (D13-D16)

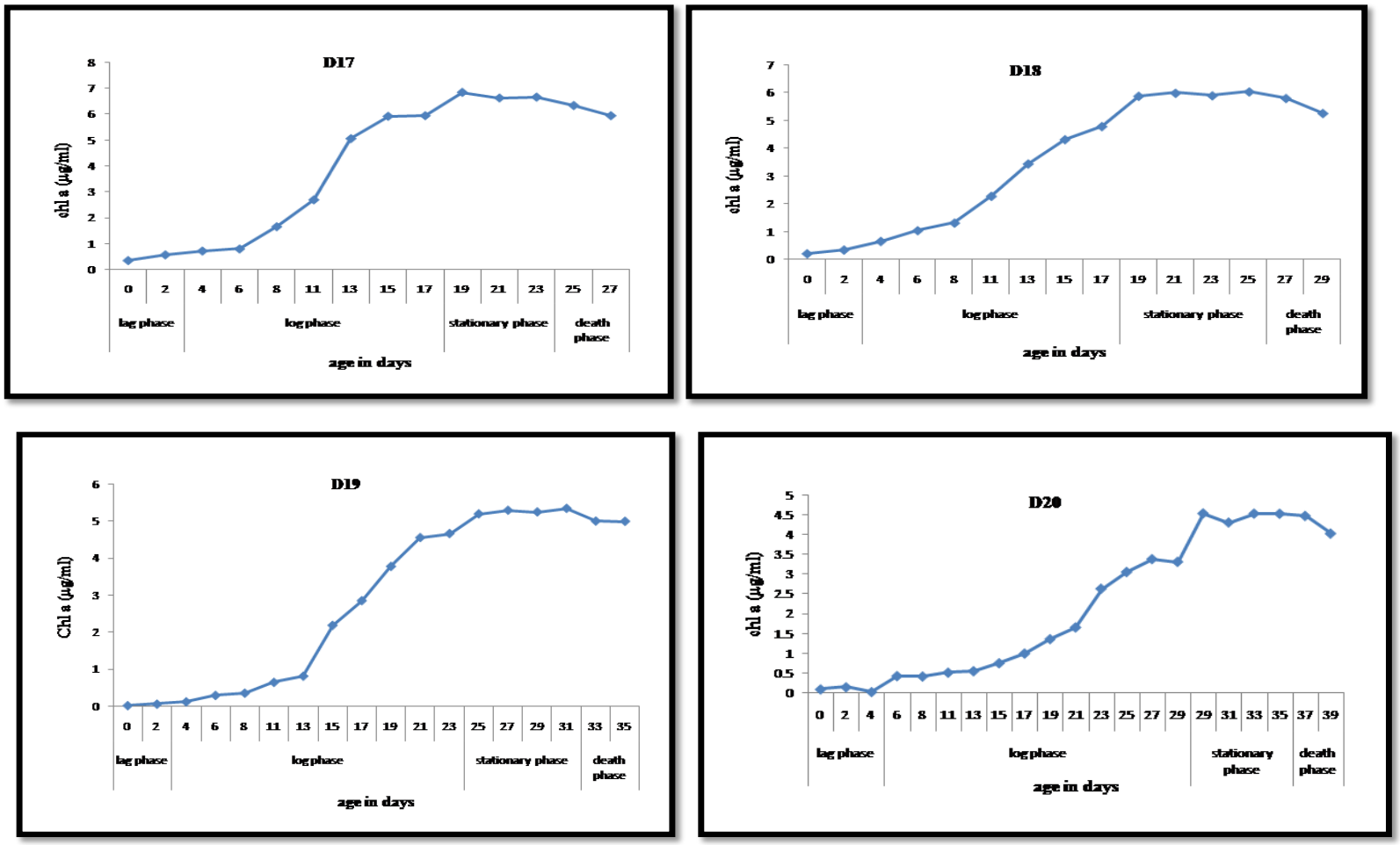


Fig. 6.5 Growth curves of the isolated cyanobacteria (D17-D20)

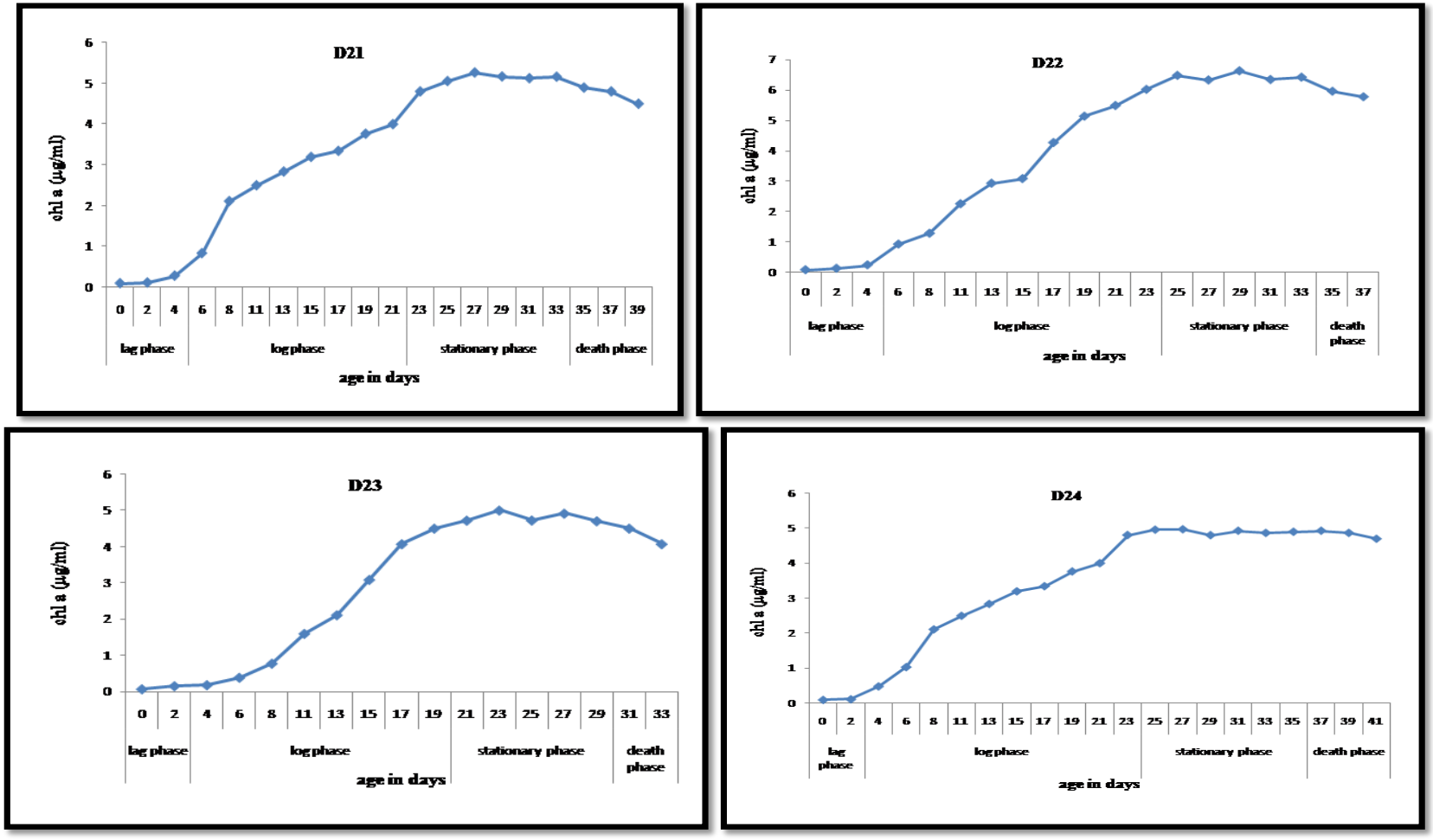
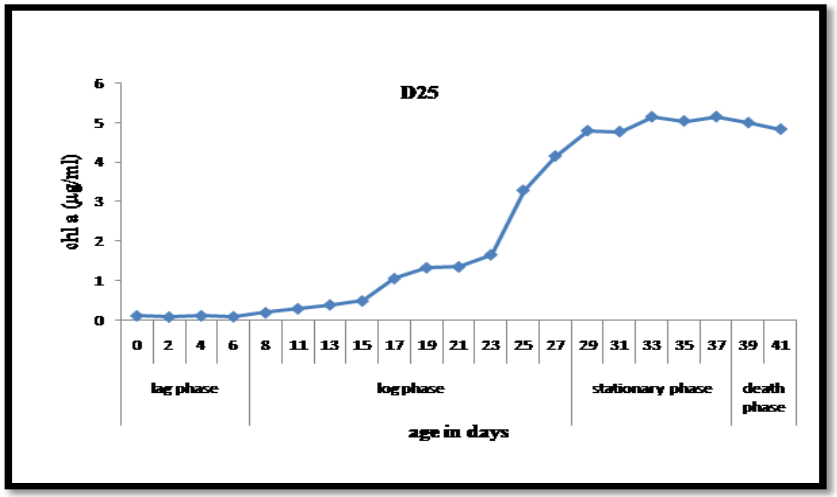
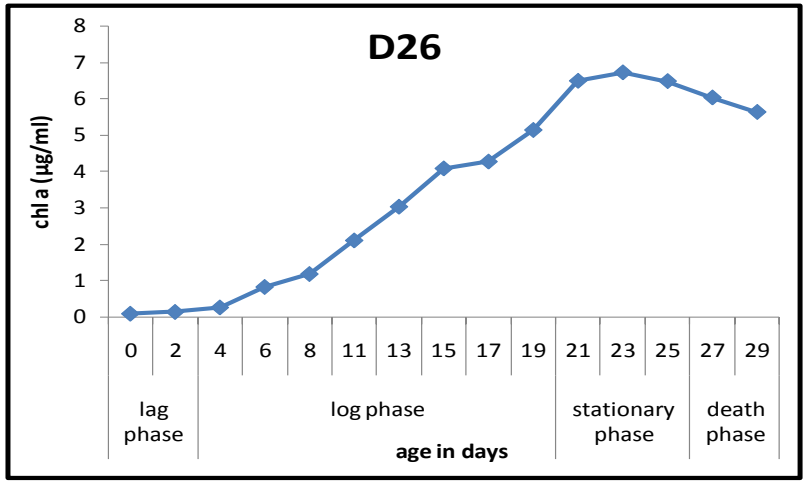


Fig. 6.6 Growth curves of the isolated cyanobacteria (D21-D24)





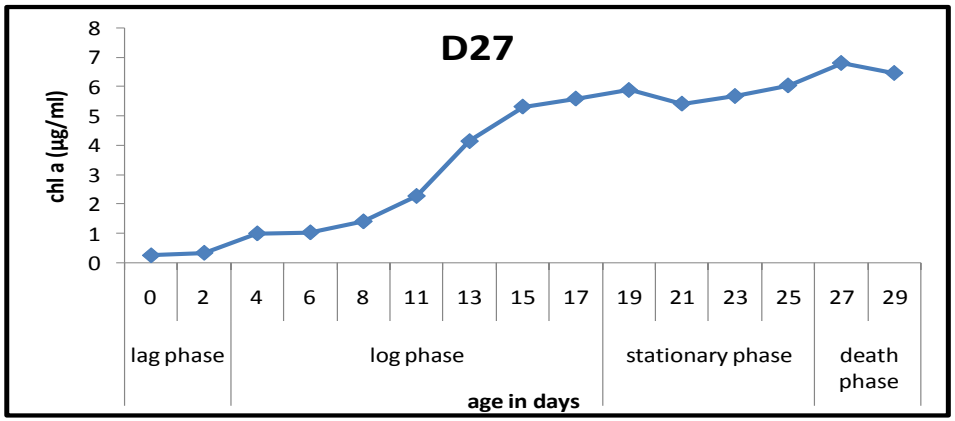


Fig. 6.7 Growth curves of the isolated cyanobacteria (D25-D27)

Table 6.2 Specific growth rate (K) and generation time (G) of the isolates

Serial no.	Strains	K(μday^{-1})	G(hr)
1	**D**1	0.491	42.712
2	**D**2	0.265	90.535
3	**D**3	0.465	51.567
4	**D**4	0.403	59.528
5	**D**5	0.109	218.222
6	**D**6	0.156	153.376
7	**D**7	0.147	162.641
8	**D**8	0.192	124.42
9	**D**9	0.149	161.037
10	**D**10	0.159	150.12
11	**D**11	0.134	178.833
12	**D**12	0.141	169.259
13	**D**13	0.15	159.235
14	**D**14	0.135	177.349
15	**D**15	0.138	173.221
16	**D**16	0.255	93.869
17	**D**17	0.418	57.389
18	**D**18	0.328	72.964
19	**D**19	0.119	200.25
20	**D**20	0.218	109.696
21	**D**21	0.151	157.943
22	**D**22	0.13	184.363
23	**D**23	0.086	277.869
24	**D**24	0.19	184.377
25	**D**25	0.366	65.535
26	**D**26	0.153	156.397
27	**D**27	0.123	194.863

6.3.2 Biochemical characterization of the isolated strains

The results showed that the tested organisms growing under the same cultural condition expressed significant differences in their growth pattern and metabolic activities. A significant variation exists for the chlorophyll *a* contents and carotenoid (**Fig. 6.10**) among the isolates. The organisms under *Oscillatoriaceae* were generally found to have higher chlorophyll *a* content. Highest chlorophyll *a* (9.43mg/l) and total carotenoid content (TCC) (3.83mg/l) were observed in *Lyngbya polysiphoniae* and *Westiellopsis prolifica*. The organism was followed by *Lyngbya limnetica* (9.35mg/l) for chlorophyll *a* content and *Scytonema tolypothrichoides* with 2.85 mg/l of carotenoid content. Lowest chlorophyll *a* was recorded for *Calothrix marchica*. *Anabaena orientalis* was recorded for lowest TCC (0.26mg/l). **Fig. 6.8-6.9** present data on protein and carbohydrates. The results of total carbohydrate content of cyanobacteria showed that the maximum contents in characterized non-heterocystous form *Nostoc linckia* (617.14 μg ml^{-1}) followed by the *Anabaena orientalis* (495.71 μg ml^{-1}) followed by *Fischerella muscicola* while the species *Nostoc spongiforme* exhibited significantly low carbohydrate content (108.09 μg ml^{-1} respectively) in comparison to the other strains examined. It was *Oscillatoria formosa* followed by *Nostoc commune* which exhibited significantly higher amount of soluble protein (301.13 μg ml^{-1} and 260.67 μg ml^{-1} respectively), followed by *Nostoc carneum* (216.07 μg ml^{-1}). Isolates screened for the potential phycobiliprotein content were found to be rich in both phycocyanin (PC) and Allophycocyanin (APC) contents **(Plate 6.11)**. The results of different concentrations of phycobilin contents of investigated cyanobacteria showed Phycocyanin (PC) to be the most dominant-phycobiliproteins in comparison to Allophycocyanin (APC) and Phycoerythrin (PE). The

maximum PC value was recorded in *Nostoc sphericum* and *Calothryx marchica* showed highest amount of APC. On the contrary *Cylindrospermum musicola* produced highest contents of phycoerythrin. Variations in the different concentrations of phycobilin contents in different species as found in this study, has been reported by other workers (Gantt, 1980; Moreno *et al*., 1995). Differential mechanisms of tolerance towards stressed environment have been reported in literature, which might be responsible for the variability observed in the parameters examined amongst the isolates (Potts & Bowman, 1985;Potts,1994**).**

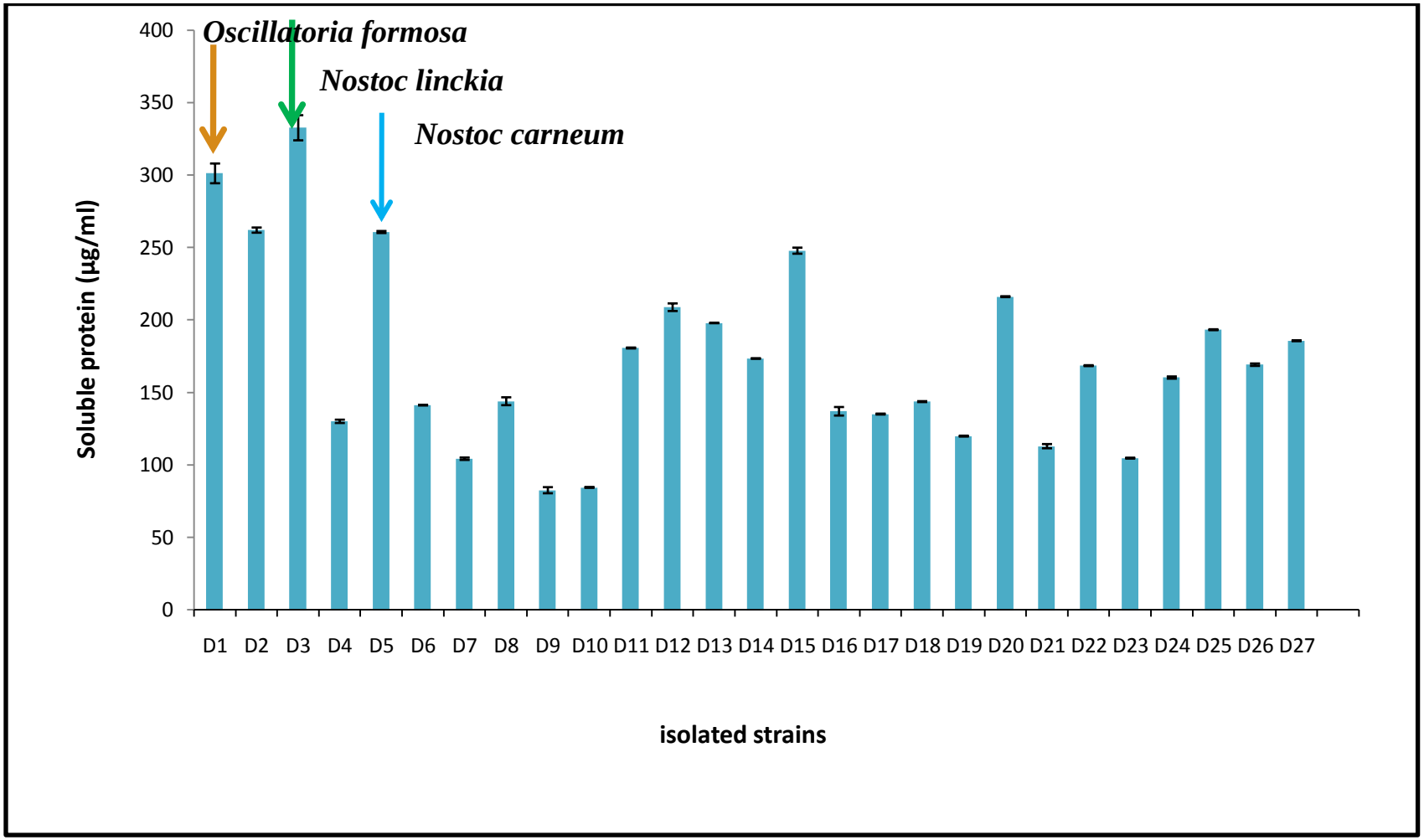


Fig. 6.8 Variation of protein contents in different isolates, P<0.05

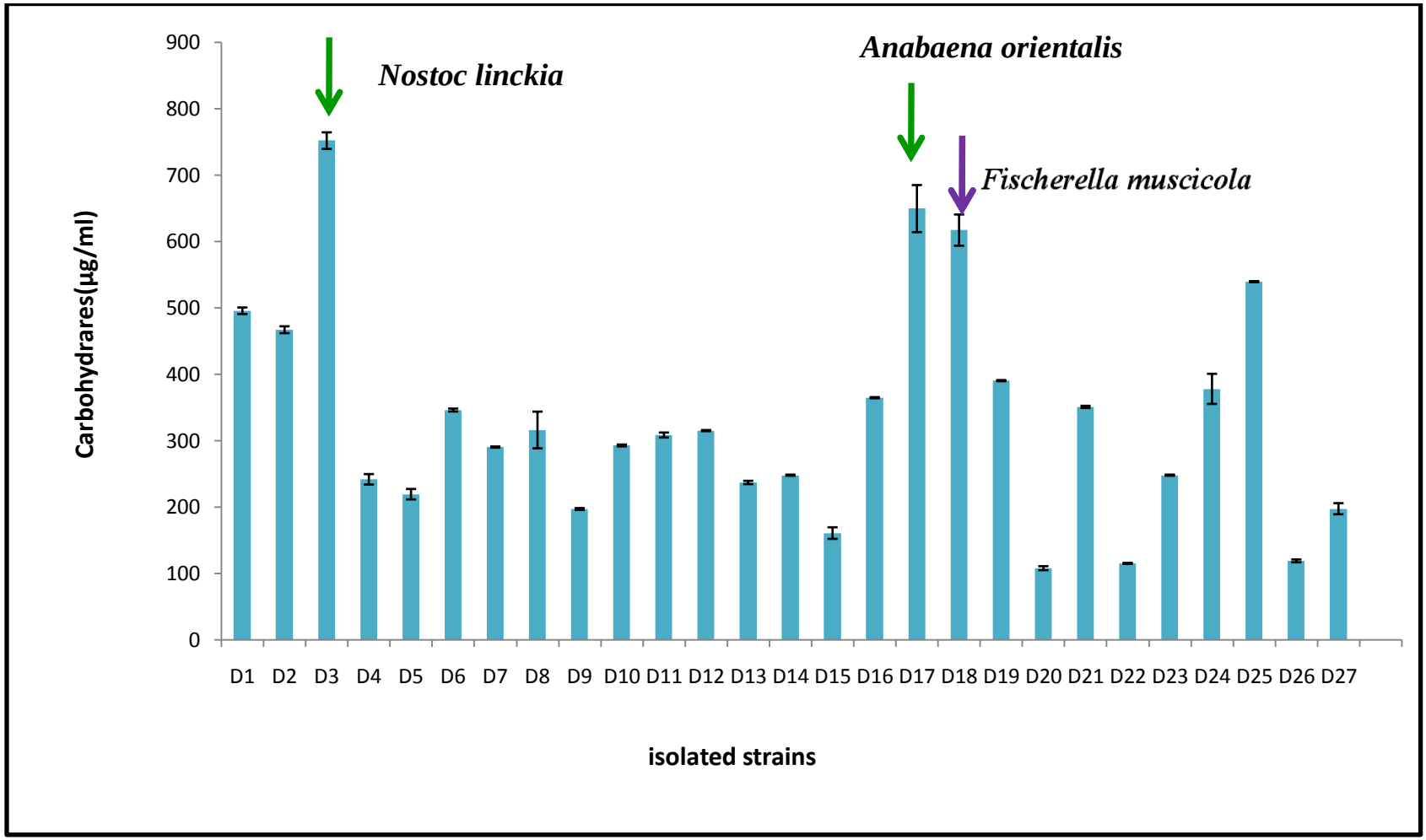


Fig. 6.9 Variation of carbohydrate contents in different isolates, $P<0.05$

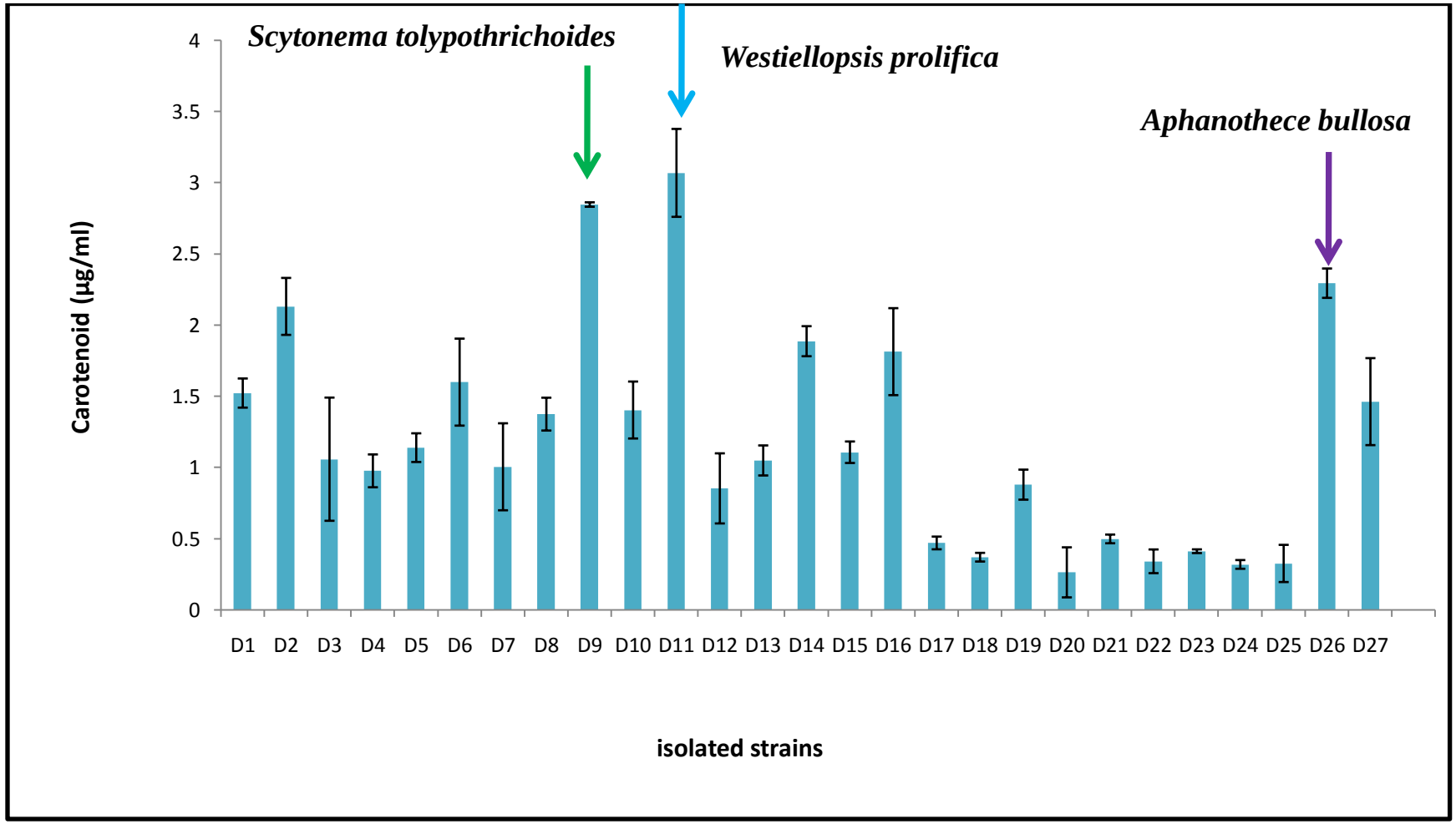


Fig. 6.10 Variation of carotenoid contents in different isolates, $P<0.05$

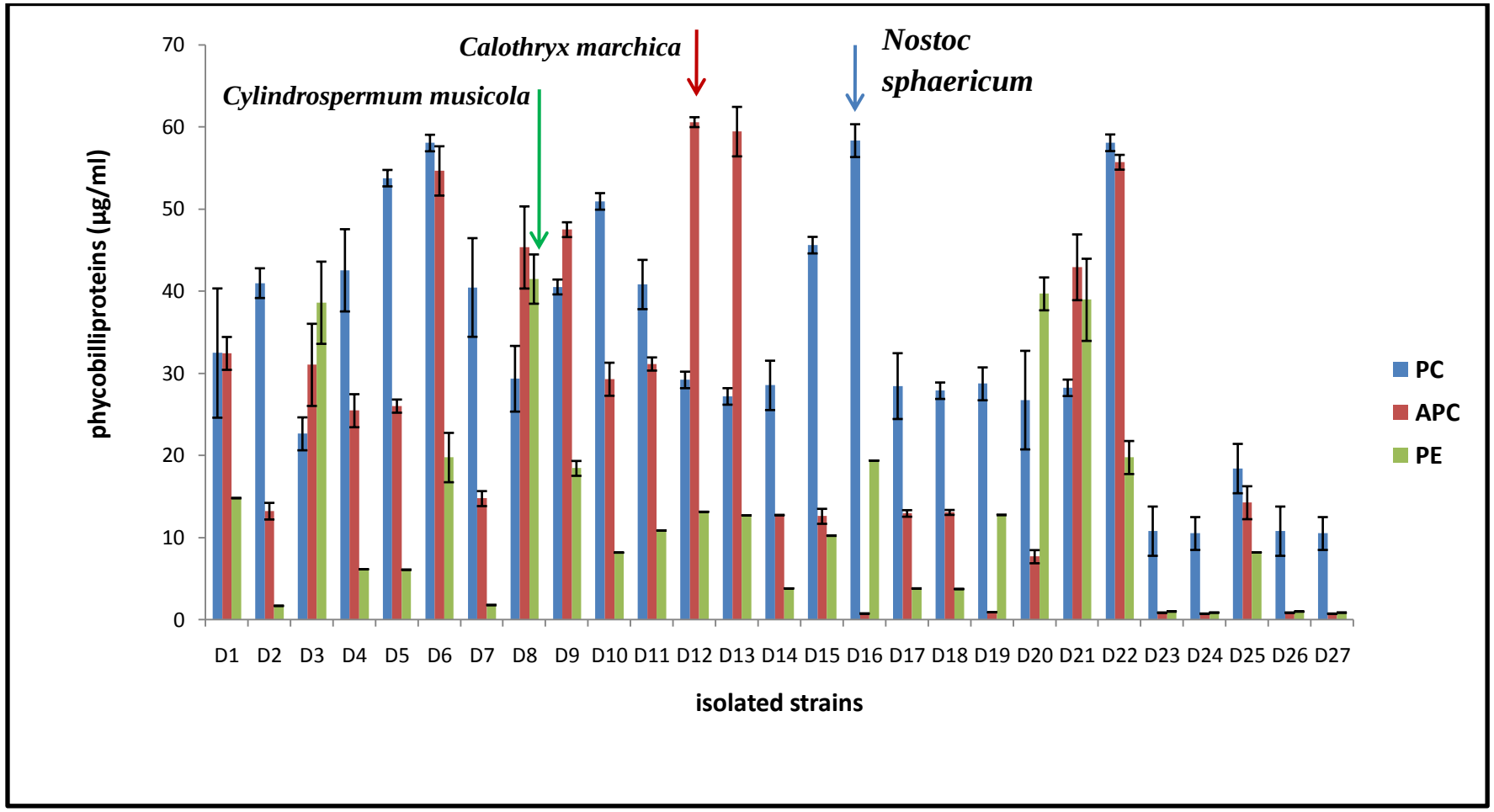


Fig. 6.11 Variation of different Phycobiliproteins (Phycocyanin, Allophycocyanin and Phycoerythrin) in different isolates, $P<0.05$

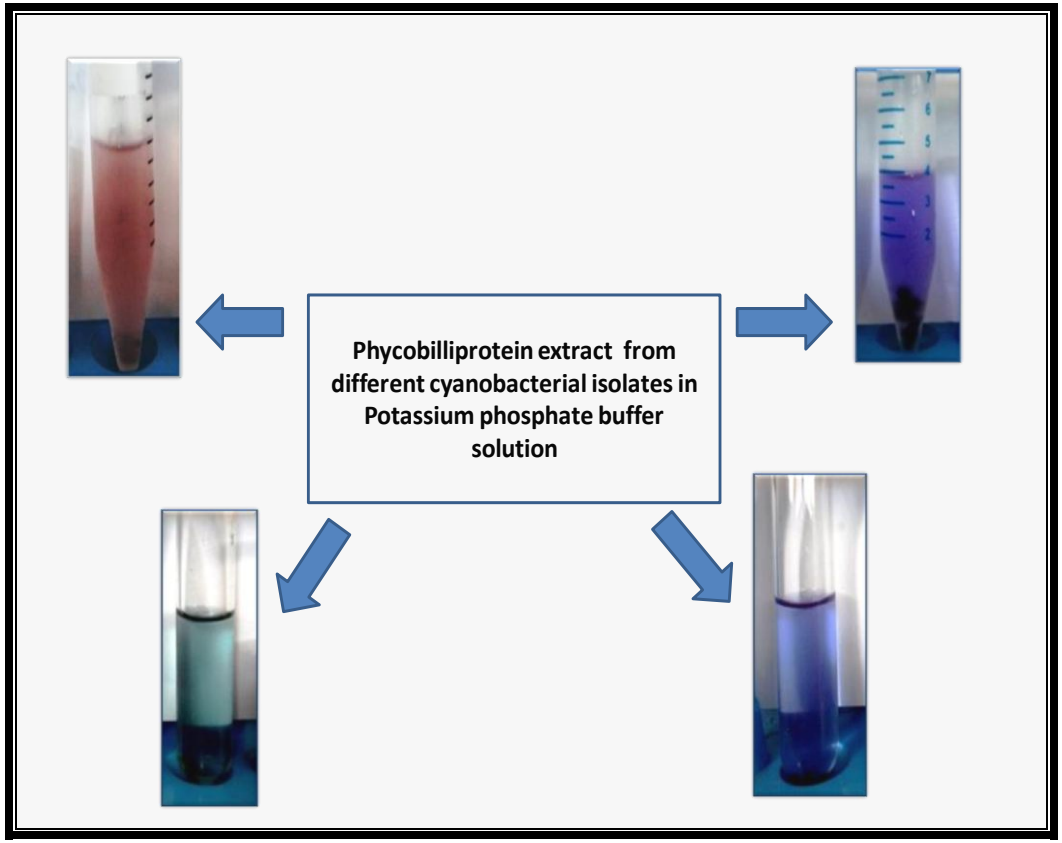


Plate 6.1: Crude extracts from the fresh algal biomass in Potassium phosphate buffer of *Calothrix marchica* (D19), *Lyngbya polysiphoniae* (D2), *Oscillatoria formosa* (D1) and *Phormidium fragile* (D21)

Table 6.3: Analysis of variance for the biochemical properties of the strains

	Sum of Squares	df	Mean Square	F	Sig.
Carbohydrate Strains Error Total	38974.420 701705.096 740679.516	1 25 26	28487.674 38974.420 28068.204	581.56	0.2894
Protein Strains Error Total	10925.692 92750.062 103675.754	1 25 26	3987.529 10925.692 3710.002	200.57.	0.003
Chlorophyll a Strains Error Total	1.787 .078 1.709	1 25 26	.069 .078 .068	654.54..	0.001.
Carotenoids Strains Error Total	2.203 13.179 15.383	1 25 26	.592 2.203 .527	299.32	0.004.
Phycocyanin Strains Error Total	615.726 4013.686 4629.412	1 25 26	178.054 615.726 160.547	321.45	0.000
Allophycocyanin Strains Error Total	1011.057 9887.000 10898.057	1 25 26	419.156 1011.057 395.480	221.34.	0.001.
Phycoerythrin Strains Error Total	2.134 3923.770 3925.904	1 25 26	150.996 2.134 156.951	342.56.	0.002

6.3.2 Conclusion

Occurrence of biologically active compounds with antiviral, antibacterial, antifungal and anticancer activities (Priyadarshani, 2012) prompted us to undertake the present investigation. Therefore, they need to be isolated from the natural habitats systematically, purified and characterized by following optimal culture conditions. In the present study some of the isolates owing to their higher biochemical contents can be considered as efficient strains and are of relevance for possible biotechnological applications.