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Growth rate, biomass production and minerals contents of some selected microalgae

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Abstract

The present study was carried out to assess the growth rate and biomass production potential and minerals content of 12 selected algal species (eight belonging to green algae, three to cyanobacteria and one to diatom). Biomass production and mineral content were measured based on weight difference and spectrophotometric method. The growth rate was determined on the basis of chlorophyll a content. The growth rate varied among different species. Lag phase was comparatively short in *Chlorella vulgaris* and *Ulothrix tenuissima,* entered exponential phase early and produced maximum biomass (422.40 and 330.0 dry wt mg/L^{-1} respectively). *Gloeocystis vesiculosa* and *Chlamydomonas reinhardtii* had longer exponential phase (45 and 39 days respectively). The nitrogen content was high in *Anabeana variabilis* (26.67 ppm). The potassium, sodium and phosphorus content were high in *Chlamydomonas reinhardtii* (30.16 ppm, 21.46 ppm and 12.85 ppm respectively). The calcium content was high in *Gloeocystis vesiculosa* (103.44 ppm). Lag phase is dynamic, organized and adaptive phase which prepared the cell for exponential phase for optimum growth. Therefore these algal species with short lag phase, high biomass and minerals content will be beneficial for the aquaculture and pharmaceutical industries.

Key words : Algae, biomass, chlorophyll, growth rate, minerals.

Microalgae are abundantly found in aquatic system and serve as a sustainable nutrient source to the heterotrophs. They are rich in protein, carbohydrate and lipid and in future may serve as an alternative to agricultural crops which are under stress due to the shortage of cultivated land. With the challenge of changing climate, algae are gaining attention as a vital source of food[21]. The microalgae with high nutritional value have proved as one of the most important food sources and feed additives in the commercial rearing of prawn larva and fishes[8,9,16]. Algae can be a sustainable, healthy and affordable food for animals and human being. Many algae are rich in protein, lipid and minerals[18,22,24] so could be the super food in future. Many algae are very rich in minerals like sodium, magnesium, phosphorus, potassium, iodine, iron and zinc so are commercially exploited all over the world.

Red algae (*Gracilaria* spp. and *Palmaria palmate*) and brown algae (*Laminaria* spp. and *Saccharina latissima*) are rich in sodium and potassium[24], while freshwater algae like *Spirulina* and *Anabaena* reportedly have good amount of mineral contents[2]. The studies on growth rate, biomass production and minerals content of green algae, blue green algae and diatom are very limited due to the difficulties in obtaining pure culture of algal species. The present study was aimed to determine the growth rate, biomass production and minerals content in some selected classes of algae.

Microalgae collection, cultivation and identification:

Freshwater algal samples were collected from the water bodies of West Garo Hills, Meghalaya (India) by using plankton net of mesh size 45 µm and then cultured on a solidified medium with 2% agar in petri plates. The media used were modified bold basal medium[26] for green algae, BG11 medium[25] for cyanobacteria and Guillard medium[12] for diatom.

The taxonomic identification up to species level was done with the help of floras and monographs which included Presscott[20]; Desikachary[11]; John *et al.,*[15] for non diatoms algae. For diatom, the Monograph of Tiffany and Britton[29] and updated online database Algae Base[13] was used.

Biomass production :

Dry weight and chlorophyll 'a' content of algae were measured as per standard method[7,28] so as to assess their biomass production potential. The algal species were grown in laboratory using appropriate culture medium. The 500 mL of Erlenmeyer flasks with 350 mL medium containing the specific algal cells were kept in culture rack at 40 µ moles m^{-2} sec^{-1} at photoperiod of 16:8 Light: Dark at room temperature. All the experiments were carried out in triplicates. Biomasses of the cultured species were recorded at three days interval. The cultures were filtered on pre-weighted filter papers. Then filter papers were oven-dried at 100°C for 2 h and their weight recorded.

The chlorophyll 'a' concentration was calculated by following the method given by Strickland and Parsons[28].

The chlorophyll a concentration in cultured sample was calculated by the following formula.

$$\text{Chlorophyll a (mg/L)} = \frac{\text{Chlorophylla(mg/L) X extract (L)}}{\text{Volume of sample (L)}}$$

Estimation of minerals in algal biomass :

Dried algal samples were digested by following standard method[19]. Dried algal sample (1 g) was digested in 10 mL triple acid mixture of nitric acid, sulphuric acid and 60% perchloric acid (10:1:1). Liquid ammonia was added into the digested sample to adjust the pH 7 and then volume was made upto 100 mL with distilled water. Then it was filtered through Whatman No.40 filter paper and the filtrates used for the analysis of sodium and potassium by flame photometer (G-301)[14], phosphorus by stannous chloride colorimetric method and Calcium was estimated by titrating against 0.01N standard EDTA with murexide as indicator[4]. Nitrogen was estimated by Kjeldahl digestion method[5].

Statistical analysis of data :

Mean and standard deviation were calculated from the experimental data of growth measurement and minerals using MS-Excel 2007.

Growth and biomass of cultured algae :

The algal cultures isolated were; eight belonged to green algae and were identified as *Chlorella vulgaris*, *Scenedesmus obliquus*, *S. dimorphus*, *Chlamydomonas reinhardtii*, *Chlorococcum infusionum*, *Gloeocystis vesiculosa; Ulothrix tenuissima* and *Desmidium swartzii*. Three algal species belonged to cyanobacteria and were identified as *Calothrix marchica*, *Anabaena variabilis* and *Leptolyngbya boryana* and one algae

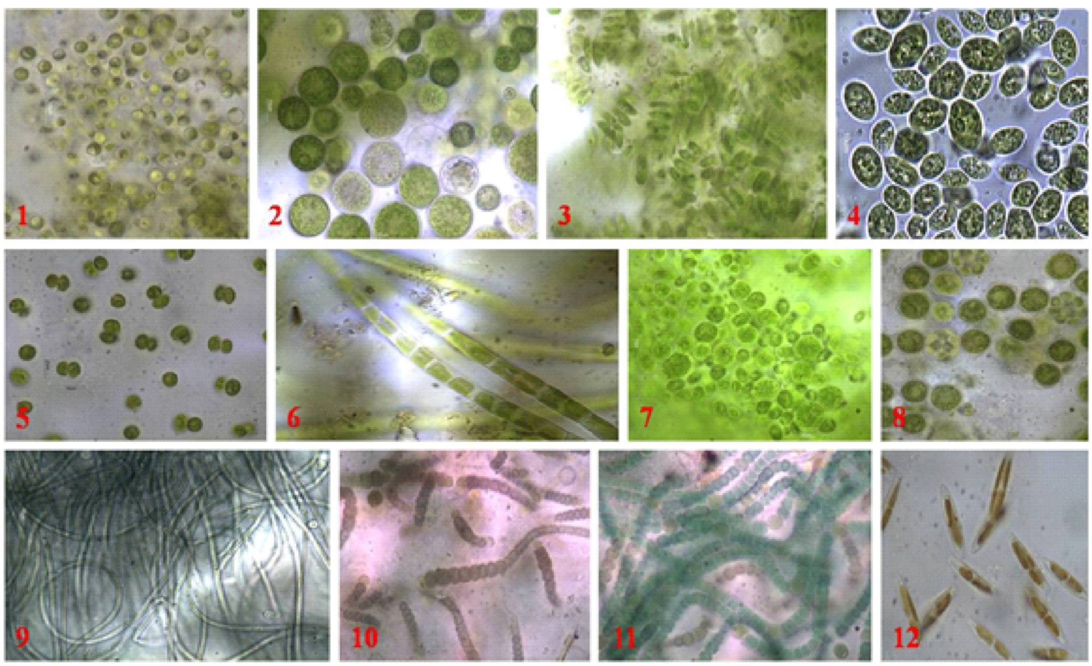
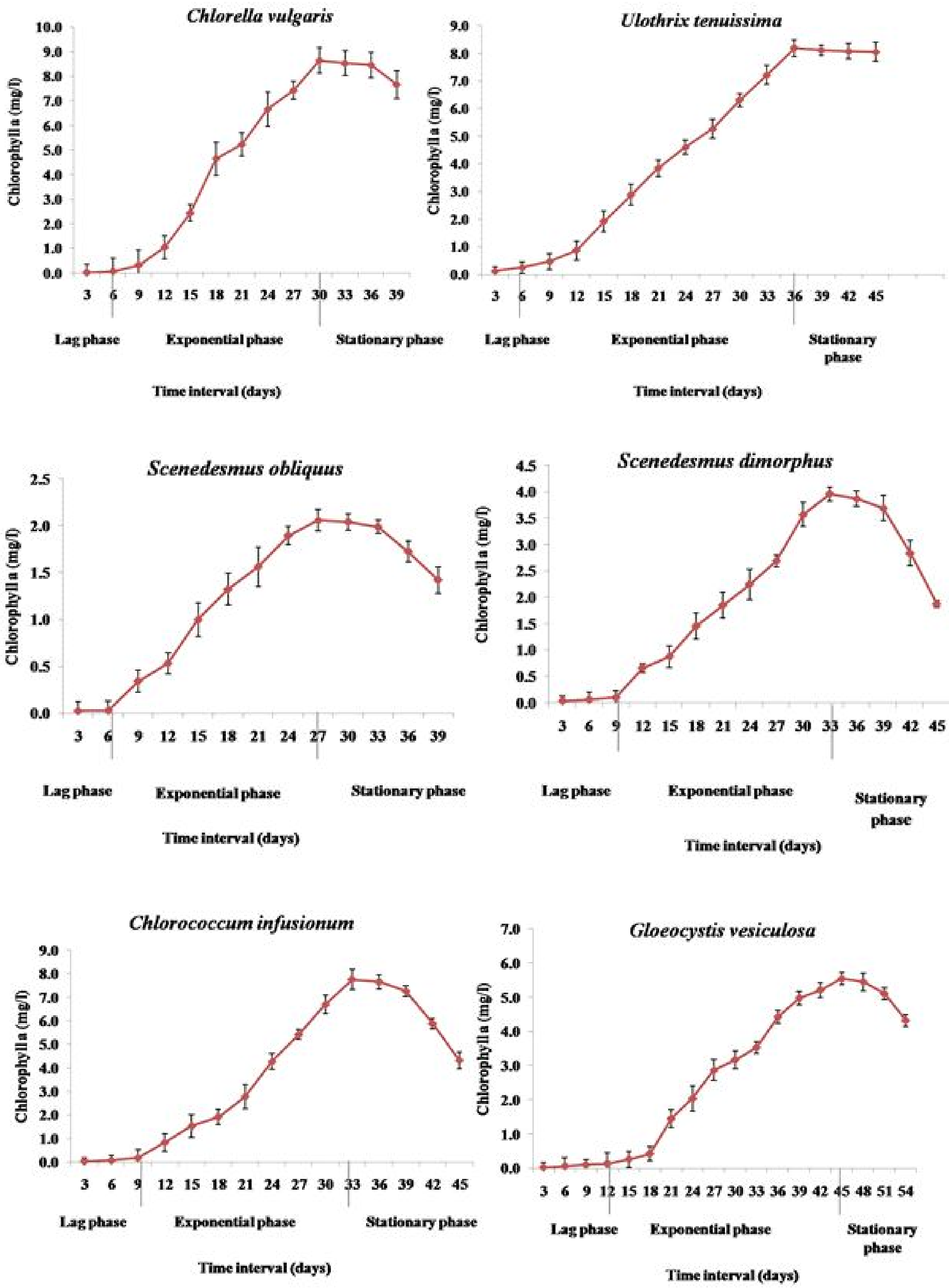


Fig. 1. The algal species identified; 1. *Chlorella vulgaris*, 2. *Chlorococcum infusionum*, 3. *Scenedesmus dimorphus*, 4. *Scenedesmus obliquus*, 5. *Gloeocystis vesiculosa*, 6. *Ulothrix tenuissima*, 7. *Chlamydomonas reinhardtii*, 8. *Desmidium swartzii*, 9. *Leptolyngbya boryana*, 10. *Calothrix marchica*, 11. *Anabaena variabilis*, 12. *Navicula veneta*.


Chlorella vulgaris
Chlorophyll a (mg/l)
Lag phase
Exponential phase
Stationary phase
Time interval (days)
Ulothrix tenuissima
Chlorophyll a (mg/l)
Lag phase
Exponential phase
Stationary phase
Time interval (days)
Scenedesmus obliquus
Chlorophyll a (mg/l)
Lag phase
Exponential phase
Stationary phase
Time interval (days)
Scenedesmus dimorphus
Chlorophyll a (mg/l)
Lag phase
Exponential phase
Stationary phase
Time interval (days)
Chlorococcum infusionum
Chlorophyll a (mg/l)
Lag phase
Exponential phase
Stationary phase
Time interval (days)
Gloeocystis vesiculosa
Chlorophyll a (mg/l)
Lag phase
Exponential phase
Stationary phase
Time interval (days)

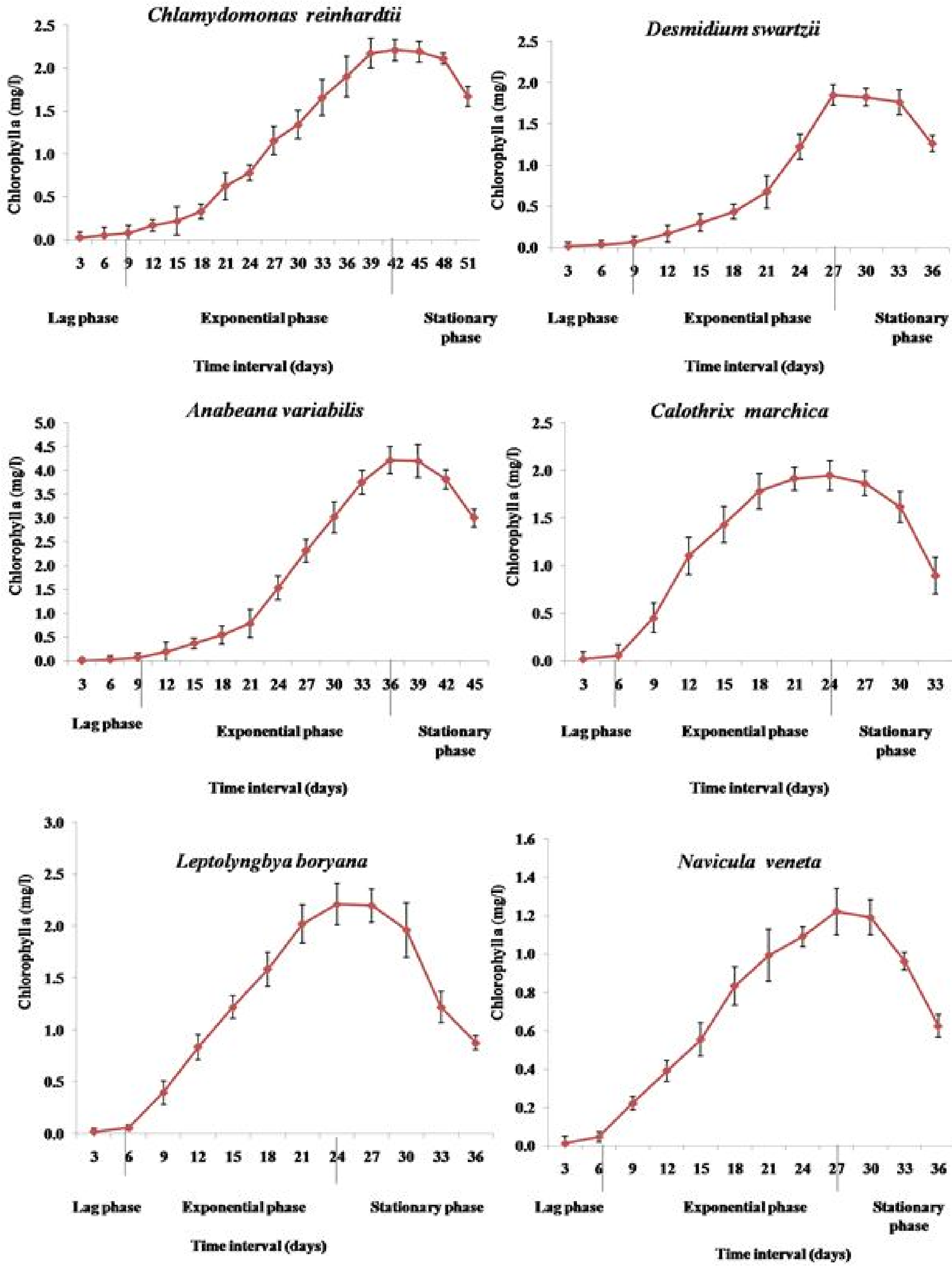


Fig. 2. Growth rate of selected algal species in culture.

Table-1. Dry weight of algal biomass obtained at 3 days interval

Dry wt mg/l	**Days interval**															
	3	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**	**30**	**33**	**36**	**39**	**42**	**45**	**48**
Chlorella vulgaris	57.2	82.5	112	178.5	234.3	275	312.7	399.3	412.5	**422.4**	418.1	399	-	-	-	-
Scenedesmus obliquus	9.0	30	75	85.8	92	115.5	132	184.8	**217.8**	214	203	148.5	135.3	9	30	75
Scenedesmus dimorphus	60	82.5	122	166.1	181.5	204.6	220.3	231.8	250.32	262.1	**264**	261.8	-	-	-	-
Chlorococcum infusionum	31.2	62.3	122.1	135.3	165	182.1	221	251	281	292	**317**	315	290.4	246	-	-
Gloeocystis vesiculosa	42.1	82.5	89.1	142.5	174.9	182.6	201.3	225.2	242.1	267.1	272.1	282.9	284.7	290	**293.7**	284.5
Ulothrix tenuissima	13.2	23.1	33	56.1	92	108.9	125	128.7	145.2	267	318	**330**	310.2	290.4	-	-
Chlamydomonas reinhardtii	36	67.32	85.21	96.21	108.9	125.4	147.2	162.1	171.6	212	228	234.8	259.2	**270.6**	267.32	188.9
Desmidium swartzii	21.4	59.4	78.21	98.46	100.34	115.2	128.5	135.2	**148.4**	142.06	134.12	-	-	-	-	-
Anabeana variabilis	27.8	56.1	82.5	115.5	132	148.5	174.9	188.1	226	254.1	271.42	**280.5**	273.9	247.5	-	-
Calothrix marchica	23.1	42.1	97	110.2	120.3	130.2	140.2	**152.1**	150.1	132	120.2	110	101	100	-	-
Leptolyngbya boryana	37	69.3	79.2	115.5	135.3	158.4	180.3	**226.1**	205.1	178.6	125.4	-	-	-	-	-
Navicula veneta	19	29.8	39.5	52.1	75.21	88.9	106.32	123.7	**128.2**	115.24	-	-	-	-	-	-

belonging to diatom was identified as *Navicula veneta*. The growth measurement is essential to know the exact harvest period of specific algae when their biomass is optimum. Among the 12 algal species, the lag phase was shorter in *Chlorella vulgaris, Ulothrix tenuissima, Scenedesmus obliquus, Calothrix marchica, Leptolyngbya boryana* and *Navicula veneta* (approx. 6 days) and was longer in *Gloeocystis vesiculosa* (12 days) (Fig. 2). In *Gloeocystis vesiculosa* and *Chlamydomonas reinhardtii* the exponential phase was longer (45 and 39 days, respectively) while *Calothrix marchica* had shortest exponential phase (15 days). Maximum biomass production both in terms of chlorophyll a content and dry weight was obtained from *Chlorella vulgaris* (8.65 and 422.40 mgL^{-1} respectively) in 30 days followed by *Ulothrix tenuissima* (8.18 and 330.0 mgL^{-1} respectively) in 36 days and *Chlorococcum infusionum* (7.75 and 317 mgL^{-1} respectively) in 33 days as compared to other species (Fig. 2; Table-1). All these algal species showed short lag phase of growth and entered exponential phase early and after exponential phase, the growth of algae declined perhaps due to the depletion of nutrients. Algae with short lag phase are beneficial because they take lesser time to adapt and exploit new environmental condition for optimum growth. According to Abdelkhalek *et al.*,[1] the quality and quantity of nutrients mainly phosphorus and nitrogen; silica for diatoms, played fundamental role in cultivation of microalgae. In addition to nutrients, others factors like pH, photon irradiation, salinity, turbulence and temperature play important role in algal growth and biomass production in culture [3,17,27].

Nitrogen, phosphorus, sodium, potassium and calcium content in 12 selected algal species:

The minerals content in selected algal species were different in different species (Fig. 3). Microalgae are important sources of minerals but very little work has been carried out for the detailed study of the minerals content in freshwater algal species because of the difficulties to get pure culture. In the present study, phosphorus and potassium content was higher in *Chlamydomonas reinhardtii* (12.85 ppm and 30.16 ppm respectively). The calcium content was high in *Gloeocystis vesiculosa* (103.44 ppm). The sodium content was also high in *Chlamydomonas reinhardtii, Chlorella vulgaris* and *Desmidium swartzii,* which ranged from 20.36 ppm-21.46 ppm. Nitrogen content was found maximum in Cyanobacteria, with highest in *Anabeana variabilis* (26.67 ppm). Beckers[6] reported microalgae as valuable source of many essential vitamins and minerals (Na, K, Ca, Mg, Fe, Zn and trace minerals). Some of the essential minerals from algae mostly red algae, green algae and brown algae were reported by Sivakumar and Arunkumar[24] from Coast of Gulf of Mannar, where they reported that Chlorophyceae (green algae) members had maximum sodium content and it was reverse in Rhodophyceae (red algae) and Pheophyceae (brown algae) where sodium content was low and potassium content was high. Csikkel-szolnoki[10] reported high calcium concentration in Red algae followed by brown algae and low in green algae. Abodaker *et al.*,[2] determined the mineral content in two cyanobacteria species namely *Spirulina platensis* with high sodium

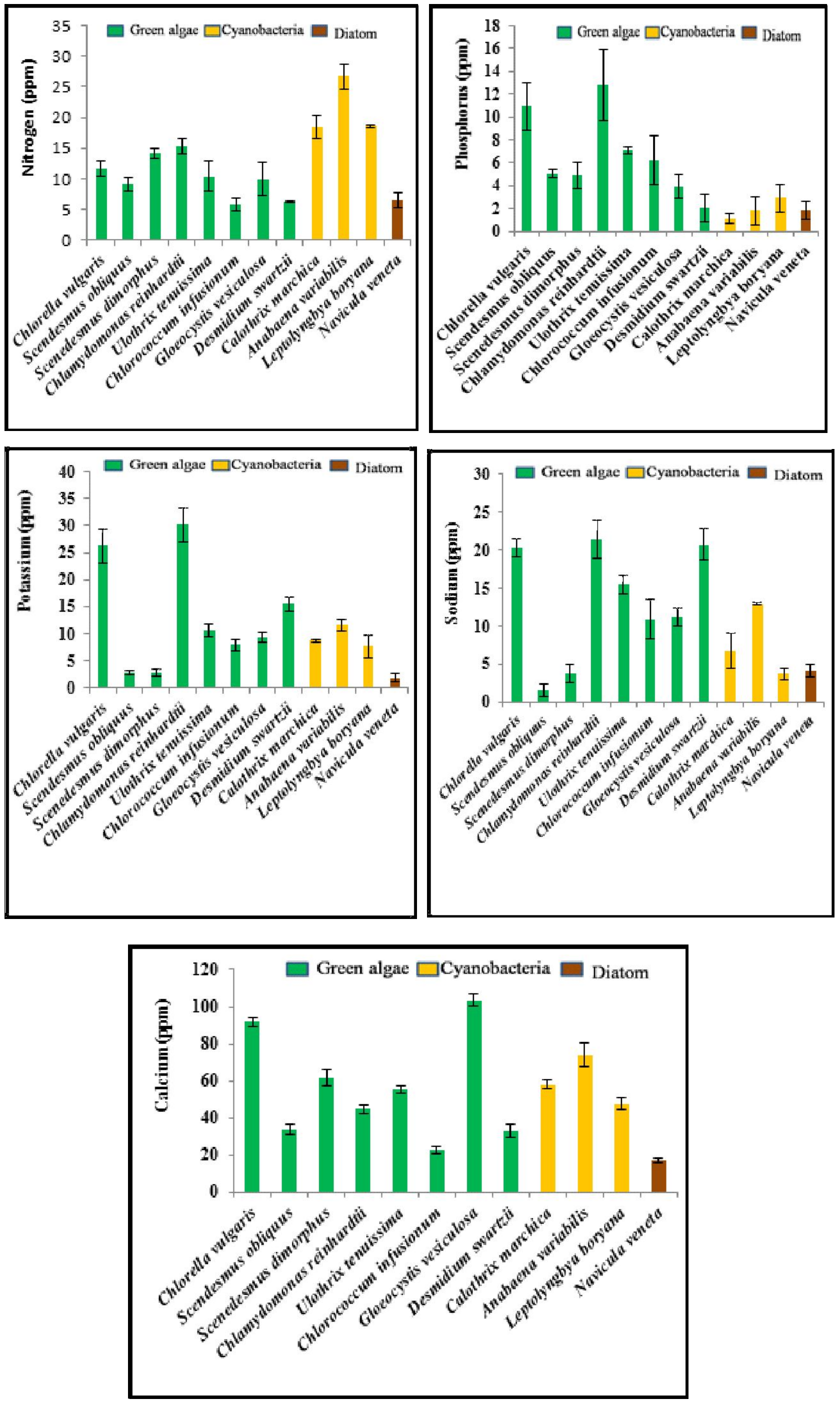


Fig. 3. Nitrogen, Phosphorus, Potassium, Sodium and Calcium in 12 selected microalgae.

content and *Anabaena* sp with high calcium, nitrogen and potassium content. Yusof *et al*.,[30] reported high calcium concentration in single cell algae (*Chlorella vulgaris*).

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