# Survey and Evaluation of Oleaferous Freshwater Algae of Dal Lake Ecosystem

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**(2010-332-D)**

**Division of Environmental Sciences**

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**Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir**

**2014**

# Survey and Evaluation of Oleaferous Freshwater Algae of Dal Lake Ecosystem

**Javeed Ahmad Lone**
**(2010-332-D)**

# Thesis

Submitted to

**The Faculty of Post-graduate Studies**
**Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir**
**in partial fulfilment of requirement for the award of the degree of**

## Doctor of Philosophy in Environmental Sciences

**2014**

# Dedicated

## To My Beloved Parents

*Whose blessings and constant*
*encouragement*
*have been always with me throughout my*

**Sher-e-Kashmir**
**University of Agricultural Sciences and Technology of Kashmir**
**Division of Environmental Sciences, Shalimar Campus, Srinagar - 190 025**

## Certificate – I

This is to certify that the thesis entitled **"Survey and Evaluation of Oleaferous Freshwater Algae of Dal Lake Ecosystem"** submitted in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy in Environmental Sciences**, to the Faculty of Postgraduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, is a record of bonafide research work carried out by **Mr. Javeed Ahmad Lone (Regd. No. 2010-332-D)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or information received during the course of investigation have duly been acknowledged.

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| Title of the Thesis | : | **"Survey and Evaluation of Oleaferous Freshwater Algae of Dal Lake Ecosystem"** |

# ABSTRACT

In the present study investigations on the various oleaferous algae of Dal Lake were carried out to identify the promising microalgal isolates for biodiesel production which is very much relevant to the need of biofuel industry. During the study 96 water samples and 96 algal samples were collected during the four seasons of the year from six different sites (DLS-I = Nehru Park, DLS-II = Bren Laam, DLS-III = Charchinar, DLS-IV = Hazratbal, DLS-V = Nagin, DLS-VI = Ranawari) of the lake. The water chemistry revealed that the lake is undergoing tremendous cultural eutrophication because of different anthropogenic activities viz., waste water from house boats and local population, agricultural runoff from the floating gardens, catchment areas and tourist activities. DLS-VI was the most polluted site of the lake during the four seasons of the year. However, the biological studies of the lentic ecosystem revealed that the lake is also rich in microalgal flora. A total of 91 algal genera were identified comprising of 217 species, 41 varieties and 8 forma. Maximum number of species belongs to Chlorophyceae (149) with 30 varieties and 8 forma. The Chlorophycean algae of Dal Lake was best represented in summer and autumn months and lowest in the winter followed by spring season. In the class Bacillariophyceae, 30 species and 5 varieties were recorded showing their peaks of standing crop during winter months. Cyanophyceae was the third largest class with 28 species and 2 varieties

and showed their peak abundance during summer and autumn seasons with the maximum standing crop at DLS-VI. Among the Euglenophyceae, 4 genera, 9 species with 4 varieties were recorded and all these species were found in appreciable numbers at DLS-VI during the autumn season. Rhodophyceae was monotypic as it was represented by only one taxon and is a new record to the phycological studies of India. To identify the most promising biodiesel microalgal strains for our future research programme thirteen microalgal isolates were also successfully isolated and purified from the six different sites of the Dal Lake and were subjected to lipid analysis using slight modified Folch method (1957). The peak lipid content in the isolates ranged from 4.63-30.99 per cent. Amongst all, two microalgae *Scenedesmus dimorphus* and *Scenedesmus quadricauda* were selected for further studies as these two isolates possess appreciable amount of lipids 30.99 per cent and 28.61 per cent respectively. The growth analysis pattern of these two robust algae in the BBM media showed that both the species are fast growing and reached a stationary phase on 14$^{th}$ day of incubation and are suitable for high-density culture. The photosynthetic pigments were calculated using two set of equations viz., Arnon's formulas and Wellburn equations. High amount of total pigments (mg $g^{-1}$ fw) were calculated when Wellburn equations were applied (*S. dimorphus* = 19.806 and *S. quadricauda* = 27.099) and Arnon's equations (*S. dimorphus* = 7.274, *S. quadricauda* = 9.713) were found to be inefficient when using DMSO as a solvent. The study also revealed that maximum protein content was found in *S. quadricauda* (13.026%) followed by *S. dimorphus* (8.284%). Based upon the fast growth and maximum lipid content, the two promising species of microalgae were selected for large scale biomass production in self made 25 liter lab scale photobioreactors having 15 liters BBM media. Both the species thrived very efficiently and biomass was harvested after a period of five weeks of incubation using different harvesting techniques like flocculation, centrifugation, lypholisation and oven dry. The lypholized biomass was subjected to lipid extraction by Soxhlet (1875) and Folch method (1957) using same solvent chloroform: methanol (2:1). In Folch method 30.99 per cent oil content was reported from *S. dimorphus* while as 28.61 per cent was reported from *S. quadricauda.* On the other hand in Soxhlet extraction 27.29 per cent oil was reported in *S. dimorphus* while as 24.75 per cent oil was reported from *S. quadricauda.* The results reveal that in case of green microalgae Folch method is more efficient as compared to Soxhlet in terms of oil extraction as in the former there is complete mechanical disruption of cells. The microalgal oil of both the species was subjected to fatty acid (FA) analysis and the fatty acid methyl ester (FAME) profiles showed that both the species possess appreciable amounts of major FA with carbon chain length of C16 to C18 viz oleic acid 21.1 per cent, 26.2 per cent, palmitic acid 18.9 per cent, 17.8 per cent, and linoleic 13.1 per cent, 13.8 per cent making them suitable feedstock for the production of good quality biodiesel. The total amount of fatty acid methyl ester of the *S. dimorphus* was 86.2 per cent and that of *S. quadricauda* was 85.7 per cent with 13.8 per cent and 14.3 per cent hydrocarbons and unidentified. The quality parameters of both the

microalgal oil like degree of unsaturation (DU), cetane number (CN), iodine value (IV), saponification value (SV) were within the limits of ASTM D6751, EN 14214 and ANP 255 standards respectively. The physico-chemical characteristics of oil obtained from these two tested microalgae viz., colour, density and viscosity were too high and did not pass the standards. The highly dense and viscous oils of both the microalgae reveal that transesterification is an important step to minimize these physico-chemical characteristics of the oil and conversion of the algal oil into biodiesel. Over all our results suggest that both the species of *Scenedesmus* are the promising isolates for producing high quality biodiesel.

**Key words:** Biodiesel, Dal Lake, Fatty acids, Lipids, Microalgae, *Scenedesmus dimorphus*, *Scenedesmus quadricauda*

Signature of Student

Dated:____________

Signature of Major Advisor

Dated:_________________

# ACKNOWLEDGEMENT

*I bow in reverence to the beautiful creator of the universe who is almighty Allah (Subhana–Wa–Tallah) for inspiration and for every success whose being benediction gave me all required zeal for the completion of this work.*

*Vocabulary in spite of all developments is still imperfect to express the feelings of gratitude, because when expressed in a word is only half its acknowledgement. Perhaps certain feelings of best expressed when felt unsaid. Yet I made an effort to express my overwhelming sense of gratitude for that has made this task possible.*

*It is a matter of immense pleasure to acknowledge my debt to my revered Chairman Dr. F. A. Lone, Associate Professor, Division of Environmental Sciences, SKUAST-Kashmir, Shalimar. It is because of his priceless intellectual innovative guidance, valuable suggestions, constructive criticism and constructive ideas for having given me complete independence, affectionate encouragement to put my desire and thought, which paved the way for the successful completion of this work. It is my fortune to get an opportunity to work under the supervision of such great personality. I also wish to extend my sincere thanks to my Chairman for suggesting me to work at Algology Laboratory, Plant Biodiversity, Systematics and Herbarium Division of National Botanical Research Institute, Lucknow.*

*I deem it to be my proud privilege to express my deep sense of gratitude and indebtedness to modest, generous and courteous personality Dr. M. R. Suseela, Scientist-F and Head, Algology Laboratory, National Botanical Research Institute, Lucknow for her attentive guidance, noble and humble advise, constructive suggestions, keen and sustained interest during the entire period of investigation. She has been a perennial source of inspiration and encouragement from the beginning till the completion of the thesis, without which this work could not have seen the light of the day.*

*I have warmest regard and am highly thankful to the members of my advisory committee, Dr. Javeed Iqbal Ahmad Bhat, Associate Professor, Division of Environmental Sciences; Dr. G. H. Dar, Professor, Division of Plant Pathology; Dr. J. A. Wani, Associate Professor, Division of Soil Science; Dr. Showkat Maqbool, Assistant Professor, Division of Agri-Statistics; Dr. Tariq Raja, Associate Professor, Division of Agri-Statistics (Dean P.G.*

*Nominee), for their talented and needful advice, moral encouragement during every stage of this study.*

*I express my special regards to Dr. Shafiq-ur-Rehman, Professor and Head, Division of Environmental Sciences, SKUAST-Kashmir, Shalimar for his ever-helping attitude, guidance and moral support during the whole study period.*

*I express my profound gratitude to Dr. Kiran Toppo, Technical Officer, Algology Laboratory, National Botanical Research Institute, Lucknow, U.P. India for her ever-helping attitude and appreciation throughout the course of study.*

*I cannot forget to express my warmest thanks to all the Faculty members of Division of Environmental Sciences for their round the clock help and cooperation. I am also thankful to all the teaching and non-teaching staff members of my Division for their valuable cooperation and help during the entire tenure of my studies.*

*I also acknowledge sincere thanks to the staff members of Central Library and ARIS, SKUAST-Kashmir, Shalimar for rendering all possible help.*

*I am highly obliged and thankful to the worthy Director Education and his Staff for their earnest help and cooperation in this endeavour.*

*It is my privilege to express my heartfelt gratitude to Dr. C. S. Nautiyal, Director, National Botanical Research Institute, Lucknow for allowing me to carry out my research work in the Institute and for providing all the infrastructural facilities.*

*I am thankful to Dr. D. K. Upreti, Scientist-G and Head, Plant Biodiversity, Systematics and Herbarium Division, National Botanical Research Institute, Lucknow for his timely advices and encouragement to carry out this work.*

*I owe a depth of gratitude to all my friends and research scholars especially Er. Ghulam Mohi-ud-Din Sofi, Dr. Syed Maqbool Geelani, Mr. Zaffer Iqbal Buhroo, Mr. Shahid, Mr. Shamsul Haq, Mr. Arif Hussain Bhat, Mr. Sachin Kumar Mandotra, Mr. Akash Kumar Patel, Minhaz Usmani, Mr. Pankaj Kumar, Mr. Manish Kumar Pandey, Ms. Neha Shrivastava, Dr. Chitralekha Nas Das Gupta, Dr. Rakhi Bajpai, Mrs. Gomta Devi and all my well wishers for their help, encouragement and moral support during my whole research work. Their suggestions and advices are unforgettable, that the light of hope never dies and hope battles with every possible dark.*

*It is my pleasant duty to thank my brothers, Mr. Mushtaq Ahmad Lone, Er. Touseef Ahmad Lone, my sister Ms. Shougfta Mohammad and my fiancee Maqsooda Akther for their loving support, kindness and constant encouragement rendered throughout my education for which I will remain indebted to them throughout my life. My special thanks to my dear Mamaji Mr. Manzoor Ahmad Lone for his unconditional support, loving cooperation and constant encouragement during the entire study.*

*Parents are the most precious gifts of God to Mankind. I have no words to express my heartfelt gratitude to my beloved and wonderful parents Mr. Gulam Mohammad Lone, Mrs. Shaha Begum and my grandmother Fatima Begum, who are the tower of strength and source of inspiration for me, who dedicated their entire life for my success, without their unconditional love, moral and emotional support I would have never been reached to this stage.*

*I extend my thanks to Mr. Younus Ahmad Bhat and Mr. M. Rafiq of M/s Universal Computers, Shalimar for their promptness and care in composing this manuscript in shortest possible time.*

*Last but not the least I thank all those whose names do not figure here but have helped me directly or indirectly in shaping up this thesis.*

*Javeed Ahmad Lone*

**Place :** Srinagar

**Date :**

# CONTENTS


| Chapter | Particulars | | Page No. |
|---|---|---|---|
| 1. | INTRODUCTION | | 1-6 |
| 2. | REVIEW OF LITERATURE | | 7-42 |
| | 2.1 | International algological studies | 7 |
| | 2.2 | National algological studies | 8 |
| | 2.3 | Jammu and Kashmir algological studies and water chemistry | 10 |
| | 2.4 | Biochemical analysis of microalgae and physico-chemical characterisation of algal oil | 17 |
| 3. | MATERIALS & METHODS | | 43-67 |
| | 3.1 | Survey, collection and preservation of samples | 43 |
| | 3.2 | Physico-chemical analysis of water samples | 44 |
| | 3.3 | Identification, isolation and screening of microalgae | 50 |
| | 3.4 | Maintenance of promising algal isolates | 55 |
| | 3.5 | Scanning and growth measurement of promising algal isolates by spectrophotometric method | 56 |
| | 3.6 | Biochemical studies of promising algal isolates | 57 |
| | 3.7 | Lab scale (self-made apparatus) photobioreactor for biomass production | 61 |
| | 3.8 | Analysis of algal oil | 62 |
| | 3.9 | The different parameters (colour, density, viscosity) of the algal oil was analyzed y standard methods of analysis (AOAC, 1995) | 64 |

| | | | |
|---|---|---|---|
| | 3.10 | Determination of fatty acid composition using gas chromatographic method (Morrison and Smith, 1964) | 65 |
| | 3.11 | Assessment of microalgal oil quality for biodiesel production | 66 |
| | 3.12 | Statistical analysis | 67 |
| 4. | EXPERIMENTAL FINDINGS | | 68-202 |
| | 4.1 | Physico-chemical analysis of water samples | 68 |
| | 4.2 | Algal diversity: Morpho-taxonomic description | 76 |
| | 4.3 | Screening of algal isolates for lipid content | 198 |
| | 4.4 | Growth measurement of two promising microalgae by spectrophotometric method | 200 |
| | 4.5 | Estimation of photosynthetic pigments | 200 |
| | 4.6 | Estimation of total protein content of two microalgal species | 202 |
| | 4.7 | Comparison of lipid content in two different methods using same solvent | 204 |
| | 4.8 | Fatty acid estimation using GC and GC-MS | 204 |
| | 4.9 | Quality parameters of microalgal oil for biodiesel production | 207 |
| | 4.10 | Characteristics of microalgal oil obtained from two tested microalgae | 207 |
| 5. | DISCUSSION | | 210-231 |
| | 5.1 | Physico-chemical characteristics of Dal Lake water samples | 210 |
| | 5.2 | Algological studies | 216 |
| | 5.3 | Lipid studies of different microalgae (qualitative and quantitative) | 220 |

| | | | |
|---|---|---|---|
| | 5.4 | Growth measurement of microalgae by spectrophotometric method | 221 |
| | 5.5 | Pigment estimation in two promising microalgae by using different equations | 223 |
| | 5.6 | Estimation of total protein content of two microalgal species | 225 |
| | 5.7 | Comparison of lipid content in two different methods using same solvent | 226 |
| | 5.8 | Fatty acid estimation using gas chromatography (GC) and chromatography-mass spectrometry (GC-MS) | 227 |
| | 5.9 | Quality parameters of oil for biodiesel production | 228 |
| | 5.10 | Characteristics of microalgal oil obtained from two tested microalgae | 230 |
| 6. | SUMMARY & CONCLUSION | | 232-233 |
| | LITERATURE CITED | | i-xlii |

# LIST OF TABLES


| Table No. | Particulars | Page No. |
|---|---|---|
| 1. | BG-11 media (Stanier *et al.,* 1971) | 51 |
| 2. | Bold's Basal Medium-BBM (Bold, 1949; Bischoff and Bold, 1963) | 55 |
| 3. | Physico-chemical characteristics (n=4) of the water samples collected from six different sites of the Dal Lake in winter season (February-2012) | 69 |
| 4. | Physico-chemical characteristics (n=4) of the water samples collected from six different sites of the Dal Lake in spring season (April-2012) | 70 |
| 5. | Physico-chemical characteristics (n=4) of the water samples collected from six different sites of the Dal Lake in summer season (July -2012) | 71 |
| 6. | Physico-chemical characteristics (n=4) of the water samples collected from six different sites of the Dal Lake in autumn season (October-2012) | 72 |
| 7. | Class-wise representation of total algae during the four seasons at six different sites of Dal Lake | 77 |
| 8. | Diversity and distribution of Chlorophyceae at six different sites of Dal Lake during four seasons of a year | 78-87 |
| 9. | Diversity and distribution of Cyanophyceae at six different sites of Dal Lake during four seasons of a year | 88-89 |
| 10. | Diversity and distribution of Eugleanophyceae at six different sites of Dal Lake during four seasons of a year | 90 |

| | | |
|---|---|---|
| 11. | Diversity and distribution of Bacillariophyceae and Rhodophyceae at six different sites of Dal Lake during four seasons of a year | 91-92 |
| 12. | Percentage lipid yields in different microalgal isolates | 199 |
| 13. | Chlorophyll estimation (mg $g^{-1}$ fw) in two microalgae by using two different set of equations | 201 |
| 14. | Total protein content in two microalgal species | 203 |
| 15. | Comparison of lipid content in two different methods using same solvent | 205 |
| 16. | Fatty acid profiles (%) of two microalgal strains | 206 |
| 17. | Quality parameters of oil from two microalgae for biodiesel production | 208 |
| 18. | Characteristics of microalgal oil obtained from two tested microalgae | 209 |

# LIST OF FIGURES


| Fig. No. | Particulars | After Page |
|---|---|---|
| 1. | Map of Dal Lake, Srinagar, India showing sampling sites | 43 |
| 2. | Box-and-Whisker plots of water parameters (n=4) during winter season (February-2012) | 72 |
| 3. | Box-and-Whisker plots of water parameters (n=4) during spring season (April-2012) | 72 |
| 4. | Box-and-Whisker plots of water parameters (n=4) during summer season (July- 2012) | 72 |
| 5. | Box-and-Whisker plots of water parameters (n=4) during autumn season (October- 2012) | 72 |
| 6. | Spectrascan of *S. quadricauda* with scan speed of 1800 nm/min screened between 400 and 1100 nm | 201 |
| 7. | Spectrascan of *S. dimorphus* with scan speed of 1800 nm/min screened between 400 and 1100 nm | 201 |
| 8. | Growth curve of two microalgae under batch mode | 201 |
| 9. | Chlorophyll estimation in two microalgal species by using two different set of equations: Arnon, 1949 and Wellburn, 1994 equations | 203 |
| 10. | Total protein content of two microalgal species | 203 |
| 11. | Comparison of lipid content in two different methods using same solvent | 203 |
| 12. | Fatty acid profile of *Scenedesmus dimorphus* and *Scenedesmus quadricauda* | 209 |

# LIST OF PLATES


| Plate No. | Particulars | After Page |
|---|---|---|
| 1. | Isolation techniques of microalgae, large scale algal biomass production and their harvesting using different methods | 51 |
| 2-43. | Digital microphotographs of Chlorophyceae, Cyanophyceae, Euglenophyceae, Bacillariophyceae and Rhodophyceae | 197 |
| 44. | Pure cultures of different microalgae | 199 |
| 45. | Nile red fluorescent yellow gold emissions by different microalgae showing presence of lipids | 199 |

**Chapter – 1**

# INTRODUCTION

The global energy demand has been increasing at an unprecedented rate with an increasing pressure on utilities of fossil based fuels. However, the non renewable nature of fossil fuels has raised numerous problems like increase in crude oil prices and global warming etc. (Malcata, 2011). Thus there is an urgent need to search for an alternative fuel source that is renewable, economical, sustainable and environmental friendly. One potential source of such an alternative fuel is biomass (any organic matter which is available on renewable basis) which can provide all forms of energy that humans need .Unlike finite deposits of coal, oil and natural gas, biomass can be produced almost anywhere and under a wide range of environmental conditions. Algae, one of the forms of biomass (microalgal biomass mainly consists of carbohydrates, proteins and oils) (Sheenan, 1998) can also be substantially used for the production of domestic bio-fuels (biodiesel, bioethanol, biohydrogen etc). Algae and plants serve as a natural source of oil, which conventional petroleum refineries can convert it into diesel fuel–a product known as 'green diesel'/biodiesel (NREL, 2006). As a biodegradable, renewable and non-toxic fuel, biodiesel doesn't contribute carbon dioxide or sulphur to atmosphere and emits less gaseous pollutants than conventional petroleum fuels. As a result it has the potential to reduce the level of pollutants and the level of potential or probable carcinogens in the environment (Chisti, 2007). In many countries biodiesel is produced mainly from jatropha oil, soybeans, canola oil, palm oil, corn oil, waste cooking oil and animal fat.

Algae are a large and diverse group of simple plants ranging from unicellular to multicellular form. They lack leaves, roots, and other organs that characterise higher plants. The branch of science that deals with the study of algae is called Phycology or Algology. The term Phycology is derived from two Greek words (Phycos-sea weeds; logos-discourse). The group includes simplest and most primitive member of thallophyta which exhibit a wide range of

reproductive strategies ranging from simple, asexual cell division to complex forms of sexual reproduction. These are considered as the first autotrophic (i.e., photosynthetic or holotrophic) plants of the planet. They comprise nearly one third part of world plant biomass of the earth.

Algae are ubiquitous, that occur in almost all habitats, ranging from marine and freshwater to desert sands and from hot springs to snow. They are very small, single celled to complex multicellular forms, such as the gaint kelps of eastern Pacific Ocean that grow to more than 60 meters in the length and form dense marine forests. The habitats occupied by fresh water algae are divided into lotic (running) and lentic (stagnant) water types. The lotic water including river, stream, canal, water fall and rivulets while lentic water includes the ditches, puddles, pools, ponds, lakes, agricultural fields and moist surface of soil, rock, stone, tree trunk, air, ice etc.

Due to the growth of algae in different habitats, they may be variable and highly diversified group of green plants i.e., phytoplanktonic (free floating), benthos (attached to sediments), epiphytic (on plants), epilithic (on stones), epipelic (on sand), endophytic (inside the plant), epizoic (on shells), and endozoic (inside sponge).

Algae are placed at the lowest rung of evolution and serve as base model for origin of land plants. They have enormous economic implications, not only as primary producers and pollution indicators (Prasad and Singh, 1996) but also as a source of several natural products, biofertilizers and fine chemicals. They are an inseparable associate of environment and also help in the purification of the environment. The early accumulation of oxygen in the earth's atmosphere was due to photosynthesis of ancient algal forms. It is estimated that, the algal photosynthesis contributes nearly 90 per cent of oxygen release in the earth's atmosphere. Globally algae are considered to fix 50 per cent of $CO_2$ and are the primary producers in aquatic habitat supporting rich food chains and oxygenate the aquatic systems (Misra *et al.,* 2001).

Most algal species exist as single cell in aqueous habitats, but some are organized in simple, filamentous, colonies. Scientists have estimated the total number of algal species to over 50,000 in the world but only 30,000 species are identified and examined (Frac *et al.,* 2010). Even less species are really tried out biotechnologically for industrial purposes. The photosynthesis in algae is similar to that found in all plants, but algae are especially effective in converting carbon dioxide and other nutrients into organic compounds. An algal facility could be sited next to a power plant or industry that burns fossil fuels and could recycle part of the carbon dioxide from flue gases into liquid fuels. Thereby shall help us to reduce its emissions in the atmosphere and combating climate change.

The recent research has proved that oil yielding algae are clearly superior to that of oleaginous terrestrial plants such as rapeseed or soybeans because they are unicellular and their faster multiplication rate in a liquid environment allows them better access to water, $CO_2$ and minerals. Due to this reason, algae are capable of synthesizing 30 times more oil per hectare than the terrestrial plants (Tickell, 2000; Chisti, 2007). The annual oil productivity of algae is around 90,000 L $ha^{-1}$ which is far greater than seed crops viz., Soybean = 450 L $ha^{-1}$, Canola = 1200 L $ha^{-1}$, Palm = 6000 L $ha^{-1}$, Jatropha = 1892 L $ha^{-1}$, and Corn = 172 L $ha^{-1}$ (Schneider, 2006; Haag, 2007). Since algae do not have to produce structural compounds such as cellulose for leaves, stems and roots, therefore have faster growth rate than terrestrial crops. As algae have short harvesting cycle as compared to terrestrial plants, their cultivation permits several harvests in a very short time frame, a strategy differing from that associated with yearly crops. Another important advantage of algae is that its doubling time is very short (within 24 hours) and during exponential growth the doubling time is as short as 3-4 hours (Schneider, 2006; Chisti, 2007). Under ideal conditions, algae can double their weight three to five times a day which makes it exceptionally good for biodiesel production. The other important characteristic of algae is that they do

not require freshwater for their growth as nutrients can also be supplied by wastewater and do not displace food crops.

Algae contain lipids and fatty acids as membrane components, storage products, metabolites and sources of energy. Under unfavourable environmental or stress conditions, they will stop growing and dividing and will transfer most of their energy into lipids as storage products for survival. Under these conditions, some strains can accumulate more than 80 per cent of their weight as lipids (Metting, 1996; Spolaore *et al.,* 2006). The oil levels of 20-50 per cent in algal cells are quite common viz., *Schizochytrium* sp. = 50-77 per cent, *Botryococcus braunii* = 25-75 per cent, *Nannochloropsis* sp. = 31-68 per cent, *Neochloris oleoabundans* = 35-65 per cent, *Nitzschia* sp. = 45-47 per cent, *Scenedesmus* sp. = 12-40 per cent, *Nannochloris* sp. = 20-35 per cent, *Chlorella* sp. = 28-32 per cent, *Spirogyra* sp. = 11-21 per cent, *Spirulina maxima* = 4-9 per cent and *Spirulina platensis* = 4-9 per cent (Becker, 1994; Chisti, 2007). Accumulation of neutral lipids mainly occurs in the form of triacylglycerides that can be processed by transesterification with primary alcohols into diesel oil or gasoline. This high yield, high density algal biomass can be an excellent source for algal oil which in turn is utilized to produce biodiesel.

Kashmir valley is one of the divisions of Jammu and Kashmir, India, which is bestowed with number of world famous lentic water bodies and Dal Lake is one such important water body of Himalayan ecosystem. This urban lake is of fluviatile origin having been formed from the ox-bows of the river Jhelum situated towards the North-east of Srinagar, Kashmir at the foot of Zabarwan mountains. The lake lies between 34°6'N -34°10'N latitude and 74°50'E-74°54'E longitude at an altitude of about 1,584 m above mean sea level (Najar and Khan, 2012). The main source of water for this lake is rainfall (Khan *et al.,* 2012). The lake is also mainly fed by a large perennial inflow streams called as *Telbal Nala* that

contributes about 80 per cent of the total inflow to the lake (ENEX, 1978; Zutshi and Vass, 1978; Trisal, 1987) as well as a number of other small streams, viz., *Peshpaw*, *Shalimar*, *Merakhsha* and *Harshikul* around the shore line (Najar and Khan, 2012). However, the lake bed abounds in number of natural springs.

The lake is divided into four basins: Hazratbal, Bod Dal, Nagin and Gagribal which differ markedly in their area, volume, depth and shoreline development indices etc. The Nagin basin is the deepest basin (maximum depth of about 6 m), and Gagribal basin the shallowest (maximum depth 2.5 m). The total water surface area of the lake is 11.50 $km^2$, of which 4.1 $km^2$ is under floating gardens, 1.51 $km^2$ is submerged land and 2.25 $km^2$ under marsh lands respectively (Jeelani and Shah, 2006). The *Telbal nala* with other small streams enter the lake at Hazratbal basin, then passes through Bod Dal basin, and finally drains into the river Jhelum from Gagribal basin side at the Dal Gate (Jeelani and Shah, 2006). The Nagin basin also receives water from the Hazratbal basin and leaves through the marshy area without any prominent outlet. Being an urban lake, the Dal Lake is surrounded by developed land and most of the lakeshore is occupied by houses, hotels, restaurants and houseboats.

The lake always remains in a eutrophic state during the summer season with highest standing crop of algae in spring-summer interphase and depression during winter. The early ecological studies conducted in the lake by many researchers (Kant and Kachroo, 1971; Kant and Kachroo, 1974; Zutshi *et al.*, 1980; Mir and Kachroo, 1982; Rather, 1994; Jeelani *et al.,* 2008; Singh *et al.,* 2008) reveal that the lake is having a great diversity and abundance of algae especially microalgae. The important oil yielding phytoplanktons found in this Himalayan Lake are *Chlamydomonas* sp., *Chlorella* sp., *Nitzschia* sp., *Navicula* sp., *Scenedesmus* sp., *Spirogyra* sp., *Spirulina* sp. etc. Hence to widen our basic understanding of the oleaferous algae in Dal lake, which is very much relevant to

the need of biofuel industry in Kashmir, it was necessary to identify and screen out potential oil yielding algal isolates and to characterize their biochemical and physicochemical studies. So the present research work was planned with the following objectives:

- ➲ Survey and collection of microalgae from different sites of Dal lake ecosystem of Kashmir.
- ➲ Identification, isolation and screening of potential oil yielding algal strains.
- ➲ In-vitro mass culturing of the pure isolates.
- ➲ Bio-chemical studies and physico-chemical analysis of algal oils.

**Chapter – 2**

# REVIEW OF LITERATURE

This part of thesis emphasizes upon work done on the biological diversity of fresh water algae, around the globe and in India with special emphasis on Jammu and Kashmir, India. The isolation, biomass production, biochemical analysis of microalgae and physicochemical anlaysis for biodiesel production has also been emphasized in the chapter.

## 2.1 International algological studies

The progress of algological studies, around the globe has been reviewed for last more than five decades. The work has been extensively done on Chlorophyceae, Cyanophyceae, Bacillariophyceae and Rhodophyceae.

The 19$^{th}$ Century witnessed a great spurt in algal studies: From 1817-1824 Agardh carried out a study on the algal flora of Scandinavia. Ralfs (1848) studied desmids of Britain. A great contribution came from Kuetzing (1845-1849) who authored a great number of genera than any phycologists before and described many species. Afterwards from 1891 Borge carried out an extensive research on the algal flora of Germany as well as on the collections received from China and Paraguay. West and West (1895, 1897-1898) have given a detailed taxonomical enumeration of fresh water algae from Madagascar, North America and Singapore. A monograph of British Desmidiaceae was prepared by West *et al.,* in 1923. The freshwater algae of South Africa particularly from Natal and Transvall cape colony were studied by Fritsch and Rich (1924, 1937).

The freshwater algal flora of Ceylon was reported by Crow (1923). Handa (1927 a, b) has made an important contribution on the fresh water algae from Rangoon. Prescott (1931, 1935, 1936 a, b and 1937) reported desmid flora of Iowa, New England, Western United States, Gatun Lake, Panama canal, Isle and Michigan. Prescott and Magnotta in 1935 gave notes on Michigan desmids.

A study of algal flora of Britian was carried out by Lund (1942-1960) who recorded species like *Chlamydomonas, Scenedesmus* and few new forms. The structure and reproduction of algae was explored by Fritsch (1945). Some freshwater algae from North America were reported by Prescott *et al.* (1949). Prescott and Scott (1945) and Prescott (1951) carried out extensive work on the fresh water algae of United States of America. The algae of Illinois were reported by Tiffany and Britton (1952).

Indonesian freshwater algae received a considerable attention from Scott and Prescott (1956, 1958 and 1960) who prepared notes on them. Coessel (1975, 1979, 1984, 1988, 1989 and 1993) made significant contributions on Dutch desmid flora and Coesel (2000) also studied the desmid flora of Thailand. Pham *et al.* (2011) prepared a checklist of the algae of Singapore. Freitas and Loverde-Oliveira (2013) prepared checklist of green algae for the State of Mato Grosso, Central Brazil.

## 2.2 National algological studies

In the eighteenth and nineteenth century, great advances have been made in the field of Algology, especially on Cyanophyceae, Chlorophyceae, Bacillariophyceae and Rhodophyceae throughout the Indian subcontinents. During the last 60 years, several standard publications on morphology and taxonomy of various algal groups were credited to prominent algologists like Desikachary (1959) on Cyanophyta, Randhawa (1959) also added new dimensions to the study of Zygnemataceae. Ramanathan (1964) on Ulotrichales, Philipose (1967) on Chlorococcales while Iyengar and Desikachary (1981) on Volvocales, Gonzalves (1982) on Oedogoniales. Likewise Anand (1989) came up with publications on Blue green algae.

Ehrenberg (1854) was the first worker who studied the geographical distribution of various species of Diatoms particularly from Bengal in India. Turner (1892) published a memoir of the East Indian fresh water algae. Turner in

his work incorporated 22 species of Myxophyceae, 542 species of desmids and 60 species of Chlorophyceae exclusive of desmids. West and West (1902) described 7 species of Rhodophyceae, 49 species of diatoms, 33 species of Myxophyceae, 246 species of desmids and 34 species of Chlorophyceae from Ceylon. West and West (1907) recorded 58 species of diatoms and 148 species of desmids as well as 53 species of blue green algae from Madras and Burma. Ghose (1923, 1927b) has given systematic and ecological accounts of blue green algae from Lahore, Shimla and Rangoon areas. Bharadwaja's (1928-64) notable contribution was on the Cyanophycean flora of Uttar Pradesh, India. The distribution of Ulotrichales algae in India was extensively studied by Ramanathan (1964) in his monographic work. Randhawa (1934, 1936b, c, 1938, 1940, 1941b, 1943, 1958, 1959) extensively studied and made significant contributions on Zygnemataceae and Chaetophoraceae. The occurrence of Oedogoniales taxa in India has been reported by Singh (1936), Gonzalves and Sonnad (1961) from Mysore, Goyal (1964b) from Rajasthan, Bharati and Pai (1972b) from Mysore, Karnataka. Misra (1937) reported Zygnematales members from Kashmir valley of Jammu and Kashmir. The Cladophorales were recorded from different places of India by Balakrishnan (1954), Randhawa and Venkataraman (1961). Goyal and Venkataraman (1964) have described culture variations in the morphology of *Anabaena cycadeae* Reink. Chaturvedi and Pandey (1976) have listed 52 taxa of Cyanophyceae and Chlorophyceae from Rohilkhand, Uttar Pradesh India. Pandey and Pandey (1980) have studied 33 taxa under 15 genera of Bacillariophyceae from Allahabad, Utter Pradesh India. Mukhopadhyay and Chatterjee (1981) have compiled the description of 57 taxa of blue green algae from Howrah district, West Bengal. Dickie (1882) described few interesting algae from Sikkim Himalayas. In 1984 Sankaran discovered a new species of genus *Batrachospermum* Roth named as *B. desikacharyil* from Tamil Nadu. Prasad *et al.* (1986) enumerated 22 taxa of Cyanophyceae from Panchmarhi, Madhya Pradesh. Desikachary *et al.* (1990, 1998) prepared a detailed account of Indian Rhodophycean algae from fresh as well as marine water habitats. A pioneer work on Chlorococcalean flora was done

by Kaushik *et al.* (1991) from Madhya Pradesh. Kant and Gupta (1998) have comprised 171 species of Cyanophyceae from Ladakh, Jammu and Kashmir. In the same year they made extensive survey of algal forms of Ladakh and recorded 286 genera, 848 species, 155 varieties, 27 forms and 6 combinations. Habib (2000) studied 25 taxa of diatoms under 10 genera described from foot hills of Garhwal Himalaya Uttaranchal. Habib (2001) has studied some Chlorococcalean taxa from foot hills of Kumaun Himalaya. Suseela and Dwivedi (2001) reported 4 taxa of Chaetophoralean members from Bundelkhand region of Uttar Pradesh. Suseela and Dwivedi (2002) have made a great contribution to fresh water algal flora of class Bacillariophyceae from Bundelkhand region Uttar Pradesh. In the same year Pattanaik and Adhikary (2002) have reported 16 taxa under 8 genera of Cyanophyceae from some archaeological sites and monuments of India. Khare and Suseela (2004) have enumerated 31 taxa of Cyanophyceae, Chlorophyceae and Bacillariophyceae from Nainital, Uttaranchal. Misra *et al.* (2004) studied 17 taxa of 15 genera in Cyanophyceae, Chlorophyceae and Bacillariophyceae from Sant Kabir Nagar, Uttar Pradesh. Suseela and Toppo (2007) enumerated the desmid flora of Sikkim Himalayas. Toppo and Suseela (2009) enumerated *Scenedesmus* species in Chhattisgarh State. Suseela and Toppo (2010) enumerated the occurance of rare desmids and their addition to Indian algal flora. Suseela and Toppo (2011) studied the occurence and diversity of *Staurastrum* species in lentic water bodies of Chattisgarh State. Kumar *et al.* (2013) studied the Cyanophycean flora of Kangra district of Himachal Pradesh.

## 2.3 Jammu and Kashmir algological studies and water chemistry

The pioneering studies on the Lakes of Kashmir have been initiated with the work of Edmondson and Hutchison (1934) who described some physico-chemical characteristics of Dal Lake and Mansbal Lake. Zutshi *et al.* (1972) while studying the limnology of Kashmir valley lakes have divided them into three categories-glacial lakes, pine forest lakes and valley lakes on the basis of origin, altitudinal location and the nature of biota they harbor. Zutshi and Vass (1973)

reported on the changing trophic status of Dal Lake and Anchar Lake and visualized the progressive increase in BOD and COD based on the water analysis data sets. Kant and Kachroo (1973) reported the peak for Myxophyceae in October, Chlorophyceae in August, and Bacillariophyceae in January and February, and Cryptomonadineae in September-October, and Dinophyceae and Euglinineae in August-September, Bacillariophyceae had a secondary peak in October and monthly distribution of dominant genera was represented graphically. Kant and Kachroo (1974) studied the diurnal changes in temperature, pH etc in Dal Lake.

According to Enex (1978) a net flow of 5.5 tons of phosphorous and 88.9 tons of nitrogen entered into the Dal Lake from immediate catchment. Zutshi and Khan (1978) reported high nutrient concentration of inshore waters as compared to offshore in Dal Lake.

Zutshi and Vass (1978) made liminological studies and revealed Dal Lake to be alkaline and slightly buffered. Kaul *et al.* (1980) investigated the physico-chemical characteristics of a high altitude forest lake Nilnag in Kashmir in comparison with the other valley lakes. Zutshi *et al.* (1980) on studying comparative limnology of nine lakes of Jammu and Kashmir Himalayas, ranked these lakes from sub-tropical monomictic to dimictic type based on the difference in their morphology and thermal behavior.

Mir and Kachroo (1982) reported that in the Dal and Nagin lakes of Kashmir Himalaya, the main bulk of the phytoplankton comprises Bacillariophyceae with highest standing crop in spring-summer interphase and depression in winter. They found that local meteorological disturbances and the shallowness of the lake cause erratic fluctuations in physico-chemical parameters and alter the biological balance of the lake water. Phosphate (max. in summer 26 $\mu g\ L^{-1}$ and min. in autumn $18.41\mu g\ L^{-1}$) and nitrate (max. in June 1.501 $mg\ L^{-1}$ and min. in September 0.154 $mg\ L^{-1}$) are in such low quantities that either may not be a limiting factor in growth of Bacillariophyceae. The silicon (max. in June

7.5 mg $L^{-1}$ and min. in December 3.2 mg $L^{-1}$) does not play a significant role in the development since the lakes are poor in its production, though factors like temperature, dissolved oxygen, etc have a profound effect on the ferri-silico-humate complex.

Zutshi and Vass (1982) studied the phytoplankton crop of the lake and reported that in general lake consist of members belonging to Chlorophyceae, Bacillariophyceae and Cyanophyceae. They also found significant site variation with regard to plankton distribution. On the basis of estimated annual production of phytoplankton, they proposed that the open area of the lake depicts low trophic level and the limnological features of various basins of the Dal Lake differ significantly as a result of which it is not justified to assign as single trophic status to the entire lake system.

Zutshi and Wanganeo (1984) evaluated the trophic status of some Kashmir lakes based on nutrient load model and reported export of phosphate and nitrate into the lakes from catchments.

Wanganeo and Wanganeo (1991) revealed that lakes in Kashmir manifested a marked change in algal assemblage with change in physical and chemical environment.

Khan (2002) consolidated first series on phycological studies in Kashmir and recorded a total of 889 documented algal species representing various groups and distributed in diverse freshwater habitats.

Kundanger and Abubakr (2004) on comparing the previous limnological data of Dal Lake reported an increase in chemical parameters but with a decrease in dissolved oxygen and pH.

Jeelani and Shah (2006) analyzed two hundred and forty water samples (in four seasons) to monitor the natural and anthropogenic influences on the water chemistry of the Dal Lake, Kashmir Himalaya. The scatter diagrams and the geological map of the study area suggest predominance of carbonate and silicate

weathering. Lower pH and high total dissolved solids, electrical conductivity and nitrate values in the Gagribal basin and in some patches of other basins reflect anthropogenic inputs in the form of sewage from surrounding population, houseboats, hotels, etc. The Dal Lake is characterized by high chemical index of alteration (CIA: 87-95), reflecting extreme weathering of the catchment area. The data suggest that the Dal Lake is characterized by differential natural and anthropogenic influences.

Jeelani *et al.* (2008) carried out limnological investigation on the Dal lake of Kashmir at eight sampling stations under different environmental conditions from 2000-2002 and later in the year 2006-2007. They found that the continued conversion of water area of this lake into land would result in the extinction of species at a rate greater than they disappeared earlier. The main purpose of their investigation was to assess the impact of climate change on the biodiversity of fresh water ecosystem of Kashmir. One of the important evidences of climate change is the successful flourishment of a tropical aquatic pteridophyte viz., *Azolla* species in Kashmir waters infesting almost all the water bodies in this Himalayan valley. It invaded the Kashmir aquatics, proliferated successfully and effectively encroached upon the native free floating species in a very short span of time. Their studies concluded that warmer temperature has affected the migrations of some phytoplankton and zooplankton species within the lake regions.

Iqbal *et al.* (2008) assessed the impact of effluents on phytoplankton population in four different basins of Dal Lake during Dec. 2001 to Nov. 2002. Clear variations were recorded for physico-chemical parameters of water. The sites in the vicinity of drains recorded low dissolved oxygen content and higher values of conductivity and major plant nutrients like phosphorus and nitrogen compared to the corresponding sites in the open water area. A total of 134 species of phytoplankton were recorded during their investigation also. The seasonality of phytoplankton depicted a definite seasonal succession being dominated by diatoms during spring, green algae during summer, blue-green algae during

autumn and diatoms again during winter. Species like *Asterionella formosa*, *Pediastrum tetras* and *Tetraedron regulari* restricted their presence only near the regions receiving sewage outfalls and species like *Pediastrum ovatum*, *Merismopedia glauca* and *Trachelomonas* sp. were found only at open water sites.

Singh *et al.* (2008) assessed water quality and eutrophication status of various lakes situated in the western Himalayan part of India. Lakes situated in Jammu and Kashmir (Mansar, Surinsar, Dal, Tsokar, Tsomaoriri lakes) and Himachal Pradesh (Renuka Lake) were studied. Their results show that most of physico-chemical parameters lied within the range for drinking and irrigation purposes in the Mansar, Surinsar and Dal lakes. The eutrophication status in all lakes was assessed using phosphate data, which showed Mansar, Surinsar and Tsomoriri under eutrophic while as Dal, Tsokar and Renuka lakes under hyper-eutrophic condition.

Yaqoob *et al.* (2008) evaluated the tropic status of three lakes viz; Dal, Nilnag and Sheshnag on the basis of physicochemical parameters criterion. It has been observed that the high altitude lake (Sheshnag) is still maintaining its pristine low tropic nature, although under stress for quite some time in view of Amarnath Yatra. While the Pine forest lake (Nilnag) is marching towards high trophic nature as a result of heavy influx of silt load containing nutrients from the catchment area. Where as, the urban valley lake (Dal) operating under tremendous anthropogenic pressures pumping heavy load of autochthonus and allochthonus material leads to its rapid trophic evolution in the form of eutrophication.

Shafiq-ur-Rehman (2009) found that the Dal Lake of the Kashmir valley has suffered with formation of a rare phenomenon of red-bloom of a new species discovered as *Euglena shafiqii*. In a series of examinations performed on the pollution scenario of the Dal Lake, the physical and chemical features like temperature, hydrogen ion concentration, dissolved oxygen, total alkalinity, nitrate, phosphate, chloride, sodium and potassium in the waters were studied. Most of the overloaded chemical features were found high in the lake. Moreover,

the levels of these events were much higher in the basins of the bloom-affected waters. The nutrients have been found important factor for the periodicity and aggregation of *Euglena shafiqii*, since during the bloom period these nutrients were reduced, thus believed to be utilized by the organisms for growth.

Ganai *et al.* (2010) carried out studies on Wular Lake located at a distance of 34 km from Srinagar city of Kashmir valley. The study was undertaken from March, 2007 to February, 2008 to assess various limnological parameters including plankton. A total of 64 phytoplankton spp. were identified. Bacillariophyceae was found to be the most dominant group at the selected site. The most abundant species in terms of population density were *Amphora spp., Cyclotella* spp.*, Longissimae longatum, Navicula* spp. and *Nitzchia* spp. Chlorophyceae formed the second most dominant group of phytoplankton with *Chlorella* spp., *Pediastrum* spp.*, Spirogyra* spp. and *Volvox* spp. as the most abundant species. Amongst cyanophyceae, *Anabaena* spp. was found to be the most dominant species at the selected site. Euglenophyceae formed the least represented group of phytoplankton which showed the peak population in spring. Chlorophyceae and Cyanophyceae showed positive correlation with water temperature respectively at the selected site whereas Bacillariophyceae and Euglenophyceae showed the negative correlation with water temperature respectively.

Siraj *et al.* (2010) reported wide seasonal and site-specific fluctuations in physico-chemical parameters of Dal Lake.

Najar and Khan (2012) applied the multivariate statistical techniques, such as cluster analysis, principal component analysis (PCA) and factor analysis (FA) to evaluate and interpret the water quality data set for 13 parameters at 10 different sites of the three lakes in Kashmir, India. Physico-chemical parameters varied significantly ($p<0.05$) among the sampling sites. Hierarchical cluster analysis grouped 10 sampling sites into three clusters of less polluted, moderately polluted and highly polluted sites, based on similarity of water quality

characteristics. FA/PCA applied to data sets resulted in three principal components accounting for a cumulative variance of 69.84, 65.05 and 71.76 per cent for Anchar Lake, Khushalsar Lake and Dal Lake, respectively. Factor analysis obtained from principal components indicated that factors responsible for accelerated eutrophication of the three lakes are domestic waste waters, agricultural runoff and to some extent catchment geology. Their study assesses water quality of three lakes through multivariate statistical analysis of data sets for effective management of these lakes.

Fazal and Amin (2012) focussed on the interaction between *'hanjis'* (Dal dwellers) and ecologically important Dal Lake and its surroundings in Srinagar city, Jammu and Kashmir. The Dal is one of the beautiful lakes of the world for which Lawrence has said, "Perhaps in the whole world there is no corner as pleasant as the *Dal* Lake". Their study finds that the *Hanjis* with increase in their population have inflected transformations in and around the lake with their activity. These transformations are leading to deterioration of the Dal Lake and its environs and there is an urgent need for intervention for the welfare of the *Hanjis* and the management of the Dal Lake.

Khan *et al.* (2012) studied the Limnology of Dal lake Kashmir and interpreted that the Lake is of mesotrophic to eutrophic nature. The water of the lake indicated average conductivity and total dissolved solids at 499 S $cm^{-1}$ and 319.16 mg $L^{-1}$ respectively with pH 7.1. Their results indicated that diverse change in water chemistry and metal concentration in the Lake takes place.

Khan *et al.* (2012) attempted to record the effect of human population on Dal lake taking into consideration the disturbed sites and undisturbed sites, involving the effect of human activities on this ecosystem. Their study also highlighted the latest pollution status of the lake by comparing the various physico-chemical parameters with the earlier data and suggested some remedial measures to save it from further deterioration.

Another study on the water chemistry of Hazratbal basin of Dal Lake in Srinagar, Kashmir was carried out by Kanue *et al.* (2013). They indicated that the world famous Dal Lake is undergoing a fast eutrophication due to pollution caused by agricultural practices in the catchment area which has subsequently enriched the lake water with enormous inputs of fertilizers, nutrient content, organic matter from both autochthonous and allochthonous modes.

The variations in the basic physical and chemical characteristics of the water in Dal lake Srinagar were determined on monthly basis by Mushatq *et al.* (2013). Their findings highlighted the deterioration of water quality in the lake due to urbanization and anthropogenic activity in the lake. They considered lake as eutrophic as evidenced by its shallow depth (1 to 4.5 m), low transparency (1 to 2.8 m), and higher concentrations of other nutrients such as phosphates, nitrates, sulphates and chloride.

### 2.4 Biochemical analysis of microalgae and physico-chemical characterisation of algal oil

Arnon (1949) presented evidence that a copper enzyme, polyphenoloxidase (otherwise known as tyrosinase or catecholase), is localized in the chloroplasts of spinach beet (chard), *Beta vulgaris*. The amount of chlorophyll-a, b and total were determined using the formulas given by Arnon based on the work of MacKinney (1941) who provided the values of extinction coefficients.

Shoaf and Lium (1976) found that dimethyl sulfoxide (DMSO) and 90 per cent acetone extracted equal amounts of chlorophyll from diatoms and blue-green algae, but DMS0 was superior to 90 per cent acetone for all green algae tested giving 2-60 times more chlorophyll depending on the species. The absorbance spectra of pure chlorophyll a and b from 600 nm to 750 nm were identical whether dissolved in 90 per cent acetone or a mixture of DMS0 and 90 per cent acetone (1: 1 v/v). Thus, several equations for estimating chlorophyll

concentration based on extinction in 90 per cent acetone are applicable with this solvent.

Palumbo *et al.* (1987) evaluated the use of dimethyl sulfoxide (DMSO) to extract chlorophyll-a from periphyton samples for use in measuring photosynthesis and the effect of stream water chemistry on stream periphyton communities. DMSO and methanol extracted equivalent amounts of chlorophyll-a from rock surfaces.

Li and Wang (1997) demonstrated that algal oils can be used as feedstock's for biodiesel production, and compared to other vegetable oils and animal fats, the production of algal oil has many advantages i.e., algae have short life cycle, less labour required, less affect by venue, season and climate, and easier to scale up.

Lee *et al.* (1998) found that of various methods for lipid recovery in *Botryococcus braunii* UTEX 572, the most effective method was disruption of the cells with a bead-beater followed by extraction with chloroform/methanol (2:1, v/v) and gave a lipid content of 28.6 per cent of dry wt. There was a significant relationship between *in vivo* fluorescence of cells stained with Nile Red and lipid content in *B. braunii* determined gravimetrically ($r^2 = 0.997$). They suggested that the Nile Red staining as a rapid method was as good as the gravimetric method commonly used for lipid determination which requires toxic solvents and considerable time-consuming manipulations.

Biodiesel is a biodegradable, renewable, and non-toxic fuel and has received considerable attention in recent years. It also contributes no net carbon dioxide or sulphur to the atmosphere and emits less gaseous pollutants than conventional diesel fuels (Lang *et al.,* 2001; Antolin *et al.,* 2002; Vicente *et al.,* 2004).

Bigogno *et al.* (2002) hypothesized that among algae of alpine environment there could be strains particularly rich in long chain polyunsaturated

fatty acids (LC-PUFA). Isolated Chlorophyte *Parietochloris incisa* from Mt. Tateyama, Japan and was found to be the richest plant source of the pharmaceutically valuable LC-PUFA, arachidonic acid (AA, 20:4ώ6). The alga is also extremely rich in triacylglycerols (TAG), which reaches 43 per cent (of total fatty acids) in the logarithmic phase and up to 77 per cent in the stationary phase. In contrast to most algae whose TAG are made of mainly saturated and monounsaturated fatty acids, TAG of *P. incisa* are the major lipid class where AA is deposited, reaching up to 47 per cent in the stationary phase. Except for the presence of AA, the PUFA composition of the chloroplastic lipids resembled that of green algae, consisting predominantly of C16 and C18 PUFAs.

Tait and Hik (2003) found that dimethyl sulfoxide (DMSO) appears to be a reliable solvent for extracting chlorophyll. However, modification of standard methods may be necessary for some species under field conditions. They found that Chlorophyll extraction of whole leaf tissue with DMSO incubated at between 25 and 40 $^{o}$C was generally similar to the 80 per cent acetone method, except for one graminoid species that required maceration. There was little effect of incubation temperature or duration of incubation beyond 7 h on extraction efficiency, but DMSO extracts were less stable than acetone extracts during one week of cold storage, especially if they thawed during this period. Since chlorophyll extraction methods may provide variable results, particularly in the field, studies using different solvents should be compared cautiously unless specific methods have been calibrated.

Kerschbaum and Rinke (2004) studied that for a proper design of our micro heat exchangers the viscosity of biodiesel must be known around a temperature of 273 K. Therefore, different samples of biodiesel were examined in the range of 258-303 K and empirical equations for the temperature dependent viscosities computed. Above temperatures of 273 K an exponential equation based on the usual Arrhenius form describes all measurements well. Below 273 K

the viscosity sharply rises within 2 K and then further increases. The reason is the precipitation of crystalline saturated methyl esters.

Pratoomyot *et al.* (2005) determined fatty acid composition of 10 species of microalgae at the exponential phase and the stationary phase. The microalgae consist of two species of diatoms (*Nitzschia ovalis*, *Thalassiosira* sp.) five species of green microalgae (*Tetraselmis* sp. *Dictyosphaerium pulchellum*, *Stichococcus* sp., *Chlorella* sp., *Scenedesmus falcatus*) and three species of blue green microalgae (*Anacystis* sp., *Synechococcus* sp., *Synechocystis* sp.). The medium for culturing of diatoms and green microalgae was F/2 and BG-11 media was used for Cyanophyceae. The microalgae were harvested for analyzing fatty acid and stored at -80ºC prior to analysis. Fatty acid composition of microalgae differed from species to species. The majority fatty acid composition of all the algae at the exponential phase and the stationary phase were in the range of C14-C20 respectively. In conclusion, they found that *Nitzschia ovalis* and *Thalassiosira* sp. would serve as good nutritional sources of high unsaturated fattyacids for aquaculture animals.

Xu *et al.* (2006) obtained high quality biodiesel from a microalga *Chlorella protothecoids* through the technology of transesterification. The technique of metabolic controlling through heterotrophic growth of *C. protothecoides* was applied and the heterotrophic *C. protothecoides* contained the crude lipid content of 55.2 per cent. They extracted efficiently the large amount of microalgal oil from the heterotrophic cells by using *n*-hexane, and then transmuted into biodiesel by acidic transesterification. The biodiesel was characterized by a high heating value of 41 MJ $kg^{-1}$, a density of 0.864 kg $L^{-1}$, and a viscosity of $5.2 \times 10^{-4}$ Pas (at 40 $^{o}$C). Their method has great potential in the industrial production of liquid fuel from microalga.

Chisti (2007) reported that autotrophic microalgae can utilize carbon dioxide as the carbon sources and sunlight as the energy for oil accumulation under some special conditions and found that many autotrophic microalgae, such

as *Chlorella* sp., *Botryococcus braunii, Crypthecodinium cohnii, Dunaliella primolecta, Monallanthus salina, Neochloris oleoabundans, Nannochloropsis* sp., *Nannochloris* sp., *Nitzschia* sp., *Phaeodactylum tricornutum* and *Schizochytrium* sp. accumulate oils. The oil content in some microalgae can exceed 80% by weight of dry biomass and oil levels of 20-50 per cent are quite common.

Patil *et al.* (2007) selected twelve algal strains representing different classes mainly from the culture collection of the Norwegian Institute for Water Research (NIVA). The growth responses and fatty acid composition were analysed. The maximum production rate was obtained with *Pseudokirchneriella subcapitata* (0.63 g $L^{-1}$ $day^{-1}$) and the lowest with *Porphyridium cruentum* 0.13 g $L^{-1}$ $day^{-1}$. Arachidonic acid (AA) and eicosapentaenoic acid (EPA) were the dominating polyunsaturated fatty acids (PUFAs) in *P. cruentum*, while only EPA accumulated in *P. tricornutum.* Docosahexaenoic acid (DHA) was the major PUFA in *Isochrysis galbana,* while *Pavlova sp*. had both EPA and DHA.

Rao *et al.* (2007) found that the growth of *Botryococcus braunii* and production of its constituents viz, hydrocarbon, carbohydrate, fatty acid and carotenoids were influenced by different levels of salinity. Under salinity at 34 mM and 85 mM, 1.7-2.25 fold increase in the relative proportion of palmitic acid and two fold increase in oleic acid were observed. A two fold increase in carotenoid content was noticed at 85 mM salinity (75% of total carotenoid) as the major carotenoid followed by β-carotene. The increase in biomass yields and changes in other constituents indicated the influence of salinity and the organism's adaptability to the tested levels of salinity (17 mM to 85 mM).

Physico-chemical properties of *Madhuka longifolia*, *Sterculia foetida* and *Hibiscus cannabinus* seed oils were investigated by Gaikwad and Swamy (2008). *M. longifolia* has significantly high oil content. The temperature dependence of density and absolute viscosity in these oils has been determined over the temperature range from 303 K to 343 K. The values of density and absolute viscosity in these oils were comparable with that reported for other similar kind of

oils. Their experiments also reveal that both density and viscosity decreased with increase in temperature.

Hossain and Salleh (2008) used common species *Oedogonium* and *Spirogyra* to compare the amount of biodiesel production. Algal oil and biodiesel production was higher in *Oedogonium* than *Spirogyra* sp. However biomass (after oil extraction) was higher in *Spirogyra* than *Oedogonium* sp. Sediments (glycerine, water and pigments) was higher in *Spirogyra* than *Oedogonium* sp. There was no difference of pH between *Spirogyra* and *Oedogonium* species and their results indicated that biodiesel can be produced from both species while as *Oedogonium* is better source than *Spirogyra* species.

Liu *et al.* (2008) investigated the effect of iron on growth and lipid accumulation in marine microalgae *Chlorella vulgaris*. In experiment I, supplementing the growth media with chelated $FeCl_3$ in the late growth phase increased the final cell density but did not induce lipid accumulation in cells. In experiment II, cells in the late-exponential growth phase were collected by centrifugation and re-inoculated into new media supplemented with five levels of $Fe^{3+}$ concentration. Total lipid content in cultures supplemented with $1.2 \times 10^{-5}$ mol $L^{-1}$ $FeCl_3$ was up to 56.6 per cent biomass by dry weight and was 3-7 fold that in other media supplemented with lower iron concentration. Moreover, a simple and rapid method determining the lipid accumulation in *C. vulgaris* with spectrofluorimetry was also developed.

Rodolfi *et al.* (2008) screened thirty microalgal strains in the laboratory for their biomass productivity and lipid content. Four strains (two marine and two freshwater) were selected because of robust, highly productive and with relatively high lipid content. Microalgal strains were cultivated under nitrogen deprivation in 0.6 L bubbled tubes. Only the two marine microalgae accumulated lipid under such conditions. One of them, *Nannochloropsis* sp. which attained 60 per cent lipid content after nitrogen starvation, was grown in a 20 L Flat Alveolar Panel photobioreactor to study the influence of irradiance and nutrient (nitrogen or

phosphorus) deprivation on fatty acid accumulation. They also evaluated its lipid production potential under natural sunlight and the strain was grown outdoors in 110 L green wall panel photo bioreactors under nutrient sufficient and deficient conditions. Lipid productivity increased from 117 mg $L^{-1}$ $day^{-1}$ in nutrient sufficient media (with an average biomass productivity of 0.36 g $L^{-1}$ $day^{-1}$ and 32 per cent lipid content to 204 mg $L^{-1}$ $day^{-1}$ with an average biomass productivity of 0.30 g $L^{-1}$ $day^{-1}$ and more than 60 per cent final lipid content in nitrogen deprived media. In a two-phase cultivation process (a nutrient sufficient phase to produce the inoculum followed by a nitrogen deprived phase to boost lipid synthesis) the oil production potential could be projected to be more than 90 kg per hectare per day. This is the first report of an increase of both lipid content and lipid productivity attained through nutrient deprivation in an outdoor algal culture. The experiments showed that this marine eustigmatophyte has the potential for an annual production of 20 tons of lipid per hectare in the Mediterranean climate and of more than 30 tons of lipid per hectare in sunny tropical areas.

Chiu *et al.* (2009) investigated that the effects of concentration of $CO_2$ aeration on the biomass production and lipid accumulation of *Nannochloropsis oculata* in a semi continuous culture. Their results show that the lipid accumulation from logarithmic phase to stationary phase of *N. oculata* NCTU-3 significantly increased from 30.8 to 50.4 per cent. In their microalgal cultures aerated with 2, 5, 10 and 15 per cent $CO_2$, the maximal biomass and lipid productivity in the semi continuous system were 0.480 and 0.142 g $L^{-1}$ $d^{-1}$ with 2 per cent $CO_2$ aeration respectively. Even the *N. oculata* NCTU-3 cultured in the semi continuous system aerated with 15 per cent $CO_2$, the biomass and lipid productivity could reach to 0.372 and 0.084 g $L^{-1}$ $d^{-1}$ respectively.

Johnson and Wen (2009) found that *Schizochytrium limacinum* is a heterotrophic microalga that is capable of producing high levels of biomass and total fatty acid. The objective of their research work was to explore the potential of producing biodiesel fuel from this alga using different biodiesel preparation

methods, including oil extraction followed by transesterification (a two-stage method) or direction transesterification of algal biomass (a one-stage method). The biodiesel prepared via the direct transesterification of dry biomass was subjected to ASTM standard tests and the results indicate the alga *S. limanicum* is a suitable feedstock for producing biodiesel via the direct transesterification method.

Ahmad *et al.* (2009) also reported the results of production and physico-chemical characterization of peanut oil biodiesel (POB). According to their study optimum conversion of POB from triglycerides was achieved by using 1:6 molar ratio (methanol: oil) at 60$^{o}$C. Fuel properties of POB were determined and compared with American standard testing material. The viscosity at 40$^{o}$C of POB (100 per cent) was 5.908, specific gravity 0.918, density at 40$^{o}$C 0.0992, flash point 192, pour point 3$^{o}$C, cloud point 6$^{o}$C, and sulfur contents 0.0087. Also the engine performance by using POB in terms of consumption, efficiency and power output was quite comparable with petro-diesel.

In an another study carried out by Gouveia and Oliveira (2009) screening of microalgae *Chlorella vulgaris*, *Spirulina maxima*, *Nannochloropsis* sp., *Neochloris oleabundans*, *Scenedesmus obliquus* and *Dunaliella tertiolecta* was done in order to choose the best one(s) in terms of quantity and quality as oil source for biofuel production. They proved that *Neochloris oleabundans* (fresh water microalga) and *Nannochloropsis* sp. (marine microalga) were suitable raw materials for biofuel production, due to their high oil content (29.0 and 28.7% respectively). Both microalgae, when grown under nitrogen shortage, showed a great increase (~50%) in oil quantity. They found that if the purpose is to produce biodiesel only from one species, *Scenedesmus obliquus* presents the most adequate fatty acid profile, namely in terms of linolenic and other polyunsaturated fatty acids. However, the microalgae *Neochloris oleabundans*, *Nannochloropsis* sp. and *Dunaliella tertiolecta* can also be used if associated with other microalgal oils and or vegetable oils.

Demirbas (2009) extracted oil from macroalga *Cladophora fracta* and a microalga *Chlorella protothecoides* samples obtained from Sera Lake in Trabzon Turkey at 100 meter altitude. He found that the oil proportion from the lipid fractions of *Chlorella protothecoides* were considerable higher than that of *Cladophora fracta*. The heating value of *Chlorella protothecoides* (25.1 MJ $kg^{-1}$) was also higher than that of *Cladophora fracta* (21.1 MJ $kg^{-1}$) and polyunsaturated fatty acids of *Chlorella protothecoides* (62.8%) also were higher than those of *Cladophora fracta* (50.9%).

Griffiths and Harrison (2009) reviewed information available in the literature on microalgal growth rates, lipid content and lipid productivities for 55 species of microalgae including 17 Chlorophyta, 11 Bacillariophyta and 5 Cyanobacteria as well as other taxa. Their collated information provides a framework for decision-making and a starting point for further investigation of species selection. The importance of lipid productivity as a selection parameter over lipid content and growth rate individually were demonstrated in their review.

Huang *et al.* (2009) investigated lyophilized alga powders as the starting material and optimized Nile red method for quantitative measurement of neutral lipids in *Chlorella* with a high correlation coefficient ($R^2$=0.99) between gravimetric and spectrofluorimetric quantification. Using this method, *Chlorella vulgaris* were screened out of several *Chlorella* strains with the highest lipid content and orthogonal design experiments were performed to search for the significant factors affecting lipid accumulation. Their data implied that the sensitivity and versatility enable this method a useful tool in optimizing culture and accumulation condition of lipid production in alga and figured out several factors significantly influenced lipid production in *C. vulgaris.*

Lamers (2009) explored the viability of growing algae in photo-bioreactors using wash water from the biodiesel plant supplemented with corn powder hydrolysate and COMBO media as a growth medium. Growing green algae *Chlorella protothecoides* under heterotrophic growth conditions, a lipid

content of 55.2 per cent dry weight can be achieved. These lipids are viable for conversion into biodiesel through transesterification and these algae-produced lipids could add 39,704 L of biodiesel to the annual Ridge town production. Meanwhile by reusing the water this has helped remediate the wash water from the biodiesel plant.

Liang *et al.* (2009) investigated the biomass and lipid productivities of *Chlorella vulgaris* under different growth conditions. While autotrophic growth did provide higher cellular lipid content (38%), the lipid productivity was much lower compared with those from heterotrophic growth with acetate, glucose, or glycerol. Optimal cell growth (2 g $L^{-1}$) and lipid productivity (54 mg $L^{-1}$ $day^{-1}$) were attained using glucose at 1 per cent (w/v) whereas higher concentrations were inhibitory. The growth of *C. vulgaris* on glycerol had a similar dose effects as those from glucose.

Mohan *et al.* (2009) cultivated a green micro alga, *Chlorella vulgaris,* isolated from industrial effluents, using a suitable growth medium in a large-scale high rate algal pond. The bio-molecules such as total protein, total carbohydrate and total lipid, and the pigments chlorophyll, β-carotene, were analyzed at regular intervals during cultivation. The algal biomass was harvested by low-cost methods such as settling using flocculants and auto-flocculation.

Widjaja (2009) studied that the fresh water microalgae *Chlorella vulgaris* was one of the proof as it contained high triacyl glyceride which made it a potential candidate for biodiesel production. The factors responsible for good growing of microalgae such as $CO_2$ and nitrogen concentration were investigated and was found that total lipid content was increased after exposing to media with not enough nitrogen concentration. However, under this nitrogen depletion media, the growth rate was very slow leading to lower lipid productivity. The productivity could be increased by increasing $CO_2$ concentration. The lipid content was found to be affected by drying temperature during lipid extraction of

algal biomass. Drying at very low temperature under vacuum gave the best result but drying at 60 $^{o}$C slightly decreased the total lipid content.

Amini *et al.* (2010) provided information on the effect of three light intensities (37.5, 62.5 and 100 mol photons $m^{-2}$ $s^{-1}$) and photoperiods (light: dark) cycle 8:16, 12:12 and 16:8 h on growth rate, duplication time and biomass production in microalga *Chlorella vulgaris*. Stock of *C. vulgaris* was separated from water samples purified and cultured in 1000 ml Erlenmeyer flasks at constant temperature of 25±0.5°C, using Zehnder medium. Cell count was conducted daily and biomass was measured at the exponential growth phase in different treatments. Analysis of variance indicated significant difference ($P<0.05$) among light regimes. The maximum growth rate 1.13 $d^{-1}$ was observed at 100 µmol photons $m^{-2}$ $s^{-1}$ and 16:8 h light duration and also the minimum duplication time 0.6 L $d^{-1}$ occurred at this treatment. The maximum biomass 2.05 g $L^{-1}$ was recorded at 62.5 µ mol photons $m^{-2}$ $s^{-1}$ and 8:16 h light period.

Allwayzy *et al.* (2010) extracted lipid from a fresh water microalgae *Chlorella vulgaris* using iron as a stress treatment to achieve high lipid content. Secondly, the physical and chemical properties of *Chlorella vulgaris* and *Chlorella protothecoides* oil were compared with diesel and biodiesel from other sources.

Lopez *et al.* (2010) measured the protein content of dry biomass of the microalgae *Porphyridium cruentum, Scenedesmus almeriensis, Muriellopsis sp*. and of the cyanobacteria *Synechocystis aquatilis, Arthrospira platensis* by the Lowry method following disruption of the cells by milling with inert ceramic particles. They found that protein content in dry biomass ranged from 30 to 55 per cent.

Mata *et al.* (2010) reviewed the current status of microalgae use for biodiesel production, including their cultivation, harvesting, and processing. The microalgae species most used for biodiesel production were presented and their

main advantages were described in comparison with other available biodiesel feed stocks.

Stanley *et al.* (2010) extracted microalgal oil from *Chaetoceros* sp. and determined the physico-chemical properties. The density, viscosity, acid value, saponification value and free fatty acids were recorded as 1.305 gm $ml^{-1}$, 6.2 $mm^2$ $s^{-1}$, 2.5339 mg $gm^{-1}$ of oil, 173.56 mg $gm^{-1}$ of oil and 0.71 gm 100 $gm^{-1}$ of algae (oleic acid). The fatty acid profile showed pentadecanoic acid (17.56%), nonadecenoic acid (20.1%), methyl palmitate (2.91%), methyl linoleate (12.07%) and palmitic acid (1.97%) as major fatty acids.

Morowvat *et al.* (2010) investigated the production of biodiesel from a naturally isolated strain of *Chlamydomonas* sp. They isolated the microalgal strain from the paddy-field soil samples during a screening program. The identification was done using physiological and molecular approaches. After reaching the stationary phase of growth, the total content of the lipids was extracted. The extracted fatty acids were primarily esterified and then identified through GC/MS analysis. Several types of fatty acid methyl esters (FAMEs) were identified in the isolated microalga and the presence of at least nine FAMEs in *Chlamydomonas* sp. MCCS 026 was shown. The total fatty acid content of the isolated strain was 25 per cent. The composition of fatty acids in the studied species of microalga was mainly docosanoic acid methyl ester, tetradecanoic acid methyl ester, hexadecanoic acid methyl ester and nonanoic acid methyl ester.

The microalgal strain of *Chlorella* species was isolated from the paddy field soil samples during a screening program by Amini *et al.* (2011). After 17 days, at the end of exponential phase of growth, the total content of the lipids was extracted. The extracted fatty acids were first esterified and then identified using GC/MS analysis. The composition of fatty acids in the studied species of microalga was mainly palmitic acid methyl ester, myristic acid methyl ester, stearic acid methyl ester and undecanoic acid methyl ester. They found that this

strain because of its highly saturated fatty acids content can be an ideal candidate for biodiesel production.

Liu *et al.* (2011) isolated 43 green algal strains from Chinese freshwaters, and then incubated in the laboratory bioreactors for the growth and oil accumulation investigations. During a 15 day incubation experiment, the accumulations of their biomass and total lipids, together with the lipid productivities for these green algal strains were systematically investigated and compared. Their results indicated that the accumulations of biomass for the 43 algal strains ranged from 0.53 g $L^{-1}$ to 6.07 g $L^{-1}$ during the experiments, with the highest biomass of 6.07 g $L^{-1}$ for green algae *Scenedesmus bijuga*. The lipid content for the tested algal strains varied from 20 per cent to 51 per cent of the dry biomass at the end of cultivation experiments. Green algae *Chlorella pyrenoidosa* was one of the best oil producers based on their investigations, with the total lipid content of 51 per cent of dry biomass. Taking the growth rates and the accumulations of intracellular lipids into the consideration, 10 strains were considered to have significant potential for biofuel applications during their experiment.

Goswami and Kalita (2011) selected the two fresh water microalgal strains, *Scenedesmus dimorphus* and *Scenedesmus quadricauda*. Both the algal strains were cultivated in different concentrations of urea as nitrogen source in the growth medium. The concentration of urea at which maximum growth rate in terms of biomass and lipid productivity obtained was at 0.1g $L^{-1}$ urea in case of both the strains. The maximum increase in biomass per day and lipid content for *Scenedesmus dimorphus* was found to be 1.523 mg $L^{-1}$ $day^{-1}$ and 34 per cent in terms of dry cell weight with a specific growth rate of 0.54 $day^{-1}$ of and for *Scenedesmus quadricauda* it was 1.266 mg $L^{-1}$ $day^{-1}$ and 31 per cent in terms of dry cell weight with a specific growth rate 0.392 $day^{-1}$.

Doan *et al.* (2011) isolated ninety-six strains of marine microalgae with an elevated biomass productivity and intracellular lipid content from the coastal

waters of Singapore using an automated flow cytometric cell-sorting technique. Cell sorting was based on the two-dimensional distribution of algal cells for red fluorescence (representing chlorophyll auto-fluorescence) against forward-light scatter (representing cell size) and red vs. green fluorescence. They further characterized twenty one strains with respect to cell growth rate, biomass concentration, lipid content (total and neutral lipid) and fatty acid profile. The growth rates of *Skeletonema costatum*, *Chaetoceros* and *Thalassiosira species* were greatest among the entire strains, but in terms of absolute lipid yield *Nannochloropsis* strains predominated. *Nannochloropsis* strains had a lipid content ranging from 39.4 to 44.9 per cent of dry weight biomass. Transesterification of the lipids yielded 25 to 51 per cent of fatty acid methyl ester (FAME) i.e. biodiesel, where as total FAME content ranged between 11 and 21 per cent of dry weight biomass. Their study demonstrated that *Nannochloropsis* is a promising species for biodiesel feedstock.

Makareviciene *et al.* (2011) investigated the growth of two robust algae strains *Chlorella* sp. and *Scenedesmus* sp. growing in Lithuanian lakes with the aim to obtain optimum conditions for biomass cultivation for biofuel production in the Lithuanian environment. Samples were taken from different nitrogen sources and of different concentrations, with addition of various concentrations of $CO_2$ and in the presence of salt. The best biomass productivity was achieved using urea as a nitrogen source or modified growing medium BG-11 with decreased concentration of $NaNO_3$. The positive impact on the growth of biomass was achieved by aeration with $CO_2$ (especially with concentration of 24%). A content of oil in *Chlorella* sp. and *Scenedesmus* sp. has suggested their potential use as biodiesel feedstock.

Shaaban *et al.* (2011) studied the total chlorophyll concentrations of the phytoplanktonic samples of Rosetta branch of the river Nile during two successive years from (August 2006 to April 2008). At all stations the total chlorophyll contents (mg $L^{-1}$) of the identified algae and the maximum quantitative algal

individuals were reached their maximum peaks during summer 2007. Peak periods of total chlorophyll coincided with peak periods of the stations recorded high algal biomass. Positive relation was observed between the fluctuations of total chlorophyll contents of the phytoplankton and those of total number of individuals at all investigated stations of Rosetta branch.

Kalita *et al.* (2011) carried a study to find out the influence of different NaCl concentration (0.04M- 0.34M) in the growth of the freshwater microalga *Ankistrodesmus falcatus* and its biochemical constituents viz. lipid, protein, carbohydrate and secondary pigment viz., chlorophyll. There was considerable variation in growth as well as biochemical constituents of the microalga with varying concentration of NaCl. Highest increase in lipid content was found to be in 0.17 M NaCl, however protein and carbohydrate content was enhanced in 0.34 M NaCl, but there was a decrease in chlorophyll content with increasing concentration of NaCl. The changes in growth and biochemical constituents indicated the influence of salinity and organism's adaptability to the tested levels of salinity.

Huerlimann *et al.* (2010) compared lipid content and composition, and lipid and biomass productivity during logarithmic, late logarithmic and stationary phase of *Nannochloropsis* sp., *Isochrysis* sp., *Tetraselmis* sp., and *Rhodomonas* sp. grown in L1, f/2, and K-medium. Of the tested species, *Tetraselmis* sp. exhibited a lipid productivity of 3.9–4.8 $g^{-2}$ $d^{-1}$ in any media type, with comparable lipid productivity by *Nannochloropsis* sp. and *Isochrysis* sp. when grown in L1-medium. The dry biomass productivity of *Tetraselmis* sp. (33.1-45.0 $g^{-2}$ $d^{-1}$) exceeded that of the other species by a factor 2-10. Of the organisms studied, *Tetraselmis* sp. had the best dry biomass and lipid production profile in large-scale cultures. Their study provides a practical benchmark, which allows comparison of microalgal production systems with different footprints, as well as terrestrial systems.

Leesing *et al.* (2011) worked to produce heterotrophic microalgal lipid in flask batch fermentation. *Chlorella* sp. supported maximum values of 0.374 $g^{-1} L^{-1} d^{-1}$, 0.478 g lipid $g^{-1}$ cells and 0.112 $g^{-1} L^{-1} d^{-1}$ for volumetric lipid production rate and specific yield of lipid and specific rate of lipid production respectively when culture was performed on BG-11 medium supplemented with 50 g $L^{-1}$ glucose. Among the carbon sources tested, maximum cell yield coefficient, maximum specific yield of lipid and volumetric lipid production rate were found of 0.728, 0.237 and 0.619 respectively using sugarcane molasses as carbon source. The main components of fatty acid from extracted lipid were palmitic acid, stearic acid, oleic acid and linoleic acid which were similar to vegetable oils and suitable for biodiesel production.

Chen *et al.* (2011) used the marine microalgae *Dunaliella tertiolecta* as a model organism and a profile of its nutritional requirements was also determined. Inorganic phosphate $PO_4^{3-}$ and trace elements: cobalt ($Co^{2+}$), iron ($Fe^{3+}$), molybdenum ($Mo^{2+}$) and manganese ($Mn^{2+}$) were identified as required for optimum growth of algae. The inorganic nitrogen in the form of nitrate $NO^{3-}$ instead of ammonium ($NH_4^+$) was required for maximal biomass production. The lipids were accumulated under nitrogen starvation growth conditions was found to be time-dependent.

Deng *et al.* (2011) found that under nutrient starvation conditions, many microalgae are known to accumulate triacylglycerols (TAG) that can be used for biodiesel production. However, few studies have been performed to analyze the effect of deficiency in nutrient elements such as sulphur, phosphorus, potassium, iron, magnesium and calcium on oil production particularly in *Chlamydomonas* and *Chlorella*. In this study, they investigated lipid content of *Chlamydomonas reinhaditti* and *Chlorella vulgaris* grown in TAP, HSM, BG-11 and SE lacking optimal concentrations of these elements. Their results show that, in high carbon HSM and TAP media, N and S starvation led to significant increase in cellular lipid content in both microalgae species. In addition, *C. reinhaditti* grown in TAP

media without P, Fe, K, Ca or Mg or in HSM media without K, Ca or Mg also accumulated detectably higher neutral lipids. In contrast, in *C. vulgaris,* such accumulation was observed only in Mg-free and Fe-free HSM media. In low carbon SE and BG-11 media, N starvation resulted in a moderate increase in the lipids content both in *C. reinhaditti* and *C. vulgaris*. On the other hand, P, S, K, Ca or Mg deficiency promoted neutral lipids accumulation in *C. vulgaris*. Finally, they analyzed and discussed the relationships among cell growth rate, lipid accumulation and nitrogen concentrations in *C. reinhaditti*.

Griffiths *et al.* (2011) found that the optical density can be used as a convenient indirect measurement of biomass concentration in microbial cell suspensions. Absorbance of light by a suspension can be related directly to cell density using a suitable standard curve. However, inaccuracies can be introduced when the pigment content of the cells changes. Under the culture conditions used, pigment content of the microalga *Chlorella vulgaris* varied between 0.5 and 5.5 per cent of dry weight with age and culture conditions. This led to significant errors in biomass quantification over the course of a growth cycle, due to the change in absorbance. Using a standard curve generated at a single time point in the growth cycle to calculate dry weight (dw) from optical density led to average relative errors across the growth cycle, relative to actual dw, of between 9 and 18 per cent at 680 nm and 5 and 13 per cent at 750 nm. When a standard curve generated under low pigment conditions was used to estimate biomass under normal pigment conditions, average relative errors in biomass estimation relative to actual dw across the growth cycle were 52 per cent at 680 nm and 25 per cent at 750 nm. Similar results were found with *Scenedesmus, Spirulina* and *Nannochloropsis*. They suggested strategies to minimise error include selection of a wavelength that minimises absorbance by the pigment.

Kumar *et al.* (2011) collected a total of six naturally occurring algal biomass bulk samples from different localities of north India. The algae identified were, one blue-green alga *Tolypothryx* and rest five were green algae *Pithophora,*

*Spirogyra, Hydrodictyon, Rhizoclonium* and *Cladophora.* Oil was extracted from the dried algal samples and fatty acid analysis was done. Physicochemical properties of algal oils such as density, viscosity, lipid content, pH and non-saponifiable fats were estimated. Gas chromatographic analysis revealed higher percentage of methyl palmitate, methyl stearate and methyloleate and methyl linoleate. The physico-chemical properties of algal oil meet all the properties given by American society for testing and materials (ASTM) D6751, ISO 15607 and EN14214-Europe and was concluded that the algal oil can be used as a potential biofuel.

Mercer and Armenta (2011) presented an overview, based on the last 10 years of advances made in technologies for extracting and purifying microalgae oil. They compared solvent extraction technologies with extraction alternatives such as mechanical milling and pressing, enzymatic and supercritical fluid extraction. They also reviewed recent advances based on molecular engineering of microbes to aid oil extraction. Downstream processing for the potential commercial production of microalgae oil not only must consider economic costs, but should also consider minimizing environmental impacts in order to attain sustainable production processes.

The biomass and nutrient uptake from *Neochloris oleoabundans* production in an open trough system was investigated by Murray *et al.* (2011). The growth medium used was BG-11, temperature ranged from 16.7 °C to 25.3 °C and pH ranged from 5.52 to 9.94 because the customary pH increase during algal biomass production was moderated by incoming $CO_2$ gas streams (atmospheric, 2, 4 and 6% $CO_2$). Peak concentrations of algal biomass ranged from 643 to 970 mg $L^{-1}$, specific growth rates ranged from 0.15 to 0.37 $d^{-1}$ and doubling times ranged from 4.8 to 1.9 days. Carbon, nitrogen and phosphorus were incorporated into the biomass at 0.05, 8.3 and 54 per cent of supplied amounts. The open growth systems supplemented with $CO_2$ should be designed to regulate medium pH within the range of 6.3 to 7.1.

Nigam *et al.* (2011) dealt with one of the method to enhance the lipid content in microalgae. *Chlorella pyrenoidosa* was grown autotrophically in batch culture and the effect of different concentrations of nitrogen source (0-0.4 g $L^{-1}$ $KNO_3$) on growth and lipid content was studied. As the nitrate concentration in the medium decreased, biomass production also decreased but the lipid content increased. Moreover, at the same concentration of nitrate source, lipid tends to accumulate more in stationary phase in comparison to exponential phase. Highest lipid accumulation of 26 per cent was recorded in the culture with 0.05 g $L^{-1}$ $KNO_3$, which is one fourth of basal nitrogen source concentration. Their study suggested that nitrogen starvation is the effective approach to enhance lipid for biofuel production.

Moazami *et al.* (2011) screened 147 microalgal strains from the Persian Gulf and the Qeshm Island (Iran) in order to choose the best ones, in terms of growth (biomass) rate and lipid content for biodiesel production. A methodology, combining experiments in lab scale and pilot plant (open pond) were used to produce and evaluate biomass and lipid productivity. The culture conditions, including photo flux (180 µE $m^{-2}$ $s^{-1}$), photoperiod (12 h light/dark), temperature (25 $^{o}$C), pH (~8), air (carbon dioxide) and growth medium were kept constant for all experiments. Microalgae were screened in two stages using optical density (for evaluation of biomass concentration) and Nile red and gas chromatography (for determination of lipid content and fatty acid fractions). In general maximum specific growth rate and the maximum biomass productivity were obtained after 8-12 day culture. *Nannochloropsis* sp. and *Neochloris* sp. were selected from the marine microalgal culture collection due to their high biomass (50 and 21.7 g $L^{-1}$ respectively) and oil content (52 and 46% respectively). If the purpose is to produce biodiesel only from one species, *Nannochloropsis* sp. presented the most adequate fatty acid profile, namely linolenic and other polyunsaturated fatty acids. However, the microalgae *Chlorella* sp. can also be used if associated with other microalgal oils.

The 45 algal cultures were isolated from the freshwater Lake at Wonju, South Korea by Abou-Shanab *et al.* (2011). Five microalgal isolates were selected based on their morphology and ease of cultivation under their test conditions. These cultures were identified as strains of *Scenedesmus obliquus* YSL02, *Chlamydomonas pitschmannii* YSL03, *Chlorella vulgaris* YSL04, *S. obliquus* YSL05, and *Chlamydomonas mexicana* YSL07 based on microscopic examination and LSU rDNA (D1-D2) sequence analysis. *S. obliquus* YSL02 reached a higher biomass concentration (1.84 ± 0.30 g $L^{-1}$) with a lower lipid content (29% w/w) than did *C. pitschmannii* YSL03 (maximum biomass concentration of 1.04 ± 0.09 g $L^{-1}$ with a 51% lipid content). The results suggest that *C. pitschmannii* YSL03 is appropriate for producing biodiesel based on its high lipid content and oleic acid proportion.

Ramachandra *et al.* (2011) conducted a study using a microalgal consortium for a period of 15 days to evaluate the feasibility of algal biomass from laboratory as well as outdoor culture conditions. Native algal strains were isolated from a tropical freshwater Lake. Preliminary growth studies indicated the relationship between the nitrates and phosphates to the community structure through the days. The lipid profile was also performed using Gas chromatography-Mass spectrometry revealed the profile of the algal community. However, they suggested that further studies on the application of the mixed population are required to make this consortium approach economically viable for producing algae biofuels.

Kong *et al.* (2011) investigated that mixotrophism might be a competitive pattern for the culture of *C. vulgaris* on a large scale based on the achieved maximum biomass and volumetric productivities of lipid and chlorophyll. Glucose was the optimal carbon source for mixotrophic cultivation of *C. vulgaris* and the effects of glucose content on the algal growth under mixotrophic conditions were considerable because lower glucose content (1 g $L^{-1}$) promoted the production of biomass and photosynthetic pigments; higher glucose contents

(≥5 g $L^{-1}$) increased the biomass and lipid accumulation but inhibited the chlorophyll biosynthesis. The microalga could not grow well without pH control when ammonium and organic nitrogen were the sole nitrogen sources in the mixotrophic cultures because of the remarkable drop in pH value, while the critical urea concentration was observed at 0.50 g $L^{-1}$. It was concluded that mixotrophic cultivation of *C. vulgaris* is a feasible approach for lipid accumulation and chlorophyll biosynthesis that are dependent on the enhancement of biomass content and volumetric productivity.

Xin *et al.* (2011) studied effect of cultivation temperature on the growth and lipid accumulation properties of a freshwater microalga *Scenedesmus* sp. LX1. *Scenedesmus* sp. LX1 could grow in a wide range of temperature (10~30 $^{o}$C) and the growth activation energy was 49.3 kJ $mol^{-1}$. The optimal temperature to produce microalgal biomass and lipid was 20 $^{o}$C and after 15 days of batch cultivation the productivities of 313.3 g biomass (g $P^{-1}$), 112 g lipid (g $P^{-1}$) and 14.7 g TAGs (g $P^{-1}$) were obtained. The content of polyunsaturated fatty acids decreased with the increase of cultivation temperature. For the first time the cultivation temperature, specific growth rate and lipid content per microalgal biomass were correlated together.

Wagenen *et al.* (2012) subjected *Nannochloropsis salina* to ranges of light intensity (5-850 µmol $m^{-2}$ $s^{-1}$) and temperature (13-40 °C) and its exponential growth rate, total fatty acids (TFA) and fatty acid composition were measured. The maximum acclimated growth rate was 1.3 $day^{-1}$ at 23 °C and 250 µmol $m^{-2}s^{-1}$. Fatty acids were detected by gas chromatography with flame ionization detection after transesterification to corresponding fatty acid methyl esters (FAMEs). A sharp increase in TFA containing elevated palmitic acid (C16:0) and palmitoleic acid (C16:1) during exponential growth at high light was observed, indicating likely triacylglycerol accumulation due to photo-oxidative stress. Lower light resulted in increases in the relative abundance of unsaturated fatty acids in thin cultures and increases were observed in palmitoleic and eicosapentaenoic acids

(C20:5ω3). As cultures aged and the effective light intensity per cell converged to very low levels, fatty acid profiles became more similar and there was a notable increase of oleic acid (C18:1ω9). The amount of unsaturated fatty acids was inversely proportional to temperature demonstrating physiological adaptations to increase membrane fluidity.

Yadavalli *et al.* (2012) studied the lipid productivity of *Chlorella pyrenoidosa* in a customized laboratory scale photobioreactor. Lipid yield increased when *C. pyrenoidosa* was subjected to stress conditions like different nitrogen sources, light intensities and modes of cultivation. They observed that the growth rate of *C. pyrenoidosa* was directly proportional to light intensity and nitrogen concentrations. Of the two nitrogen sources tested, sodium nitrate proved better than urea in terms of lipid yield. The study also demonstrated that at lower nitrogen concentrations fed batch mode of cultivation resulted in maximum lipid productivity of 0.103 g $d^{-1}$ at 135 µmol $m^{-2}s^{-1}$ when compared to batch mode.

Sankar and Ramasubramanian (2012) studied the effective nutrient medium for the growth of *Chlorella vulgaris*. Their research investigated that six different types of growth media viz., CFTRI media, OFERR media, Revised media- 6, Bangladesh medium No. 3, Zarrouk's media and Bold's Basal media used for culturing of *C. vulgaris*. They carried experiment for a period of 20 days. The illumination of light was maintained at 2000 lux and temperature at 28°C ± 2 °C. At the end of the experiment period protein, chlorophyll-a, chlorophyll-b, total chlorophyll content of the algae was examined and it was observed that the algae grown in Bold's Basal media have grown well with protein 36.16±0.03 mg $L^{-1}$, chlorophyll-a 2.05±0.00 mg $L^{-1}$, chlorophyll-b 0.56±0.00 mg $L^{-1}$, total Chlorophyll 2.66±0.00 mg $L^{-1}$. Bangladesh medium No. 3, showed similar results when compared with Bold's Basal medium and it can be useful for the effective culturing of *C. vulgaris.*

Sathya *et al.* (2012) isolated *Chlorella pyrenoidosa* from the water bodies of Madurai, Tamil Nadu and maintained in CHU-10 medium with a photoperiod

of 12 hours light/12 hours dark, light intensity of 2000 lux at a temperature of 25$^{o}$C. Growth rate, pH, dry weight and pigments such as chlorophyll-a, carotenoids were monitored at an interval of 10 days until the 50$^{th}$ day of growth. The amount of chlorophyll-a and carotenoids was maximum on 40$^{th}$ day of growth. Nile red fluorescence indicated more number of lipid bodies on 40$^{th}$ day. At 460-480 nm excitation light, the Nile red fluorescence of lipid vesicles exhibited an emission maximum of 465 nm and a quantum yield of 0.65. Lipid content (48.8 per cent dry weight) was maximum on 40th day of growth. Fatty acid analysis by GC indicated more amount of saturated fatty acids in *C. pyrenoidosa*. Hydrocarbon analysis by GC-MS was done using the hexane extract of the algal strain. Linear regression analysis showed a significant relationship between OD and dry weight, OD and pigments, dry weight and lipid weight.

Seenivasan *et al.* (2012) analysed the biochemical composition (protein, sugar, lipid), photosynthetic pigments like chlorophyll, carotenoid and mineral composition of three species of seaweeds *Codium adhaerens* Anderson (green algae) *Sargassum wightii* Greville (brown algae), *Acanthophora spicifera* (Vahl.) Boergs (red algae) from intertidal region of the Mandapam coastal water. The results indicated that the maximum protein content (6.396±0.97%) was recorded in the brown alga *S. wightii*. The maximum lipid content (1.213±0.02%) was recorded in green alga *C. adharens*. The maximum chlorophyll-a (0.347±0.051), total chlorophyll (0.438±0.061) and carotenoid (0.670±0.225) were recorded in the brown seaweed *S. wightii* whereas chlorophyll-b (0.107±0.016) was highest in *C. adharens*.

Griffiths *et al.* (2012) selected eleven species of microalgae on the basis of available literature data, were tested for lipid productivity, gravity sedimentation and the suitability of their fatty acid profiles for biodiesel production. The response to nitrogen limitation was species specific. Lipid yields and productivity were higher at 150 mg $L^{-1}$ nitrate than at 1,500 mg $L^{-1}$ for all species tested except *Spirulina platensis.* Particularly *Chlorella vulgaris* and *Scenedesmus* had the

highest growth rates and showed the greatest increase in lipid content in response to nitrogen limitation. *Cylindrotheca fusiformis*, *S. platensis, Scenedesmus* and *Tetraselmis suecica* had the fastest settling rates and highest biomass recoveries after 24 h of gravity sedimentation. For most species the fuel would need to be blended or culture conditions to be optimised to achieve the correct lipid profile in order for microalgal fuel to meet the European standards for biodiesel production (EN 14214). The most promising species overall were the freshwater algae *Scenedesmus* and *C. vulgaris* and the marine algae *C. fusiformis* and *Nannochloropsis.*

Jena *et al.* (2012) screened three brackish water microalgal strains (*Chlorococcum* sp., *Chlorella* sp. and *Scenedesmus* sp.) of Odisha coast for the suitability of biodiesel production. Among all, *Scenedesmus* sp. seems to be the best one for high lipid productivity (24.66 mg $L^{-1}day^{-1}$) with high biomass yield of 0.9 g $L^{-1}$ at stationary phase. The *Scenedesmus* sp. also possesses the most adequate fatty acid profile. Their study suggested that *Scenedesmus* sp. is appropriate for bio-diesel production due to its high lipid content and this strain was selected for higher scale studies.

Ilavarasi *et al.* (2012) demonstrated the total lipid content, fatty acid profile and biodiesel production from a naturally isolated fresh water strain of *Chlorella* sp. and the total lipid content was found to be 8 per cent under normal nutrient conditions. Gas Chromatography of FAME was analyzed and the major fatty acids observed were palmitic acid, stearic acid, oleic acid, linoleic acid and alpha linolenic acid. The algal oil was transesterfied and pH of the biodiesel was found to be 8.1.

Kawachi *et al.* (2012) investigated the relationship between hydrocarbons and the molecular phylogeny of *Botryococcus braunii* by using 31 axenic strains isolated in Japan. By gas chromatography/mass spectrometric analysis, nine hydrocarbon species were detected and categorized as 4 types: the three known races A, B, and L and the tentatively named race S comprising epoxy-n-alkane

and saturated n-alkane chains with carbon numbers 18 and 20, respectively. The phylogenetic relationship of *Botryococcus* strains also appeared to be in considerable agreement with unique hydrocarbon synthesis pathways.

Ananadhi and Stanley (2012) identified *Chaetoceros* sp. for the research work and studied its lipid, carbohydrate and protein content. The main aim of their experiment was to make use of the algae present in the water bodies and to extract the useful algal oil meant for biodiesel production to meet the challenges of fuel requirement in the present scenario. Microalgal oil was extracted from *Chaetoceros* sp. and the physicochemical properties were determined. The density, viscosity, acid value, saponification value and free fatty acids were recorded as 1.305 g $ml^{-1}$, 6.2 $mm^2$ $s^{-1}$, 2.5339 mg $g^{-1}$ of oil, 173.56 mg $g^{-1}$ of oil, and 0.71gm 100 $g^{-1}$ of algae (oleic acid). The fatty acid profile showed pentadecanoic acid (17.56%), nonadecenoic acid (20.1%), methyl palmitate (2.91%), methyl linoleate (12.07%) and palmitic acid (1.97%) as major fatty acids.

Muthukumar *et al.* (2012) obtained high quality biodiesel from microalgae *Chlorella marina* and *Nannochloropsis salina* through transesterification. Growth studies revealed that maximum cell growth rate was obtained at $15^{th}$ day of the culture. The flocculation activity result showed that pH 11 was optimum for cell flocculation at 37°C. In their study, 60.26 per cent of biodiesel yielded from 0.752 g $L^{-1}$ contains 30 per cent oil content from *N. salina*, whereas 50 per cent yielded from 0.527 g $L^{-1}$ contains 20 per cent oil content. The crude lipid content found in *C. marina* and *N. salina* was found to be 20.33±1.82 per cent and 32.13±1.40 per cent of dry biomass. The density and viscosity of the biodiesel obtained from the crude lipid of *N. salina* and *C. marina* were 0.992 and 0.971 (kg $L^{-1}$), viscosity 3.2 and 4.8 (Pa s) at 40°C respectively. The method they implemented in their study could be novel approach and could have great potential in the industrial production of liquid fuel from microalgae.

Ahmad *et al.* (2012) collected mixed algae culture *Microspora* sp., Diatoms, *Lyngbya* sp., *Cladophora* sp., *Spirogyra* sp. and *Rhizoclonium* sp. from Botanical Garden of Government College University Lahore and were grown in artificial ponds of 13.5 L capacity. Algal growth was monitored for six days by measuring its fresh and dry weight which showed almost similar results of 3.34 g $day^{-1}$ and 3 g $day^{-1}$ respectively. The yield of biodiesel produced by transesterification of dried algal biomass was calculated to be 15.13 per cent on an average. Quality of biodiesel was analyzed for kinematic viscosity (4.5 $mm^2$ $s^{-1}$), flash point (167°C), specific gravity (0.895 g $ml^{-1}$), iodine value (80 mg $g^{-1}$), acid number (0.65 mg KOH $g^{-1}$) and water contents (32 mg $kg^{-1}$). They found that the quality of biodiesel was according to the ASTM standards for biodiesel.

Lone *et al.* (2013) found that *S. platensis* exhibited significant higher growth in standard CFTRI medium containing 90 ppm phosphorus as nanoparticles of tricalcium phosphate and hydroxy apatite. On the other hand calcium phosphate nanoparticles caused significant reduction in nitrate reductase activity as well as in protein content of the alga. Marked change in chlorophyll-a/b ratio was also noted when phosphorus was supplied through nano tricalcium phosphate and nano hydroxy apatite particles as compared to ionic form ($K_2HPO_4$). Their study also revealed that the growth of *Spirulina* in the presence of ZnO nanoparticles was retarded, while no growth was observed with CuO nanoparticles.

Chapter – 3

# MATERIALS AND METHODS

A comprehensive research programme was carried out to characterize the oleaferous algae of Dal Lake as per the following technical details:

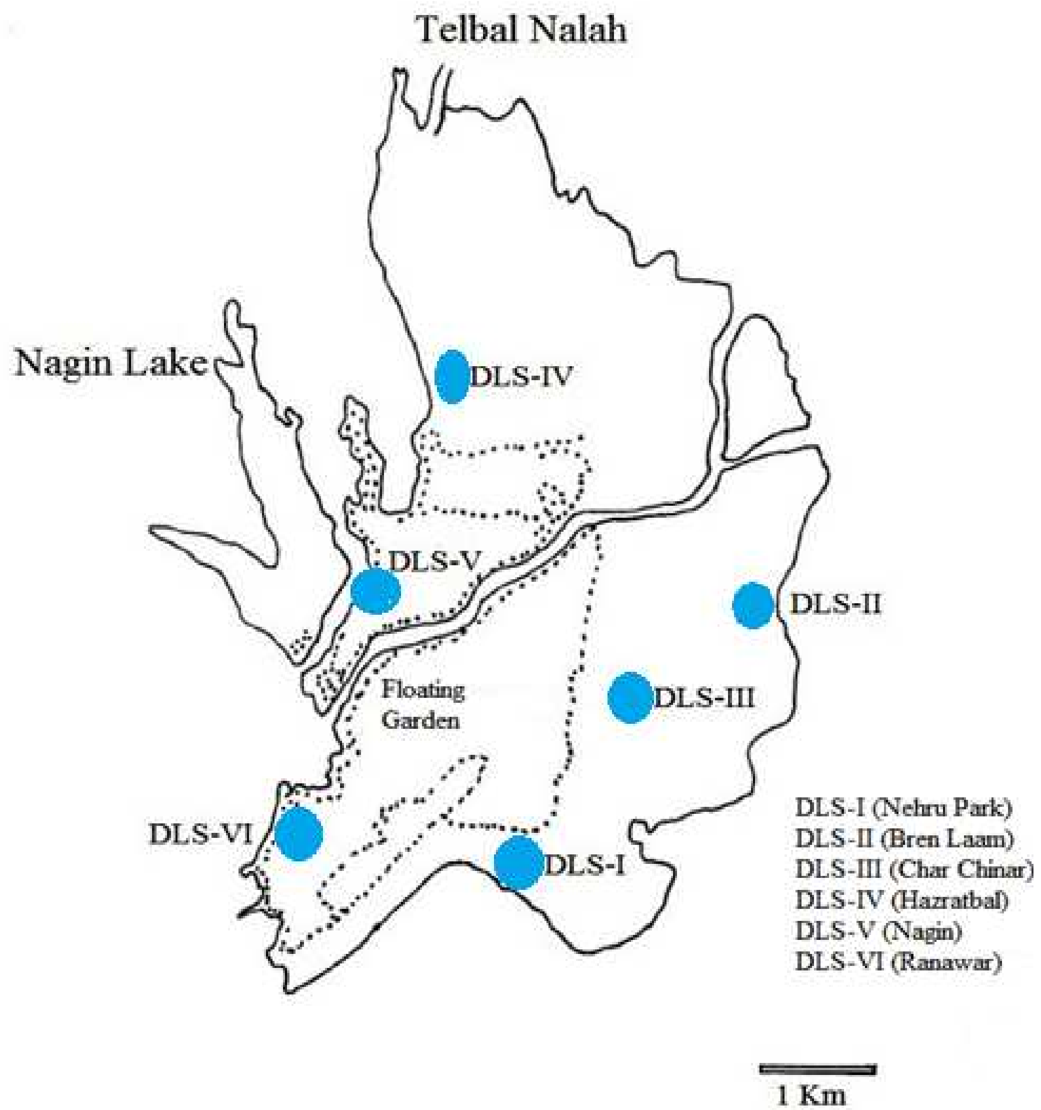
## 3.1 Survey, collection and preservation of samples

### 3.1.1 Study sites

For the convenient monitoring, systematic field study and regular collection of water and algal samples, six permanent sampling sites were selected in the Dal Lake (Fig. 1). These sites were selected according to differences in degrees of human interferences within different parts of the lentic ecosystem and also as zones of special ecological interests. These sites were designated as Dal Lake Sites (DLS)-DLS-I (Nehru Park), DLS-II (Bren Laam), DLS-III (Char Chinar), DLS-IV (Hazratbal), DLS-V (Nagin), and DLS-IV (Ranawari) respectively.

### 3.1.2 Sampling of water and algal samples

Water and fresh algal samples were collected separately from the six different sampling sites of Dal Lake between 8:30-13:30 in sample collection bottles made of polyethylene and polypropylene (100 ml for algal samples and 500 ml for water samples) with four replications for each sample. Sampling was done during the four seasons of a year i.e., spring (April), summer (July), autumn (October), and winter (January). Water samples were collected from the Lake between 15th and 20th of every seasonal month from January 2012 to December 2012. The fresh algal samples were collected with the help of sample collecting spoons, forceps etc and were immediately fixed by using suitable amount of preservative (7 % formaldehyde) for the biodiversity studies (Wetzel and Lickens, 2000).




(Adapted and modified from Zutshi and Ticku, 1990.)

**Fig. 1 : Map of Dal Lake, Srinagar, India showing sampling sites**

### 3.2 Physico-chemical analysis of water samples

The physico-chemical parameters (pH, electrical conductivity, temperature, total dissolved solids, total hardness, total alkalinity, phosphate, nitrate, and silicate) of the surface water samples collected from six sites of Dal Lake were analysed as per "American Public Health Association" (APHA, 1995). The parameters including temperature and total dissolved solids were determined on spot. While as pH and conductivity were measured within 5 hours of sampling, the rest of the parameters were determined in the laboratory within 24 hours.

#### 3.2.1 pH

pH was measured with the help of LABINDA pH meter (Pico PH05070305) by calibrating the instrument with buffer tablets of pH 7 and 4 in 100 ml of distilled water.

#### 3.2.2 Electrical conductivity

Electrical conductivity was measured with the help of direct reading conductivity meter (Systronics-304), where cell constant was maintained at 1.00.

#### 3.2.3 Total dissolved solids and temperature

Total dissolved solids and temperature were measured with the help of digital TDS meter (Himedia) attached with digital thermometer.

#### 3.2.4 Total hardness

Requirement: The titration assembly and three reagents required in this method were :

1. Standard EDTA titrant (0.01M): 3.723 g of disodium salt of ethylene diamine tetra acetate was dissolved in distilled water to prepare 1 L of titrant and was stored in polythene bottle.
2. Ammonia buffer solution: 114 ml concentrated $NH_4OH$ was added to 13.5 g of $NH_4Cl$ and the volume was made up to 200 ml.

3. Eriochrome Black-T indicator: 0.5 g dye was dissolved in 100 ml of 80 per cent ethyl alcohol

**Method**

50 ml of the water sample was taken in a flask, then1 ml of ammonia buffer and 5 drops of indicator solution was added. The colour of the sample turned wine-red and was titrated with EDTA solution, until a clear blue colour appeared. The readings were noted and total hardness was calculated.

**Calculations**

$$\text{Total hardness as mg L}^{-1} \text{ as } CaCO_3 = \frac{\text{ml of titrant}}{\text{ml of sample}} \times 1000$$

### 3.2.5 Total alkalinity

Requirement: The titration assembly and three reagents required in this method were

1. Sulphuric acid titrant (0.02 N): The stock solution of 0.1 N was prepared by diluting 2.8 ml of concentrated sulphuric acid to 1 L. 200 ml of this stock solution was diluted to 1 L to obtain 0.02 N acid titrant and was standardized.

2. Phenolphthalein indicator: 1.25 g phenolphthalein was dissolved in 125 ml ethyl alcohol and to it 125 ml distilled water was added. 0.02 N NaOH drop wise was added to it until a faint pink colour appeared.

3. Methyl orange indicator: 182 g $MnSO_4.H_2O$ was dissolved in distilled water, filtered and diluted to 500 ml.

**Method**

50 ml of sample was taken in Erlenmeyer flask and two drops of phenolphthalein indicator were added. If a slight pink colour appeared, sample was titrated with acid titrant to a colourless end point and the reading was noted as

"p" (ml of titrant used for phenolphthalein alkalinity). Now 2 drops of methyl orange were added in the same flask and continued to titrate further till the colour changed from yellow to orange. This reading was noted as "t" (total volume of the titrant used for both the titrations).

**Calculations**

$$\text{Phenolphthalein alkalinity (P) as mg L}^{-1}\ \text{CaCO}_3 = \frac{\text{ml of titrant "p"}}{\text{ml of sample}} \times 100$$

$$\text{Total alkalinity (T) as mg L}^{-1}\ \text{CaCO}_3 = \frac{\text{ml of titrant "t"}}{\text{ml of sample}} \times 100$$

### 3.2.6 Silicates

Requirement: The spectrophotometer (X-ma 1000, Human Corporation) and reagents required in this method were:

1. Glassware used was thoroughly cleaned that had been soaked overnight in chromic acid prior to cleaning. All the reagents were kept in polythene bottles and hard (corning) glassware was used to avoid leaching of silica from glass wares. Chemicals used were Analar-grade of Himedia and low in silica.
2. Acid ammonium molybdate: 2 g of ammonium molybdate $\{(NH_4)_6MO_7O_{24}.4H_2O\}$ was dissolved in about 70 ml distilled water and to it 6 ml of HCl was added and diluted to 100 ml with distilled water. pH was adjusted between 7 to 8 with silica free NaOH and stored in polyethylene bottle for not more than 1 month.
3. Oxalic acid $(COOH)_2.2H_2O$: 10 g of oxalic acid was dissolved in distilled water and diluted to 100 ml.
4. Metol-sulphite solution: 6 g of sodium sulphite $(Na_2SO_3.7H_2O)$ was dissolved in water; 5g of metol was added and warmed to

dissolve. The volume was made up to 250 ml, filtered with a fine filter paper and stored in a dark bottle.

5. Reducing agent: Carefully 30 ml concentrated sulphuric acid was added to 100 ml distilled water and cooled. 100 ml of metol sulphite solution with stirring and 60 ml oxalic acid solution were added, diluted to 300 ml. The stopper was inserted without delay and mixed well. A fresh solution was made after two weeks.

6. Silicon standard solution ($Na_2SiF_6$): 0.6714 g of dry sodium fluoro-silicate was dissolved in water and made up to 1 L (1 ml of this solution contains 100 µg silicon)

**Method**

3 ml of acid ammonium molybdate reagent was taken in a 50 ml flask and 25 ml of water sample was added to it. The sample was mixed well by inverting at least six times and was left to stand for 5 to 10 minutes. Then 15 ml of the reducing agent was added and the volume was made upto 50 ml by addition of distilled water. The whole solution was shaken thoroughly and allowed to stand for three hours. The absorbance at 810 nm was read against distilled water as a blank and $SiO_4$-Si was expressed in mg $L^{-1}$.

**Interference**

Tannin, large amounts of iron, colour, turbidity, sulfide, and phosphate interferes. Treatment with oxalic acid eliminated interference from phosphate and decreases interference from tannin.

### 3.2.7 Total Phosphorus

Requirement: The spectrophotometer (X-ma 1000, Human Corporation) and the reagents required in this method were:

1. Ammonium molybdate strong acid solution: 5 g ammonium molybdate $(NH_4)_6MO_7O_{24}.4H_2O)$ was dissolved in 35 ml distilled

water. Cautiously 62 ml concentrated $H_2SO_4$ was added to 80 ml distilled water. After cooling the molybdate solution was added and diluted to 200 ml.

2. Stannous chloride solution: 0.5 g fresh $SnCl_2.2H_2O$ was dissolved in 2 ml concentrated HCl, diluted to 20 ml with distilled water. A freshly prepared solution was used whenever required.

3. Standard phosphate solution: 0.1757 g of potassium dihydrogen phosphate (oven dried at 105 $^oC$) was dissolved in distilled water and diluted to 1 L. 1 ml of this solution contains 40 µg $PO_4$.

4. Sodium hydroxide solution (1N): 4.0 g sodium hydroxide was added to 100 ml distilled water.

5. Perchloric acid ($HClO_4.2H_2O$).

**Method**

A suitable volume of sample (25 ml) was evaporated, cooled and the residue was dissolved in 1 ml perchloric acid. Gently the flask was heated on hot plate until the residue becomes colourless. The remaining perchloric acid was fumed off but not to dryness, and cooled. 10 ml of distilled water was added, followed by a drop of phenolphthalein indicator. The sample was then titrated with sodium hydroxide solution to slight pink end point. The volume was made up to 25 ml.

Now 25 ml of this prepared sample was taken in an Erlenmeyer flask (distilled water blank was simultaneously prepared). 1 ml ammonium molybdate solution and 0.12 ml (3 drops) stannous chloride solution was added. Blue colour gradually appeared. Rate of colour development and intensity of colour depends on temperature of the final solution, each 1$^oC$ increase producing about 1 per cent increase in colour, Hence, all the samples, standards, and reagents were held within 2$^oC$ of one another and in the temperature range between 20 and 30 $^oC$.

The reading on spectrophotometer was noted at 690 nm after 10 minutes but before 15 minutes using distilled water as a blank.

**Interference**

Positive interference is caused by silica and arsenate only if the sample is heated. Negative interferences are caused by arsenate, fluoride, thorium, bismuth, sulphide, thiosulfate, thioisocyanate or excess molybdate. Blue colour is caused by ferrous iron but does not affect results if ferrous iron concentration is less than 100 mg $L^{-1}$. Most of the ions do not interfere in concentration upto 1000 mg $L^{-1}$.

### 3.2.8 Nitrate

Requirement: The spectrophotometer (X-ma 1000, Human Corporation), casserole and the reagents required in this method were:

1. Phenol disulphonic acid: 25 g pure white phenol was dissolved in 150 ml concentrated $H_2SO_4$. Cautiously 75 ml fuming sulphuric acid (15 per cent free $SO_3$) was added; stirred well, and heated on water bath for two hours.
2. Potassium hydroxide solution (12 N): 336.5 g KOH was dissolved in distilled water and diluted to 500 ml.
3. Ammonium hydroxide (32% w/w $NH_3$).
4. Standard potassium nitrate solution: 7.22 g anhydrous potassium nitrate was added in nitrate free distilled water and made up to 1 L. 1 ml of this solution contains 1 mg $NO_3^-N$ i.e., 4.43 mg $No_3^-$ ions.

**Method**

25 ml water sample was taken in a casserole (dilution necessary if $NO_3$-N exceeds 2.0 mg per liter) and evaporated to dryness on hot water bath. The residue was rubbed thoroughly with 0.5 ml Phenol disulphonic acid reagent to dissolve all solids (use glass spatula). 5 ml distilled water and 1.5 ml concentrated $NH_4OH$ (or 12 N KOH) was added one after the other. The sample was stirred and a yellow

colour developed. The supernatant was taken avoiding the flocks if any, and was read in spectrophotometer at 410 nm against distilled water as a blank. The value of nitrates was found out with the help of standard curve and the results were expressed in mg $L^{-1}$.

**Precaution**

The sample must be optically clear sample. However the turbid sample was filtered through 0.45 µm pore diameter membrane filter.

## 3.3 Identification, isolation and screening of microalgae

### 3.3.1 Diversity and identification of algae by using microscopy

Each of the 100 ml sample collected with four replications were immediately preserved and phytoplanktons were studied for the biodiversity details. The identification of microalgae was carried out by using advanced microscope (LEICA DM 500, U.K) connected with computer having digital image analyzer and software (LAS EZ 1.8.0) and microphotographs were taken with attached camera LEICA EC3. The identification of the microalgae was also authenticated based upon standard keys given by Desikachary (1959) for blue green algae; Tiffany (1952) for blue green, green algae and diatoms; Prescott (1970) for blue green and green algae; Phillipose (1967) for green algae etc. for morphological characteristics. The attributes recorded for morphological parameters through microscopic examination were size and shape of vegetative cells, spines, flagella, heterocyst and colour of thallus.

### 3.3.2 Isolation of pure algal cultures

The isolation of selected algal strains was performed as per different Isolation techniques given by Kaushik (1987).

#### 3.3.2.1 Culture enrichment

The fresh algal samples collected from all sampling sites were transported to laboratory. An aliquot of 20 ml of the samples were first enriched in 150 ml

Erlenmeyer flasks containing 80 ml sterilised BG-11 medium (Table 1). The culture flasks were then incubated in culture room at 27°C±0.5°C under light intensity of 3000 lux and 16:8 h light/dark cycle (Plate 1a). After three weeks of incubation culture broths were later transferred to petri plates.

**Table-1 : BG-11 media (Stanier *et al.,* 1971)**

| Stock | Stock solution ($g\ L^{-1}$) | $ml\ L^{-1}$ |
|---|---|---|
| $NaNO_3$ | 150 | 10 ml |
| $K_2HPO_4.3H_2O$ or $K_2HPO_4$ | 40 or 30 | 1 ml |
| $MgSO_4.7H_2O$ | 75 | 1 ml |
| $CaCl_2.\ 2H_2O$ | 36 | 1 ml |
| Citric acid | 6 | 1 ml |
| Ferric ammonium citrate | 6 | 1 ml |
| EDTA | 1 | 1 ml |
| $Na_2CO_3$ | 20 | 1 ml |
| Trace metal solution | See below | 1 ml |

This medium is successfully used for most of the Green algae and Cyanobacteria. Vitamin $B_{12}$ may be added for those species that require it.

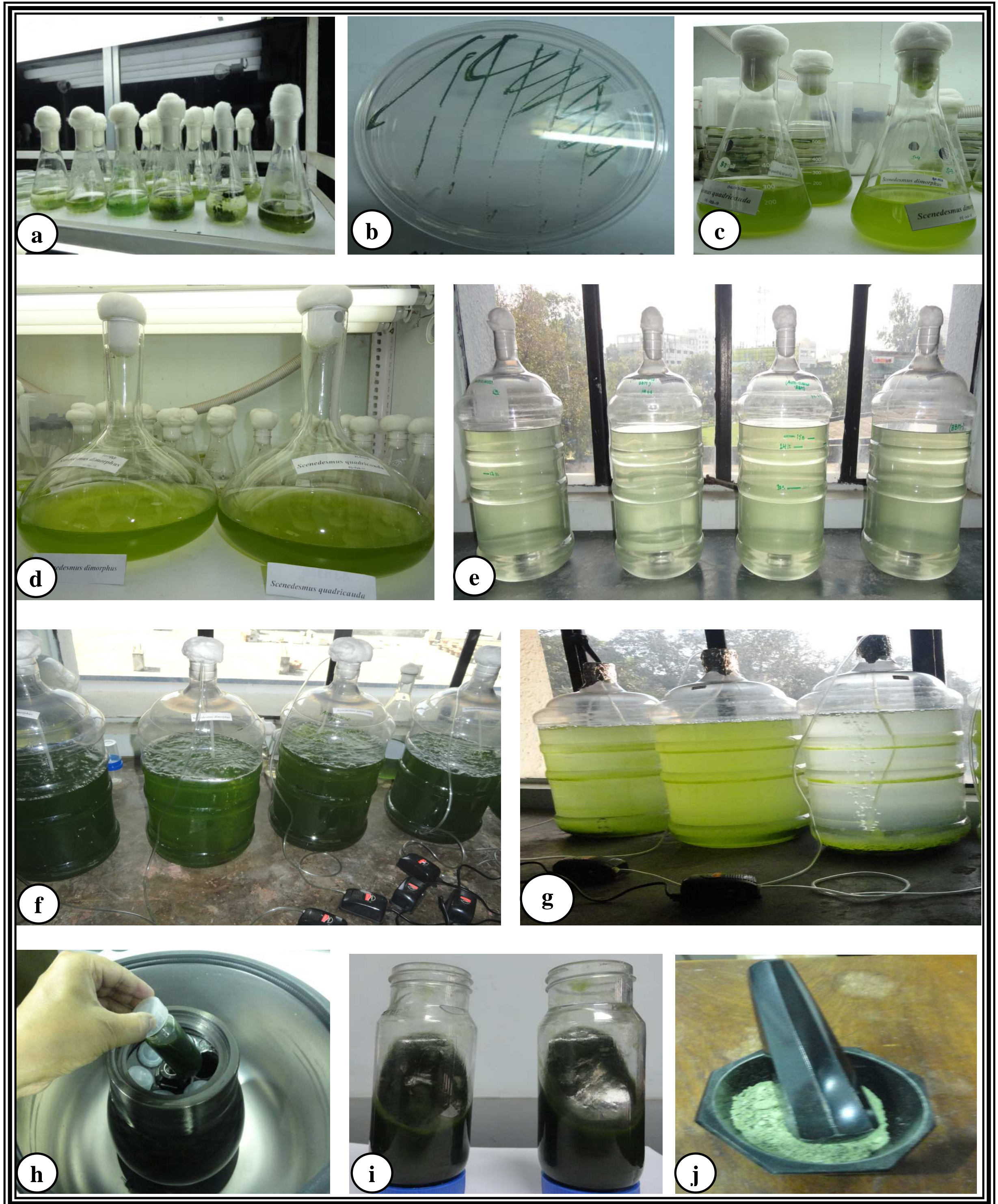
pH was adjusted to approximately 7.5 (Initial pH is approximately 8.5) when making solid media, agar can be added directly to medium (10 $g\ L^{-1}$).

**Trace metal solution**

| Substance | $g\ L^{-1}$ |
|---|---|
| $H_3BO_3$ | 2.86 |
| $MnCl_2.4H_2O$ | 1.81 |
| $ZnSO_4.7H_2O$ | 0.222 |
| Na $MoO_4.5H_2O$ | 0.390 |
| CuSO4.$5H_2O$ | 0.079 |
| $Co(NO_3)_2.6H_2O$ | 0.0494 |

Each of the above substance was dissolved separately prior to adding the next on the list.

# PLATE 1




**a:** Culture enrichment, **b:** Streak plate, **c:** Pure cultures, **d:** Inoculum preparation in Haffkine flasks, **e:** 25 L capacity tanks with 15 L BBM media, **f:** Bulk biomass production of microalgae, **g:** Flocculation using Alum, **h:** Centrifugation method, **i:** Microalgal biomass, **j:** Powder form of algal biomass

#### 3.3.2.2 Streaking

The enriched cultures were streaked with a loop on to the surface of petri plates containing BG-11 growth media solidified with agar and incubated at 27± 0.5°C at 3000 lux light intensity. These plates were also incubated for 2-3 weeks and morphologically distinct colonies developed.

The objective of the streaking was to produce pure discrete colonies of microalgae from the concentrated suspensions of cells. During inoculation, the closely packed cells at the start of the streak form colonies that run together, but as the streak continues, fewer and fewer cells remain in the clump being carried on the needle. As these fall off and grow on the surface of the petri plate, well separated colonies develop. Thus successive streaking was performed from one petri plate to another that usually yielded us purification of unialgal or axenic cultures (Plate 1b).

#### 3.3.2.3 Inoculation

When an organism is grown in a sterilized medium a number of algal cells (inoculums) are transferred (inoculated) in the medium with special precautions to maintain the purity of the culture (Kaushik, 1987). The streaking needle was first heated to redness by flaming immediately before and after making the transfer and cooled it by jabbing it into the edge of the fresh agar plate. This flaming destroys any living forms on the surface of the needle. Many individual colonies from plates were picked up and inoculated in 150 ml flasks containing 50 ml BG-11 medium.The purity of the cultures was examined by microscopic observations at different stages of growth.

After the purification, unialgal cultures were incubated in culture room under controlled temperature range of 27±0.5°C and under continuous illumination of 3000 lux with 14/10 h light/dark photoperiod. The mouths of the plates and Erlenmeyer flasks from which cultures were taken and into which they were transferred were allowed to be passed through the burner flame immediately

before and after the needle is introduced and removed. In addition to destroying any organism on the lip of the tube, flaming tends to create outward convection currents, thus decreasing the chance of contamination.

These microalgae were then grown in a standard culture media of BG-11 using culture flasks having two replicates (Erlenmeyer flasks 150mL, 250mL, 500mL) plugged with non-absorbent cotton (Plate 1c). All these pure cultures were maintained by repeated transfer to liquid medium or by preparing slants for their long term use. Culture media and culture vessels were sterilized by heat at 121$^{o}$C and 15 lb/inch pressure for 15 minutes using autoclave (Kaushik, 1987). All the isolation techniques were carried out under controlled conditions using laminar air flow (Thermadyne, India).

### 3.3.3 Screening of algal isolates for lipid content

All the four weeks old cultures of microalgae were subjected to Nile red technique for neutral lipid observations under fluorescent microscope and folch method for quantitative lipid screening as below**:**

#### 3.3.3.1 Nile red staining technique

Nile red is an excellent vital fluorescence stain used for the detection of intracellular algal lipid droplets by fluorescent microscope (Green-span *et al.,* 1985). Nile red a red phenoxazine dye (9-dimethylamino-5H-benzo (α) phenoxazine-5- one) was obtained from Sigma (USA). Organic solvent (acetone) was of Analar grade.

**Procedure**

Nile red solution, 40 µl, in acetone (250 mg $L^{-1}$) was added to 10 ml of algal suspension into test tubes. The mixture was vigorously agitated on a vortex mixer and incubated in dark for 10 minutes. The fluorimetric analysis (microphotography) of Nile red stained cells were observed in a fluorescence microscope (Leica DM 4.3.0 2500, Germany) under blue light with a 100 watts xenon voltage lamp (ebq 100-04). The instrument was equipped with a digital camera (Leica DFC 450 C) using a 450-490 nm narrow band excitation filter and

a 585 nm narrow band emission filter with 100X objective lens under immersion oil.

#### 3.3.3.2 Determination of total lipid by slight modified Folch method (Folch *et al.,* 1957)

After a period of four weeks, the algal cells were harvested by centrifugation (Thermo Scientific, Germany; Sorvall ST 16R) at 10,000 rpm for 10 minutes and screened for lipid/oil content. 80 ml of algal culture from all these culture flasks were centrifuged to form an algal pellet. The algal pellet was then washed twice with double distilled water to remove the media salts. The pellet in petri plates was dried at 58 $^{o}$C in oven for 24 hours and biomass was deduced.

The algal tissue was homogenized with 15 ml chloroform/methanol (2:1) by using Ultrasonic Homogenizer (JY 96-11N, Ningbo Scinetz Biotechnology co. Ltd. China, with noise isolating tamber) for 20 minutes. This homogenization process was repeated two times for the complete disruption of cells. After dispersion of algal cells, the whole mixture was agitated during 15-20 minutes in Kuhner shaker (Lab-Therm LT-X, Switzerland) at room temperature. The homogenate was then centrifuged at 8,000 rpm for about 7 minutes to recover the liquid phase (supernatant) and discard the pellet. The solvent (liquid phase) was washed by addition of 0.2 volumes (2 ml for 10 ml) of 0.9 per cent sodium chloride solution. After vortexing for some seconds, the mixture was centrifuged at low speed (3000 rpm) for 5 minutes to separate the two phases. The upper phase was removed with the help of micropipette and the lower chloroform phase containing lipids, was evaporated under vacuum in a rotary evaporator. The weight of lipids was deduced in milligrams by subtracting the weight of the empty flask from the weight of flask with lipids. The percentage of oil was calculated as per the following equation (Abubakar *et al.,* 2012).

$$\text{Lipid content (\%)} = \frac{\text{Weight of lipids in mg}}{\text{Weight of sample in mg}} \times 100$$

### 3.4 Maintenance of promising algal isolates

The two promising species of green microalgae *Scenedesmus dimorphus* and *Scenedesmus quadricauda* were selected based upon their maximum lipid content for large scale biomass production using Haffkines flasks of 4 L and transparent tanks 25 L (lab scale photobioreactor) using BBM media plugged with non-absorbant cotton (Table 2). 2 L unialgal inoculum was prepared as a starter inoculum in 4 L capacity Haffkines with the culture conditions same as above in culture room (Plate 1d). After the late log phase i.e. approximately after two week's time, 10 per cent inoculum was used for the lab scale photobioreactor.

**Table-2: Bold's Basal Medium-BBM (Bold, 1949; Bischoff and Bold, 1963)**

| Component | 1 Liter Stock Solution | Add quantity below per liter of medium |
|---|---|---|
| Major Stock Solutions | | |
| $NaNO_3$ | 25.00 g $L^{-1}$ $dH_2O$ | 10 ml |
| $CaCl_2 \cdot 2H_2O$ | 2.50 g $L^{-1}$ $dH_2O$ | 10 ml |
| $MgSO_4 \cdot 7H_2O$ | 7.50 g $L^{-1}$ $dH_2O$ | 10 ml |
| $K_2HPO_4$ | 7.50 g $L^{-1}$ $dH_2O$ | 10 ml |
| $KH_2PO_4$ | 17.50 g $L^{-1}$ $dH_2O$ | 10 ml |
| NaCl | 2.50 g $L^{-1}$ $dH_2O$ | 10 ml |
| Alkaline EDTA Stock Solution | | add 1 ml of this solution per liter of medium |
| EDTA anhydrous | 50 g $L^{-1}$ $dH_{2O}$ | |
| KOH | 31 g $L^{-1}$ $dH_2O$ | |
| Acidified Iron Stock Solution | | add 1 ml of this solution per liter of medium |
| $FeSO_4 \cdot 7H_2O$ | 4.98 g $L^{-1}$ $dH_2O$ | |
| $H_2SO_4$ | 1.0 ml | |
| Boron Stock Solution | | add 1 ml of this solution per liter of medium |
| $H_3BO_3$ | 11.42 g $L^{-1}$ $dH_2O$ | |
| Trace Metal Stock Solution | | add 1 ml of this solution per liter of medium |
| $ZnSO_4 \cdot 7H_2O$ | 8.82 g $L^{-1}$ $dH_2O$ | |
| $MnCl_2 \cdot 4H_2O$ | 1.44 g $L^{-1}$ $dH_2O$ | |
| $MoO_3$ | 0.71 g $L^{-1}$ $dH_2O$ | |
| $CuSO_4 \cdot 5H_2O$ | 1.57 g $L^{-1}$ $dH_2O$ | |
| $Co(NO_3)_2 \cdot 6H_20$ | 0.49 g $L^{-1}$ $dH_2O$ | |

The recipe will result in a final solution of 1005 ml.

This is a widely used artificial freshwater medium, especially for growing green algae. The medium lacks vitamins and some of the trace metal concentrations are relatively high, making the medium unacceptable for growth of many non-green algae. Six macronutrient stock solutions, an alkaline EDTA solution, an acidified iron solution, a boron solution and a trace metals solution were individually prepared. The final medium was prepared by adding 10 ml of the first six stock solutions to 940 ml of distilled water. 1 ml each of the alkaline EDTA, acidified iron, boron and trace metals solutions were also added. The final pH was adjusted to 6.6 and the medium was autoclaved.

### 3.5 Scanning and growth measurement of promising algal isolates by spectrophotometric method

3 ml culture was taken from the flask in the first cuvette and 3 ml BBM media was used as blank in second cuvette. The maximum absorbance was inspected by scanning a culture sample between 400 and 1100 nm using double beam UV Vis spectrophotometer (Chemito Spectrascan UV 2700, Thermo Scientific,) loaded with Spectrum PC software. The highest absorbance peak value was then used to measure the optical density.

Six Erlenmeyer flasks (500ml capacity) were arranged in two series, each containing 250 ml BBM media and 12 per cent (30 ml) inoculum. These flasks were arranged in two series, each series containing three flasks. The first series was containing the inoculum of *Scenedesmus dimorphus* and the second was containing the inoculum of *Scenedesmus quadricauda.* Growth rate of cultures was determined by measuring the optical density ($OD_{680nm}$) after every 24 hours at 11:00 am. For the measurement of OD, 3 ml culture was drawn from the flask and BBM media was used as a blank. OD was measured at 680 nm as per the scanning process initially using a double beam spectrophotometer. The sample cultures were diluted to an OD of less than one, to fall within the linear range of measurement. The actual OD was determined by multiplying the OD value with the dilution factor (Griffiths *et al*., 2011).

### 3.6 Biochemical studies of promising algal isolates

The bio-chemical analysis (chlorophyll-a, chlorophyll-b, total chlorophyll, total carotenoids, total pigment and protein) of three weeks old culture was determined by following advanced laboratory methods.

#### 3.6.1 Pigment quantification (Wellburn, 1994)

**Instruments used**

Double beam UV VIS spectrophotometer, hot plate with high speed stirrer and temperature control, oven, centrifuge (TC4815D Eltek microspin India) and lypholizer (Labconco Freezone 2.5, USA).

**Method of extraction and materials required**

Dimethyl sulphoxide (DMSO) solvent was used for the extraction of pigments.DMSO, small 10 ml centrifuge tubes, aluminium foil.

**Method**

Pigments were extracted from algal cells using dimethyl sulphoxide (DMSO 1 ltr=1.10 kg, M 78.13 g $mol^{-1}$, Purity≥99.8%, Merck). Culture samples (2 ml) were centrifuged in eppendorf tubes at 10,000 rpm for 5 minutes and the supernatant was discarded. Hot ($60^oC$) DMSO (2 ml) was added and cells were resuspended by vortexing. Samples were incubated at $60^o$ C, with occasional shaking, for 10 minutes before centrifuging. The supernatant pigment extract was removed and diluted with DMSO to an OD of less than one. The OD at 649, 665 and 480 nm was determined and the pigment content was calculated using the equations below (Wellburn, 1994).

Chlorophyll-a (Chl-a) (mg $L^{-1}$) = 12.47 ($OD_{665}$)-3.62 ($OD_{649}$)

Chlorophyll-b (Chl-b) (mg $L^{-1}$) = 25.06 ($OD_{649}$)-6.5 ($OD_{665}$)

Total chlorophyll (mg $L^{-1}$) = Chl-a + Chl-b

Total carotenoids (mg $L^{-1}$)= [1000($OD_{480}$)-1.29 (Chl-a)-53.78 (Chl-b)]/220

Total pigment mg $L^{-1}$ = Sum of the above

All the measurements were carried out in duplicates. DMSO solution was used as blank and the results were converted into mg $g^{-1}$ fw.

### 3.6.2 Estimation of photosynthetic pigments (Hiscox and Israelstam, 1979)

A suitable amount of microalgae was taken in centrifuge tubes and was centrifuged at 10,000 rpm for 10-15 minutes. Now supernatant from the tubes was removed and the microalgae (sediment) were transferred into 25 ml beakers for Lyophilisation in lypholizer for 3 hours.

100 mg of microalgae was weighed and placed in the test tubes. 20 ml of DMSO was added in the same tubes. The tubes were covered with the aluminium foil (to avoid photo- oxidation of pigments) and kept in an oven at 65°C for 5 hours or kept overnight at room temperature. The absorbance of chlorophylls contained in solution at 663, 645, and 630 were recorded. Chl-a, Chl-b, Chl-c and total Chl were calculated by using the formulas given by Arnon (1949) based on the work of Mac Kinney (1941) who provided the values of extraction coefficients. DMSO solution was used as blank and the results were expressed in terms of mg $g^{-1}$ fw.

$$\text{Chl-a} = \frac{[(12.7 \times A_{663}) - (2.69 \times A_{645})] \times V}{1000 \times W}$$

$$\text{Chl-b} = \frac{[(22.9 \times A_{645}) - (4.68 \times A_{663})] \times V}{1000 \times W}$$

$$\text{Total Chl-`a+b'} = \frac{[(8.02 \times A_{663}) + (20.2 \times A_{645})] \times V}{1000 \times W}$$

Where A=Absorbance at given wavelength, V= Volume of DMSO and W= Weight of microalgae in milligrams.

The above extract was also used for the quantification of carotenoids. The absorbance of the carotenoids at 480 nm was determined using the equation provided by Price and Hendry (1991) and Venkatarayappa *et al.* (1984).

$$\text{Total Carotenoids} = \frac{A_{480} + [(0.114 \times A_{663}) - (0.638 \times A_{645})] \times V}{1000 \times W}$$

### 3.6.3 Protein estimation by slight modified Lowry's Method (Lowry *et al.*, 1951)

**Instruments used**

Ultra-Sonicator for the dispersion and disruption of algal-cells to release proteins and other constituents. Double beam UV VIS spectrophotometer (Thermo Scientific, Chemito Spectrascan UV2700) for use at 750 nm.

**Reagents:** The following reagents were prepared as

Lysis buffer: Triton X-100: 5 ml $L^{-1}$; EDTA: 0.3722 g $L^{-1}$; PMSF (Protease inhibitor): 0.0348g $L^{-1}$; distilled water: 995ml.

Reagent A: 2 per cent sodium carbonate in 0.1 N sodium hydroxide.

Reagent B: 0.5 per cent copper sulphate ($CuSO_4.5H_2O$) in 1 per cent potassium sodium tartrate.

Reagent C: Alkaline copper solution: Mix 50 ml of A and 1 ml of B prior to use. If precipitate is formed by mixing, then discard the solution.

Reagent D: Folin Ciocalteau Reagent: Generally (phosphomolybdate and phosphotungstate) available commercially is 2 N. It was diluted by adding equal volume of water so that 1N is formed prior to its use. The reagent was stored in an amber coloured bottle in cold.

Protein Solution (Stock Standard): 50 mg of bovine serum albumin was weighed and dissolved in distilled water and the volume was made in a 50 ml standard flask.

Working Standard: 10 ml of the stock solution was diluted with distilled water in a standard flask. One ml of this solution contained 200 µg proteins.

**Extraction of protein from sample**

Extraction was usually carried out with the help of ultrasonic homogenizer. 20 ml of algal culture was centrifuged at 8000 rpm for about 5-7 minutes, supernatant was discarded and pellet formed was dried and weighed. 5 ml of lysis buffer and 5 ml of distilled water was added to the pellet, vortexed for 5 minutes and incubated at room temperature for 40-50 minutes and the same was ultra-sonicated for about 10-20 minutes. The extract so formed was used for protein estimation.

**Estimation of protein**

0.1, 0.2, 0.3, 0.4 and 0.5 ml of the working standard was pipetted out into a series of test tubes. 0.1 ml of the sample extract was also pipetted out in other test tube. The volume was made up to 1 ml in all the test tubes. A tube with 1 ml of water served as the blank. 5 ml of reagent C was added to each tube including the blank. It was then mixed well and allowed to stand for 10 minutes. Then 0.5 ml of reagent D was added, mixed well and incubated at room temperature in the dark (to prevent degradation of the Folin reagent) for 30 minutes. The blue colour was developed and the readings were taken at 750 nm against a blank. A standard graph was drawn and the amount of protein in sample was calculated (from linear regression equation, obtained from the standard curve) as µg protein per ml, that is, the spectrophotometric absorbance was converted to protein concentration using a calibration curve established with BSA. The protein content of the biomass was calculated using the following equation (Lopez *et al.,* 2010).

$$\text{Protein} \left(\% \frac{w}{w}\right) = \frac{CV}{M} \times 100$$

Where C is the protein concentration (mg $L^{-1}$) obtained from the calibration curve, V is the volume of the lysis buffer (L) used to resuspend the biomass and m is the amount of biomass (mg). All the samples and standards were prepared in duplicates.

## 3.7 Lab scale (self-made apparatus) photobioreactor for biomass production

This experimental setup was done for large scale biomass production and oil extraction from two species of microalgae i.e., *Scenedesmus dimorphus* and *Scenedesmus quadricauda.* Four transparent plastic tanks (lab scale photobioreactor with 25 L capacity each) containing 15 L of BBM nutrient medium adjusted to pH 6.6 and 2 L inoculum of microalgae were taken (Plate 1e). These plastic tanks were arranged in two series, each series containing two tanks. To avoid the sedimentation of microalgae and to speed up the biomass production aeration was provided with the help of aerators (Plate 1f). These photobioreactors were kept under the indirect sunlight (8000 to10000 lux measured by lux meter) at the temperature range of 28 to $32^0$ C. After five weeks the two strains were kept for 4 days under dark period and closed environment and upon microscopic examination it was observed that cells are much larger in size and shape and showed a different morphology.

### 3.7.1 Harvesting of microalgal biomass

The algal biomass was harvested using different methods like flocculation, centrifugation, lypholisation and then oven dried.

#### 3.7.1.1 Flocculation

This is one of the best methods used for harvesting of microalgae. In this process algal cells were forced to form lumps by the use of chemicals. Flocculation of both the *Scenedesmus species* was done using alum as a flocculating agent. With the help of thread alum was dipped into the tanks for about 10 minutes with simultaneous shaking of supernatant for complete sedimentation of microalgae. After 1 hour of time the upper phase (supernatant) in plastic tanks (transparent without algae) was removed carefully with the help of transparent plastic tubes and the bottom phase containing microalgae were centrifuged (Plate 1g).

#### 3.7.1.2 Centrifugation

Dewatering of flocculated microalgal biomass was carried out with the help of centrifuge (Thermo Scientific, Germany; Sorvall ST 16R) loaded with six transparent centrifuge tubes each having capacity of 100 ml. Centrifugation was carried out at 8,000 rpm for 8 minutes(Plate 1h). Microalgal biomass was finally washed with distilled water three times to remove the salts (Plate 1i).

#### 3.7.1.3 Lypholisation and oven dry

Biomass was first lypholized (Labconco Freezone 2.5, USA) to remove the moisture content at -50 $^{o}$C for 4 hours and then oven dried by setting temperature with the help of digital temperature controller at 58 $^{o}$C for 24 hours. The dried biomass was grinded using mortar pestle, converted to powder form and was subjected to oil extraction (Plate 1j).

## 3.8 Analysis of algal oil

### 3.8.1 Oil extraction from dried algal biomass using automatic soxhlet extraction technique (Soxhlet, 1879)

The dried algal biomass (8.84 g of *S. dimorphus* and 6.48 g of *S. quadricauda* powder form) was placed in a porous cellulose thimble and was placed in an extraction chamber, which was suspended above a 250 ml round bottom (RB) flask containing 150 ml solvent mixture (chloroform: methanol i.e., 2:1 vol. /vol.) and below a condenser. The top of the thimble was covered with the filter paper. Also several boiling stones (Chemware, Ultra-pure PTFE, Saint-Gobain) were placed into a RB-flask. The water flow to the condenser was turned on. The RB-flask was heated with the help of heater at 60$^{o}$C and the solvent evaporated and moved up into the condenser where it was converted into a liquid that trickles into the extraction chamber containing the algal sample. The extraction was allowed to run for 6 hours (approximately 40 cycles). The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask.

At the end of the extraction process, which lasts a few hours, the RB-flask containing the solvent and lipid was removed.

### 3.8.2 Comparison of oil extraction from two different methods

Algal biomass harvested from the large scale cultivation of two tested microalgae in indigenous made photobioreactor was subjected to oil extraction by two different methods i.e., Folch *et al.* (1957) and Soxhlet (1879). The total percentage of oil was obtained by these two methods and compared for their lipid extraction efficiency.

### 3.8.3 Algal oil preparation for analysis

After the extraction process, the contents were cooled and filtered by using whatman filter paper to separate the biomass. The biomass was washed with 25 ml of same solvent twice to extract the residual lipids present in the biomass. The extracts were pooled, taken in a separating funnel and washed with 1% sodium chloride solution (50 ml) twice. The solvent layer formed was allowed to pass through anhydrous sodium sulphate (sodium sulphate was taken in a glass funnel with cotton plug) and the solvent in the RB-flask was then removed by using vaccum rota-evaporator (Buchi type) attached with temperature controlled water bath to get the algal oil. The mass of algal oil was measured to determine the oil content in biomass.

The different parameters of oil such as colour, viscosity and density were analyzed by standard methods of analysis (AOAC, 1995) and algal oil characters were compared with biofuel standards contained in IS 15607 and EN 14214.

### 3.9 The different parameters (colour, density, viscosity) of the algal oil were analyzed by standard methods of analysis (AOAC, 1995)

#### 3.9.1 Colour

Colour of algal oil was determined as per the colour codes, standards and nomenclature of Ridgway (1912).

#### 3.9.2 Viscosity

Viscosity of the algal oils was estimated with the help of advanced digital Viscometer (Bohlin Visco 88, Malvern, U.K) attached with computer having latest digital Bohlin software (Visco 88 Julabo, V06.51)

Measuring system (cone plate) CP 5.4$^{o}$/30 was used and gap between upper cone (CP) and lower plate (base) was 0.15 mm. Shear rate applied was 10-1103 sec$^{-1}$ and 10 samples with delay time 30 sec and integration time 60 sec were taken in this shear range at each temperature. The viscosity of algal oils was measured at a shear rate of 497.3 sec$^{-1}$ and the temperature was maintained at 30$^{o}$C by using attached temperature controlled oil bath (Julabo 77960, Germany).

#### 3.9.3 Density

Density, ρ, measurements as a function of temperature on the present microalgal oils has been performed by gravimetric method (Sankarappa *et al.,* 2005). Density of microalgal oil to the density of water was measured, when both were at the same temperature. The density bottle was taken and its mass was measured using the electronic balance (Sartorius CP124S, d= 0.1 mg) and this weight was recorded as X. Then 2ml of distilled water was taken in the bottle and its weight was recorded as Y. Now 2 ml of microalgal oil was taken in the same bottle and its mass was measured and this weight was taken as Z. The density of microalgal oil was calculated using two replicates for each sample by the following formula:

$$\text{Density} = (z\text{-}x) / (y\text{-}x)$$

$$\text{Density } \rho = \rho_1 \text{ (microalgal oil)}/\rho_2 \text{ (distilled water)}$$

Where,

$\rho_1$ (sub) = density of the substance.

$\rho_2$ (water) = density of water.

## 3.10 Determination of fatty acid composition using gas chromatographic method (Morrison and Smith, 1964)

The lipid extracts (algal oil) were converted into its fatty acid methyl esters (FAME) by transesterification using 2 per cent sulphuric acid in methanol reagent i.e., 30 ml of methanol and 5 drops of sulphuric acid were added to the algal oil and refluxed in Soxhlet unit for about 4 hours. 10 ml of water and 10 ml of petroleum ether was added to refluxed algal oil in a separating funnel and shaked slightly. The two layers (aqueous layer and oil layer) were formed in a separating funnel. Aqueous layer was extracted from the separating funnel into the Erlenmeyer flask. The same process was repeated three times to obtain the petroleum ether layer. The oil layer was washed with dry anhydrous sodium sulphate (sodium sulphate was taken in a glass funnel with cotton plug) to remove the moisture content. The oil layer was then allowed to evaporate under vacuum in a rota-evaporator to get the FAME. The reaction products were analyzed by gas chromatography (GC) and mass spectrometry (GC-MS).

### 3.10.1 GC-MS / GC analysis protocol

The fatty acid composition of algal fatty acid methyl esters was analyzed qualitatively using GC-MS and quantitatively using GC. The GC/GC-MS analyses were carried out using Agilent 6890N Gas chromatography connected to an Agilent 5973 mass selective detector at 70 eV (m/z 50-550; sources at 230 °C and quadruple at 150 °C) in the electron impact mode with a HP-5 capillary column (30 m x 0.25 mm I.d. X 0.25 μm film thickness). The oven temperature was programmed for two min at 160 °C and raised to 300 °C at 5 °C $min^{-1}$ and maintained for 20 min at 300 °C. The carrier gas, helium was used at a flow rate of 1.0 ml $min^{-1}$. The inlet temp was maintained at 300 °C with a split ratio of 50:1. Structural assignments were based on interpretation of mass spectrometric

fragmentation and confirmed by comparison of retention times as well as fragmentation patterns of authentic compounds.

GC analysis was performed on HP 6850 Series gas chromatography equipped with flame ionisation detector (FID detector) and fused silica capillary column DB -225 (30 m x 0.25 mm I.d. x 0.25 µm film thickness). The injector and detector temperatures were maintained at 300 and 325 °C, respectively. The oven temperature was programmed for two min at 160 °C and raised to 300 °C at 5 $^{o}$C min$^{-1}$ and maintained for 20 min at 300 °C. The carrier gas, nitrogen was used at a flow rate of 1.5 ml min$^{-1}$ the injection volume was 1 µL, with a split ratio of 50:1. The identification of individual fatty acids was based on retention time of authentic fatty acid and the area percentages were recorded with a standard HP Chemstation data system.

### 3.11 Assessment of microalgal oil quality for biodiesel production

The quality of microalgal oil was determined by assessing the saponification value (SV), iodine value (IV), cetane number (CN) and degree of unsaturation (DU) using standard protocols of IUPAC 2.201(1979), AOAC method 920.159 and AOAC method 920.160 respectively. These values were calculated by using empirical equations 1-4 (Francisco *et al.,* 2010; Osunkedo *et al.,* 2013).

$$SV = \Sigma (560 \times F) / MW \quad (1)$$

$$IV = \Sigma (254 \times F \times D) / MW \quad (2)$$

$$CN = (46.3 + 5458 / SV) - (0.225 \times IV) \quad (3)$$

$$DU = (MUFA, wt \%) + (2 \times PUFA, wt \%) \quad (4)$$

Where F is the percentage of each fatty acid, MW is the molecular mass of fatty acid (Sheppard, 1992), D is the number of double bonds, MUFA is monounsaturated fatty acids and PUFA is polyunsaturated fatty acid in wt., per cent.

### 3.12 Statistical analysis

The different standard statistical procedures were followed (Gomez and Gomez, 1984) and are presented in the tables, figures and results for each of the experiment. In all the experimental setups, the measurements of the values were done in duplicates, triplicates and four replicates and the mean ± standard deviation (SD), mean ± standard error (SE) were calculated using GraphPad Prism 5 statistical software. The Box-and-Whisker plots for each of the water parameter were carried out by using R- software.

Chapter – 4

# EXPERIMENTAL FINDINGS

This chapter embodies the results obtained from research programme conducted at Division of Environmental Sciences, SKUAST-Kashmir and Algology laboratory of NBRI-Lucknow, India. The data obtained from measurements of each variable were subjected to statistical analysis and the results are presented as follows:

## 4.1 Physico-chemical analysis of water samples

The data on the variations in the various physico-chemical characteristics viz temperature, pH, electric conductivity, total dissolved solids, total hardness, total alkalinity, silicate, nitrate and phosphate (mean values of four replicates) of the selected sites in the Dal Lake ecosystem were observed during various seasons (winter, spring, summer and autumn) of year 2012 and the results are presented in Tables 3, 4, 5 and 6. The Box–and-Whisker plots statistical analysis was also carried out for each of the water parameter as shown in the Figs. 2, 3, 4 and 5.

### 4.1.1 Temperature

During the winter season water temperature ranged from 12.53 $^{o}$C at Char Chinar site (DLS-III) to 14.1 $^{o}$C at Ranawari site (DLS-VI). Similarly the water temperature varied from 16.2 $^{o}$C at Nehru Park and Nagin sites (DLS-I, DLS-V) to 16.8 $^{o}$C at Ranawari site (DLS-VI) respectively in the spring season. The highest water temperature was observed in summer season at site Ranawari site (DLS-VI) exhibiting highest value of 25.6 $^{o}$C. On the other hand water temperature during the autumn season again declined with values ranging from 20.9 $^{o}$C at Nehru Park site (DLS-I) to 21.55 $^{o}$C at Ranawari site (DLS-VI). In general, highest water temperature of the lake ecosystem was observed in summer followed by autumn season while as lowest water temperature was observed in winter season.

**Table-3 : Physico-chemical characteristics (n=4) of the water samples collected from six different sites of the Dal Lake in winter season (February-2012)**

| S. No. | Name of site | Temperature ($^oC$) | pH (scale 1-14) | EC ($\mu S\ cm^{-1}$) | TDS (ppm) | Total Hardness (ppm) | Total Alkalinity (ppm) | Silicate (ppm) | Nitrate (ppm) | Phosphate (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | DLS-I | 13.65 | 7.97 | 192.5 | 101.0 | 106.3 | 80.0 | 1.23 | 1.12 | 0.50 |
| 2 | DLS-II | 13.45 | 7.50 | 212.8 | 123.8 | 87.5 | 72.5 | 1.53 | 0.75 | 0.48 |
| 3 | DLS-III | 12.53 | 7.58 | 192.5 | 107.5 | 93.8 | 80.0 | 0.22 | 0.12 | 0.11 |
| 4 | DLS-IV | 13.60 | 7.69 | 310.0 | 170.8 | 162.5 | 107.5 | 2.90 | 1.24 | 0.45 |
| 5 | DLS-V | 13.55 | 7.95 | 325.0 | 174.5 | 162.5 | 117.5 | 0.15 | 0.88 | 0.48 |
| 6 | DLS- VI | 14.10 | 7.38 | 540.0 | 275.8 | 181.3 | 157.5 | 11.65 | 2.10 | 1.13 |

DLS stands for Dal Lake Sites: DLS-I (Nehru Park), DLS-II (Bren Laam), DLS-III (Char Chinar), DLS-IV (Hazratbal), DLS-V (Nagin), DLS- IV (Ranawari)

**Table-4 : Physico-chemical characteristics (n=4) of the water samples collected from six different sites of the Dal Lake in spring season (April-2012)**

| S. No. | Name of site | Temperature (°C) | pH (scale 1-14) | EC (μS $cm^{-1}$) | TDS (ppm) | Total Hardness (ppm) | Total Alkalinity (ppm) | Silicate (ppm) | Nitrate (ppm) | Phosphate (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | DLS-I | 16.20 | 7.75 | 125.0 | 108.3 | 93.8 | 105.0 | 2.12 | 0.91 | 0.43 |
| 2 | DLS-II | 16.23 | 8.88 | 137.5 | 114.8 | 100.0 | 111.3 | 2.15 | 1.11 | 0.53 |
| 3 | DLS-III | 16.18 | 8.85 | 90.0 | 76.0 | 75.0 | 92.5 | 4.50 | 0.19 | 0.13 |
| 4 | DLS-IV | 16.50 | 8.65 | 135.0 | 118.5 | 112.5 | 131.3 | 7.50 | 0.96 | 0.48 |
| 5 | DLS-V | 16.20 | 7.75 | 117.5 | 99.5 | 100.0 | 116.3 | 4.13 | 0.89 | 0.40 |
| 6 | DLS- VI | 16.80 | 7.10 | 162.5 | 137.5 | 118.8 | 158.8 | 15.17 | 1.98 | 1.25 |

DLS stands for Dal Lake Sites: DLS-I (Nehru Park), DLS-II (Bren Laam), DLS-III (Char Chinar), DLS-IV (Hazratbal), DLS-V (Nagin), DLS- IV (Ranawari)

**Table-5 :** **Physico-chemical characteristics (n=4) of the water samples collected from six different sites of the Dal Lake in summer season (July -2012)**

| S. No. | Name of site | Temperature ($^{o}C$) | pH (scale 1-14) | EC ($\mu S\ cm^{-1}$) | TDS (ppm) | Total Hardness (ppm) | Total Alkalinity (ppm) | Silicate (ppm) | Nitrate (ppm) | Phosphate (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | DLS-I | 24.78 | 8.57 | 80.0 | 59.3 | 75.0 | 57.5 | 2.54 | 1.50 | 0.58 |
| 2 | DLS-II | 24.33 | 9.47 | 82.5 | 63.0 | 75.0 | 47.5 | 8.50 | 1.88 | 0.71 |
| 3 | DLS-III | 24.28 | 8.69 | 85.0 | 63.5 | 75.0 | 55.0 | 5.28 | 1.13 | 0.47 |
| 4 | DLS-IV | 24.63 | 7.84 | 180.0 | 127.8 | 125.0 | 87.5 | 7.25 | 2.25 | 0.96 |
| 5 | DLS-V | 24.70 | 7.89 | 112.5 | 81.0 | 87.5 | 75.0 | 8.84 | 2.25 | 0.53 |
| 6 | DLS- VI | 25.60 | 7.69 | 195.0 | 143.3 | 137.5 | 140.0 | 19.12 | 3.95 | 1.80 |

DLS stands for Dal Lake Sites: DLS-I (Nehru Park), DLS-II (Bren Laam), DLS-III (Char Chinar), DLS-IV (Hazratbal), DLS-V (Nagin), DLS- IV (Ranawari)

**Table-6 : Physico-chemical characteristics (n=4) of the water samples collected from six different sites of the Dal Lake in autumn season (October-2012)**

| S. No. | Name of site | Temperature (°C) | pH (scale 1-14) | EC (µS $cm^{-1}$) | TDS (ppm) | Total Hardness (ppm) | Total Alkalinity (ppm) | Silicate (ppm) | Nitrate (ppm) | Phosphate (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | DLS-I | 20.90 | 7.68 | 166.0 | 79.3 | 106.3 | 72.5 | 3.75 | 1.13 | 0.53 |
| 2 | DLS-II | 21.33 | 9.28 | 152.4 | 68.0 | 75.0 | 62.5 | 7.18 | 1.69 | 0.75 |
| 3 | DLS-III | 21.20 | 7.65 | 76.0 | 60.0 | 68.0 | 50.0 | 3.29 | 0.86 | 0.38 |
| 4 | DLS-IV | 21.10 | 7.59 | 186.6 | 88.3 | 93.8 | 67.5 | 7.25 | 1.92 | 0.60 |
| 5 | DLS-V | 21.15 | 7.36 | 229.0 | 98.0 | 106.3 | 92.5 | 8.15 | 1.22 | 0.48 |
| 6 | DLS- VI | 21.55 | 6.96 | 308.5 | 143.0 | 137.5 | 150.0 | 17.75 | 2.75 | 1.45 |

DLS stands for Dal Lake Sites: DLS-I (Nehru Park), DLS-II (Bren Laam), DLS-III (Char Chinar), DLS-IV (Hazratbal), DLS-V (Nagin), DLS- IV (Ranawari)

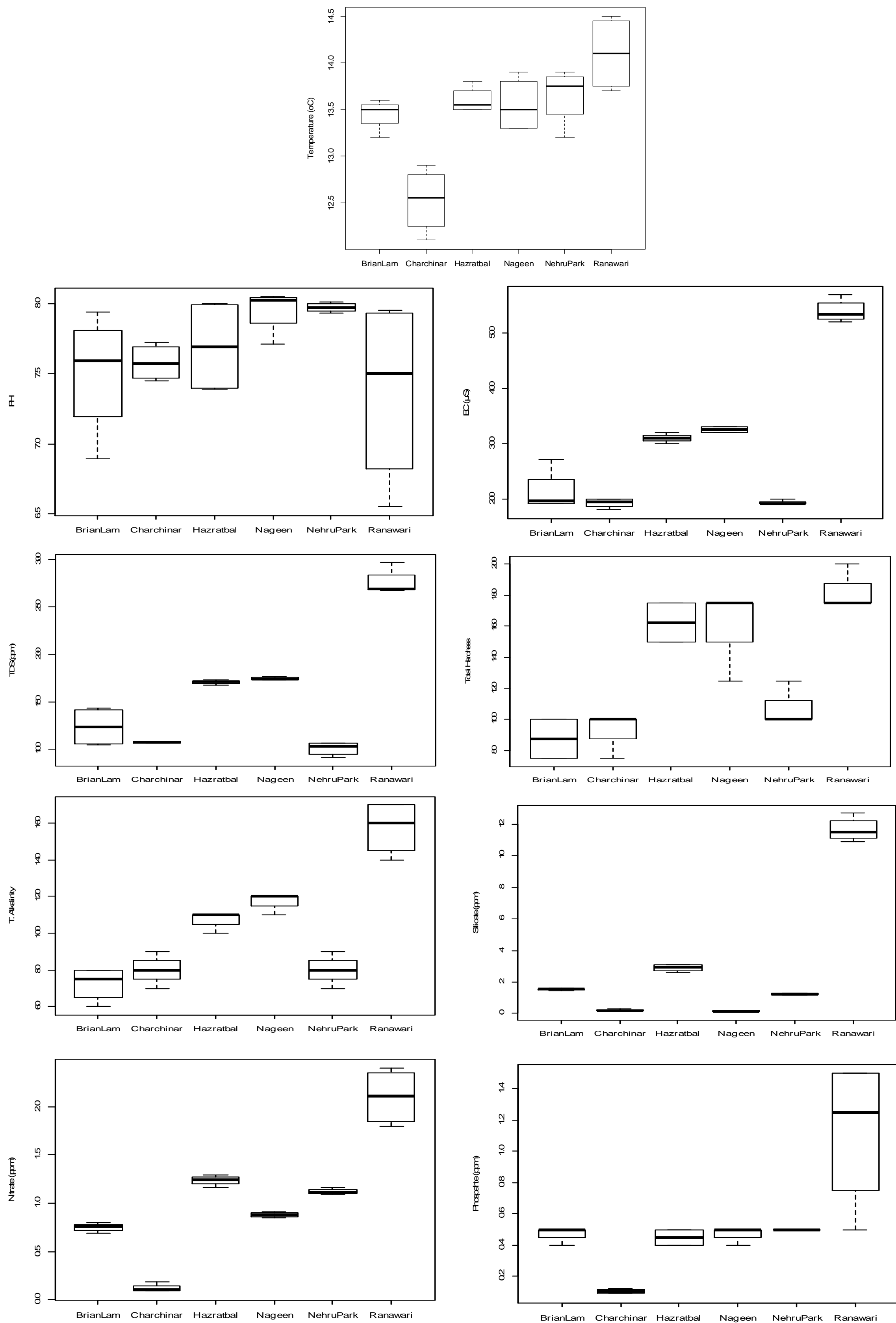



**Fig. 2 : Box-and-Whisker plots of water parameters (n=4) during winter season (February-2012)**

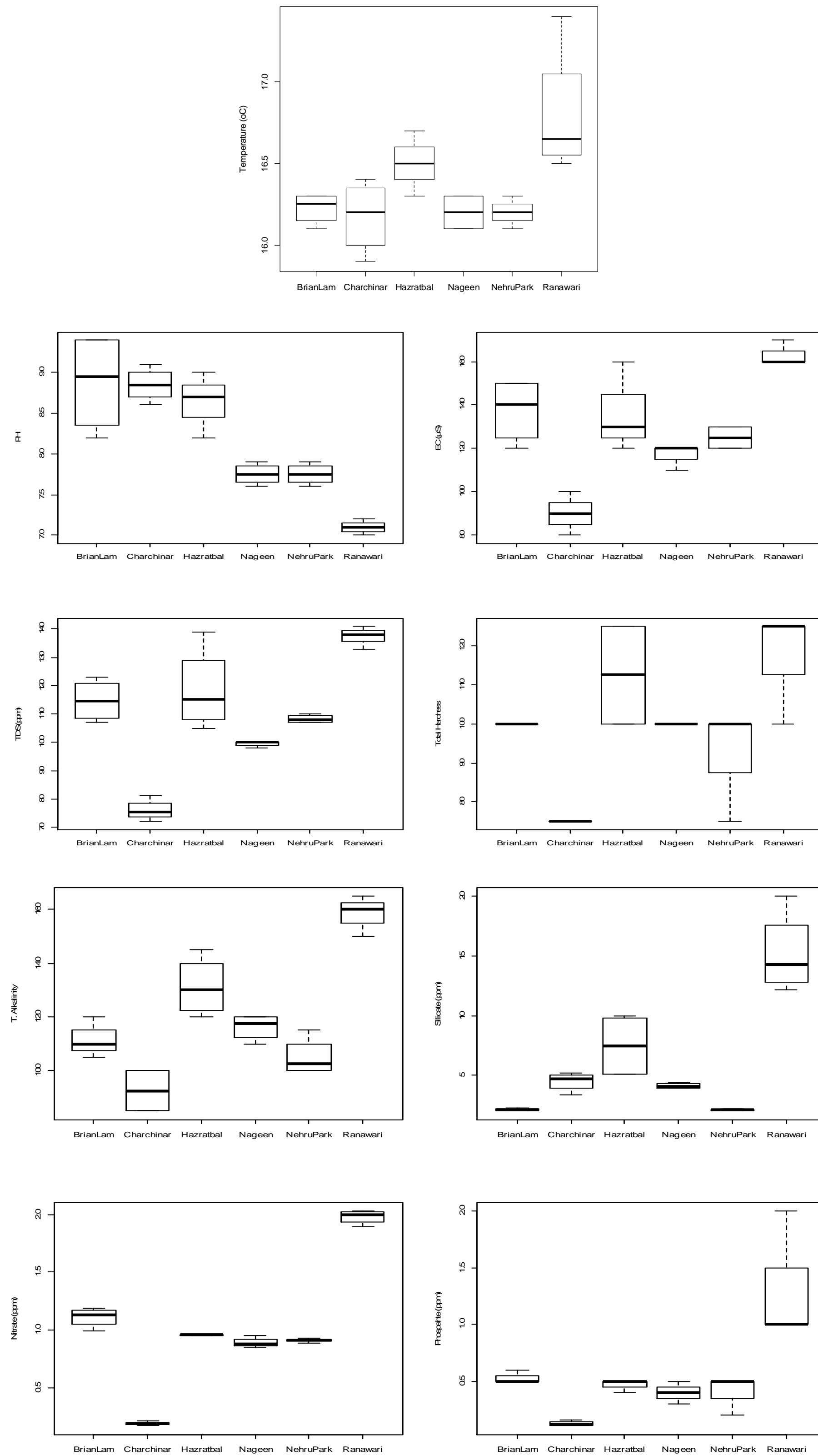



**Fig. 3 : Box-and-Whisker plots of water parameters (n=4) during spring season (April-2012)**

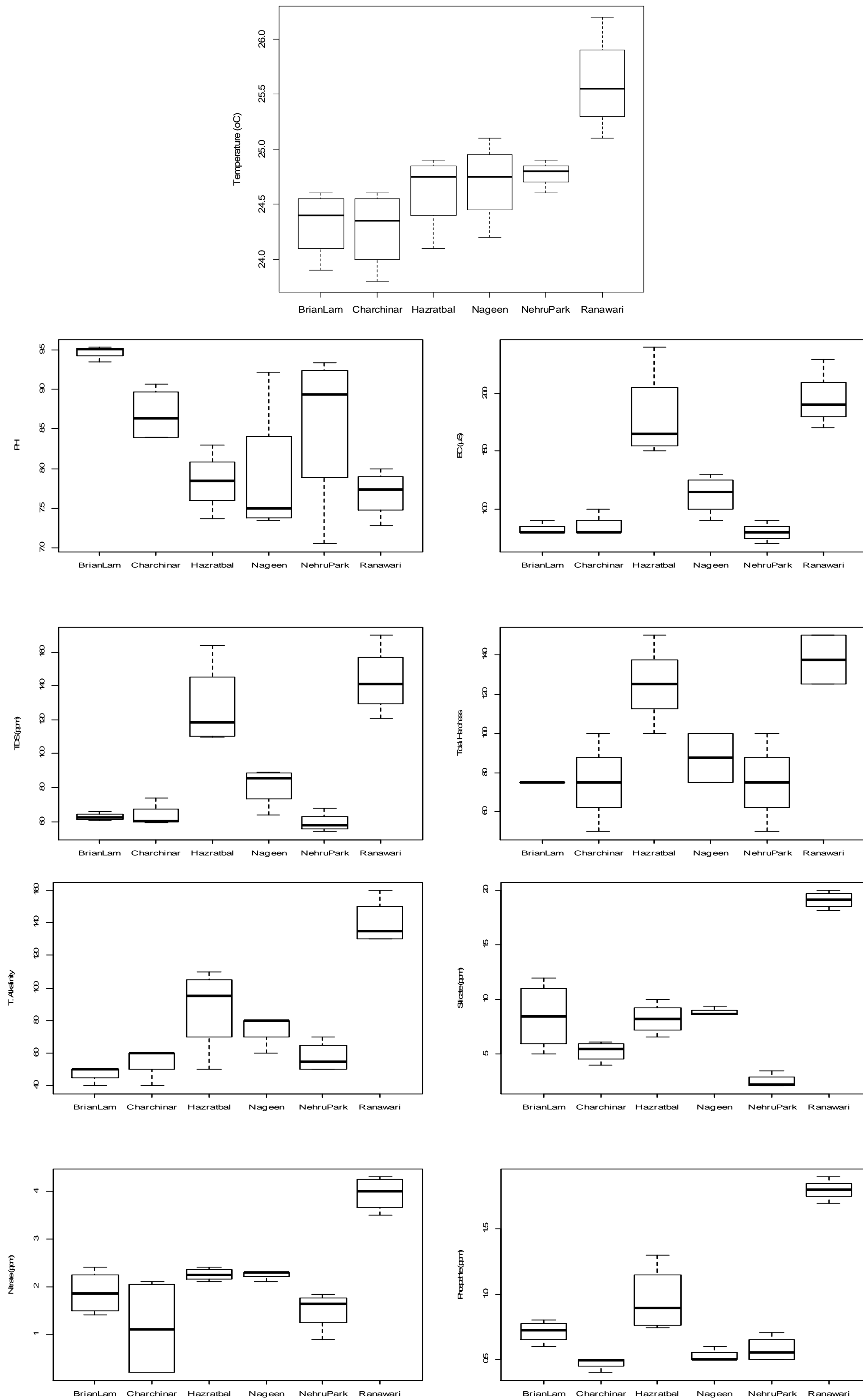



**Fig. 4 : Box-and-Whisker plots of water parameters (n=4) during summer season (July- 2012)**

#### 4.1.2 pH

The pH shows lower values of 6.96 at Ranawari site (DLS-VI) during autumn season which however exhibited increased trends of 7.1 at the same site during spring season. The data also shows that highest values of pH 9.47 were reported from Bren Laam site (DLS-II) during summer season followed by 9.28 again at Bren Laam site (DLS-II) during autumn season. In general pH values fluctuated from 7.38 to 7.97 in winter, 7.10 to 8.88 in spring, 7.69 to 9.47 in summer and 6.96 to 9.28 in autumn season in all the study sites.

#### 4.1.3 Electrical conductivity

Amongst all seasons electrical conductivity (EC) exhibited a minimum value of 76.0 µS $cm^{-1}$ in the autumn season at Char Chinar site (DLS-III) followed by 80.0 µS $cm^{-1}$ at Nehru Park site (DLS-I) in the summer season. The results also show that maximum value of 540.0 µS $cm^{-1}$ was observed at Ranawari site (DLS-VI) in winter season followed by 325.0 µS $cm^{-1}$ at Nagin site (DLS-V) during winter season. From the results it is evident that values of electrical conductivity in winter season showing lowest value of 192.5 µS $cm^{-1}$ at Nehru Park and Char Chinar sites (DLS-I, DLS-III) and exhibiting highest value of 540.0 µS $cm^{-1}$ at Ranawari site (DLS-IV). Similarly in spring, summer and autumn season the values range from 90.0 µS $cm^{-1}$ to 162.5 µS $cm^{-1}$, 80.0 µS $cm^{-1}$ to 195.0 µS $cm^{-1}$, 76.0 µS $cm^{-1}$ to 308.5 µS $cm^{-1}$ respectively.

#### 4.1.4 Total dissolved solids

Through investigation of water samples the data on the concentration of the total dissolved solids (TDS) shows that Ranawari site (DLS-VI) exhibits higher values of 275.8 ppm followed by Nagin site (DLS-V) showing concentration of 174.5 ppm during winter season. On the other hand (DLS-I) lower values of 59.3 ppm of TDS were found at Nehru Park site (DLS-I) during summer season followed by 60.0 ppm at Char Chinar site (DLS-III) during autumn season. In general TDS values fluctuated from 101.0 to 275.8 ppm in

winter, 76.0 to 137.5 ppm in spring, 59.3 to 143.3 ppm in summer and 60.0 to 143.0 ppm in autumn season in all the study sites.

### 4.1.5 Total hardness

The values for total hardness during the present study period shows greater variation in different seasons at the different sites. The water samples of Ranawari site (DLS-VI) shows high values for total hardness 181.3 ppm followed by the values of 162.5 ppm at Hazratbal and Nagin sites (DLS-IV, DLS-V) during winter season and moderate values for total hardness (68.0 ppm) were found at Charchinar site (DLS-III) during autumn season. From the results it is evident that values of total hardness in winter season showing lowest value of 87.5 ppm at Bren Laam site (DLS-II) and exhibiting highest value of 181.3 ppm at Ranawari site (DLS-VI). Similarly in spring, summer and autumn season the values range from 75.0 to 118.8 ppm, 75.0 to 137.5 ppm and 68.0 to 137.5 ppm respectively.

### 4.1.6 Total alkalinity

The results show that the maximum concentration of total alkalinity 158.8 ppm was recorded at Ranawari site (DLS-VI) in spring season followed by 157.5 ppm during winter season again at Ranawari site (DLS-VI). On the other hand the minimum concentration of 47.5 ppm was recorded in summer season at Bren Laam site (DLS-II) followed by 50.0 ppm at Char Chinar site (DLS-III) during autumn season. In general total alkalinity values fluctuated from 72.5 to 157.5 ppm in winter, 92.5 to 158.8 ppm in spring, 47.5 to 140.0 ppm in summer and 50.0 to 150.0 ppm in autumn season in all the study sites.

### 4.1.7 Silicate

During the winter season silicates ranged from 0.15 ppm at Nagin site (DLS-V) to 11.65 ppm at Ranawari site (DLS-VI). Similarly the silicates varied from 2.12 at Nehru Park site (DLS-I) to 15.7 at Ranawari site (DLS-VI) in the spring season. The highest silicate content was observed in summer season at Ranawari site (DLS-VI) exhibiting highest value of 19.12 ppm. On the other hand

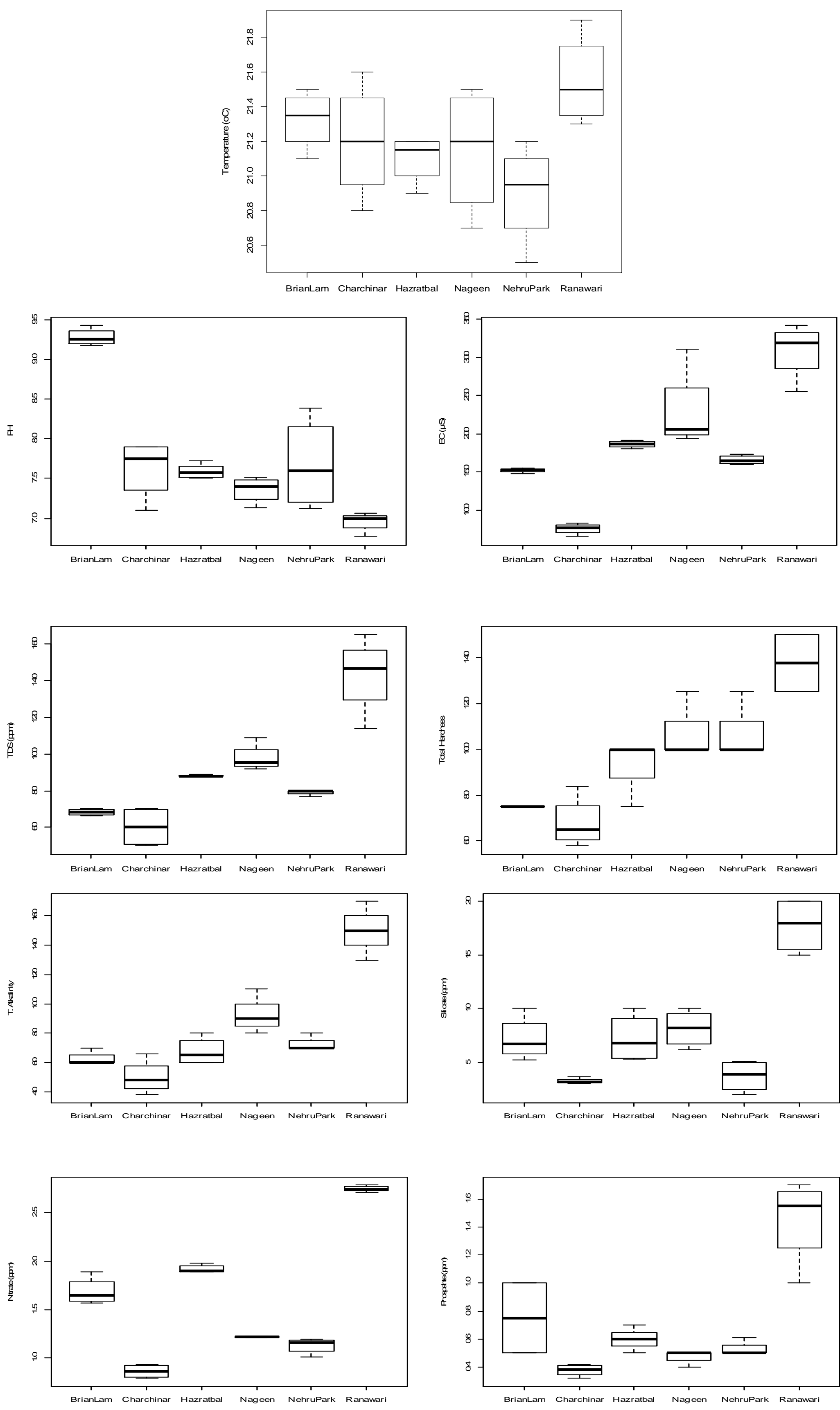



**Fig. 5: Box-and-Whisker plots of water parameters (n=4) during autumn season (October- 2012)**

silicates during the autumn season again declined with values ranging from 3.29 ppm at Char Chinar site (DLS-III) to 17.75 ppm at Ranawari site (DLS-VI). In general highest silicate content was observed in summer season at all the sites followed by autumn season while as lowest silicate was observed in winter season.

### 4.1.8 Nitrate

During the winter season nitrate ranged from 0.12 ppm at Char Chinar site (DLS-III) to 2.10 ppm at Ranawari site (DLS-VI). Similarly the nitrate concentration varied from 0.19 ppm at Char Chinar site (DLS-III) to 1.98 at Ranawari site (DLS-VI) in the spring season. The highest chemical nutrient was observed in summer season at Ranawari site (DLS-VI) exhibiting highest value of 3.95 ppm. On the other hand nitrates during the autumn season again declined with values ranging from 0.86 ppm at Char Chinar site (DLS-III) to 2.75 ppm at Ranawari site (DLS-VI). In general highest nitrate content was observed in summer season at all the sites followed by autumn season however, the nitrate showed fluctuations during winter and spring season.

### 4.1.9 Phosphate

The data on the seasonal concentration of phosphate at various sites of Dal lake ecosystem shows that the values ranged from 0.11 to 1.13 ppm at Char Chinar site (DLS-III) and Ranawari site (DLS-VI) respectively in the winter season. Similarly the phosphate varied from 0.13 ppm at Char Chinar site (DLS-III) to 1.25 at Ranawari site (DLS-VI) in the spring season. The highest phosphate content was observed in summer season at site Ranawari site (DLS-VI) exhibiting highest value of 1.80 ppm. On the other hand phosphates during the autumn season again declined except Bren Laam site (DLS-II) with values ranging from 0.38 ppm at Char Chinar site (DLS-III) to 1.45 ppm at Ranawari site (DLS-VI). In general highest phosphate was observed in summer season followed by autumn

season however the phosphate showed fluctuations during winter and spring seasons.

## 4.2 Algal Diversity : Morpho-taxonomic Description

In the present comprehensive research programme fresh water algal flora of Himalayan Dal lake ecosystem have been studied with the help of digital photo imagery and using advanced Leica software for measurements. A total of ninety six algal samples during the four seasons of the year were collected for biological studies from six different sites of Dal Lake. During the study, a total of 91 algal genera were identified comprising of 217 species, 41 varieties and 8 forma and their class-wise representation during the four seasons at six different sites of Dal Lake is presented in Table 7. The digital micro photoimagery and measurements were performed at X 630 except few species where the magnification has been mentioned.

The taxa have been arranged in various orders of each class Chlorophyceae, Cyanophyceae, Eugleanophyceae, Bacillariophyceae and Rhodophyceae. The orders of Chlorophyceae and Eugleanophyceae have been arranged after the Fritsch (1935), orders of Cyanophyceae have been arranged according to Fritsch (1945) and Desikachary (1959) and the orders of Bacillariophyceae have been arranged according to Hendey (1964). The diversity and distribution of each Class (Chlorophyceae, Cyanophyceae, Eugleanophyceae, Bacillariophyceae and Rhodophyceae) at six different sites of Dal Lake during four seasons of a year are presented in the Tables 8, 9, 10 and 11. The Chlorophycean algae of Dal Lake were best represented in summer and autumn months and lowest in the winter followed by spring season. Bacillariophyceae showed their peaks of standing crop during winter months while as Cyanophyceae showed their peak abundance during summer and autumn seasons and in case of Euglenophyceae autumn season was favourable. The species of each genus are alphabetically arranged and in some cases, only a variety or forma of a particular species was encountered. The description has been given for each taxon and is

**Table-7 : Class-wise representation of total algae during the four seasons at six different sites of Dal Lake**

| S. No. | Class | Genera | Species | Varieties | Forma |
|---|---|---|---|---|---|
| 1 | Chlorophyceae | 56 | 149 | 30 | 08 |
| 2 | Cyanophyceae | 16 | 28 | 02 | - |
| 3 | Bacillariophyceae | 14 | 30 | 05 | - |
| 4 | Eugleanophyceae | 04 | 09 | 04 | - |
| 5 | Rhodophyceae | 01 | 01 | - | - |
| | **Total** | **91** | **217** | **41** | **08** |

**Table-8 : Diversity and distribution of Chlorophyceae at six different sites of Dal Lake during four seasons of a year**

| Phytoplanktons | Winter | | | | | | Spring | | | | | | Summer | | | | | | Autumn | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI |
| *Actinastrum hantzschii* | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | + | ++ | ++ | - | ++ | - | +++ | - | + |
| *Actinastrum hantzschii* var. *fluviatile* | - | - | - | - | - | - | - | - | - | - | - | - | ++ | + | + | + | - | - | ++ | - | - | - | + | - |
| *Actinastrum lagerh* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | ++ | - | ++ | +++ | - | - | ++ | - |
| *Ankistrodesmus falcatus* | - | - | - | - | - | - | - | - | - | - | - | - | ++ | + | + | ++ | - | +++ | - | + | - | ++ | + | - |
| *Ankistrodesmus falcatus* var. *radiatus* | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | ++ | - | - | +++ | - | - | ++ | - |
| *Ankistrodesmus falcatus* var. *spirilliformis* | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | ++ | - | - | +++ | - | - | + | ++ | ++ |
| *Ankistrodesmus spiralis* | - | - | - | - | - | - | - | - | - | - | - | - | ++ | ++ | - | - | - | - | +++ | - | - | - | - | - |
| *Arthrodesmus octocornis* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | +++ | - | - | + |
| *Asterococcus limneticus* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - | - | +++ | - | - |
| *Botryococcus brauni* | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | ++ | - | - | - | - | - |
| *Bulbochaete mirabilis* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | - | - |
| *Chaetosphaeridium* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | | + |
| *Characium acuminatum* | - | - | - | - | - | - | - | ++ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Closterium acutum* | - | - | - | - | - | - | - | - | - | - | - | ++ | ++ | + | ++ | + | ++ | + | + | + | + | + | + | +++ |
| *Closterium braunii* | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | | +++ | | | - | | ++ | - | | - |
| *Closterium ehrenbergii* | - | - | - | - | - | - | - | - | - | - | - | - | - | | ++ | | + | + | - | ++ | | - | +++ | - |
| *Closterium gracile* | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | | ++ | ++ | | + | + | - | +++ |

**Contd….**

**Table-8 Contd….**

| Phytoplanktons | Winter | | | | | | Spring | | | | | | Summer | | | | | | Autumn | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI |
| *Closterium lunula* f. *biconvexum* | - | - | - | - | - | - | - | - | - | - | - | - | +++ | | ++ | - | - | - | - | ++ | - | + | - | - |
| *Closterium moniliferum* | - | - | - | - | - | - | - | - | - | - | - | - | + | ++ | - | - | - | ++ | + | - | - | - | +++ | + |
| *Closterium punctulatum* | - | - | - | - | - | - | - | - | - | - | - | - | + | + | ++ | + | ++ | ++ | ++ | - | - | ++ | - | - |
| *Closterium parvulum* | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | ++ | ++ | - | +++ | - | ++ | - | - | - |
| *Closterium venus* | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | + | + | +++ | - | ++ | ++ | + | + |
| *Coelastrum microporum* | - | - | - | - | - | - | - | - | - | - | - | - | ++ | + | - | +++ | + | ++ | + | ++ | - | ++ | + | +++ |
| *Coelastrum sphaericum* | - | - | - | - | - | - | - | - | - | - | - | - | | +++ | + | ++ | + | + | + | - | - | - | +++ | - |
| *Coenococcus planctonicus* | - | - | - | - | - | - | - | - | - | - | - | - | + | ++ | - | + | + | + | +++ | - | + | + | + | + |
| *Coenococcus polycoccus* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | +++ | - | - | - | - | + |
| *Cosmarium auriculatum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | ++ | + | + | - | + | - | ++ | - |
| *Cosmarium botrytis* | - | - | - | - | - | - | - | - | - | + | - | - | + | + | ++ | + | - | - | +++ | - | - | - | | - |
| *Cosmarium botrytis* var. *mediolaeve* | - | - | - | - | - | - | - | - | - | | + | - | - | - | - | - | ++ | - | - | - | + | - | ++ | - |
| *Cosmarium candianum* var. *depressum* | - | - | - | - | - | - | - | - | + | - | - | - | ++ | + | + | ++ | + | - | - | - | + | ++ | + | ++ |
| *Cosmarium capense* | - | - | - | - | - | - | - | - | + | - | - | - | - | + | - | + | - | + | + | + | + | + | ++ | + |
| *Cosmarium connatum* | - | - | - | - | - | - | - | - | - | - | - | + | - | ++ | + | + | - | - | - | ++ | - | + | - | |
| *Cosmarium contractum* f. *jacobsenii* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | +++ | - | - | - | - | + |
| *Cosmarium granatum* | - | - | - | - | - | - | - | - | - | + | + | + | - | - | + | ++ | - | - | ++ | - | - | - | ++ | - |
| *Cosmarium leave* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | ++ | - | - | + | + | - |
| *Cosmarium lundellii* | | | - | - | - | - | - | + | - | - | - | + | + | + | + | + | - | - | - | - | +++ | - | | ++ |
| *Cosmarium margaritatum* | - | - | - | - | - | - | - | - | - | - | + | | + | + | - | - | | - | + | + | ++ | | + | + |

Contd….

**Table-8 Contd….**

| Phytoplanktons | Winter | | | | | | Spring | | | | | | Summer | | | | | | Autumn | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI |
| *Cosmarium moniliforme* | | | | | | | + | + | | - | - | - | + | + | +++ | - | + | - | ++ | + | - | | + | |
| *Cosmarium pachydermum* | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | - | + | - | - | - | ++ | + | |
| *Cosmarium pardalis* | - | - | - | - | - | - | - | - | - | | - | - | - | - | - | - | - | - | ++ | | - | ++ | - | - |
| *Cosmarium perfissum* | - | - | - | - | - | - | - | - | - | - | - | - | + | ++ | - | + | + | - | +++ | | + | + | | - |
| *Cosmarium phaseolus* var. *omphalum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | ++ | - | ++ | - |
| *Cosmarium Polygonum* | - | - | - | - | - | - | - | - | + | - | - | - | - | + | + | + | + | + | + | + | + | - | ++ | - |
| *Cosmarium portianum* | - | - | - | - | - | - | - | - | - | - | - | + | - | - | ++ | - | - | - | ++ | - | + | - | - | - |
| *Cosmarium pseudobroomei* | - | - | - | - | - | - | - | - | - | - | - | - | - | | + | + | +++ | + | + | + | - | - | - | + |
| *Cosmarium pseudogranatum* | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | ++ | | ++ | - | - | + | + | - |
| *Cosmarium punctulatum* | - | - | - | - | - | - | - | - | + | + | + | - | - | - | - | - | - | + | + | - | +++ | - | ++ | - |
| *Cosmarium reniforme* | - | - | - | - | - | - | - | - | - | - | + | + | - | + | + | + | + | + | + | + | +++ | - | + | - |
| *Cosmarium pygmaeum* | - | - | - | - | - | - | - | - | - | - | - | - | ++ | ++ | - | - | ++ | - | - | - | ++ | - | - | - |
| *Cosmarium quadrum* | - | - | - | - | - | - | - | - | - | - | - | - | + | + | ++ | - | + | - | - | - | - | + | ++ | + |
| *Cosmarium retusiforme* | - | - | - | - | - | - | - | - | - | - | | - | - | ++ | ++ | - | ++ | - | - | - | - | - | + | + |
| *Cosmarium subgranatum* var. *borgei* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | - | - | - | + | + | ++ | + |
| *Cosmarium subimpressulum* | - | - | - | - | - | - | - | - | - | - | - | - | + | ++ | + | + | = | - | ++ | - | - | - | - | |
| *Cosmarium subtumidum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | ++ | - | - |
| *Cosmarium subundulatum* | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | ++ | - | - | +++ | - | - | - | - | |

**Contd….**

**Table-8 Contd….**

| Phytoplanktons | Winter | | | | | | Spring | | | | | | Summer | | | | | | Autumn | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI |
| *Cosmarium turpinii* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | +++ | - | + | + |
| *Cosmarium vermae* | - | - | - | - | - | - | - | - | - | ++ | - | - | + | + | + | ++ | - | - | ++ | - | + | - | - | - |
| *Crucigenia crucifera* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | + | - | - | +++ | - |
| *Crucigenia rectangularis* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | +++ | - | - | - | - | + | - | - | - | ++ |
| *Crucigenia tetrapedia* | - | - | - | - | - | - | - | - | - | - | - | - | +++ | - | - | - | - | - | | + | + | - | - | ++ |
| *Cylindrocapsa conferta* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | | - | | - | + |
| *Cylindrocapsa geminella* var. *minor* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | - | - | - |
| *Desmidium bengalicum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | - | ++ | - | +++ | - |
| *Dichotomosiphon tuberosus* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| *Dictyosphaerium pulchellum* | | | | + | | + | - | + | + | + | + | + | + | + | + | + | + | + | +++ | + | ++ | + | + | + |
| *Dictyosphaerium reniforme* f. *major* | - | - | - | - | - | - | + | + | - | - | - | +++ | + | - | + | + | ++ | + | + | + | ++ | + | + | ++ |
| *Dimorphococcus lunatus* | - | - | - | - | - | - | - | - | - | - | - | - | +++ | - | - | - | - | - | ++ | - | - | - | + | - |
| *Euastrum denticulatum* var. *rectangular* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | ++ | - | - | ++ | - | - | - |
| *Euastrum insulare* | - | - | - | - | - | - | - | - | - | - | - | - | +++ | - | + | - | + | - | - | - | - | + | - | - |
| *Euastrum interminus* var. *burmense* | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | + | - | | - | - | ++ | + | + | - |
| *Euastrum spinulosum* | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | | - | +++ | - | - | - | ++ | - |
| *Euastrum sublobatum* var. *sumatranum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | +++ | + | + | - | - | - | - | - |
| *Eudorina elegans* | - | - | - | - | - | - | - | - | - | - | - | - | | + | + | +++ | + | - | + | + | + | - | +++ | + |

**Contd….**

**Table-8 Contd….**

| Phytoplanktons | Winter | | | | | | Spring | | | | | | Summer | | | | | | Autumn | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI |
| *Gloeotaenium loitlesbergerianum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | - | - | - | - | - | - | ++ | + |
| *Golenkinia radiata* | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | ++ | - | - | - | - | - | |
| *Gonium pectorale* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | ++ |
| *Hydrodictylon reticulatum* | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | + | ++ | - | - | +++ | - | - | | + | + |
| *Kirchneriella contorta* var. *elegans:* | - | - | - | - | - | - | - | - | - | - | - | - | +++ | - | - | ++ | - | - | - | - | ++ | ++ | - | - |
| *Kirchneriella lunaris* | - | - | - | - | - | - | - | - | - | - | - | - | | - | ++ | - | - | - | +++ | - | - | - | - | + |
| *Lagerheimia wratislawiensis* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - |
| *Micractinium pusillum* | - | - | - | - | - | - | - | - | - | - | - | - | + | - | | - | - | ++ | - | - | - | - | + | + |
| *Micractinium pusillum* var. *elegans* | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | + | - | - | - | ++ | - | + |
| *Micrasterias pinnatifida* | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | + | ++ | - | - | + | - | + |
| *Micrasterias radians* | - | - | - | - | - | - | - | - | - | - | - | - | | - | - | + | - | - | ++ | - | - | - | + | + |
| *Monoraphidium griffithii* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | ++ | - | - | - | + | - |
| *Nephrocytium agardhianum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | - | - | - | - | ++ | + |
| *Nephrocytium lunatum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - |
| *Oedeogonium* species | + | - | - | - | - | - | +++ | - | - | - | - | - | - | +++ | - | +++ | +++ | - | - | - | - | - | - | - |
| *Oedeogonium Intermedium* | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | + | + | - | - | ++ | - | - | + | - | + |
| *Oedeogonium undulatum* f. *senegalense* | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - |
| *Oocystis elliptica* | - | - | - | - | - | - | - | - | - | - | - | + | + | ++ | - | - | ++ | - | ++ | | + | - | ++ | - |

**Contd….**

**Table-8 Contd….**

| Phytoplanktons | Winter | | | | | | Spring | | | | | | Summer | | | | | | Autumn | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI |
| *Oocystis gigas* | - | - | - | - | - | - | - | - | - | - | + | - | + | + | - | - | - | - | ++ | + | + | - | - | - |
| *Oocystis lacustris* | - | - | - | - | - | - | - | - | - | - | + | - | ++ | | - | - | - | - | - | + | + | - | - | - |
| *Oocystis solitaria* | - | - | - | - | - | - | - | - | - | - | ++ | - | + | + | - | - | - | - | - | + | + | - | - | - |
| *Palmellococcus saccharophilus* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | + | - | + | - |
| *Pandorina morum* | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | ++ | - | + | - | + | + | + | - | ++ | + |
| *Pediastrum angulosum* | - | - | - | - | - | - | - | + | - | - | + | - | - | ++ | - | - | | - | + | - | - | - | - | +++ |
| *Pediastrum boryanum* var. *longicorne* | - | - | - | - | - | - | - | + | - | - | - | - | | ++ | + | + | + | + | ++ | - | - | - | + | - |
| *Pediastrum duplex* var. *clathratum* | - | - | - | - | - | - | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | +++ | + |
| *Pediastrum duplex* var. *reticulatum* | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | | +++ | - | - | - | + | + |
| *Pediastrum tetras* | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | + | + | + | - | - | + | + | + | +++ | - |
| *Pediastrum tetras* var. *tetraodon* | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | ++ | - | +++ | - | - | - | ++ | - | - |
| *Penium margaritaceum* | - | - | - | - | | | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - |
| *Planktosphaeria gelatinosa* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | | + | - | - | ++ | ++ | - |
| *Pleurotaenium ehrenbergii* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| *Polyedriopsis spinulosa* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | + | - | - | - | - | - | - |
| *Rhizoclonium hieroglyphicum* | | + | - | - | - | - | + | + | +++ | - | - | - | + | + | + | + | + | + | | +++ | - | - | - | - |
| *Spirogyra* species | | + | | +++ | | | + | | + | - | - | - | + | | + | + | + | | +++ | - | - | - | - | - |

**Contd….**

**Table-8 Contd….**

| Phytoplanktons | Winter | | | | | | Spring | | | | | | Summer | | | | | | Autumn | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI |
| *Scenedesmus armatus* var. *boglariensis* f. *bicaudatus* | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - |
| *Scenedesmus abundans* | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | - | - | - | ++ | - | - | - | ++ | - |
| *Scenedesmus abundans* var. *longicauda* | - | - | - | - | - | - | - | ++ | - | - | - | - | - | - | - | - | ++ | - | - | - | + | - | - | - |
| *Scenedesmus acutiformis* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | + | + | ++ | - | - | + |
| *Scenedesmus arcuatus* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | + | + | + | - | - | - | ++ | - |
| *Scenedesmus armatus* var *dispar* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | - | ++ | - | - |
| *Scenedesmus armatus* var. *bicaudatus* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | ++ | - | - |
| *Scenedesmus bijugatus* var. *graevenitzii* | - | - | - | - | - | - | - | - | - | + | + | + | + | + | + | - | - | - | - | + | + | + | ++ | + |
| *Scenedesmus cumbricus* | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | - | - | - | - | - | - | - |
| *Scenedesmus denticulatus* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | + | + |
| *Scenedesmus denticulatus* var. *australis* | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | + | - | - | - | + |
| *Scenedesmus dimorphus* | - | - | - | - | - | - | - | - | - | - | - | + | + | + | ++ | +++ | - | + | ++ | - | +++ | ++ | + | ++ |
| *Scenedesmus obliquus* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | ++ | - | - | - | - | - |
| *Scenedesmus opoliensis* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | | |
| *Scenedesmus perforates* | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Scenedesmus quadricauda* | - | - | - | - | - | - | - | - | + | - | + | + | - | ++ | - | +++ | - | ++ | + | + | ++ | ++ | ++ | +++ |

**Contd….**

**Table-8 Contd….**

| Phytoplanktons | Winter | | | | | | Spring | | | | | | Summer | | | | | | Autumn | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI |
| *Scenedesmus quadricauda* var. *longispina* | - | - | - | - | - | - | - | - | - | + | - | + | + | - | - | - | - | - | ++ | - | - | - | - | - |
| *Scenedesmus quadricauda* var. *quadrispina* | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | - | ++ | - | - | + | ++ | - | ++ |
| *Scenedesmus quadricauda* var. *westii* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | ++ | - | - | - | +++ | + | ++ |
| *Scenedesmus serratus* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - |
| *Selenastrum gracile*: | - | - | - | - | - | - | - | - | - | - | - | - | - | + | ++ | + | - | - | + | - | - | - | - | ++ |
| *Sphaerozosma filiforme* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - |
| *Spondylosium nitens*. var. *triangular* f. *javanicum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - |
| *Spondylosium planum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | ++ | |
| *Staurastrum* species | - | - | - | - | - | - | - | - | - | - | | | + | ++ | + | - | - | - | - | - | - | - | - | - |
| *Staturastrum pseudotetracerum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | + | ++ | + | + |
| *Staurastrum arachne* var. *sumatranum* | - | - | - | - | - | - | - | - | - | - | + | + | ++ | + | + | - | - | + | - | - | - | + | - | + |
| *Staurastrum cuspidatum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | - | + | + |
| *Staurastrum dickiei* var. *circulare* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | - | ++ |
| *Staurastrum furcatum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | ++ | - | - | ++ |
| *Staurastrum gemelliparum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | + | - | - | - |
| *Staurastrum gladiosum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | + | - | - | - |

**Contd….**

**Table-8 Contd….**

| Phytoplanktons | Winter | | | | | | Spring | | | | | | Summer | | | | | | Autumn | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI |
| *Staurastrum gracile* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | ++ | + | + | ++ | - | ++ | - | ++ | - |
| *Staurastrum gracile* f. *iyengar* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | ++ | - | - | - | - | - |
| *Staurastrum granulosum* | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | + | + | ++ | - | - | - | ++ | - | + |
| *Staurastrum hexacerum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | ++ | | + | ++ |
| *Staurastrum kalapanii* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - |
| *Staurastrum longibrachiatum* var. *intermedium* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | + | - | + | - |
| *Staurastrum pachyrhynchum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - | ++ | - | - | - |
| *Staurastrum punctulatum* | - | - | - | - | - | - | - | - | - | - | - | - | ++ | + | - | + | ++ | ++ | - | - | - | - | - | - |
| *Staurastrum retusum* | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | ++ | ++ | + | - | - | - | - | - | ++ |
| *Staurastrum rhynchoceps* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | ++ | - | - | - | - | - |
| *Staurastrum setigerum* | - | - | - | - | - | - | - | - | + | + | - | - | - | - | + | - | ++ | - | - | - | - | + | - | - |
| *Staurastrum tetracerum* | - | - | - | - | - | - | - | - | - | - | - | + | - | - | + | - | ++ | - | - | - | + | - | - | - |
| *Staurodesmus* species | - | - | - | - | - | - | - | - | - | - | - | + | - | - | + | - | ++ | - | - | - | - | - | - | - |
| *Staurodesmus cuspidatus* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - | +++ | - | - | |
| *Tetraedron gracile* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | | - | - | ++ | - | - | + |
| *Tetraedron minimum* f. apiculatum | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | + | + | - | - | ++ | - | - | + |
| *Tetraedron muticum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | +++ | | - | - | - | + | - |
| *Tetraedron trigonum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | | +++ | | - | - | - | - | - |

**Contd….**

**Table-8 Contd….**

| Phytoplanktons | Winter | | | | | | Spring | | | | | | Summer | | | | | | Autumn | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **I** | **II** | **III** | **IV** | **V** | **VI** | **I** | **II** | **III** | **IV** | **V** | **VI** | **I** | **II** | **III** | **IV** | **V** | **VI** | **I** | **II** | **III** | **IV** | **V** | **VI** |
| *Tetraedron tumidulum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | | +++ | - | - | - | - | - |
| *Tetrallantos lagerheimii* | - | - | - | - | - | - | - | - | - | - | - | - | | - | - | | | ++ | | - | - | - | - | - |
| *Tetraspora lamellose* | - | - | - | - | - | - | - | - | - | - | - | - | +++ | - | - | - | - | | - | - | | - | - | - |
| *Tetraspora lubrica* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | - | - | +++ | - | - | - |
| *Tetrastrum heteracanthum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | - |
| *Trochiscia aciculifera* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | | - | - |
| *Ulothrix aequalis* | - | - | - | - | - | - | - | - | - | - | - | - | +++ | | - | - | | | - | | - | | - | - |
| *Uronema confervicolum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | +++ | | - | | - | | | |
| *Xanthidium acanthophorum* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | + | + | +++ | |
| *Xanthidium antilopaeum* var. *polymazum* | - | - | - | - | - | - | - | - | - | - | +++ | - | - | + | + | - | - | - | - | - | - | - | - | + |
| *Zygnema* species | - | - | - | - | - | - | + | | + | | ++ | - | - | - | ++ | - | - | - | +++ | - | - | - | - | - |
| *Zygnema collinsianum* | - | - | - | - | - | - | | | + | + | | - | - | - | - | - | - | - | +++ | - | - | - | - | - |

**Abbreviations:** Where I, II, III, IV, V and VI are Dal Lake Sites: DLS-I (Nehru Park), DLS-II (Bren Laam), DLS-III (Char Chinar), DLS-IV (Hazratbal), DLS-V (Nagin) and DLS-VI (Ranawari). **Frequency status:** (-, **0%**) Absent; (+, **< 20%)** Present and Rare; (++, **>20-50%)** Common; (+++, **>50%**) Dominant; (++++, **100%**) Abundant.

## Table-9 : Diversity and distribution of Cyanophyceae at six different sites of Dal Lake during four seasons of a year

| Phytoplanktons | Winter | | | | | | Spring | | | | | | Summer | | | | | | Autumn | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI |
| *Anabaena circinalis* | - | - | - | - | - | - | - | - | - | - | - | - | - | +++ | - | - | ++ | +++ | + | +++ | ++ | ++ | - | ++ |
| *Anabaena doliolum* | - | - | - | - | - | - | - | - | - | - | - | - | | ++ | - | - | + | ++ | + | | - | - | - | +++ |
| *Aphanizomenon flos-aquae* | - | - | - | - | - | - | - | - | - | - | - | - | + | +++ | - | - | - | - | - | - | - | - | - | - |
| *Arthrospira jenneri* | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | + | ++ | ++ | - | - | - | + | +++ |
| *Chroococcus minutes* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | | - | - | - | - | - | - | - |
| *Chroococcus schizodermaticus* | - | - | - | - | - | - | - | - | - | - | - | - | | - | ++ | - | - | - | - | - | +++ | - | - | ++ |
| *Chroococcus tenax* | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | +++ | - | - | - |
| *Chroococcus turgidus* | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | ++ | - | ++ | +++ | - | - | + | - | - |
| *Chroococcus turgidus* var. *maximus* | - | - | - | - | - | - | - | - | - | - | - | - | + | ++ | - | - | - | - | +++ | - | - | - | - | ++ |
| *Coelosphaerium collinsii* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | +++ | - | - | - | - | - |
| *Dactylococcopsis acicularis* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | + | - | - | - | - | +++ | - |
| *Gloeocystis ampla* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | +++ | - |
| *Gloeothece rupestris* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | +++ | - |
| *Gloeothece samoensis* var. *major* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | + | - | - | - | - | +++ | + |
| *Gomphosphaeria naegeliana* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | +++ | - | - | - | - | + |

**Contd…..**

**Table-9 Contd….**

| Phytoplanktons | Winter | | | | | | Spring | | | | | | Summer | | | | | | Autumn | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI |
| *Gomphosphaeria* species | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | +++ | +++ |
| *Lyngbya birgei* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | +++ | - | + | - | - | - |
| *Lyngbya contorta* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | + | - | +++ |
| *Merismopedia glauca* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | +++ | - | - | - | - | - |
| *Microcystis aeruginosa* | - | - | - | - | - | - | - | - | - | - | - | - | ++ | ++++ | ++ | - | - | - | - | ++++ | ++ | | - | ++ |
| *Oscillatoria curviceps* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | + | | ++ | ++ | + | - | ++ | | +++ |
| *Oscillatoria chalybea* | + | - | - | + | | + | - | - | - | - | - | + | - | - | + | + | + | ++ | + | + | - | ++ | + | +++ |
| *Oscillatoria granulata* | - | - | - | - | - | - | - | - | - | + | - | ++ | - | - | + | ++ | + | ++ | ++ | + | - | ++ | + | +++ |
| *Oscillatoria chlorina* | + | - | - | + | | + | - | - | - | - | - | ++ | - | - | + | ++ | + | ++ | ++ | + | - | ++ | + | +++ |
| *Oscillatoria limosa* | - | - | - | - | - | - | - | - | - | + | - | - | - | - | + | | + | +++ | ++ | - | - | ++ | | +++ |
| *Oscillatoria irrigua* | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | + | ++ | + | - | - | ++ | + | +++ |
| *Oscillatoria formosa* | + | - | - | + | | + | - | - | - | - | - | + | - | - | ++ | | + | ++ | ++ | + | - | ++ | +++ | - |
| *Phormidium purpurascens* | - | - | - | - | - | - | - | - | - | + | - | - | +++ | - | - | - | - | ++ | +++ | ++ | - | ++ | ++ | +++ |
| *Spirulina meneghiniana* | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | ++ | - | - | - | ++ | - | +++ |
| *Trichodesmium lacustre* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | - |

**Table-10 : Diversity and distribution of Eugleanophyceae at six different sites of Dal Lake during four seasons of a year**

| Phytoplanktons | Winter | | | | | | Spring | | | | | | Summer | | | | | | Autumn | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI |
| *Euglena acus* | - | - | - | - | - | - | - | + | - | + | - | - | + | - | - | - | ++ | +++ | - | - | - | + | ++ | +++ |
| *Euglena acus* var. *rigida* | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | ++ | ++ | ++ | - | - | - | ++ | +++ |
| *Euglena deses* | - | - | - | - | - | - | - | - | - | + | - | - | + | - | - | - | + | +++ | - | - | - | +++ | ++ | +++ |
| *Euglena proxima* | - | - | - | - | - | - | - | - | - | + | - | - | + | - | - | - | ++ | + | ++ | - | - | +++ | ++ | ++ |
| *Euglena spirogyra* | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | ++ | - | - | - | +++ | ++ | ++ |
| *Lepocinclis fusiformis* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - | - | + | + |
| *Lepocinclis fusiformis* var. *minor* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - | - | + | + |
| *Phacus acuminatus* | - | - | - | - | - | - | - | - | - | + | - | - | + | - | - | + | +++ | + | - | - | | + | - | +++ |
| *Phacus anacoelus* | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | + | ++ | - | - | - | - | + | + | +++ |
| *Phacus anacoelus* var. *undulata* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | ++ | + | - | - | - | ++ | - | +++ |
| *Phacus longicauda* | - | - | - | - | - | - | - | - | - | + | - | - | ++ | - | - | + | ++ | +++ | ++ | - | - | + | - | +++ |
| *Trachelomonas hispida* var. *coronate* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | + | - | + |

**Table-11 : Diversity and distribution of Bacillariophyceae and Rhodophyceae at six different sites of Dal Lake during four seasons of a year**

| Phytoplanktons | Winter | | | | | | Spring | | | | | | Summer | | | | | | Autumn | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI | I | II | III | IV | V | VI |
| *Achnanthes coarctata* var. *parallela* | + | + | ++ | + | ++ | + | - | - | - | + | - | - | - | - | ++ | + | + | - | - | - | - | ++ | - | - |
| *Amphora coffeoeformis* var. *africana* | ++ | + | + | + | ++ | ++ | - | - | - | + | - | - | - | - | - | - | - | - | | ++ | - | - | - | - |
| *Amphora* species | + | ++ | ++ | + | +++ | ++ | - | - | - | + | ++ | - | - | - | + | + | - | - | - | - | - | - | ++ | - |
| *Caloneis silicula* | + | + | ++ | + | + | ++ | - | - | - | + | ++ | - | - | - | - | - | + | - | - | ++ | - | - | - | - |
| *Cocconeis placentula* | ++ | + | ++ | ++ | + | ++ | - | - | - | - | - | - | + | ++ | - | - | - | - | - | ++ | - | - | - | - |
| *Cyclotella meneghiniana* | + | ++ | + | + | ++ | ++ | - | - | - | - | ++ | - | - | - | - | - | - | ++ | - | - | - | - | - | ++ |
| *Cymbella affinis* | + | + | ++ | + | +++ | ++ | - | - | - | ++ | - | + | - | - | - | - | - | - | - | - | - | - | ++ | - |
| *Cymbella aspera* | + | ++ | + | +++ | ++ | + | - | - | - | ++ | - | + | - | - | + | - | + | - | - | - | - | ++ | - | - |
| *Cymbella lanceolata* | + | + | + | +++ | + | ++ | - | - | - | + | ++ | + | + | - | ++ | - | - | - | - | - | +++ | - | - | - |
| *Cymbella parva* | +++ | ++ | ++ | ++ | ++ | ++ | - | - | - | + | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - |
| *Cymbella tumida* | ++ | ++ | +++ | ++ | +++ | ++ | - | - | - | + | - | + | - | + | ++ | - | - | - | - | - | ++ | - | - | - |
| *Eunotia monodon* | ++ | + | + | + | ++ | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | ++ | - |
| *Eunotia parallela* | ++ | + | + | + | ++ | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ |
| *Fragilaria biceps* | + | ++ | + | + | +++ | ++ | - | - | - | - | ++ | + | - | - | - | - | + | - | - | - | - | - | ++ | - |
| *Fragilaria capucina* | + | ++ | ++ | + | ++ | ++ | | - | - | ++ | - | + | ++ | - | + | - | - | - | ++ | - | - | - | - | - |
| *Fragilaria crotonensis* | ++ | ++ | + | + | ++ | ++ | - | - | - | ++ | + | + | - | - | - | - | - | - | ++ | - | - | - | - | - |
| *Fragilaria intermedia* var. *robusta* | ++ | + | + | + | ++ | + | - | - | - | ++ | - | + | - | - | - | - | - | - | - | - | - | ++ | - | - |
| *Gamphonema acuminatum* | + | + | +++ | + | +++ | ++ | + | - | ++ | ++ | - | + | +++ | + | - | - | ++ | - | - | - | - | ++ | - | - |
| *Gamphonema constrictum* | + | + | +++ | +++ | +++ | ++ | + | - | ++ | ++ | - | + | +++ | + | - | - | ++ | - | - | - | ++ | - | - | - |

**Contd….**

**Table-11 Contd…**

| Phytoplanktons | Winter | | | | | | Spring | | | | | | Summer | | | | | | Autumn | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **I** | **II** | **III** | **IV** | **V** | **VI** | **I** | **II** | **III** | **IV** | **V** | **VI** | **I** | **II** | **III** | **IV** | **V** | **VI** | **I** | **II** | **III** | **IV** | **V** | **VI** |
| *Gamphonema telegraphicum* | ++ | + | +++ | + | +++ | ++ | + | - | + | + | ++ | + | - | - | - | - | - | - | - | - | - | - | - | ++ |
| *Gamphonema truncatum* | + | + | +++ | ++ | ++ | ++ | + | - | + | - | - | + | +++ | ++ | - | - | - | - | - | ++ | - | - | - | - |
| *Hantzschia* species | + | + | ++ | +++ | + | + | + | - | - | + | ++ | + | - | - | - | - | - | - | - | - | - | - | ++ | - |
| *Navicula cryptocephaloides* | +++ | ++ | ++ | ++ | ++ | ++ | + | - | - | - | + | + | - | - | - | - | - | - | ++ | - | - | - | - | - |
| *Navicula radiosa* | +++ | + | + | + | +++ | ++ | + | - | - | - | + | ++ | - | - | - | - | +++ | - | ++ | - | - | - | - | - |
| *Navicula viridis* | +++ | - | +++ | + | ++ | ++ | + | - | - | - | - | ++ | - | - | - | - | +++ | - | - | - | - | - | - | +++ |
| *Nitzschia acicularis* | +++ | - | +++ | - | +++ | + | + | - | - | + | + | - | ++ | - | - | - | +++ | - | - | - | - | - | - | ++ |
| *Rhopalodia gibba* | + | - | +++ | + | ++ | - | - | - | - | ++ | + | - | - | - | - | - | - | - | - | - | ++ | - | - | - |
| *Synedra capitata* | + | + | + | - | + | + | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | - | - | - | - |
| *Synedra delicatissima* | + | ++ | + | - | ++ | + | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | - | - | - |
| *Synedra dorsiventralis* | ++ | + | + | - | ++ | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - |
| *Synedra tenera* | ++ | ++ | - | ++ | ++ | - | - | - | - | - | - | - | - | - | - | + | + | - | | | ++ | - | - | - |
| *Synedra ulna* | ++ | ++ | - | - | ++ | + | - | - | - | + | + | - | - | - | - | + | + | - | ++ | - | - | - | - | - |
| *Synedra ulna* var. *contracta* | - | +++ | - | - | ++ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | |
| *Synedra ulna* var. *amphirhynchus* | + | - | + | - | ++ | - | - | - | - | + | - | - | ++ | - | - | - | - | - | - | - | - | - | - | - |
| **Rhodophyceae** | | | | | | | | | | | | | | | | | | | | | | | | |
| *Glaucosphaera vacuolata* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - |

**Abbreviations:** Where I, II, III, IV, V and VI are Dal Lake Sites: DLS-I (Nehru Park), DLS-II (Bren Laam), DLS-III (Char Chinar), DLS-IV (Hazratbal), DLS-V (Nagin) and DLS-VI (Ranawari). **Frequency status:** (-, **0%**) Absent; (+, **< 20%)** Present and Rare; (++, **>20-50%)** Common; (+++, **>50%**) Dominant; (++++, **100%**) Abundant

followed by list of references (for comparison), collection number and accession number.

### 4.2.1 *Chlorophyceae*

Chlorophyceae (green algae) constitute one of the major groups of algae occurring in various habitats. The plants are unicellular to multicellular, colonial or filamentous, free floating or attached and usually contain one or more chromatophores that are highly variable among different species. Chromatophores are single to numerous in each cell with axial or parietal in position. They may be reticulate, cup shaped, discoid, stellate and laminate or having a variety of other shapes.

**Genus*: Pandorina* Bory, 1824**

Order: *Chlamydomonadales*
Family: *Volvocaceae*
Genus: *Pandorina*
Species: *morum*

***Pandorina morum* (Muell.) Bory**

**Plate - 26c**

Colony ovate or obovoid and motile, composed of 8-16-(32) globose or pyriform cells. Cells are mutually compressed and enclosed in a prominent gelatinous envelope. Cells are with the broad anterior end directed outwards. The cells have a parietal cup shaped chloroplast with one basal pyrenoid, pigment-spots anterior and lateral. Each cell has two flagella arising from the anterior end of the cell and diverging widely after emerging from the colonial envelope. The colony swims in a rolling or tumbling fashion. Cells are 17.36 µm long and 15.05 µm in diameter, colony 73.70 µm diameter.

Prescott, G.W. 1951, p. 75, pl. 1, Fig. 23.
Collection No: DLS201290, Accession No: 02016

### Genus: *Eudorina* Ehrenberg 1832b*:*

Order: *Chlamydomonadales*
Family: *Volvocaceae*
Genus: *Eudorina*
Species: *elegans*

***Eudorina elegans*** **Ehrenberg**

**Plate - 26d**

A free swimming ovate, obovoid, or globose colony in which 16-32-64 ovoid or ovate cells are inclosed within a gelatinous envelope. Cells sometimes arranged in transverse series, sometimes evenly disposed throughout the colonial mucilage. The two long flagella are present which diverge widely beyond the periphery of the colonial envelope. Cells are often with one or two anterior beaks or papillae where the flagella arise. There are two minute contractile vacuoles at the base of each flagellum. The chloroplast is cup-shaped and parietal with one to several pyrenoids. Red pigment-spot is laterally placed at the anterior end of the cell. Cytoplasmic strands connecting the cells are sometimes visible. Cells are 13.30 µm in diameter and colony 85.33 µm diameter.

Prescott, G.W. 1951, p. 76, pl. 1, Figs. 24-26.

Collection No: DLS201290, Accession No: 02016

### Genus: *Asterococcus* Scherffel, 1908*:*

Order: *Chlamydomonadales*
Family: *Palmellopsidaceae*
Genus: *Asterococcus*
Species: *limneticus*

***Asterococcus limneticus*** **G. M. Smith**

**Plate - 17i**

Cells spherical, arranged at some distance from one another in free-floating colonies of 4-16 within a colourless homogenous investing mucilage. Each cell

has a stellate chloroplast with 4-16 lobes radiating from a central core. The lobes become flattened against the cell wall. Cells are 23.92 µm in diameter and colonies 119. 60 µm in diameter.

Prescott, G.W. 1951, p. 86, pl. 4, Fig. 11.

Collection No: DLS201285, Accession No: 02011

**Genus: *Gonium* Mueller, 1773**

Order: *Chlamydomonadales*
Family: *Goniaceae*
Genus: *Gonium*
Species: *pectorale*

***Gonium pectorale* Mueller**

**Plate - 26f**

This green alga lives in colonies of 4-8-16 ellipsoid, sub spherical or sometimes ovoid cells closely arranged in a flat, quadrangular plate, usually with four inner cells bordered by a series of twelve marginal ones which have their anterior ends projected outward and parallel with the plane of the colony. The inner cells are directed at right angles to the plane. Cells are inclosed by individual sheaths, which are connected to neighbouring sheaths by very short processes. Cells are 6.65 µm in diameter and colony 64.55 µm in diameter.

Prescott, G.W. 1951, p. 75, pl. 1, Fig. 21.
Collection No: DLS201294, Accession No: 02020

**Genus: *Cylindrocapsa* Reinsch, 1867**

Order: *Chlamydomonadales*
Family: *Cylindrocapsaceae*
Genus: *Cylindrocapsa*
Species: *conferta, geminella* var. *Minor*

***Cylindrocapsa conferta* W. West 1892**

**Plate - 21a**

Short unbranched filaments of oblong, ovoid or quadrate cells, uniserate (rarely biseriate or palmelloid) in arrangement and inclosed by a wide, tough gelatinous sheath with distinct lamellations about the individual cells. Chloroplast (one to each cell) a massive, dense body containing a central pyrenoid. Filaments attached when young by the adherence of the mucilaginous tube to the substrate. Cells short, quadrangular-ovate, enclosed by a wide sheath of lamellate mucilage. Cells are 17.16 µm long and 16.73 µm in diameter.

Prescott, G.W. 1951, p. 110, pl. 9, Figs. 5, 6.

Collection No: DLS201272, Accession No: 001998

***Cylindrocapsa geminella* var. *minor* Hansgirg 1888**

**Plate – 21b**

Unlike the above species *C. geminella* var. *minor* has narrower and ovate or ellipsoid cells. The filaments are sometimes twisted and contorted. Cells are 19.08 µm long and 18.17 µm in diameter with sheath.

Prescott, G.W. 1951, p. 110, pl. 9, Figs. 1, 2.

Collection No: DLS201255, Accession No: 001981

**Genus: *Dimorphococcus* A. Braun, 1855**

Order: *Chlorococcales*

Family: *Dictyosphaeriaceae*

Genus: *Dimorphococcus*

Species: *lunatus*

***Dimorphococcus lunatus* A. Braun**

**Plate – 42h**

Colonies are irregular and free-floating. Cells are in groups of four and arranged

alternatively in a zigzag fashion. Outer cells of each group reniform or somewhat crescent-shaped, inner cells elongate-ovoid to ellipsoid. The ends of cells are rounded. Cells are 10.07 µm long, 5.91 µm in diameter and colonies up to 82.66 µm in diameter.

Phillipose, M.T. 1967, p. 205, Figs. 115 a, b.

Collection No: DLS201203, Accession No: 001928

**Genus: *Dictyosphaerium* Naegeli, 1849**

Order: *Chlorococcales*
Family: *Dictyosphaeriaceae*
Genus: *Dictyosphaerium*
Species: *pulchellum*, *reniforme* f. *major*

***Dictyosphaerium pulchellum* Wood**

**Plate – 29e, g**

Colonies are free floating, nearly spherical and of 4-64 or more cells. The colonies are enclosed within a hyaline homogenous gelatinous envelope. Cells spherical to ovoid, with a single parietal cup-shaped chloroplast having a single pyrenoid. Daughter colonies are formed by the fragmentation of larger colonies. Cells are 8.22 µm in diameter and colonies up to 69.26 µm in diameter.

Phillipose, M.T. 1967, p. 200, Figs. 110 a, b.

Collection No: DLS201275, Accession No: 02001

***Dictyosphaerium reniforme* f. *major***

**Plate – 29f**

The mature cells of this species are kidney shaped and grouped in a bundle, the cells being held together by slender brown filament. Cells are 49.62 µm long and 12.93 µm in diameter.

Kant, S. and Gupta, P. 1998, p. 88, pl. 21, 91. Figs. 5, 12.

Collection No: DLS201244, Accession No: 001970

**Genus: *Characium* A. Braun in Kuetzing, 1849**

Order: *Chlorococcales*
Family: *Characiaceae*
Genus: *Characium*
Species: *acuminatum*

***Characium acuminatum* A. Braun**

**Plate – 21g**

Cells are oblong, narrowed anteriorly to form a short apiculation, acuminate; stipe short and attached to filamentous algae. Cell is 26.51 µm long and 14.50 µm in diameter.

Prescott, G.W. 1951, p. 216, pl. 46, Fig. 7.

Collection No: DLS201229, Accession No: 001955

**Genus: *Polyedriopsis* Schmidle, 1900a**

Order: *Chlorococcales*
Family: *Hydrodictyaceae*
Genus *Polyedriopsis*
Species: *spinulosa*

***Polyedriopsis spinulosa* Schmidle**

**Plate – 27e**

It is a unicellular, free-floating, tetragonal or pyramidal green algae. The angles are truncately rounded and furnished with a tuft of 3-10 long tapering setae. The sides are concave and the chloroplast is like a parietal plate covering most of the cell wall (more massive in old cells) with one pyrenoid. The cell is 13.39 µm in diameter and with 39.07 µm long setae.

Prescott, G.W. 1951, p. 272, pl. 62, Figs. 2, 3.

Collection No: DLS201269, Accession No: 001995

### Genus: *Botryococcus* Kuetzing, 1849

Order: *Chlorococcales*

Family: *Botryococcaceae*

Genus: *Botryococcus*

Species: *braunii*

***Botryococcus braunii*** **Kuetzing**

**Plate – 43a**

Thallus of this alga is free floating and consists of spherical to indefinite (irregular) shaped colonies, often united in aggregates by delicate mucilaginous integuments or projections. Cells are ovoid to ellipsoid, radiate arranged and without a conspicuous gelatinous envelope but completely enclosed in a tough, dark coloured gelatinous membrane having irregular wrinkles. Colonies are often united in compound net-like aggregates by means of long delicate mucilaginous projections from the colonial envelope. Cells are ovoid to ellipsoid and arranged radially at the periphery of the colony and each cell with a parietal cup shaped chloroplast. Simple colonies are up to 100 µm in diameter and compound colonies upto 1.5 mm in diameter.

Tiffany, L.H. and Britton, M.E. 1952, p. 207, pl. 57, Figs. 642-643.

Prescott, G.W. 1951, p. 232, pl. 52, Figs. 1, 2, 11.

Phillipose, M.T. 1967, p. 195, Figs. 108 a, f.

Collection No: DLS201273, Accession No: 001999

### Genus: *Coenococcus* Korshikov

Order: *Chlorococcales*

Family: *Radiococcaceae*

Genus *Coenococcus*

Species: *planctonicus, polycoccus*

***Coenococcus planctonicus* Korshikov 1953**

**Plate -28a**

The algae lives in coenobia of 4, 8, 16 or 32-64 cells and forming dense tetrads lying within a spherical, ellipsoidal or irregularly-shaped mucilaginous envelope. The cells are spherical, broadly oval in young colonies, smooth-walled. The chloroplast is cup shaped to goblet shaped. Cells are 9.79 µm broad.

John, D.M., Whitton, B.A. and Brook, A.J. 2005, p. 352, pl. 86, Figs. c, f.

Collection No: DLS201274, Accession No: 02000

***Coenococcus polycoccus* (Korshikov) Hindak 1977**

**Plate – 28b**

Coenobia are 4-celled to multicellular (8, 16 or 32 cells) embedded within a spherical to irregularly spherical mucilaginous envelope. The chloroplast is parietal and dense. Cells are 4.89 µm in diameter.

John, D.M., Whitton, B.A. and Brook, A.J. 2005, p. 352, pl. 86, Fig. d.

Collection No: DLS201274, Accession No: 02000

**Genus: *Golenkinia* Chodat, 1894a**

Order: *Chlorococcales*
Family: *Micractiniaceae*
Genus *Golenkinia*
Species: *radiata*

***Golenkinia radiata* Chodat**

**Plate – 43c, d**

Cells are usually solitary, spherical and rarely in 4-celled colonies, with the entire cell wall covered by a number of (usually ten) long bristles which are not thickened at the base. Chloroplast is single cup-shaped and with a pyrenoid. Cells

are usually 9.05 - 15.33 μm in diameter and bristles 23.94 - 25.38 and upto 44.76 μm long.

Phillipose, M.T. 1967, p. 102, Fig. 27.

Collection No: DLS201249, Accession No: 001975

**Genus: *Tetraedron* Kuetzing 1845**

Order: *Chlorococcales*
Family: *Hydrodictyaceae*
Genus *Tetraedron*
Species: *gracile*, *minimum* f. *Apiculatum, muticum, trigonum, tumidulum*

***Tetraedron gracile* (Reinsch) Hansg.**

**Plate – 12b**

Cells are flat, cruciform, quadrangular and sides deeply concave between the processes, angles 3-forked, ultimate divisions slender and acute. The cell-wall is smooth. Cells with processes are 40.54 μm in diameter and without processes 12.01 μm in diameter.

Prasad, B.N. and Misra, P.K. 1992 II, p. 14, pl. 2, Fig. 8.

Collection No: DLS201280, Accession No: 02006

***Tetraedron minimum* (A. Braun) Hansgirg f. *apiculatum* (Reinsch) De Toni**

**Plate - 12e**

Cells are small and quadrangular with the sides concave and angles rounded. Cell wall is smooth. Cells are with a very short fine papilla from each angle. Cells are 9.05 μm in diameter.

Phillipose, M.T. 1967, p 138, Figs. 53, a, c, d.

Guarrera, S.A. and Echenique, R.O. 1992, 257-272.

Collection No: DLS201281, Accession No: 02007

***Tetraedron muticum* (A. Braun) Hansgirg**

**Plate – 12d**

Cells of this species are small, flat and triangular with the sides slightly concave and angles broadly rounded or truncate. Cell wall is smooth. Cells are 9.60 µm long and 11.27 µm in diameter.

Phillipose, M.T. 1967, p. 137, Figs. 51 a, b.

Collection No: DLS201269, Accession No: 001995

***Tetraedron trigonum* (Naegeli) Hansgirg**

**Plate – 12a**

Cells of this species are flat, triangular with somewhat concave sides and rounded corners each ending in a stout spine. Cells are without spines 28.17 µm in diameter and spines 7.15 µm long.

Phillipose, M.T. 1967, p. 142, Figs. 58 a, b, p.

Collection No: DLS201270, Accession No: 001996

***Tetraedron tumidulum* (Reinsch) Hansgirg**

**Plate – 12c**

Cells are tetragonal with margins more or less concave and angles rounded, or sometimes with knob-like projections. Cells are 33.52 µm in diameter.

Phillipose M.T. 1967, p. 139, Fig. 54.

Smith, G.M. 1926, p. 173, pl. 6, Figs. 24-27.

Collection No: DLS201273, Accession No: 001999

**Genus: *Tetraspora* Link, 1809**

Order: *Chlorococcales*

Family: *Tetrasporaceae*

Genus *Tetraspora*

Species: *lamellose*, *lubrica*

***Tetraspora lamellose* Prescott 1944**

**Plate - 12g**

Thallus is irregularly lobed and saccate, free floating. Cells are spherical in 2's, with thick walls and gelatinous, lamellate sheaths which are distinct and not confluent with the colonial mucilage. Pseudo cilia are very fine and 20-30 times the diameter of the cell in length. Chloroplast with a dense parietal plate covering almost the entire wall. This species differs from the others by the possession of distinct lamellate cell sheaths and the extraordinarily long pseudo cilia. Cells are 10.44 µm in diameter.

Prescott, G.W. 1951, p. 88, pl. 5, Fig. 6.

Collection No: DLS201273, Accession No: 001999

***Tetraspora lubrica* (Roth C. A. Agardh)**

**Plate - 12f**

In this species cells are tubular with repeatedly split envelopes usually attached to aquatic macrophytes. Cells are 8.22 µm in diameter and colony is 28.54 µm in diameter;

Tiffany, L.H. and Britton, M.E. 1952, p. 22, pl. 3, Figs. 30, 31.

Collection No: DLS201281, Accession No: 02007

**Genus: *Trochiscia* Kuetzing, 1845**

Order: *Chlorococcales*

Family: *Oocystaceae*

Genus *Trochiscia*

Species: *aciculifera*

***Trochiscia aciculifera* (Lagerh.) Hansgirg**

**Plate - 27f**

Cells spherical to ovoid, solitary or in colonies of 2-4 embedded in mucilage. Cell wall thick and covered by numerous sharply pointed spines. Cells are 14.87 µm in

diameter and spines 2.35 µm long.

Phillipose, M.T. 1967, p. 99, Figs. 58, a-h.

Collection No: DLS201268, Accession No: 001994

**Genus: *Tetrastrum* Chodat, 1895a**

Order: *Chlorococcales*

Family: *Scenedesmaceae*

Genus *Tetrastrum*

Species: *heteracanthum*

***Tetrastrum heteracanthum* (Nordstedt) Chodat**

**Plate - 27a**

Colonies are 4-celled and flat with the cells quadrately arranged. Cells are nearly heart-shaped with a long and short seta from the outer surface. Setae are straight or curved. Cells are 7.20 µm in diameter and short setae 12.10 µm long.

Phillipose, M.T. 1967, p. 244, Fig. 156.

Collection No: DLS201268, Accession No: 001994

**Genus: *Tetrallantos* Teiling, 1916**

Order: *Chlorococcales*

Family: *Scenedesmaceae*

Genus *Tetrallantos*

Species: *lagerheimii*

***Tetrallantos lagerheimii* Teiling**

**Plate - 43f**

Colonies are usually four-celled, but sometimes 8-16 celled. The cells are being held together by the remnants of the mother cell wall and usually enclosed by a delicate mucilaginous envelope, which is often visible only after staining. Cells are crescent to sausage shaped with their ends rounded. Cells are 7.76 µm broad and 16.62 µm long.

Phillipose, M.T. 1967, p. 291, Fig. 188.

Collection No: DLS201294, Accession No: 02020

**Genus: *Crucigenia* Morren, 1830**

Order: *Chlorococcales*
Family: *Scenedesmaceae*
Genus: *Crucigenia*
Species: *crucifera, rectangularis, tetrapedia*

***Crucigenia crucifera* (Wolle) Collins**

**Plate - 27d**

Colonies are 4-celled, rhomboidal with slightly concave sides and a small rectangular space at the centre and often bound together by an inconspicuous gelatinous mass and also frequently joined together in multiple colonies of sixteen or more cells. Cells are elongate with the outer side concave and the inner side straight or slightly convex. The chloroplasts are 1-4, parietal or disc-shaped and usually with a pyrenoid. Cells are 4.62 µm broad and 6.93 µm long. Four celled colonies are 12.19 µm broad and 11.51 µm long.

Phillipose, M.T. 1967, p. 240, Figs. 138, 149.

Collection No: DLS201288, Accession No: 02014

***Crucigenia rectangularis* (A. Braun) Gay 1891**

**Plate - 27c**

Colony is free-floating, consisting of ovate or oblong cells, very regularly arranged about a rectangular central space in two pairs, with the apices adjoining. The chloroplasts are one-four, parietal discs, with a pyrenoid in each. Cells are 13.02 µm long and 8.03 µm in diameter.

Prescott, G.W. 1951, p. 285, pl. 65, Figs. 7, 8.

Collection No: DLS201232, Accession No: 001958

***Crucigenia tetrapedia* (Kirch.) West and West**

**Plate - 27b**

Colony is free floating, consisting of four triangular cells, arranged with a minute central space with acute angles. The chloroplast is parietal with a single pyrenoid, forming a rectangular plate of sixteen cells in four quarters. Cells are 8.60 μm in diameter.

Prescott, G.W. 1951, p. 285, pl. 66, Fig. 1.

Anand, N. 1998, p. 36, Fig. 110.

Collection No: DLS201248, Accession No: 001974

**Genus: *Hydrodictyon* Roth, 1800**

Order: *Chlorococcales*

Family: *Hydrodictyaceae*

Genus: *Hydrodictyon*

Species: *reticulatum*

***Hydrodictyon reticulatum* (Linn.) Lagerh**

**Plate - 42d**

Colonies are reticulate, free floating and macroscopic, meshes pentagonal or hexagonal. The cells are elongate-cylindrical with large central vacuole and cell-wall is two layered. The reticulation of colony is 3-12 (generally 5-6) sided. Cells are 56.40 μm long and 12.97 μm in diameter.

Phillipose, M.T. 1967, p. 135, Fig. 48.

Toppo, K. 2007, p. 50, pl. 8, Fig. 12.

Collection No: DLS201272, Accession No: 001998

**Genus: *Dichotomosiphon* Ernst, 1902**

Order: *Chlorococcales*

Family: *Dichotomosiphonaceae*

Genus: *Dichotomosiphon*

Species: *tuberosus*

***Dichotomosiphon tuberosus* (A. Braun) Ernst**

**Plate - 21d**

Algae are dichotomously branched with long coenocytic filaments and colorless rhizoids. The akinetes are straight and elongate or clavate and curved. The filaments are 5.82 µm in diameter.

Tiffany, L.H. and Britton, M.E. 1952, p. 110,124, pl. 36, Figs. 375-377.

Collection No: DLS201293, Accession No: 002019

**Genus: *Coelastrum* Naegeli, 1849**

Order: *Chlorococcales*
Family: *Coelastraceae*
Genus *Coelastrum*
Species: *microporum*, *sphaericum*

***Coelastrum microporum* Naegeli**

**Plate - 19b, c**

Colonies are spherical and of 8-16-32-64 (usually 16-32) cells with small intercellular spaces. Cells are also spherical to ovoid, enclosed by delicate gelatinous sheaths and interconnected by almost imperceptible gelatinous processes. Cells with sheath are 11.54 µm in diameter and colony is 61.69 µm in diameter.

Phillipose, M.T. 1967, p. 228, Fig. 135, a.

Collection No: DLS201292, Accession No: 002018

***Coelastrum sphaericum* Naegeli**

**Plate - 19a, d**

Colonies are spherical to ellipsoid and of 4-8-16-32 regularly arranged cells. The cells are ovoid with the narrow end directed outwards. The sides of cells where they are in contact with each other flattened and the outer free sides are strongly

curved. The intercellular spaces are about half the diameter of the cells or larger. Cells are 12.01 µm in diameter and colony is 44.42 µm in diameter.

Phillipose, M.T. 1967, p. 229, Fig. 136.
Collection No: DLS201253, Accession No: 001979

**Genus: *Nephrocytium* Naegeli, 1849**

Order: *Chlorococcales*
Family: *Oocystaceae*
Genus: *Nephrocytium*
Species: *agardhianum, lunatum*

***Nephrocytium agardhianum* Naegeli**

**Plate - 29b**

Cells are uniform with rounded ends and usually in colonies of 4, 8, or rarely 1, 2 or 16 cells, within a gelatinous envelope. The cells are arranged somewhat spirally in young and irregularly in old colonies. Cells are 17.21 µm long, 9.42 µm in diameter, and colony is upto 51.67 µm in diameter.

Phillipose, M.T. 1967, p. 189, Figs. 190, a, b.

Collection No: DLS201288, Accession No: 02014

***Nephrocytium lunatum* W. West**

**Plate - 29d**

Cells are half moon to sickle-shaped with one side convex and the other concave and ends pointed. Cells are spirally arranged within an ellipsoid to oblong hyaline gelatinous envelope to form 4-8 celled colonies. Cells are 12.47 µm long, 5.63 µm in diameter. Colonies are 47.56 µm long and 34.08 µm broad.

Phillipose, M.T. 1967, p. 189, Fig. 103.
Collection No: DLS201288, Accession No: 02014

**Genus*: Lagerheimia* Chodat 1895**

Order: *Chlorococcales*

Family: *Oocystaceae*

Genus: *Lagerheimia*

Species: *wratislawiensis*

***Lagerheimia wratislawiensis* Schroder**

**Plate - 26e**

Cells are solitary, free floating, spherical, tetrahedral and ellipsoidal or sub spherical with broadly rounded poles. Cell wall is delicate but distinct and slightly gelatinized on the surface. Cells are with subpolar or both subpolar and equatorial setae (bristles). Setae are with a distinct tubercle, which is sometimes dark brown at the base. In many of the cells there were two chloroplasts, each with a well defined pyrenoid. Cells are 9.14 µm long without spine, 5.42 µm in diameter without spine and spine is 16.01 µm long.

Smith, G.M. 1926, p. 180. pl. 12, Figs. 10-14.

Collection No: DLS201270, Accession No: 001996

**Genus: *Oocystis* Naegeli in A. Braun, 1855**

Order: *Chlorococcales*

Family: *Oocystaceae*

Genus: *Oocystis*

Species: *elliptica*, *gigas, lacustris, solitaria*

***Oocystis elliptica* W. West**

**Plate - 24a, b**

The species is usually in 4-8 celled colonies with the envelope narrow rarely solitary. Cells are usually free-floating, solitary or enclosed within the expanded mother cell membrane to form temporary colonies. Cells are elongate-ellipsoid, about two times as long as broad and with broadly rounded ends which are not

thickened. Cells are 20.87 µm long, 16.08 µm in diameter and colony is 46.73 µm in diameter.

Phillipose, M.T. 1967, p. 186, Fig. 100.

Collection No: DLS201272, Accession No: 001998

***Oocystis gigas*** **Archer**

**Plate - 24c**

It is usually in colonies of 2-4 cells. The envelope is round and narrow. Cells are broadly ellipsoid, longer than broad with the ends broadly rounded and not thickened. Cells are 22.16 µm long, 18.56 µm in diameter and colony is 40.36 µm long.

Phillipose, M.T. 1967, p. 183, Fig. 94a.

Collection No: DLS201275, Accession No: 002001

***Oocystis lacustris*** **Chodat**

**Plate - 24d**

Cells are ellipsoid with somewhat pointed ends, about one times longer than broad and usually broad in 4-8 celled colonies with polar nodules. Cells are 14.07 µm long and 7.15 µm in diameter. Eight-celled colonies are 62.89 µm long and 48.67 µm in diameter.

Phillipose, M.T. 1967, p. 181, Fig. 90.

Collection No: DLS201249, Accession No: 001975

***Oocystis solitaria*** **Wittrock**

**Plate - 24e**

Cells are solitary or in colonies of 2, 4 or 8 cells enclosed within the old mother cell wall and are ovoid to ellipsoid, thick walled and with markedly thick polar nodules. Cells are 37.07 µm long and 23.92 µm in diameter.

Phillipose, M.T. 1967, p 180, Fig. 89.

Collection No: DLS201241, Accession No: 001967

**Genus: *Gloeotaenium Hansgirg*, 1890**

Order: *Chlorococcales*

Family: *Oocystaceae*

Genus: *Gloeotaenium*

Species: *loitlesbergerianum*

***Gloeotaenium loitlesbergerianum* Hansgirg**

**Plate - 29c**

Colony is broadly ellipsoid in front view and oblong in side view. Cells are spherical to ovoid and completely filling the space inside the mother cell wall. The gelatinous bands are broad. Cell wall is thick and lamellated. Chloroplast is parietal, filling the cell and is with or without a pyrenoid. Cells are 29.65 µm in diameter and colonies (2-4) celled are 77.85 µm in diameter.

Phillipose, M.T. 1967, p. 178, Fig. 88.

Collection No: DLS201288, Accession No: 002014

**Genus: *Palmellococcus* Chodat, 1894**

Order: *Chlorococcales*

Family: *Oocystaceae*

Genus: *Palmellococcus*

Species: *saccharophilus*

***Palmellococcus saccharophilus* (Krueger) Chodat**

**Plate - 25f**

Cells are ellipsoid, ovoid or rarely spherical, cylindrical or pear shaped. Cell membrane is thin, colorless and slimy. The mature cells are with one to several discoid or plate-like chloroplasts which are generally devoid of a pyrenoid and structure of chloroplast is often masked by the accumulation of reddish oil within the chloroplast. Cells are 36.11 µm long and 23.55 µm in diameter. Cell membrane is up to 2.77 µm thick.

Phillipose, M.T. 1967, p. 176, Fig. 86.

Kant, S. and Gupta, P. 1998, p. 84, pl. 84 Fig. 2.

Kumar, S. 2005, p. 106, pl. 10, Figs. 8, 10

Collection No: DLS201280, Accession No: 02006

**Genus: *Planktosphaeria,* G. M. Smith 1918**

Order: *Chlorococcales*
Family: *Chlorococcaceae*
Genus: *Planktosphaeria*
Species: *gelatinosa*

***Planktosphaeria gelatinosa* G. M. Smith 1918**

**Plate - 28c, d**

It is a free-floating colony of spherical cells compactly grouped within a mucilaginous, homogeneous envelope. In colonies the cells may become somewhat loosely arranged also, but usually they are closely clustered. The single parietal cup-shaped chloroplast is present. Cells are 18.38 µm in diameter.

Prescott, G.W. 1951, p. 240, pl. 53, Fig. 23.

Collection No: DLS201286, Accession No: 002012

**Genus: *Actinastrum* Lagerheim, 1882**

Order: *Chlorococcales*
Family: *Scenedesmaceae*
Genus *Actinastrum*
Species: *hantzschii*, *hantzschii* var. *fluviatile*, *lagerh*

***Actinastrum hantzschii* Lagerheim**

**Plate - 17f**

Colonies are of 4 or 8 radially arranged cells, sometimes joined together to form multiple colonies and are spindle-shaped, 3-6 times as long as broad. The middle of cell is twice as broad as the apices, apices are attenuated or slightly rounded.

Cells are 18.66 µm long, 2.90 µm in diameter and colonies are 33.15 µm in diameter.

Kant, S. and Gupta, P. 1998, p. 89, pl. 92, Fig. 11.

Collection No: DLS201253, Accession No: 001979

***Actinastrum hantzschii* var. *fluviatile* Schroeder**

**Plate - 17h**

This variety is colonial, planktonic, composed of 4-16 truncate-fusiform or basidia-like cells, sometimes cigar-shaped with sub acute poles, radiating in all planes from a common center. A variety differing from the typical in the cells are sharply pointed. Cells are 45.45 µm long and 3.97 µm in diameter.

http://www.algaebase.org/search/species/detail/?

Collection No: DLS201273, Accession No: 001999

***Actinastrum lagerh* Lagerh**

**Plate - 17g**

Colonies are of 4 or 8 radially arranged cells sometimes joined together to on multiple colonies, spindle-shaped, 3-6 times as long as broad. The center of cells is elongate-cylindrical, tapering slightly to abruptly truncate ends. Cells are 49.74 µm long and 2.75 µm in diameter.

Kant, S. and Gupta, P. 1998, p. 89, pl. 92, Fig. 11.

Toppo K. 2007, p. 1, pl. 6, Fig. 1.

Collection No: DLS201278, Accession No: 02004

### Genus: *Ankistrodesmus* Corda, 1838

Order: *Chlorococcales*

Family: *Selenastraceae*

Genus: *Ankistrodesmus*

Species: *falcatus*, *falcatus* var. *spirilliformis, falcatus* var. *radiates, spiralis*

***Ankistrodesmus falcatus* (Corda) Ralfs**

**Plate - 17d, e**

Cells are acicular to narrowly fusiform with the ends tapering to acute spines, usually in fasciculate bundles of 2-4-8 or more. The cells are rarely solitary usually not enclosed within a mucilaginous envelope. Cells are 48.65 - 52.11 µm long and 2.74 - 4.53 µm in diameter.

Phillipose, M.T. 1967, p. 211, Figs. 121 a, e.

Collection No: DLS201269, Accession No: 001995

***Ankistrodesmus falcatus* (Corda) Ralfs var. *spirilliformis* G. S. West**

**Plate - 17c**

Cells are solitary, spirally curved and with pointed ends. Cells are 46.82 µm long and 2.86 µm in diameter.

Phillipose, M.T. 1967, p. 213, Fig. 121, b.

Collection No: DLS201272, Accession No: 001998

***Ankistrodesmus falcatus* (Corda) Ralfs var. *radiates* (Chodat) Lemmermann**

**Plate - 17a**

Cells are in radiating bundles, straight or curved. Cells are 51.42 µm long and 1.48 µm in diameter.

Phillipose, M.T. 1967, p. 213, Fig. 121, d.

Collection No: DLS201278, Accession No: 02004

***Ankistrodesmus spiralis* (Turner) Lemmermann**

**Plate - 17b**

Cells are acicular with acute apices, in colonies of usually 4-8-16 rarely two. Cells are spirally twisted round one another in the median region, but free at the ends. Cells are 43.96 µm long and 2.59 µm in diameter.

Phillipose, M.T. 1967, p. 209, 210, Fig. 119, d.

Collection No: DLS201275, Accession No: 002001

### Genus: *Selenastrum* Reinsch, 1867

Order: *Chlorococcales*

Family: *Selenastraceae*

Genus: *Selenastrum*

Species: *gracile*

***Selenastrum gracile*** **Reinsch**

**Plate - 29a**

Colonies are free floating made up of 4-8-16 cells usually without an outer mucilaginous envelope. Sometimes several such groups joined together to form larger colonies containing as many as hundred or more cells. Cells are lunate to sickle-shaped and quite narrow in proportion to the length. The apices of cells are acutely pointed rarely bifid. Cells are 24.57 µm long and 6.56 µm in diameter.

Phillipose, M.T. 1967, p. 219, Fig. 128.

Collection No: DLS201255, Accession No: 001981

### Genus*: Kirchneriella* Schmidle, 1893

Order: *Chlorococcales*

Family: *Selenastraceae*

Genus: *Kirchneriella*

Species: *contorta* var. *elegans, lunaris*

***Kirchneriella contorta*** **var.** ***elegans*** **(Playfair) Komarek**

**Plate - 20b**

Colonies are free-floating with lunate to sickle-shaped, curved or spirally twisted cells showing pointed or rounded ends. Cells lie close to each other with an irregular arrangement within the homogenous gelatinous envelope. Colonies are of 4, 8, 16 or 32, cells in gelatinous sheath. Cell body is long, strongly curved or twisted. Cells are 9.42 µm long and 3.42 µm in diameter.

http://www.algaebase.org/search/species/detail/?

Collection No: DLS201251, Accession No: 001977

***Kirchneriella lunaris* (Kirchner) K. Moebius**

**Plate - 20a**

Colonies are spherical to ellipsoid within an outer gelatinous envelope. Cells are irregularly within the envelope in groups of four or eight, flattered and crescent-shaped with pointed ends and about twice as long as broad. Cells are 10.09 µm long, 6.12 µm in diameter and colonies are 59.64 µm in diameter.

Phillipose, M.T. 1967, p. 222, Fig. 131.

Toppo, K. 2007, p. 75, pl. 7, Fig. 3.

Collection No: DLS201272, Accession No: 001998

## Genus: *Micractinium* Fresenius, 1858

Order: *Chlorococcales*
Family: *Micractiniaceae*
Genus: *Micractinium*
Species: *pusillum*, *pusillum* var. *elegans*

***Micractinium pusillum* Fresenius**

**Plate - 20c**

Colonies are quadrate, tetrahedric and pyramidate with 4-8-16 or more cells arranged in groups of four, each group being quadrate or pyramidate. Cells are spherical with a thin firm cell membrane and with one or more (2-5) long hyaline setae, which are not thickened at the base, from the outer free surface. Cells are 6.37 µm in diameter and setae are 43.41 µm long rarely upto 60 µm, and are 1.62 µm broad at the base.

Phillipose, M.T. 1967, p. 104, Fig. 29

Collection No: DLS201271, Accession No: 001997

***Micractinium pusillum* Fresenius var. *elegans* G. M. Smith**

**Plate - 20d, e**

A free-floating coloney of 4-16 spherical cells are arranged in a pyramid or in a square, groups of 4 in association with other similar groups. The free walls are beset with 1-5 finely tapering setae. A variety differing from the typical by having more numerous and longer setae on the free walls of the cells. The setae are 3-5 in number. Cells are 7.02 µm in diameter and setae are 34.41 µm long.

Prescott, G.W. 1951, p. 288, pl. 66, Fig. 7.

Collection No: DLS201284, Accession No: 02010

**Genus: *Monoraphidium* Komarkova-Legnerova, 1969**

Order: *Chlorococclaes*

Family: *Selenastraceae*

Genus: *Monoraphidium*

Species: *griffithii*

***Monoraphidium griffithii* (Berkely) Komarkova-Legnerova**

**Plate - 22c**

The algae are unicellular, not embedded in mucilage envelope. Cell body is elongated spindle in shape helically twisted, straight or curved sharply pointed at both ends. Cell walls are smooth. Cells are 42.02 µm long and 3.60 µm in diameter.

Komarkova-Legnerova, J. 1969, p.75-144: http://www.algaebase.org/search/genus

Collection No: DLS201274, Accession No: 02000

**Genus: *Pediastrum* Meyen, 1829**

Order: *Chlorococcales*

Family: *Hydrodictyaceae*

Genus: *Pediastrum*

Species: *angulosum*, *boryanum* var. *longicorne, duplex* var. *reticulatum, duplex* var. *clathratum, tetras, tetras* var. *tetraodon*

***Pediastrum angulosum* (Ehr.) Meneghini**

**Plate - 16e**

Colonies are without perforations, usually single layered and round, elliptical or kidney-shaped, sometimes large and two-layered with small irregular perforations. The internal cells are 4-6 angled, broader than long with the outer side slightly sinuous. Marginal cells are broad, outer face slightly emarginated, lobes with or without short processes. Cell wall is hyaline, yellowish or reddish, sometimes thickened and with reticulate ridges, rarely smooth or coarsely granulate. Colonies are 8-128 celled with variable arrangement of cells. Cells are 11.18 µm long, 12.56 µm in diameter and colonies are 87.55 µm in diameter.

Phillipose, M.T. 1967, p. 118, Fig. 39, g.

Collection No: DLS201292, Accession No: 002018

***Pediastrum boryanum* var. *longicorne* Reinsch**

**Plate - 15b**

Colonies are circular to oval and usually of 16-32 (rarely 4-8 or upto 128) cells arranged in concentric rings without intercellular spaces. Inner cells are polygonal with straight sides. Outer face of marginal cells is slightly to deeply emarginate and with two short processes ending in stumpy spines. Cell wall is usually granulate, sometimes smooth. Cells are 11.05 µm long, 14.41 µm in diameter, and sixteen-celled colonies are up to 60.03 µm in diameter. Horns (processes) are 5.07 µm long.

Phillipose, M.T. 1967, p. 119, Fig. 40, b.

Collection No: DLS201253, Accession No: 001979

***Pediastrum duplex* var. *reticulatum* Lagerheim**

**Plate - 16c**

Cells are H-shaped with sides of processes of marginal cells nearly parallel. The intercellular spaces are large and oval. Cells are 20.32 µm long, 20.57 µm in diameter, and 16 celled colonies are 95.12 µm in diameter.

Phillipose, M.T. 1967, p. 124, Fig. 43, g.

Collection No: DLS201274, Accession No: 02000

***Pediastrum duplex* var. *clathratum* (A. Braun) Lagerheim**

**Plate - 16f**

Cells are with deeply emerginate sides and with larger intercellular spaces than in *Pediastrum duplex*. Colonies are 8-64 celled. Cells are 20.20 µm long, 18.30 µm in diameter and diameter of colony is 63.82 µm.

Phillipose, M.T. 1967, p. 123, Fig. 43.

Collection No: DLS201288, Accession No: 002014

***Pediastrum tetras* (Ehr.) Ralfs**

**Plate - 16d**

Colonies are rectangular, oval or circular of 4-8-16 (-32) cells without intercellular spaces. The marginal cells are divided into two lobes by deep linear to cuneate incision on the outer side reaching to the middle of the cell. Each lobe is truncate, slightly emarginated, or further divided into two lobes. Inner cells are 4-6 sided with a single linear incision. Cells are 10.87 µm long, 16.70 µm in diameter and colony is 26.15 µm in diameter.

Phillipose, M.T. 1967, p. 128, Fig. 45.

Collection No: DLS201288, Accession No: 002014

***Pediastrum tetras* var. *tetraodon* (Corda) Hansgirg**

**Plate - 16a**

Colonies are 4-8-16 celled. The incision of cells is deep with the lobes adjacent to the incision of the marginal cells that are very pronounced. Cells are 13.29 µm long, 10.05 µm in diameter and colony is 36.57 µm in diameter.

Phillipose, M.T. 1967, p. 129, Fig. 45, d.

Collection No: DLS201271, Accession No: 001997

## Genus: *Scenedesmus* Meyen, 1829

Order: *Chlorococcales*

Family: *Scenedesmaceae*

Genus: *Scenedesmus*

Species: *abundans*, *abundans* var. *longicauda, acutiformis, arcuatus, armatus* var. *dispar, armatus* var. *boglariensis* f. *bicaudatus, armatus* var. *bicaudatus, bijugatus* var. *graevenitzii, denticulatus*, *cumbricus, denticulatus* var. *australis, dimorphus, obliquus, opoliensis, perforates, quadricauda, quadricauda* var. *westii, quadricauda* var. *longispina*, *quadricauda* var. *quadrispina, serratus*

***Scenedesmus abundans* (Kirchner.) Chodat**

**Plate - 2b, 4c, 5f**

Colonies are usually 2 - 4 celled, rarely eight celled and arranged in a linear series. Cells are ovoid to oblong-ovoid. The external cells are with one or more median lateral spines from the outer face in addition to spines from the four corners of the colony and internal cells with 1-2 spines from the poles. Cells are 9.70 µm long and 6.00 µm in diameter with 4.43 µm long spine.

Phillipose, M.T. 1967, p. 279, Figs. 184 a-d.

Collection No: DLS201273, Accession No: 001999

***Scenedesmus abundans* var. *longicauda* G. M. Smith 1916a**

**Plate - 2a**

Cells are smaller than the typical, with relatively longer spines. Cells are 10.62 µm long and 3.42 µm in diameter with spines 6.93 µm long.

Prescott, G.W. 1951, p. 274, pl. 62, Figs. 4, 5.

Collection No: DLS201229, Accession No: 001955

*Scenedesmus acutiformis* **Schroeder 1897**

**Plate - 5a**

Cells are arranged in a single series of 4 (2-8), fusiform-elliptic, with poles sharply pointed. The inner cells are with a single facial longitudinal ridge and outer cells are with 2-4 longitudinal ridges. Cells are 25.12 µm long and 10.44 µm in diameter.

Prescott, G.W. 1951, p. 275, pl. 62, Figs. 6, 7.

Collection No: DLS201274, Accession No: 02000

*Scenedesmus arcuatus* **Lemmermann**

**Plate - 5d, e**

Colonies are usually eight celled rarely 4 or 16, curved and with intercellular space. Cells in eight celled colonies are in two series, oblong ovoid, sometimes slightly angular at the base due to mutual pressure. Cell wall is smooth and without teeth or spines. Cells are 10.62 µm long and 5.82 µm in diameter, colony is 22.44 µm in diameter.

Phillipose, M.T. 1967, p. 256, Figs. 166, a-c

Collection No: DLS201289, Accession No: 02015

*Scenedesmus armatus* **(Chodat) G. M. Smith. var.** ***dispar*** **Nov.**

**Plate - 4a**

Colonies are four celled with the cells arranged in a sub alternating series. Cells are oblong-ellipsoid with broadly rounded ends and with a delicate longitudinal rib extending from pole to pole. Each terminal cell is with a fairly long outwardly oblique spine from one pole, a shorter erect or slightly curved spine from the opposite pole and a third very short outwardly directed spine near the same pole. The long and short spines of one terminal cell are alternating with those of the other terminal cell. The internal cells are with a short recurved spine from

alternating poles. Cells are 15.61 µm long and 7.20 µm in diameter. Polar spines are 6.53 µm long and lateral spines 1.72 µm long.

Phillipose, M.T. 1967, p. 261, 265, Fig. 171, l.

Collection No: DLS201286, Accession No: 02012

***Scenedesmus armatus* (Chodat) G. M. Smith var. *boglariensis* Hortobagyi f. *bicaudatus* Hortobagyi**

**Plate - 2e**

This variety differs from the type in all the cells possessing well developed longitudinal ribs extending from pole to pole. The ribs are smooth or with slightly undulate margin. Terminal cells are with a fairly long spine from each pole, usually from the outer edge of the pole, one of the spines somewhat straight and the other slightly curved. The internal cells are without spines or with small spines from the pole. The colony is 2-4 celled, flat or sometimes slightly curved, and with the cells arranged in a linear series.

In case of forma the colonies are four to eight celled. Cells are with prominent longitudinal ribs which are smooth or rugged. The terminal cells are with a long spine from the outer edge of one their poles and the spines of the two terminal cells are alternating with each other. The other pole of the terminal cells and the poles of internal cells are without spines or with one. Cells are 16.25 µm long, 4.89 µm in diameter and with spine 10.50 µm long.

Phillipose, M.T. 1967, p. 264, Figs. 171 g, j, f.

Collection No: DLS201262, Accession No: 001988

***Scenedesmus armatus* (Chodat) G. M. Smith var. *bicaudatus* (Guglielmetti) Chodat**

**Plate - 3c**

Colonies are usually two to four celled. Cells are oblong-ellipsoid with acute spices and arranged in a linear series. Terminal cells are with a long spine from

each pole. All cells are with a median lateral longitudinal rib. The variety differs from the type in having a long spine from one of the poles of the terminal cell only and the spines of the two terminal cells are also alternating with each other. The longitudinal ribs are usually seen only in the internal cells. Cells are 13.39 µm long and 4.62 µm in diameter with spine12.79 µm long.

Phillipose, M.T. 1967, p. 262, Figs. 171 d, f, m.

Collection No: DLS201284, Accession No: 02010

***Scenedesmus bijugatus* (Turpin) Kuetzing var. *graevenitzii* (Bernard) comb. Nov.**

**Plate - 2h, 4e**

Colonies are flat or slightly curved of 2-4-8 cells arranged in a single linear series. Cells are oblong-ellipsoid to ovoid with the ends broadly rounded. Cells are fusiform, ellipsoid, oblong-ellipsoid to ovoid with obtuse poles and arranged in an alternating series with adjacent cells in contact only along a short portion of their length. Cells are 18.75-20.45 µm long, 8.68-9.51 µm in diameter and colony is 48.39 µm in diameter.

Phillipose, M.T. 1967, p. 254, Figs. 164 a, b.

Collection No: DLS201288, Accession No: 02014

***Scenedesmus cumbricus* (G. M. Sm.) Chodat**

**Plate - 2c**

Colonies are circular arranged in two tiers and cells are also circular. Cells are 11.91 µm long and 7.76 µm in diameter.

Toppo, K. 2007, p. 87, pl. 8, Fig. 15.

Collection No: DLS201268, Accession No: 001994

***Scenedesmus denticulatus* Lagerheim**

**Plate - 2g**

Colonies are usually four-celled with the cells arranged in a cruciate to sub alternate manner. Cells are ovoid-oblong to ellipsoid with 1-4 (usually 2) teeth from each pole. Teeth sometimes are absent from one end of the inner cells. Cells are 13.85 µm long and 6.93 µm in diameter.

Phillipose, M.T. 1967, p. 268, Fig. 176 b.

Collection No: DLS201268, Accession No: 001994

***Scenedesmus denticulatus* Lagerheim var. *australis* Playfair**

**Plate - 3a**

Colonies are two to four celled. Cells are arranged in a single linear series, oblong cylindrical with more or less rounded ends and with one (very rarely two) short teeth from the poles of all cells. Cell membrane is somewhat thick. Cells are 20.69 µm long and 7.76 µm broad. Teeth are 1.94 µm long.

Phillipose, M.T. 1967, p. 268, Figs. 176 f, h.

Collection No: DLS201272, Accession No: 001998

***Scenedesmus dimorphus* (Turpin) Kuetzing**

**Plate - 4d**

Colonies are 4-8 celled with the cells arranged in a linear or sub alternating series (eight-celled colonies always in sub-alternating series). Cells are 26.23 µm long and 7.76 µm in diameter and colony is 51.44 µm in diameter.

Phillipose, M.T. 1967, p. 249, Figs. 164 a, c.

Collection No: DLS201256, Accession No: 001982

***Scenedesmus obliquus* (Turpin) Kuetzing**

**Plate - 5b**

Cells are fusiform with delicately pointed apices, in a linear or alternating series. The inner cells are asymmetric, outer cells are lunate or symmetric. The cell-wall

is smooth without spines. Cells are 29.28 µm long and 7.20 µm in diameter.

Tiffany, L.H. and Britton, M.E. 1952, p. 123, pl. 35, Fig. 370.

Collection No: DLS201274, Accession No: 02000

***Scenedesmus opoliensis* P. Richter**

**Plate - 3e**

Colonies are two to four celled with cylindrical to subfusiform cells and arranged in a linear series. The adjacent cells are in contact only along about a third of their length. Internal cells remain tumid in the median region and attenuated towards the ends. Terminal cells are often narrower and sub rectangular. Poles of all cells are semi truncate to rostrate, sometimes ending in one or two very short spines. Poles of terminal cells are with a long, more or less recurved spines. Cells are 18.10 µm long and 3.97 µm in diameter with spine 22.50 µm long.

Phillipose, M.T. 1967, p. 276, Figs. 181 a, b.

Collection No: DLS201285, Accession No: 02011

***Scenedesmus perforates* Lemmermann 1904**

**Plate - 2d**

Cells are sub rectangular with convex end walls and concave lateral walls, thus forming biconvex intercellular spaces. The end cells of the colony bears a single long curved spine at each pole arising from the corner of the cells and the outer lateral walls of the end cells are straight. Cells are 14.31 µm long and 5.45 µm in diameter with spine 20.98 µm long.

Prescott, G.W. 1951, p. 279, pl. 46, Figs. 24, 25.

Collection No: DLS201262, Accession No: 001988

***Scenedesmus quadricauda* (Turpin) Brebisson**

**Plate - 3b, 4b, f**

Colonies are usually four-celled, sometimes 2 or 8 celled. Cells are oblong cylindrical with rounded ends and arranged in a linear series. Poles of terminal

cells possess a long, more or less straight or curved spine. Cell wall is smooth and without ridges. Cells are 17.45 µm long and 6.83 µm in diameter with spine 13.06 µm long.

Phillipose, M.T. 1967, p. 284, Fig. 187.
Collection No: DLS201253, Accession No: 001979

***Scenedesmus quadricauda* (Turpin) Brebisson var. *westii* G. M. Smith**

**Plate - 3d**

Colonies are usually four to eight celled. Cells are 16.16 µm long and 6.00 µm in diameter with spine 14.45 µm long,

Phillipose, M.T. 1967, p. 286, Figs. 187, h-I.
Collection No: DLS201285, Accession No: 02011

***Scenedesmus quadricauda* (Turpin) Brebisson var. *longispina* (Chodat) G. M. Smith**

**Plate - 2f**

Colonies are usually 2-4 celled, rarely 8 celled. Cells are ovoid to cylindrical with the cells narrower than in the type and the spines proportionately longer, compared to the length of the cells. Internal cell sometimes possess very short delicate spines from some of their poles. Cells are 15.79 µm long, 5.08 µm in diameter and with spine 14.89 µm long

Phillipose, M.T. 1967, p. 285, Figs. 187 b, c.
Collection No: DLS201274, Accession No: 02000

***Scenedesmus quadricauda* (Turpin) Brebisson var. *quadrispina* (Chodat) G. M. Smith**

**Plate - 5c**

Colonies are usually 2-4 celled. Cells are broadly ovoid and about twice as long as broad. Poles of terminal cells are with a single short recurved spine. Cells are 17.27 µm long, 6.28 µm in diameter and with spine10.65 µm long

Phillipose, M.T. 1967, p. 285, Fig. 187 d, j.
Collection No: DLS201293, Accession No 02019

***Scenedesmus serratus* (Corda) Bohlin**

**Plate - 3f**

Colonies are four celled. Cells are oblong ovoid with truncate or tapering ends, in contact with adjacent cells for the greater part of their length. Terminal cells possess a single uninterrupted row of small spines extending from pole to pole. Internal cells are with two such rows. Apices of all cells are with 3-4 denticulations. Cells are 18.84 µm long and 7.48 µm in diameter.

Phillipose, M.T. 1967, p. 268, Fig. 174.

Collection No: DLS201284, Accession No: 02010

**Genus: *Bulbochaete* Agardh 1817**

Order: *Oedogoniales*

Family: *Oedogoniaceae*

Genus: *Bulbochaete*

Species: *mirabilis*

***Bulbochaete mirabilis* Wittrock 1817**

**Plate - 22f**

Filaments remain usually attached, branched and the branches are unilateral. The vegetative cells are uninucleate, normally widening upwards. The basal cell is ordinarily the only one capable of division in formation of main axis, first new cell forming a long tubular bristle with swollen bulb-like base, subsequent cells intercalated between basal cell and next one above. The terminal cell of each branch always furnishes with a bristle. Cells are 27.35 µm long and 20.36 µm in diameter with 75.48 µm long spine.

Tiffany, L.H. and Britton, M.E. 1952, p. 50, pl. 14, Fig. 95.

Collection No: DLS201265, Accession No: 001991

**Genus: *Uronema* Lagerheim, 1887**

Order: *Chaetophorales*

Family: *Chaetophoraceae*

Genus: *Uronema*

Species: *confervicolum*

***Uronema confervicolum* Lagerheim**

**Plate - 20f**

Filaments are long, unbranched uniseriate, slightly curved and consisting of many cells constricted at septa. The cells are cylindrical, slightly longer than broad. The apical cell is acuminating with pointed apex that may be straight or bent and basal cell is narrow and long. Each cell is with one laminate chloroplast occupying a part of the cell. Cells are 12.47 µm long and 6.10 µm in diameter.

Prasad, B.N. and Misra, P.K. 1992 II, p. 46, pl. 6, Figs. 3, 4.

Collection No: DLS201264, Accession No: 001990

**Genus*:* *Ulothrix* Kuetzing, 1833**

Order: *Ulotrichales*

Family: *Ulotrichaceae*

Genus: *Ulothrix*

Species: *aequalis*

***Ulothrix aequalis* Kuetzing 1845**

**Plate - 21e**

Filaments are very long, unbranched, not apically attenuated, with special hold fast cell generally attached in some species free floating in later stages. The vegetative cells are composed of cylindrical cells or sometimes barrel-shaped and are without constrictions at the cross walls. The filaments form bright green masses in shallow waters of several lakes and swamps and scatter among other algae. Cells are 21.09 µm long and 12.80 µm in diameter.

Prescott, G.W. 1951, p. 96, pl. 6, Figs. 1.

Collection No: DLS201272, Accession No: 001998

## Genus: *Oedeogonium* Link 1820

Order: *Oedogoniales*

Family: *Oedogoniaceae*

Genus: *Oedeogonium*

Species: *intermedium, undulatum* f. *senegalense*

***Oedeogonium* species Link**

**Plate - 41c**

The filaments are attached, unbranched (sometimes becoming free-floating in age). Cells are cylindrical or enlarged towards the anterior end, where one or more ring-like scars resulting from cell division are usually apparent. The swollen female cells (oogonia) are present at maturity, one to several in each filament. The male cells (antheridia) are either short, compartment-like cells, each bearing one or two antherozoids, occurring in filaments the same size as those which bear the oogonia, or minute male filaments growing epiphytically on the female plants. The fertilization is by the entrance of an antherozoid through a pore or lid of the oogonium wall resulting oospore of various shapes, surrounded by a wall of two or three layers, which may be smooth or variously decorated.

In the identification of *Oedeogonium* species, the size of the vegetative cells, the shape and size of the oogonia, the form and decoration of the oospore wall and the location the of the antheridial cells are the more important differentiating and specific characters.

***Oedeogonium intermedium* Wittr. Ex Hirn**

**Plate - 41d**

The oogonia are alternating with vegetative cells and antheridia are intercalary far away from oogonia. Cells are 15.70 µm in diameter.

Kant, S. and Gupta, P. 1998, p. 114, pl. 37, 107, Figs. 6, 1, 2.

Collection No: DLS201275, Accession No: 02001

***Oedeogonium undulatum* (de Breb.) A. Braun in DeBary f. *senegalense* (Nordst.) Hirn 1900**

**Plate - 41a, b**

The vegetative cells are capitellate and possess 4-undulations, except the basal cell which is elongate and smooth.

**f. *senegalense* (Nordst.) Hirn 1900**

The vegetative cells possess 3 median undulations and of somewhat narrower proportions than in the typical plant. Cells are 62.10 µm long and 19.07 µm in diameter.

Prescott, G.W. 1951, p. 209, pl. 40, Figs. 3, 5.

Collection No: DLS201247, Accession No: 001973

**Genus: *Chaetosphaeridium* Klebahn, 1892**

Order: *Coleochaetales*
Family: *Chaetosphaeridaceae*
Genus: *Chaetosphaeridium*

***Chaetosphaeridium* species Klebahn**

**Plate - 24f**

Thallus consists (Branched filaments) of globose or flask-shaped cells and sometimes appears as gelatinous colony of unicells. Cells are enclosed by a colorless sheath which forms a neck through which a long seta extends. Each cell bears a single sheathed hair or seta and in case of cultured clones, it has been observed that they produce more than one hair per cell. One or two plate like, parietal massive chloroplasts each with single pyrenoid are present per cell. Cell is 16.16 µm in diameter.

Prescott, G.W. 1951, p. 130, pl. 14.

http://www.algaebase.org/search/genus/detail/

Collection No: DLS201232, Accession No: 001958

## Genus: *Rhizoclonium* Kuetzing, 1843

Order: *Cladophorales*

Family: *Cladophoraceae*

Genus: *Rhizoclonium*

Species: *hieroglyphicum*

***Rhizoclonium* species Kuetzing**

**Plate - 41e**

The green alga is fresh water or marine, filamentous, coarse and wiry, unbranched or with 1-2 celled rhizoidal branches, slightly or not at all constricted at septa. Cells are multinucleate and cell wall is thick and lamellate. Chloroplast is parietal, reticulate with several pyrenoids.

***Rhizoclonium hieroglyphicum* (Ag.) Kuetz**

**Plate - 41h, 42a**

The filaments are long, wiry, unbranched, unconstructed at septa. Cells are long, cylindrical and multinucleate. Chloroplast is parietal and reticulate with several pyrenoids, rhizoids are primary, long and colourless formed from the direct prolongation of the lower and of filaments. Cells are 149.68 µm long and 56.98 µm in diameter.

Prasad, B.N. and Misra, P.K. 1992 II. p. 56, pl. 7, Figs. 7, 8.

Collection No: DLS201276, Accession No: 02002

## Genus: *Zygnema* C. A. Agardh, 1824

Order: *Zygnematales*

Family: *Zygnemataceae*

Genus: *Zygnema*

Species: *collinsianum*

***Zygnema* species C. A. Agardh**

**Plate - 41g**

The filaments are simple with cells 1-9 diameters long, usually with 2 distinctly stellate chromatophores (rarely in some cells 1-4), each with a prominent central pyrenoid, connected by a cytoplasmic isthmus containing the nucleus. Cells are 39.03 µm long and 18.62 µm in diameter.

Collection No: DLS201272, Accession No: 001998

***Zygnema collinsianum* Transeau**

**Plate - 41f**

The filaments are simple with long cells, usually with 2 distinctly stellate chromatophores, each with a prominent central pyrenoid, connected by a cytoplasmic pyrenoid, connected by a cytoplasmic isthmus containing the nucleus. The vegetative cells are 36.76 µm long and 18.38 µm in diameter.

Tiffany, L.H. and Britton, M.E. 1952, p.136, pl. 40, Figs. 428, 429.

Collection No: DLS201272, Accession No: 001998

**Genus: *Pleurotaenium* Naegeli, 1849**

Order: *Desmidiales*
Family: *Desmidiaceae*
Genus: *Pleurotaenium*
Species: *ehrenbergii*

***Pleurotaenium ehrenbergii* (Brebisson) DeBary**

**Plate - 21c**

Cells are 15-30 times longer than wide, elongate, straight, slightly constricted in the middle and possess sinus a shallow depression. The semicells are cylindric, somewhat variable in form, lateral margins slightly attenuated, particularly in the apical region, with a distinct basal inflation and above it 1-2 (rarely more) undulations, apices are truncate with rounded margins, bearing a ring of 7-10 conical or rounded tubercules. Cell-wall is colorless, punctuate or smooth. Cells

are 412.77 µm long, 26.22 µm in diameter, isthmus 24.01 µm and apices 18.95 µm wide.

Tiffany, L.H. and Britton, M.E. 1952, p. 180, pl. 55, Figs. 607, 608.

Collection No: DLS201288, Accession No: 02014

**Genus: *Closterium* Nitzsch 1817**

Order: *Desmidiales*
Family: *Desmidiaceae*
Genus: *Closterium*
Species: *acutum*, *braunii*, *ehrenbergii*, *gracile, moniliferum*, *parvulum*, *venus*, *lunula* f. *biconvexum*, *punctulatum*

***Closterium acutum* (Lyngbye) Brebisson**

**Plate - 18g**

Cells are 5-33 times longer than wide, slightly curved. The outer margin has 30-60 degrees of arc, inner margin is not tumid and gradually attenuated to the acute apices. The cell wall is smooth, colorless. The sides are concave or slightly convex, ends concave, angles produced into conical projections. Cells are 188.86 µm long, 9.70 µm in diameter and apices 3.23 µm wide.

Tiffany, L.H. and Britton, M.E. 1952, pl. 52, Fig. 555.

Collection No: DLS201282, Accession No: 02008

***Closterium braunii* Reinsch**

**Plate - 18h**

Cells are 16-22 times longer than wide, very slightly curved and median portion is cylindric, not tumid, tapering abruptly to obtusely round and slightly recurved apices. The cell wall is yellow to brownish with 4-6 costae consisting of double rows of punctate. The wall between costae is irregularly porous or with 6-10 more or less distinct striations. Cells are 731.93 µm long, 65.16 µm in diameter and 14.55 µm broad at the apex.

Tiffany, L.H. and Britton, M.E. 1952, p. 176, pl. 51, Fig. 541

Collection No: DLS201263, Accession No: 001989

***Closterium ehrenbergii* Mengh**

**Plate - 18f**

Cells are longer than broad, moderately curved, inner margin concave but inflated in the middle, gradually attenuated obtusely rounded apices. The cell wall is smooth. Cells are 202.44 µm long and 39.16 µm in diameter.

Tiffany, L.H. and Britton, M.E. 1952, p. 172, pl. 52, Fig. 558.

Toppo, K. 2007, p. 113, pl. 13, Fig. 3.

Collection No: DLS201289, Accession No: 02015

***Closterium gracile* Breb**

**Plate - 18d**

Cells are linear, longer than broad, almost straight for about 2/3 of length. The margins are parallel gradually narrowed to the obtuse apices. The cell wall is smooth and colourless. Cells are 182.21 µm long and 6.37 µm in diameter.

Tiffany, L.H. and Britton, M.E. 1952, p. 174, pl. 14, Fig. 3.

Toppo, K. 2007, p. 113, pl. 14, Fig. 3.

Collection No: DLS201282, Accession No: 02008

***Closterium moniliferum* (Bory) Ehrenberg**

**Plate - 18b**

Stout is 6-7 times longer than broad and moderately curved. Cells are uniformly narrowed to the obtusely rounded apices. Cell wall is smooth and terminal vacuoles are with numerous moving granules. Cells are 255.07 µm long, 36.95 µm in diameter and apices 7.20 µm wide.

Tiffany, L.H. and Britton, M.E. 1952, p. 172, pl. 52, Fig. 549.

Collection No: DLS201290, Accession No: 02016

***Closterium parvulum* Naegeli**

**Plate - 18c**

Cells are 9-15 times longer than broad, strongly curved, inner margin not tumid, gradually attenuated to the acutely rounded apices. Cell wall is smooth, colorless

or rarely yellowish brown. Cells are 93.18 µm long, 9.24 µm in diameter and apices 2.82 µm wide.

Tiffany, L.H. and Britton, M.E. 1952, p. 173, pl. 51, Fig. 543.

Collection No: DLS201291, Accession No: 002017

***Closterium venus* Kuetzing ex Ralfs**

**Plate - 18e**

Cells are small, 9-10 times longer than broad, strongly curved, outer margin with 155- 170 degrees of arc and inner margin curved like outer. Cell is gradually attenuated to acute or sub acute apices. Cell wall is smooth. Cells are 81.55 µm long, 11.08 µm in diameter and apices 1.94 µm wide.

Prasad, B.N. and Misra, P.K. 1992 II, p. 119, pl. 16, Fig.s.18.

Tiffany, L.H. and Britton, M.E. 1952, p. 173, pl. 51, Fig. 542

Gerrath, J.F. and John, D.M. 1998, p. 206, pl. 3, Figs. 6-9

Collection No: DLS201275, Accession No: 02001

***Closterium lunula* (Mull) Nitzsch f. *biconvexum* (Schmidle) Kossinskaja**

**Plate - 18i**

Cells are oval to obtuse, upper end is much broader and attenuated at the top, the lower end is longer and rounded. Cells are 39.04 µm long, 11.83 µm in diameter and 3.23 µm broad at the apex.

Kant, S. and Gupta, P. 1998, p. 130, pl. 119, Fig. 3.

Collection No: DLS201249, Accession No: 001975

**Closterium *punctulatum* Breb.**

**Plate - 18a**

Cells are 10-11 times longer than broad, lateral margins almost straight and converging from centre to narrow acute apices. Cell wall is smooth. Cells are 559.27 µm long, 55.62 µm in diameter and 12.59 µm broad at the apex.

Collection No: DLS201263, Accession No: 001989

### Genus: *Penium* Brebisson, 1844

Order: *Desmidiales*

Family: *Desmidiaceae*

Genus: *Penium*

Species: *margaritaceum*

***Penium margaritaceum* (Ehrenberg) Brebisson**

**Plate - 42b**

Cells are 6-12 times longer than wide, cylindric or subfusiform, very slightly attenuated to the truncately rounded apices. Cell wall is reddish brown with longitudinal rows of minute granules. Cells are 73–170 µm long and 12.50-26.0 µm in diameter.

Tiffany, L.H. and Britton, M.E. 1952, p. 176, pl. 51, Fig. 540.

Collection No: DLS201224, Accession No: 001950

### Genus: *Euastrum* Ehrenberg

Order: *Desmidiales*

Family: *Desmidiaceae*

Genus: *Euastrum*

Species: *denticulatum* var. *rectangular, insulare, interminus* var. *burmense spinulosum, sublobatum* var. *sumatranum*

***Euastrum denticulatum* (Kirchh.) Gay. var. *rectangular* West and G. S. West**

**Plate - 23b**

Cells are small with deep constrictions. Semi-cells are three lobed, polar lobes are short and broad with deep medium incision. The apical angles are furnished with short spines and lateral lobes bilobulate. Cells are 39.85 µm long, 24.63 µm in diameter and with 4.43 µm isthmus.

Prasand, B.N. and Mehrotra R.K. 1977a, p. 70, pl. 3, Fig. 74.

Collection No: DLS201280, Accession No: 02006

***Euastrum insulare* (Wittr.) Roy**

**Plate - 23e**

Cells are small, slightly longer than broad with linear and closed sinus. The basal angles are narrowly rounded, lateral margins slightly retuse, deeply concave to form the expanded polar lobes. The apical angles are rounded, apex is recurved with a shallow median depression and its vertical view is elliptical. Cells are 26.51 µm long, 16.90 µm in diameter and with 3.51µm isthmus.

Dwivedi, S. 2001, p. 120, pl. 12, Fig. 5.

Taylor, W.R. 1934-1935, pl. 39, Fig. 5.

Collection No: DLS201280, Accession No: 001975

***Euastrum interminus* (Nords.) Turner var. *burmense* West**

**Plate - 23c**

Cells are small, slightly wider than long, deeply constricted. The semicells are 5 lobed, each lobes furnished with 4-5 small acute spines, polar lobes are small with a shallow median notch and angles furnished with three to four small spines. Cell wall is granulated in the polar and lateral lobes and each semicell is with two big pyrenoids. Cells are 62.43 µm long, 49.59 µm in diameter and with 8.59 µm isthmus.

Prasad, B.N. and Misra, P.K. 1992 II, p. 231, pl. 19, Fig. 10.

Dwivedi, S. 2001, p. 121, pl. 12, Fig. 7.

Collection No: DLS201280, Accession No: 02006

***Euastrum spinulosum* Delphy**

**Plate - 23a**

Cells are rather small, slightly longer than broad, deeply constricted. The sinus is narrow and linear. The semi-cells possess five lobes, lateral lobes rounded and furnished with 5-6 small and acute spines, polar lobe are small, broadly truncate with a shallow median notch and angles furnished with three small and acute spines. Cell wall is granulating within the polar and lateral lobes, each semi-cell

is with a rounded central protuberance consisting of two rows of relatively larger granules. Cells are 58.83 µm long, 52.64 µm in diameter and with 14.59 µm isthmus.

Scott, A.M. and Prescott, G.W, 1961, p. 132.

Toppo, K. 2007, p. 123, pl. 15, Fig. 3.

Collection No: DLS201275, Accession No: 02001

***Euastrum sublobatum* Breb var. *sumatranum* Scott *et* Prescott**

**Plate - 23d**

Cells are small, slightly longer than wide with linear and closed sinus. The basal angles remain narrowly rounded, lateral margins are almost straight and slightly divergent for about one and half of the height than deeply concave to form the expanded polar lobe. The apical angle is broadly rounded, apex is recovered with median depression and vertical view is elliptical. Cells are 23.83 µm long, 16.90 µm in diameter and with 3.42 µm isthmus.

Dwivedi, S. 2001, p. 122, pl. 12, Fig. 6.

Scott, A.M. and Prescott, G.W. 1961, pl. 14, Fig. 14.

Collection No: DLS201265, Accession No: 001991

### Genus *Micrasterias* C. A. Agardh

Order: *Desmidiales*

Family: *Desmidiaceae*

Genus: *Micrasterias*

Species: *pinnatifida, radians*

***Micrasterias pinnatifida* (Kuetz.) Ralfs Formae.**

**Plate - 19f**

Cells usually solitary, medium size, deeply constricted and sinus is with apical portion linear and outer open. Semi-cells are lobed with deep, radial and widely open, polar lobe with sub-parallel sides showing retusely emarginated and somewhat expanded apex with furcated, acuminate extremity. Each lobe has

incision and lobules are with furcated, acuminate extremities. Cell wall is smooth. Cells are 60.21 µm long, 74.44 µm in diameter and isthmus is 11.36 µm.

Turner, W.B. 1892, p. 91, pl. 5, Fig. 6, a.

Scott, A.M. and Prescott, G.W. 1961, p. 51, pl. 23, Fig. 1.

Collection No: DLS201273, Accession No: 001999

***Micrasterias radians* Turner**

**Plate - 19e**

Cells are also of medium size, sub-circular, very deeply constricted and sinus is with apical portion linear and outer open. Semi-cells are five lobed with deep, radical and widely open, polar lobe with sub-parallel sides showing retusely emarginated and expanded apex with furcated, acuminate extremity. Each lateral lobe is divided into two lobes by incision as deep as between polar and lateral lobes and lobules are with furcated, acuminate extremities. Cell wall is smooth. Cells are 128.73 µm long, 119.85 µm in diameter and isthmus is 15.42 µm.

Toppo, K. 2007, p. 126, pl. 15, Figs. 9.

Collection No: DLS201272, Accession No: 001998

### Genus: *Cosmarium* Corda 1834

Order: *Desmidiales*
Family: *Desmidiaceae*
Genus: *Cosmarium*
Species: *auriculatum, botrytis*, *botrytis* var. *mediolaeve, candianum* var. *depressum, capense, connatum, contractum* f. *jacobsenii, granatum, leave, lundellii, margaritatum, moniliforme, pseudogranatum, polygonum, pardalis, perfissum, pseudobroomei, pachydermum* var. *minus, phaseolus* var. *omphalum, portianum, punctulatum, pygmaeum, quadrum, reniforme*, *retusiforme, subtumidum*, *subgranatum* var. *borgei*, *subimpressulum, vermae, turpinii*, *subundulatum*

***Cosmarium auriculatum* Reinsch**

**Plate - 11b**

Cells are small, constriction not deep and sinus open outwards with rounded apex. Semicells are elliptical and sides having 5-undulations with sharp and pointed ridges, apex is narrow and straight. Cell wall with punctuations arranged in transverse series. Cells are 56.15 µm long, 55.23 µm in diameter and isthmus is 27.15 µm.

Toppo, K. 2007, p. 138, pl. 19, Fig. 7.

Prasad, B. N. and Misra, P. K. 1992 II, p. 153, pl. 22, Fig. 14.

Collection No: DLS201264, Accession No: 001990

***Cosmarium botrytis* Menegh**

**Plate - 7a, 8f**

Cells are of medium size, slightly longer than broad with deep constriction, sinus narrowly towards apex and slightly open outwards. Semi-cells are sub-semi-circular with sides crenate, apex truncate with less straight margin. Cells are 52.64 µm long, 48.02 µm in diameter and isthmus is 13.11 µm.

Kant, S. and Gupta, P. 1998, p. 135, pl. 117, Fig. 5.

Toppo, K. 2007, p. 140, pl. 21, Fig. 7.

Collection No: DLS201272, Accession No: 001998

***Cosmarium botrytis* Menegh var. *mediolaeve* W. West**

**Plate - 9e**

Cells are of medium size, slightly longer than broad, constriction deep, semi-cells semi-circular with sides crenate, apex truncate with less straight margin. Cells are 54.30 µm long, 46.27 µm in diameter and isthmus 14.31µm

Prescott, G.W. 1937, p. 205, pl. 2, Figs. 2, 3.

Collection No: DLS201290, Accession No: 002016

***Cosmarium candianum* Delp. var. *depressum* Croasd**

**Plate - 11f**

Cells are elongate with relatively broader apices and deep constriction, sinus linear. Cell wall is smooth. Cells are 34.82 µm long, 38.60 µm in diameter and isthmus is 9.24 µm.

Kumar, S. 2005, p. 143, pl. 18, Fig. 11.

Therezien, Y. 1985, p. 526, pl. 9, Fig. 2.

Collection No: DLS201249, Accession No: 001975

***Cosmarium capense* De Toni**

**Plate - 9b**

Cells are of medium size, semi-cells semi-circular, sinus narrowly linear and close. Cell wall is punctate. Cells are 76.28 µm long, 60.03 µm in diameter and isthmus is 26.69 µm.

Toppo, K. 2007, p. 141, pl. 19, Fig. 8.

Collection No: DLS201273, Accession No: 002017

***Cosmarium connatum* Breb. Ex Ralfs**

**Plate - 8c**

Cells are rather small, moderately constricted, isthmus broad, sinus very widely open. Semi-cells are transversely sub-elliptic with broad and flat apex. Cell wall is finely scorbiculate. Each semi-cell is with a chloroplast disposed in relation to two large pyrenoids. Cells are 70.10 µm long, 50.70 µm in diameter and isthmus is 38.05 µm.

Gontcharov, A.A., Watanabe, M. and Watanabe, M.M. 1999, pl. 3, Fig. 1.

Toppo, K. 2007, p. 142, pl. 17, Fig. 2.

Collection No: DLS201276, Accession No: 002002

***Cosmarium contractum* Kirchn. f. *jacobsenii* (Roy) W. et G. S. West**

**Plate - 7e, 9c**

Cells are of medium size, a little longer than broad, deeply constricted, sinus broadly open outwards. Semi-cells are circular and cell wall is smooth. Cells are 31.12 µm long, 24.66 µm in diameter and isthmus is 5.08µm.

Toppo, K. 2007, p. 143, pl. 23, Fig. 4.

Collection No: DLS201274, Accession No: 002000

***Cosmarium granatum* Brebisson**

**Plate - 9a**

Cells are longer than wide, elliptic, deeply constricted, sinus linear, slightly dilated at the apex. Semicells are truncate-pyramidate, basal angles rounded, sides straight, slightly convex or rarely slightly concave, subparallel at the base and converging toward the apex, upper angles are obtuse and apex is narrowly truncate and straight. The vertical view is elliptic and lateral view of semicell is elliptic-ovate. Cell-wall is finely punctate. Cells are 40.08 µm long, 29.28 µm in diameter and isthmus is 6.74 µm wide.

Tiffany, L.H. and Britton, M.E. 1952, p. 186, pl. 53, Fig. 565.

Collection No: DLS201272, Accession No: 001998

***Cosmarium leave* Rabenh**

**Plate - 6f**

Cells are sub-pyramidal with apex slightly retuse in the middle. Cell wall is finely punctate. Cells are 27.52 µm long, 24.75 µm in diameter and isthmus is 6.19 µm.

Yamagishi, T. and Kanetsuma, Y. 1990, p. 48, pl. 7, Fig. 15

Toppo, K. 2007, p. 152, pl. 17, Fig. 12.

Collection No: DLS201273, Accession No: 001999

***Cosmarium lundellii*** **Delphy**

**Plate - 8e**

Cells are of medium size, sinus open outwards, semi-cells are sub circular with rounded basal angles and truncate apices. Cell wall is scorbiculate and top view is elliptic. Cells are 68.82 µm long, 53.51 µm in diameter and isthmus is 21.22 µm.

Prescott, G.W. 1931, p. 121, pl. 31, Fig. 26.

Collection No: DLS201280, Accession No: 002006

***Cosmarium margaritatum*** **(Lund.) Roy *et* Biss**

**Plate - 9d**

Cells are rather large 1.3-1.4 times longer than broad, deeply constricted. Semicells are sub-rectangular, apex and sides slightly convex, basal and apical angles broadly rounded. Cell wall is uniformly granulated with punctae between them (34-38 granules seen round the margin). Cells are 63.54 µm long, 51.90 µm in diameter and isthmus is 16.07 µm.

Prasad, B.N. and Misra, P.K. 1992 II, p. 165, pl. 23, Figs. 3.

Collection No: DLS201280, Accession No: 002006

***Cosmarium moniliforme*** **(Turpin) Ralfs**

**Plate - 6e, 7f, 11c**

Cells are deeply constricted, sinus acute and widely open. Semicells are circular or subcircular in all views. Cell wall is smooth. Cells are 34.45 µm long, 22.90 µm in diameter and isthmus is 10.07 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 184, pl. 53, Fig. 577.

Collection No: DLS201256, Accession No: 001982

***Cosmarium pseudogranatum***

**Plate - 10b**

Cell are small, semicells broadly truncate exhibiting rather prominently convex sides and truncate-rounded apex. Cell wall is minutely punctate. Cells are 37.31 µm long, 25.95 µm in diameter and isthmus is 6.83 µm.

Prasad, B.N. and Misra, P.K. 1992 II, p. 175, pl. 21, Fig. 22.

Collection No: DLS201275, Accession No: 002001

***Cosmarium polygonum* (Naeg.) Arch**

**Plate - 8b**

Cells are small, slightly longer than broad, deeply constricted, sinus narrow. Semicells are broadly hexagonal, angles rounded, lateral angles slightly pronounced, sides and apex faintly retuse. Cell wall is smooth**.** Cells are 20.23 µm long, 19.12 µm in diameter and isthmus is 4.25 µm.

Prasad, B.N. and Misra, P.K. 1992 II, p.171, pl. 21, Fig. 11.

Collection No: DLS201290, Accession No: 002016

***Cosmarium pardalis* Cohn 1874**

**Plate - 7d**

Cells are deeply constricted, sinus gradually opening from a dilated extremity. Semicells are reniform. Cell wall is granulated and granules arranged in horizontal and indistinct vertical series. Cells are 60.58 µm long, 51.26 µm in diameter and isthmus is 14.50 µm.

http://www.algaebase.org/search/species/detail

Scott, A.M. and Prescott, G.W. 1961, p. 64, pl. 29, Fig. 1, 2.

Collection No: DLS201289, Accession No: 002015

***Cosmarium perfissum*** **G. S. West**

**Plate - 7c, 11a**

Cells are small, somewhat broad, dumble shaped, slightly broader than long, deeply constricted, sinus narrowly opening outwards. Semi-cells are with concave sides and cell wall is minutely punctate. Cells are 23.92 µm long, 22.72 µm in diameter and isthmus is 10.07 µm.

Scott, A.M. and Prescott, G.W. 1961, p. 65, pl. 26, Fig. 98.
Toppo, K. 2007, p. 162, pl. 18, Figs. 11.
Collection No: DLS201241, Accession No: 001967

***Cosmarium pseudobroomei*** **Wolle**

**Plate - 11e**

Cells are small about as long as a broad, very deeply constricted, sinus narrowly linear with dilated extremity. Semicells are oblong-rectangular with rounded angles and slightly convex sides and apex. Cell wall possesses small and solid granules arranged in somewhat curved, horizontal series with 21-31 seen at the margin of each semicell. The side-view is semicircular and top view is oblong with sub parallel sides. Cells are 49.32 µm long, 40.45 µm in diameter and isthmus is 10.16 µm.

Prasad, B.N. and Misra, P.K. 1992 II, p. 173, pl. 23, Figs. 6, 11, 12.
Turner, W.B. 1892, p. 66, pl. 9, Fig. 41.
Collection No: DLS201267, Accession No: 001993

***Cosmarium pachydermum*** **E. E. Lund var.** ***minus***

**Plate - 7b**

Cells are large, slightly longer than broad, deeply constricted, sinus linear with a dilated apex. Semi-cells are sub-circular with depressed apex. Cell wall is punctate with big punctations. Cells are 65.02 µm long, 50.42 µm in diameter and isthmus is 22.07 µm.

Toppo, K. 2007, p. 162, pl. 21, Fig. 2.
Collection No: DLS201284, Accession No: 002010

***Cosmarium phaseolus* Breb. var. *omphalum* (Schaarsehm.) Racib**

**Plate - 10g**

Cells are small, nearly as long as broad. Semi-cells are semi-circular with swollen apices, each semi-cell with the one chloroplast and one pyrenoid. Cell wall is punctate. Cells are 23.37 µm long, 21.80 µm in diameter and isthmus is 8.03µm.

Scott, A.M. and Prescott, G.W. 1961, pl. 31, Fig. 17.

Toppo, K. 2007, p. 163, pl. 16, Fig. 7.

Collection No: DLS201290, Accession No: 002016

***Cosmarium portianum* Archer**

**Plate - 10f**

Semicells are circular, ellipsoid, reniforme and cell surface is granulated. Cells are 33.15 µm long, 26.04 µm in diameter and isthmus is 7.57 µm.

Turner, W.B. 1892, p. 59, pl. 8, Fig. 51.

Collection No: DLS201273, Accession No: 001999

***Cosmarium punctulatum* Brebisson**

**Plate - 8d**

Semi cells are truncate to sub-semicircular or oblong-trapeziform with deep constriction, lateral margin crenate and apex broadly truncate. Cells are 35.83 µm long, 29.74 µm in diameter and isthmus is 8.50 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 188, pl. 54, Fig. 592

Prasad, B.N. and Misra, P.K. 1992 II, p. 176, pl. 23, Fig. 21.

Collection No: DLS201280, Accession No: 002006

***Cosmarium pygmaeum* W. Archer**

**Plate - 10e**

Cells are very small, a little longer than broad, deeply constricted, sinus narrowly linear. Semi-cells are oblong, hexagonal, basal and apical angles are sharp, apex

widely truncate with straight margin and center of each semi-cell is with a faint protuberance. Cell wall is smooth. Cells are 12.74 µm long, 12.38 µm in diameter and isthmus is 2.40 µm.

Turner, W.B. 1892, pl. 8, Fig. 20.

West, W. and West, G.S. 1908, p. 73, pl. 71, Figs. 24-31.

Toppo, K. 2007, p. 168, pl. 16, Fig. 14.

Collection No: DLS201249, Accession No: 001975

***Cosmarium quadrum*** **Lundell**

**Plate - 9f**

Cells are about as long as wide or slightly longer, quadrate in outline, deeply constricted, sinus linear and slightly dilated at the apex. Semi cells are sub rectangular, basal angles rounded, apical angles broadly rounded, sides almost straight or slightly convex, apex usually slightly retuse, sometimes straight. The lateral view of semicell is subcircular and vertical view is oblong –elliptic, the sides are straight and parallel. Cell-wall is granulated and the granules are arranged in decussating oblique series and somewhat less distinct vertical series, 34-37 showing at margin of each semicell, slightly reduced in middle of the apex. Cells are 74.71 µm long, 60.95 µm diameter and isthmus is 21.70 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 193, pl. 53, Fig. 580.

Collection No: DLS201288, Accession No: 002014

***Cosmarium reniforme*** **(Ralfs) Arch**

**Plate - 6b**

Cells are of medium size, slightly longer than broad with deep constriction, sinus narrow and linear with widely dilated extremity. Semicells are reniform and cell wall is granulated, granules are fairly regular, horizontal and in indistinct vertical series (about 25-31), granules seen at margin of a semicell. Cells are 57.54 µm long, 47.93 µm in diameter and isthmus is 13.85 µm.

Prasad, B.N. and Misra, P.K. 1992 II, p.181, pl. 23, Fig. 8.

Collection No: DLS201282, Accession No: 002008

***Cosmarium retusiforme* (Wille) Gutw**

**Plate - 11d**

Cells are small, slightly longer than broad, hexagonal with deep constrictions, sinus narrowly linear with dilated apex. Semi-cells with upper part of lateral margins are converging and retuse, apices faintly retuse with rounded apex. Cell wall is thick and smooth. Cells are 39.06 µm long, 27.61 µm in diameter and isthmus is 8.77 µm.

Scott, A.M. and Prescott, G.W. 1961, p. 68, pl. 32, Fig. 15.

Toppo, K. 2007, p. 171, pl. 16, Fig. 17.

Collection No: DLS201256, Accession No: 001982

***Cosmarium subtumidum* Nordst**

**Plate - 6c**

Cells are of medium size, slightly longer than broad. Semi-cells are semi-circular, sinus linear and open and cell wall is smooth. Cells are 39.16 µm long, 33.34 µm in diameter and isthmus is 8.50 µm.

Toppo, K. 2007, p. 117, pl. 21, Fig. 8.

Collection No: DLS201280, Accession No: 002006

***Cosmarium subgranatum* var. *borgei***

**Plate - 8a**

This variety belongs to a group of small sized cosmaria marked by a smooth cell wall with (in frontal view) an undulate margin. Semicells are about pyramidal in outline and undulations of the margin are pretty irregular. Variety *borgei* differing in broader, more flattened cell apices. Cells are 27.52 µm long, 23.0 µm in diameter and isthmus is 5.73 µm.

Coessel, P.F.M and Meesters, K.J. 2007:
http://www.algaebase.org/search/genus/detail/

Collection No: DLS201289, Accession No: 002015

***Cosmarium subimpressulum* Borge**

**Plate - 6d**

Cells are longer than wide with deep constriction, sinus narrow and linear. Semicells are transversely rectangular in lower part, pyramidate-truncate above, apex is prominent, sides parallel below, attenuated upward, crenate with four crenations on each side, in part acute and nearly forming right angles. The vertical view is elliptic with a broad inflation on each side and lateral view of semicell is ovate, apex truncate, tumid in the middle on both sides. Cell wall is smooth. Cells are 38.97 µm long, 27.61 µm in diameter and isthmus is 7.20 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 186, pl. 54, Fig. 588.

Collection No: DLS201272, Accession No: 001998

***Cosmarium subundulatum* Wille**

**Plate - 6a**

Cells are of medium size and are longer than broad, deeply constricted, sinus narrowly linear. Semi-cell is sub-circular and cell wall is crenate. Cells are 40.36 µm long, 30.66 µm diameter and isthmus is 7.39µm.

Prescott, G.W. 1937, p. 211, pl. 4, Figs. 2, 16.

Collection No: DLS201274, Accession No: 002000

***Cosmarium turpinii* Brebisson**

**Plate - 10a**

Cells are slightly longer than wide, very deeply constricted, sinus narrowly linear with a slightly dilated apex and somewhat open outward. Semicells are pyramidate-trapeziform, rapidly converging from a broad base with basal angles rounded, sides concave especially above, apical angles obtuse, apex slightly retuse. The lateral view of semicell is ovate with a granulated inflation on each side near the base having rounded apex. The vertical view is narrowly elliptic, with a pair of adjacent granulate tumors at the middle on each side. Cell-wall is densely granulated with granules irregularly arranged, slightly reduced in size

toward the center, with a pair of small, central tumors arising from a common base, covered with large irregularly arranged granules, and surrounded by a small ill-defined clear space. Cells are 59.60 µm long, 56.28 µm in diameter and isthmus is 13.47 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 191, pl. 54, Fig. 593.

Collection No: DLS201280, Accession No: 002006

***Cosmarium vermae* B. N. Prasad et R. K. Mehrotra**

**Plate - 10d**

Cells are small, slightly longer than broad, slightly constricted, sinus linear and closed. Semi-cells are trapeziform with sides undulated, sharply angled, forming projecting lobes at angles having side lobes wide, each lobe and undulation furnished with three - four granules and apex is slightly undulated. Cells are 26.78 µm long, 21.61 µm in diameter and isthmus is 5.73 µm.

Prasad, B.N. and Mehrotra, R. K. 1977a, p. 68, 70, pl. 1, 3, Fig.s, 46, 82.

Collection No: DLS201275, Accession No: 002001

**Genus: *Arthrodesmus* Ehrenberg, 1838**

Order: *Desmidiales*

Family: *Desmidiaceae*

Genus: *Arthrodesmus*

Species: *octocornis*

***Arthrodesmus octocornis* Ehrenberg**

**Plate - 26a**

Cells are solitary, usually small and as long as broad, generally compressed and bilaterally symmetrical in front view. The median constriction is pronounced with widely open to linear sinus. Semicells are generally triangular and are rarely elliptic, sub-trapeziform or sub-rectangular with a simple, straight or curved spine on lateral angles. Cell wall is without any ornamentation. The lower spines of the two semicells are so convergent that they overlap slightly at their tips. Cells are 32.78 µm long with spines, without spines 19.76 µm long and diameter with

spines are 33.80 µm, without spines 16.72 µm and isthmus is 4.53 µm.

Scott, A.M. and Prescott, G.W. 1961, p. 77, pl. 35, Figs. 9-12

Collection No: DLS201281, Accession No: 02007

**Genus: *Staurastrum* Meyen 1829**

Order: *Desmidiales*

Family: *Desmidiaceae*

Genus: *Staurastrum*

Species: *arachne* var. *sumatranum, cuspidatum, dickiei* var. *circulare, furcatum, gemelliparum, gladiosum, gracile, gracile* f. *iyengar, granulosum, hexacerum, kalapanii, longibrachiatum* var. *intermedium, pachyrhynchum, pseudotetracerum, punctulatum, retusum, rhynchoceps, setigerum*, *tetracerum*

***Staurastrum* species Meyen**

**Plate - 14a**

Cells are variable in size, usually longer than broad (excluding processes) and bilaterally symmetrical in front view with median constriction usually deep, generally with acute sinus and narrow isthmus. Semi-cells are cylindrical, ellipsoid, triangular, hexagonal, campanulate, cyathiform or trapiziform, with the angles frequently produced into processes of different sizes usually showing some sort of ornamentation and terminating in bi or trifurcate acute processes. Cell wall is smooth, punctuate, scrobiculate, granulate, denticulate, clothed with spines or verrucae, with one kind or combination of these ornamentations. The top view is usually triangular or 4-8 angular but may be upto 11-angular.

Collection No: DLS201252, Accession No: 001978

***Staurastrum arachne* Ralfs var. *sumatranum* Scott and Prescott**

**Plate - 14d**

Cells are small, longer than broad (excluding the processes) depressed, constriction shallow with an acute notch. Semicells are somewhat broadening towards the slightly convex apex. The short and emarginated processes are tipped

with 2-3 minute spines and the top view shows five processes. Cells are 35.23 µm long, 15.61 µm in diameter without processes and 34.26 µm in diameter with processes.

Scott, A.M. and Prescott, G.W. 1961, p. 110, pl. 59, Fig. 7.

Collection No: DLS201241, Accession No: 001967

***Staurastrum cuspidatum* Breb**

**Plate - 13i**

Cells are little longer than broad, deeply constricted, sinus opening widely and with apex broadly rectangular, isthmus narrow, rectangular, greatly elongated and semicells are fusiform. The ventral margin is slightly convex and the dorsal slightly concave, the lateral angles are acute and terminating in a straight, acute spine. The spines are parallel, convergent or divergent. The vertical view is usually triangular rarely quadrangular, sides concave, each with a straight spine, cell wall is smooth. Cells are 21.98 µm long, 18.75 µm in diameter and isthmus is 4.62 µm.

Scott, A.M. and Prescott, G.W. 1961, p. 197, pl. 25, Fig. 3.

Collection No: DLS201275, Accession No: 02001

***Staurastrum dickiei* var. *circulare***

**Plate - 13e**

Cells are small circular slightly longer than broad, semicells are semicircular with open sinus. The vertical view is usually triangular with concave sides, each with a straight spine. Cell wall is smooth. Cells are 37.49 µm long, 32.88 µm in diameter and isthmus is 4.22 µm.

Dwivedi, S. 1967, p. 150, pl. 16, 17, Figs. 12, 8.

Turner, W.B. 1892, pl. 16, Figs. 5a, b.

Collection No: DLS201267, Accession No: 001993

***Staurastrum furcatum* (Ehrenb.) Breb**

**Plate - 15d**

Cells are small, slightly longer than broad, deeply constricted and acute sinus. Cell wall is smooth and each semi-cell is with nine bifid processes. The vertical view is triangular with angles continued into short processes ending in a spine and sides with a pair of bifid processes on each lateral margin. Cells are 26.41µm long with spines, without spines 18.19 µm long, diameter with spines are 25.86 µm and without spines 11.73 µm and isthmus is 7.39 µm.

Iyengar, M.O.P and Bai, V. 1940, p. 93, Fig. 75.

Toppo, K. 2007, p. 199, pl. 27, Fig. 11.

Collection No: DLS201281, Accession No: 02007

***Staurastrum gemelliparum* Nordst**

**Plate - 13d**

The processes of these algae were tipped with tree spines. However, the vertical view of these cells was clearly triangular with widely concaved margins between the processes. Cells are 29.92 µm long with process and 18.75 µm long without process, 32.32 µm in diameter with process and 17.82 µm in diameter without processes.

Gontcharov, A.A. 1997, p. 75, Fig., 45.

Collection No: DLS201266, Accession No: 001992

***Staurastrum gladiosum* Turner**

**Plate - 14e**

Cells are of medium size, about as long as broad or slightly longer than broad, sinus acute and not very widely open. Semi-cells are elliptic reniform and cell wall is uniformly covered with stout spines, more or less arranged in circles and scattered further away. The vertical view is triangular with sides slightly concave and angles broadly rounded, about 6-8 spines on each side. Cells are 27.98 µm in

diameter without spines, 36.94 µm in diameter with spines, 25.77 µm long and having 5.61 µm long spine.

Iyengar, M.O.P. and Bai, B.V. 1940, p. 92, 94, Figs. 72, 73.
Collection No: DLS201280, Accession No: 002006

***Staurastrum gracile* Ralfs**

**Plate - 15b**

Cells are deeply constricted, sinus with an acute apex and opening widely, isthmus narrow. Semicells are broadly triangular to cup-shaped with dorsal margin slightly concave to convex with a row of small emarginated verrucae. The angles are continued in processes of variable length which are slightly alternated and horizontal. The vertical view is triangular, the sides are slightly concave and angles continued in straight processes. Cell wall is with an intramarginal row of pairs of granules and the center without ornamentation, the processes with transverse rings of granules and terminating in three or four small spines. Cells are 44.88 µm in diameter, 32.23 µm with long and isthmus is 20.23 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 199, pl. 55, Fig. 613.
Collection No: DLS201290, Accession No: 002016

***Staurastrum gracile* Rolfs. f. *iyengar et* Vimala Bai**

**Plate - 15f**

Cells are small, about 1.5 times longer than broad with slight constriction in the form of an acute notch. Semicells are slightly broading towards the faintly convex apex and the upper angles produced in to more or less horizontally disposed long processes tipped with three minute spines and showing many concentric series of denticulations. The top view is triangular. Cells are 19.30 µm in diameter without process, 36.02 µm with process, 33.06 µm long and isthmus 8.40 µm broad.

Dwivedi, S. 2001, p. 151, pl. 16, Figs. 6, 13.
Iyengar, M.O.P. and Bai, V. 1941, p. 96, Figs. 64-66.
Prasad, B.N. and Misra, P.K. 1992 II, p. 197, pl. 25, Figs. 14, 18
Collection No: DLS201273, Accession No: 001999

***Staurastrum granulosum* (Ehrenberg) Ralfs**

**Plate - 14c**

Cells are small, almost as long as broad, deeply constricted, sinus open with sub acute apex. Semicells are more or less obsemicircular with convex apex, lateral angles scarcely rounded and furnished with acute spines. The top view is triangular with slightly retuse median portion. Cell wall is granulated, granules minute, arranged in concentric series near the lateral angles and scattered in the middle portion. Cells are 28.81 µm long and 31.68 µm in diameter.

Prasad, B.N. and Misra, P.K. 1992 II, p. 197, pl. 25, Figs. 20, 22.

Collection No: DLS201241, Accession No: 001967

***Staurastrum hexacerum* Turner**

**Plate - 13h**

Cells are small, slightly broader than long, deeply constricted, sinus open. Semi-cells are sub-triangular with both margins being convex and tapering towards the angles, forming very short processes ending in about 3 spines. Cell wall is rough with tiny granules arranged in concentric series. The vertical view is triangular with lateral margins concave. Cells are 25.30 µm long, diameter with processes 30.20 µm, without processes 23.83 µm in diameter and isthmus 7.57 µm.

Iyengar, M.O.P. and Bai, V. 1940, p. 93, Figs. 58, 59, 61.

Toppo, K. 2007, p. 201, pl. 28, Fig. 14.

Collection No: DLS201274, Accession No: 02000

***Staurastrum kalapanii B*. N. Prasad *et* P. K. Misra**

**Plate - 13a**

Cells are small, slightly longer than broad, deeply constricted with open sinus and acute angles. Semi-cells are narrowly cuneate and the ventral margin is somewhat more convex than dorsal margin. The lateral angles are round and upwardly turned. The top view is triangular with concave sides. Cell wall is finely punctate

with punctuations arranged in concentric series around the angles. Cells are 18.66 µm long, 15.79 µm in diameter and isthmus is 5.45µm.

Prasad, B.N. and Misra, P.K. 1992 II, p. 156, pl. 1, 2, Figs. 8, 8a.
Collection No: DLS201269, Accession No: 001995

***Staurastrum longibrachiatum* (Borge) Gutw. var. *intermedium* Iyengar and Bai**

**Plate - 15c**

Colony is single, medium sized, fairly constricted, sinus widely open. Semi-cells are truncated gradually and attenuated towards the apex with four big verrucae at the apex, two big in the center and two small on either side. The angles of the semi-cells produced into long hollow slender processes with sharply denuate upper and lower margins with ends of the processes bifurcated, short verrucae at the base of the process on each side of the semi-cells. The vertical view is elliptic, poles continued into long processes with slightly undulate margins and about four verrucae in the top view. Cell wall is punctate. Cells are 26.04 µm in diameter without process, 68.06 µm in diameter with processes, 37.59 µm long, and isthmus is 7.76 µm.

Iyengar, M.O.P and Vimla, B. 1941, pl .91, Figs. 77, 80, 81.
Toppo, K. 2007, p. 202, pl. 28, Figs. 1, 4.
Collection No: DLS201290, Accession No: 002016

***Staurastrum pachyrhynchum* Nordst**

**Plate - 154e**

Cells are as long as broad, deeply constricted, sinus open and acute angled. Semicells are sub elliptic with dorsal margin strongly convex and angles thickened, obtusely rounded and slightly produced with a faint upward tilt. The top view is triangular showing convex sides. Cell wall is smooth. Cells are 29.37 µm long and 27.15 µm in diameter.

Prasad, B.N. and Misra, P.K. 1992 II, p. 198, pl. 25, Figs. 19, 21.

Collection No: DLS201280, Accession No: 002006

***Staturastrum pseudotetracerum* (Nordst.) W. *et* G. S. West**

**Plate - 15a**

Cells are very small, slightly broader than long (including the processes), deeply constricted, sinus widely open and broadly triangular in outline. Semicells are cuneate and apex nearly straight or faintly convex, lateral angles produce to form strongly divergent processes, tipped with 3 minute spines and furnished with many indistinct concentric rings of sub acute granules. The top-view is triangular with slightly concave sides. Cells are 10.71 µm long, 12.01 µm in diameter without processes, 22.44 µm in diameter with processes and isthmus is 4.06 µm.

Prasad, B.N. and Misra, P.K. 1992 II, p. 14, pl. 2, Fig. 8.

Collection No: DLS201277, Accession No: 002003

***Staurastrum punctulatum* Breb**

**Plate - 14b**

Cells are solitary, consists of two semicells joined by a central isthmus. Semicells are showing a radial symmetry about their vertical axis so triangular in apical view. Semicells are sub rhomboidal-elliptical and upper and lower margins are almost equally convex with angles acutely rounded and alternating with adjacent semicells, deeply constricted, sinus open and acute-angled. Cell wall is with concentric series of granules around angles. Cells are 30.94 µm long, 28.08 µm in diameter and isthmus is 9.79 µm.

Ralfs, J. 1848, pl. XXIV, Fig. 1.

Collection No: DLS201267, Accession No: 001993

***Staurastrum retusum* W. B. Turner**

**Plate - 14f**

Cells are small, as long as broad, very deeply constricted, sinus narrowly linear with a slightly dilated extremity. Semicells are shortly pyramidate-trapeziform with angles little rounded and lateral margins straight, convex, or concave, apex

very slightly concave. The vertical view is triangular, angles rounded and sides are concave. Cells are 31.77 µm long and 35.37 µm in diameter.

Turner, W.B. 1892, p. 121, pl. 13, Fig. 13b.

Collection No: DLS201265, Accession No: 001991

***Staurastrum rhynchoceps* Kreig. 1933**

**Plate - 13g**

Cells are small, slightly longer than broad. In front view it differs in the possession of a ring of minute sharp teeth above and below the isthmus. In vertical view differs in the longer and more slender processes which are gracefully curved in a clockwise direction. Cells are 36.48 µm long, 28.17µm in diameter with processes and isthmus is 8.59 µm.

Scott, A.M. and Prescott. G.W. 1961, p. 105, pl. 56, Figs. 8, 9.

Collection No: DLS201275, Accession No: 002001

***Staurastrum setigerum* Cleve**

**Plate - 13b**

Cells in vertical view are radiating, triangular and quadrangular with numerous spines. Cells are 30.29 µm in diameter and with 11.08 µm long spine.

Anand, N. 1998, p. 58, Fig. 183.

Toppo, K. 2007, p. 207, pl. 28, Fig. 12.

Collection No: DLS201265, Accession No: 001991

***Staurastrum tetracerum* Ralfs**

**Plate - 13c**

The front view shows four slender diverging processes which are entire at the apex and end view compresses with a process at each extremity. Cells are 18.10 µm long, 12.93 µm in diameter without processes, 25.95 in diameter with processes and isthmus is 4.06 µm.

Ralfs, J. 1848, p. 226, pl. XXXV.

Collection No: DLS201264, Accession No: 001990

## Genus: *Staurodesmus* Teiling, 1948

Order: *Desmidiales*

Family: *Desmidiaceae*

Genus: *Staurodesmus*

Species: *cuspidatus*

***Staurodesmus* species Teiling**

**Plate - 13f**

Cells are solitary, small to medium sized, with shallow or deep median constriction (isthmus) where semicell walls overlap. The end view of cell is elliptical or more commonly triangular or multiangular. Cell angles each with single stout or tiny spine, small granule or cell wall incrassation. Cell wall is elsewhere unornamented but with scattered pores through which a broad gelatinous sheath often produced. Cells are 40.45 µm long, 36.57 µm in diameter and isthmus is 3.39 µm. Spines are µm 9.52 long.

Collection No: DLS201267, Accession No: 001993

***Staurodesmus cuspidatus* (Brebisson) Teiling**

**Plate - 26b**

Cells are little longer than broad, deeply constricted, sinus opening widely and with apex broadly rectangular, isthmus narrow, rectangular, greatly elongated. Semi cells are fusiform, ventral margin slightly convex, the dorsal convex slightly concave, lateral angles acute and terminating in a straight, acute spine. The spines are parallel, convergent or divergent. The vertical view is usually triangular rarely quadrangular, sides concave each with a straight spine. Cell wall is smooth. Cells 21.98 µm long, 18.75 µm in diameter and isthmus 4.62 µm.

Scott, A.M. and Prescott, G.W. 1961, p. 89, pl. 53, Fig. 13.

Toppo, K.2007, p. 197, pl. 25, Fig. 3.

Collection No: DLS201280, Accession No: 02006

**Genus*: Desmidium* C. A. Agardh, 1824:**

Order: *Desmidiales*

Family: *Desmidiaceae*

Genus: *Desmidium*

Species: *bengalicum*

***Desmidium bengalicum* Turner**

**Plate - 43b**

Cells are small, usually broader than long and closely united into straight filaments embedded in thick gelatinous sheath, middle constriction is shallow, sinus open. Semicells are narrowly elliptic with straight or slightly retuse apices and more or less convex sides. Cell wall is smooth. Cells are 19.76 µm long, 24.57 µm in diameter and isthmus is 21.98 µm.

Prasad, B.N. and Misra, P.K. 1992 II, p. 203, pl. 26, Fig. 514.

Collection No: DLS201265, Accession No: 001991

**Genus: *Spondylosium* Breb.**

Order: *Desmidiales*

Family: *Desmidiaceae*

Genus: *Spondylosium*

Species: *nitens* var. *triangular* f. *javanicum, planum*

***Spondylosium nitens* (Wall) Ach. var. *triangular* Turner f. *javanicum* Gutw**

**Plate - 22d**

Cells usually small, or of medium size and united in long filaments which are generally not twisted and lacking gelatinous sheath. Cells are almost slightly longer than broad, moderately constricted, sinus open with rounded to sub-acute angles. Semi-cells are oblong to triangular with slightly protruding and rounded apex and broadly rounded lateral angles. Cell wall is smooth. Cells are 32.14 µm long, 25.21 µm diameter and isthmus is 8.31 µm.

Toppo, K. 2007, p. 215, pl. 29, Fig. 14.

Collection No: DLS201266, Accession No: 001992

***Spondylosium planum*** **(Wolle) W. and G. S. West**

**Plate - 22e**

Cells are slightly wider than long, compressed, deeply constricted, sinus widely open with the apex broadly rounded, isthmus narrow, united in filaments without a gelatinous sheath, not twisted. Semi-cells are oblong-elliptic with ventral margin more rounded than the dorsal, lateral margins broadly rounded, apices flat, region of contact between adjacent cells very broad. The vertical view is oblong-elliptic and cell wall is smooth. Cells are 9.60 µm long, 10.90 µm diameter and isthmus is 6.56 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 201, pl. 56, Fig. 625.

Collection No: DLS201264, Accession No: 001990

**Genus*: Sphaerozosma* Corda ex Ralfs, 1848**

Order: *Desmidiales*

Family: *Desmidiaceae*

Genus: *Sphaerozosma*

Species: *filiforme*

***Sphaerozosma filiforme*** **Ralfs**

**Plate - 22a**

Cells united in short or long filaments, small, compressed (biradiate) with deep median constriction (isthmus). Each semicell apex is with pair of rod-like processes serving as anchor points for pieces of primary wall holding cells together in a filament. Apical processes arising close together or widely separated. Cell walls with pores often arranged in horizontal or oblique lines. One chloroplast per semicell, with central pyrenoid. Cells are 13.39 µm in diameter.

Coesel, P.F.M and Meesters, K. J. 2007.

Ralfs, J. 1848: http://www.algaebase.org/search/genus

Collection No: DLS201280, Accession No: 02006

## Genus: *Xanthidium* Ehrenberg ex Ralfs, 1848

Order: *Desmidiales*
Family: *Desmideacae*
Genus: *Xanthidium*
Species: *acanthophorum, antilopaeum* var. *polymazum*

***Xanthidium acanthophorum* Nordst**

**Plate - 28e**

Cells are solitary, slightly compressed (biradiate) with deep median constriction (isthmus) where semicell walls overlap and furnished with a series of numerous spines along the outer margin. The semi cell centre is more or less inflated and sculptured by granules or large scrobicles. Cell wall is scattered with cylindrical pores through which a narrow or broad gelatinous sheath is secreted. Cells are 36.66 µm long, 31.95 µm in diameter, isthmus is 9.42 µm and spines are 8.31 µm long.

Scott, A.M. and Prescott, G.W. 1961, p. 79, pl. 36, 37, Fig. 12.
Collection No: DLS201288, Accession No: 02014

***Xanthidium antilopaeum* (Breb.) Kutz. var. *polymazum* Nordst**

**Plate - 28f**

It is marked by hexagonal semicells the apical and lateral angles of which as a rule are furnished with two stout spines each, disposition of the marginal spines and the central pattern of scrobiculae. Cells are 56.70 µm long, 51.62 µm in diameter, isthmus is 16.53 µm, and spines are 8.68 µm long.

Turner, W.B. 1892, p. 184, pl. XIII, Fig. 1.
Collection No: DLS201241, Accession No: 001967

### 2.2.2 *Cyanophyceae*

Cyanophyceae comprises of prokaryotic organisms, popularly called blue green algae and due to the nature of their cell wall, cell structure and capacity to fix the atmospheric nitrogen, these are also known as cyanobacteria. The cyanophyceaen members can be broadly classified into coccoid and filamentous forms, which may be unicellular or multicellular, without true nucleus and chromatophore. Protoplasm differentiated into peripheral zone with photosynthetic pigments (chromatoplasm) and central colourless portion with generative centroplasm. The assimilatory pigments are chlorophyll, phycocyanin, phycoerythrin, carotenoids. Cell contents are blue green, olive green or yellowish brown. Cell wall is thin or after gelatinization very thick, colourless or often yellow to brown, blue or violet colored.

**Genus*: Dactylococcopsis:***

Order: *Chroococcales*
Family: *Chroococcaceae*
Genus: *Dactylococcopsis*
Species: *acicularis*

***Dactylococcopsis acicularis* Lemmermann 1900, Ber. D. Deutsch**

**Plate - 22b**

A free-floating colony (rarely solitary) acicular or straight cells with extremely finely pointed poles, inclosed by a wide gelatinous envelope. Cell contents are nearly colorless to light blue-green and homogeneous. Cells are 60.40 µm long and 4.71 µm in diameter.

Prescott G.W. 1951, p. 463, pl. 105, Figs. 1, 2.

Collection No: DLS201258, Accession No: 001984

## Genus: *Gloeothece* Nag. 1849

Order: *Chroococcales*

Family: *Chroococcaceae*

Genus: *Gloeothece*

Species: *rupestris, samoensis* var. *major*

***Gloeothece rupestris* (Lyngb.) Bornet**

**Plate - 33b**

Cells are ellipsoidal to cylindrical, straight or bent and 1.5 times as long as broad, the contents are mostly blue green, 2-4 rarely 8 together in oval to subglobose colonies. The envelopes are colorless or brownish at the peripheries, lamellated or unlamellated. Cells are 9.05 µm long, 6.56 µm in diameter and colonies 26.32 µm in diameter.

Desikachary, T.V. 1959, p. 127, pl. 25, Fig. 4.

Collection No: DLS201288, Accession No: 02014

***Gloeothece samoensis* Wille var. *major* Wille**

**Plate - 33a**

Cells are ellipsoidal, yellowish or bluish green in round colonies, often many uniting, mostly 2-4 in a common envelope. The envelope is colorless sometimes colored brownish and unlamellated. Cells without sheath are 10.90 µm long and 10.99 µm in diameter. Colony is 49.41 µm in diameter.

Desikachary, T.V. 1959, p. 128, pl. 23, Fig. 3.

Collection No: DLS201288, Accession No: 02014

## Genus: *Gloeocystis* Naegeli, 1849

Order: *Chroococcales*

Family: *Chroococcaceae*

Genus: *Gloeocystis*

Species: *ampla*

***Gloeocystis ampla* Kuetzing**

**Plate - 33c**

Cells are ovoid, enclosed in unstratified gelatinous sheath and colonies are usually fragmenting. Cells are 12.38 µm long and 10.34 µm in diameter.

Tiffany, L.H. and Britton, M.E. 1952, p. 21, pl. 3, Fig. 23.

Collection No: DLS201288, Accession No: 02014

**Genus: *Gomphosphaeria* Kutz., 1836**

Order: *Chroococcales*
Family: *Gomphosphaeriaceae*
Genus: *Gomphosphaeria*
Species: *naegeliana*

***Gomphosphaeria* species**

**Plate - 33e**

Colonies are ellipsoidal or cordate, rarely subglobose, seldom very spherical, often in groups of four but also in 2 - 8, with or without individual envelopes and are arranged in a hollow spherical mucilaginous free swimming colony, attached to radially arranged, branched mucilaginous stalks. Cells are 4.71 µm in diameter and colony is 51.44 µm in diameter.

Desikachary, T.V. 1959, p. 148, pl. 28.
Collection No: DLS201272, Accession No: 001998

***Gomphosphaeria naegeliana* (Unger) Lemmermann, Kryptogamenflora der Mark Brandenburg**

**Plate - 33d**

Colony is spherical or ellipsoidal, reniform or irregularly shaped with broad radial mucilage stalks. Cells are oval or ellipsoidal, closely arranged, with gas vacuoles, often with indistinct individual mucilaginous envelopes. Cells are 4.89 µm long, 4.43 µm in diameter and colony is 47.84 µm in diameter.

Desikachary, T.V. 1959, p. 147, pl. 28 Figs. 9, 16, 22.

Collection No: DLS201272, Accession No: 001998

### Genus: *Merismopedia* Meyen, 1839

Order: *Chroococcales*

Family: *Chroococcaceae*

Genus: *Merismopedia*

Species: *glauca*

***Merismopedia glauca*** **(Ehrenberg) Nag. / Kuetzing**

**Plate – 33g**

Colonies are mostly small with 16-32-64 cells. Cells are oval or spherical, homogenous, closely arranged, pale blue-green, forming rectangular colonies. Cells are free floating, arranged in a in a single plane within homogeneous mucilage. Cells are 4.53 µm in diameter and colony is 39.25 µm in diameter.

Desikachary, T.V. 1959, p. 155, pl. 29, Fig. 5.

Tiffany, L.H. and Britton, M.E. 1952, p. 334, pl. 91, Fig. 1052.

Collection No: DLS201273, Accession No: 001999

### *Genus*: *Chroococcus* Naegeli, 1848

Order: *Chroococcales*

Family: *Chroococcaceae*

Genus: *Chroococcus*

Species: *minutes, turgidus* var. *maximus, tenax, schizodermaticus*

***Chroococcus minutes*** **(Kutz.) Nag**

**Plate - 25d**

Cells are spherical or oblong, single or in groups of 2-4, light blue-green. Sheath is not lamellated and is colorless. Cells with sheath are 12.38 µm in diameter and without sheath 9.60 µm in diameter. Colonies are 22.72 µm long and 30.66 µm in diameter.

Desikachary, T.V. 1959, p. 105, pl. 26, Fig. 15.

Collection No: DLS201274, Accession No: 02000

***Chroococcus turgidus* var. *maximus* (Kutz.) Nygaard**

**Plate - 25a, e**

Cells are spherical or ellipsoidal single or in groups. Cells in groups of 2-4 or 8 are blue-green, olive green or yellowish and sheath is colorless, much lamellated in the inner portions. Cells without sheath are 5.91 µm in diameter and with sheath 9.05 µm in diameter. Colonies are 35.83 µm long and 27.61 µm in diameter.

Desikachary, T.V. 1959, p. 102, pl. 26, Fig. 8.

Collection No: DLS201272, Accession No: 001998

***Chroococcus tenax* (Kirchn.) Hieron**

**Plate - 25c**

Cells are mostly in groups of 2-4, sometimes 8-16, blue-green or olive coloued. Sheath is colorless or yellow to brown, very thick and very distinctly lamellated with 3 - 4 lamellae. Cells without sheath are 13.76 µm in diameter and with sheath 23.83 µm in diameter.

Desikachary, T.V. 1959, p. 103, pl. 26, Figs. 7, 16.

Collection No: DLS201280, Accession No: 02006

***Chroococcus schizodermaticus* West**

**Plate - 25b**

Cells are in groups of 2-4, blue-green, sheath is yellow to brown, very distinct, lamellated, with 5-10 lamellae, and outer layers often broken. Cells without sheath are 8.03 µm in diameter and with sheath are14.68 µm in diameter. Colony is 35.92 µm in diameter.

Desikachary, T.V. 1959, p. 103, pl. 26, Figs. 17.

Collection No: DLS201281, Accession No: 02007

### Genus: *Coelosphaerium* Naegeli, 1848

Order: *Chroococcales*

Family: *Gomphosphaeriaceae*

Genus: *Coelosphaerium*

Species: *collinsii*

***Coelosphaerium collinsii*** **Drouet and Daily**

**Plate - 34b**

Colony is microscopic, many-celled, spherical, ovoid and constricted. Cells are ovoid to obcylindric, blue-green or yellowish green, without pseudovacuoles. Gelatinous matrix is diffluent. Cells never form extensive water-blooms. Cells are 5.26 µm long, 3.51 µm in diameter, and coloney is 42.30 µm in diameter.

Tiffany, L.H. and Britton, M.E. 1952, p. 332, pl. 90, Fig. 1044.

Collection No: DLS201274, Accession No: 02000

### Genus: *Microcystis* Kutzing, 1846

Order: *Chroococcales*

Family: *Chroococcaceae*

Genus: *Microcystis*

Species: *aeruginosa*

***Microcystis aeruginosa*** **Kutz**

**Plate - 33f**

Colonies when young are round or slightly longer than broad, solid, when old becoming clathrate, with distinct hyaline colonial mucilage. Cells are spherical or elongated, many in spherical, ellipsoid or irregularly overlapping or net-like colony generally with gas-vacuoles. Cells are free-swimming and often with attached daughter colonies. Cells are in homogeneous colourless mucilage and individual envelopes are absent. Cells are mostly very densely arranged. It is generally found in plankton forming water-blooms in stagnant waters. Cells are 5.91 µm in diameter.

Desikachary, T.V. 1959, p. 93, pl. 17, 18, Figs. 1, 2, 6, 10.

Collection No: DLS201253, Accession No: 001979

### Genus: *Trichodesmium* Ehrenberg, 1830:

Order: *Nostocales*
Family: *Oscillatoriaceae*
Genus: *Trichodesmium*
Species: *lacustre*

***Trichodesmium lacustre* Klebahn**

**Plate - 34c**

Trichomes are straight, cylindrical without sheath arranged in parallel and forming free floating bundles, or flocculent masses, not attenuated or slightly attenuated. The end cells are rounded, not capitate. Cells are shorter than broad and short barrel-shaped. Cells are 6.46 µm long and 10.16 µm in diameter.

Desikachary, T.V. 1959, p. 246, pl. 42, Figs. 1, 2, 6, 22

Collection No: DLS201251, Accession No 001977

### Genus: *Lyngbya* Ag., 1824

Order: *Nostocales*
Family: *Oscillatoriaceae*
Genus: *Lyngbya*
Species: *birgei*, *contorta*

***Lyngbya birgei* Smith, G. M**

**Plate - 34g**

Filaments are straight, seldom coiled, free floating. Sheath is firm, colorless and mostly unlamellated, seldom lamellated. Trichome is not constricted at the cross walls, ends are rounded, not attenuated, not capitate. Cells are shorter than broad and sometimes with gas vacuoles. Cells are 2.62 µm long and 20.36 µm in diameter.

Desikachary, T.V. 1959, p. 296, pl. 50, Figs.7, 8. 22.

Collection No: DLS201248, Accession No 001974

***Lyngbya contorta* Lemm**

**Plate - 34f**

Filaments are single, free floating and regularly spirally coiled with delicate, nearly circular coils. Sheath is narrow and colorless. Cells are not restricted at the cross walls and granulated with a single granule or without them. The end cells are rounded and not attenuated. Cells are 4.57 µm long, 5.81 µm in diameter and colony is 99.64 µm in diameter.

Desikachary, T.V. 1959, p. 290, pl. 48, 50, Figs. 5, 5, 9.

Collection No: DLS201292, Accession No: 02018

**Genus*: Phormidium* Kutz., 1843:**

Order: *Nostocales*

Family: *Oscillatoriaceae*

Genus: *Phormidium*

Species: *purpurascens*

***Phormidium purpurascens* (Kutz.) Gomont**

**Plate - 34e**

Thallus is compact, leathery and purple to brownish violet. Thallus is attached by the lower side or floating in water with torn margins. Trichome is cylindrical, strongly bent, entangled, not constricted at the cross-walls, ends are not attenuated, dark violet. Sheath is more or less diffluent. Cells are nearly quadrate or upto nearly two times longer than broad and cross walls are marked by two granules on either side, end-cells are rounded and calyptra is absent. Cells are 4.43 µm long and 2.49 µm in diameter.

Desikachary, T.V. 1959, p. 262, pl. 44, 45, Figs. 1-4.

Collection No: DLS201289, Accession No: 02015

## Genus: *Anabaena* Bory, 1822

Order: *Nostocales*

Family: *Nostocaceae*

Genus: *Anabaena*

Species: *doliolum, circinalis*

***Anabaena doliolum*** **Bharadwaja**

**Plate - 32a**

Thallus is soft mucilaginous and pale blue-green. Trichome is single, free swimming, straight, curved or slightly coiled. Cells are slightly tapering at the ends with conical apical cell possessing almost pointed apex, cells are barrel-shaped, as long as broad or a little longer or shorter than broad. Heterocysts are barrel shaped and formed near the heterocysts or in between the heterocysts. Cells are 6.28 µm in diameter. Heterocyst is 8.96 µm long and 7.48 µm in diameter.

Desikachary, T.V. 1959, p. 410, pl. 78, Figs. 3, 22.

Collection No: DLS201294, Accession No: 02020

***Anabaena circinalis*** **Rabenhorst ex Born. et Flah**

**Plate - 32b**

Filament is frothy, floating, circinate and seldom straight mostly without a sheath. Cells are barrel-shaped or spherical, somewhat shorter than broad, with gas vacuoles. Heterocyst is subspherical and spores are cylindrical, sometimes curved. The ends are rounded, ordinarily away from the heterocyst. Cells are 7.02 µm long and 6.19 µm in diameter. Heterocyst is 8.62 µm long and 9.02 µm in diameter.

Desikachary, T.V. 1959, p. 414, pl. 77, Fig. 2.

Collection No: DLS201278, Accession No: 02004

**Genus*: Arthrospira* Stizenberger, 1852**

Order: *Nostocales*

Family: *Oscillatoriaceae*

Genus: *Arthrospira*

Species: *jenneri*

***Arthrospira jenneri* (Kuetz.) Stizenberger**

**Plate - 32c**

Trichomes are blue-green, multicellular, cylindrical, without sheath, scattered or gregarious, loosely coiled and not tapering toward the apices. Cells are quadrate, granular and terminal cell is rounded, calyptras absent. Cells are 3.71 µm long and 6.15 µm in diameter.

Prescott G.W. 1951, p. 481, pl. 108, Figs. 22, 12.

Collection No: DLS201295, Accession No: 02021

**Genus: *Aphanizomenon* Morren**

Order: *Nostocales*

Family: *Nostocaceae*

Genus: *Aphanizomenon*

Species: *flos-aquae*

***Aphanizomenon flos-aquae* (Linn.) Ralfs ex Born. et Flah**

**Plate - 32d**

Trichomes are in a bundle, seldom single, straight or bent and forms feathery, plate- like or spindle-shaped bundles. Heterocysts are nearly cylindrical and spores are cylindrical with rounded ends away from the heterocyst. Cells are 2.83 µm long. Heterocyst is 6.72 µm long, 4.87 µm in diameter and spores are 22.86 µm long and 5.89 µm in diameter.

Desikachary, T.V. 1959, p. 359, pl. 107, Fig. 6.

Collection No: DLS201278, Accession No: 02004

### Genus*: Spirulina* Turpin em. Gardner, 1827

Order: *Nostocales*

Family: *Oscillatoriaceae*

Genus: *Spirulina*

Species: *meneghiniana*

***Spirulina meneghiniana*** **Zanard. ex Gomont**

**Plate - 34d**

Trichome is flexible, multicellular cylindrical, irregularly spirally coiled and bright blue-green forming a thick blue-green thallus. The sheath is absent and the terminal cell is rounded without calyptras. Cells are 7.92 µm long and 4.51 µm in diameter.

Desikachary, T.V. 1959, p. 195, pl. 36, Fig. 4, 8.

Collection No: DLS201293, Accession No: 02019

### Genus: *Oscillatoria* Vaucher 1892

Order: *Nostocales*

Family: *Oscillatoriaceae*

Genus: *Oscillatoria*

Species: *chalybea, chlorina, curviceps, irrigua, formosa, granulate, limosa*

***Oscillatoria chalybea*** **Mertens in Jurgens 1822**

**Plate - 35b**

Trichomes are aggregated to form a dark blue-green plant mass and straight for a portion of their length but much entangled and sometimes spirally twisted, gradually tapering toward the apex. Trichome forms a flat or spongy free-swimming thallus, sheath absent, rarely with a more or less very delicate sheath, motile mostly by a creeping movement causing rotation on the longitudinal axis. Cells are not granular and apical cell is conical with a smooth unthickened outer membrane. Cells are 8.60 µm in diameter and 4.95 µm long.

Prescott, G.W. 1951, p. 468, pl. 109, Fig. 89

Collection No: DLS201294, Accession No: 02020

***Oscillatoria chlorina* Kuetzing**

**Plate - 35d**

Cells are with scarcely granulose protoplasm. Trichomes are straight or bent, not apically tapering and not constricted at pellucid and ungranulated cross-walls, yellow green, end cell is rounded and without outer thickening, forming very thin, yellow-green masses. Cells are 6.10 µm long and 6.37 µm in diameter.

Tiffany, L.H. and Britton, M.E. 1952, p. 344, pl. 94, Figs. 1088, 1089.

Collection No: DLS201295, Accession No: 02021

***Oscillatoria curviceps* C. A. Agardh 1824**

**Plate - 35a**

Trichomes forms an expanded blue-green plant mass and straight for at least a portion of their length, twisted and much entangled, scarcely tapering to the apex. The apical cell is broadly rounded, not capitate, without a calyptra. Cells are not constricted at the cross walls, which may be granulate. Trichomes always form floating clots. Cells are 3.78 µm long and 19.49 µm in diameter.

Prescott, G.W. 1951, p. 487, pl. 108, Figs. 17, 18.

Collection No: DLS201292, Accession No: 02018

***Oscillatoria irrigua* (Kutz.) Gomont**

**Plate - 35f**

Thallus is blackish blue-green and trichome is light bluish purple, when dried hyaline or pale blue, straight, flexuous, not torulose, apex is slightly attenuated, subcapitate and straight. Cells are quadrate with septa ordinarily not granulated and apical cell is convex, with an evident thickened outer wall. Cells are 6.74 µm long and 5.73 µm broad.

Desikachary, T.V. 1959, p. 224, pl. 42, Figs. 7, 9, 42.

Collection No: DLS201293, Accession No: 02019

***Oscillatoria formosa* Bory 1827**

**Plate - 35g**

Trichomes are aggregated to form a dark blue-green plant mass, straight and rather firm, curved and slightly tapering toward the apex. Cells are granular with apical cell conical, not capitate and without a calyptra. Cells are 4.16 µm long and 4.16 µm in diameter.

Prescott, G.W. 1951, p. 487, pl. 109, Figs. 10, 11.

Collection No: DLS201289, Accession No: 02015

***Oscillatoria granulate* Gardner 1927**

**Plate - 35c**

Trichomes are aggregated to form an expanded plant mass, straight or somewhat curved, especially at the apex, which is slightly attenuated. The apical cell is not capitate and without a calyptra. Cells are 3.60 µm long and 4.71 µm in diameter.

Prescott, G.W. 1951, p. 487, pl. 109, Fig. 12.

Collection No: DLS201292, Accession No: 02018

***Oscillatoria limosa* Ag. Ex Gomont**

**Plate - 35e**

Thallus is dark blue-green to brown. Trichome is more or less straight, dull blue-green, brown or olive-green, not constricted at the cross-walls, or only slightly constricted. The cross walls are frequently granulated and end-cell is flatly rounded with slightly thickened membrane. Cells are 20.78 µm in diameter and 5.63 µm long.

Desikachary, T.V. 1959, p. 206, pl. 42, Fig. 11.

Tiffany, L.H. and Britton, M.E. 1952, p. 344, pl. 93, Fig. 1076.

Collection No: DLS201294, Accession No: 02020

### 4.2.3 *Eugleanophyceae*

Cells are solitary, variously shaped, motile (by means of flagella or amoeboid movement) or rarely epiphytic, or forming immobile dendroid colonies, uninucleate, with 1-3 flagella or rarely none. The protoplast may be naked or enclosed either within a wall or a lorica. The lorica consists of a transparent, colorless gelatinous material that becomes opaque and coloured yellow or brown with age due to impregnation with iron salts. The protoplast is colorless or pigmented and the outer portion is differentiated as a flexible or rigid periplast that is frequently ridged or striate. There may be a single forward projecting flagellum with a basal bifurcation, or two flagella without a bifurcation and both projecting forward, or one forward and the other trailing. An eyespot is present in most of the pigmented species and in some of the colorless ones.

**Genus: *Phacus* Dujardin, 1841**

Order: *Euglenales*

Family: *Phacaceae*

Genus : *Phacus*

Species: *anacoelus* var. *undulata, acuminatus, anacoelus, longicauda*

***Phacus anacoelus* var. *undulata* Skvortzow**

**Plate - 30b**

Cells are broadly ovoid, produced posteriorly into a long (or short) sharply pointed caudus, oblique to the longitudinal axis of the cell, anteriorly broadly rounded but slightly bilobed because of the gullet groove. The margin of the cell is with 2-3 bulges and 1 large centrally located paramylon disc is present, pigment-spots are usually present. Cells are103.90 µm long and 81.64 µm in diameter.

Prescott, G.W. 1951, p. 397, pl. 87, Fig. 3.

Collection No: DLS201294, Accession No: 02020

***Phacus acuminatus* Stokes 1855a**

**Plate - 30d**

Cells are suborbicular in outline, broadly rounded posteriorly, with a short, blunt apiculation and paramylon bodies are 1-2 ring like discs. Cells are 20.69 µm long and 16.53 µm in diameter.

Prescott, G.W. 1951, p. 396, pl. 88, Fig. 4.

Collection No: DLS201264, Accession No: 001990

***Phacus anacoelus* Stokes 1888**

**Plate - 30a**

Cells are broadly ovoid, narrowed abruptly posteriorly to form a short caudus, which turns to the left and paramylon bodies are 1-2 circular plates. The lateral margins of cells are with 2-3 creases or folds and the membrane convex between the indentations. Cells are 35.19 µm long and 31.77 µm in diameter.

Prescott, G.W. 1951, p. 397, pl. 87, Figs. 7, 8.

Collection No: DLS201292, Accession No: 02018

***Phacus longicauda* (Ehrenb.) Dujardin 1841**

**Plate - 30c**

Cells are broadly ovoid to pyriform, tapering gradually posteriorly to form a long, straight, sharply pointed caudus and anteriorly broadly rounded. The flagellum is shorter than the cell in length and paramylon body is usually in the form of a single large (or small) circular plate. Cells are 75.82 µm long and 38.42 µm in diameter.

Prescott, G.W. 1951, p. 397, pl. 87, Figs. 7, 8.

Collection No: DLS201269, Accession No: 001995

## Genus: *Lepocinclis* Perty, 1849:

Order: *Euglenales*

Family: *Phacaceae*

Genus: *Lepocinclis*

Species: *fusiformis, fusiformis* var. *minor*

***Lepocinclis fusiformis*** **(Carter) Lemmermann 1901**

**Plate - 30e**

Cells are broadly fusiform or pyriform, slightly produced posteriorly to form a blunt basal point and membrane is spirally striated. The reserve food is in the form of two to several large paramylon bodies (circular plates), the two together sometimes nearly encircling the cell and flagellum is about as long as the cell, chloroplasts are numerous parietal discs**.** Cells are 37.22 µm long and 25.21 µm diameter.

Prescott, G.W. 1951, p. 406, pl.89, Figs. 1-4.

Collection No: DLS201293, Accession No: 02019

***Lepocinclis fusiformis*** **var.** ***minor***

**Plate - 30f**

Cells are broadly elliptical and anterior pole is without an obtuse nipple, opening of apical channel and posterior pole is slightly acuminated. The pellicle is rigid, hyaline, striae very delicate difficult to see and numerous chloroplasts are present. The paramylon bodies are 2, lateral ring-shaped. Cells are 39.25 µm long and 25.58 µm in diameter. The variety differs from the typical species in its cell dimensions and without caudal process.

http://www.algaebase.org/search/genus/detail/

Collection No: DLS201295, Accession No: 002021

### Genus: *Trachelomonas* Ehrenberg, 1835

Order: *Euglenales*

Family: *Euglenaceae*

Genus: *Trachelomonas*

Species: *hispida* var. *coronate*

***Trachelomonas hispida* var. *coronate* Lemmermann**

**Plate - 31g**

In this genus euglenoid cells are enclosed in a firm gelatinous shell which has an opening for the flagellum. The test is brown, often opaque, or tan to nearly colorless, according to the amount of iron compounds deposited in it. The test may be smooth or decorated with spines, warts, reticulations, punctaions, or combinations of these. The protoplast inside is highly metabolic and has the general features of the euglenoids. There is 1 flagellum, a red pigment-spot, and numerous ovoid disc-like chloroplasts which may have pyrenoids. Cells are oblong-oval and flagellum aperture is surrounded by a short collar with the margin bearing a circle of spines. The cell wall is uniformly beset with short spines and test is 32.60 µm long and 21.16 µm in diameter.

Prescott, G.W. 1951, p. 414, pl. 83, Fig. 30.

Collection No: DLS201290, Accession No: 02016

### Genus: *Euglena* Ehrenberg, 1838

Order: *Euglenales*

Family: *Euglenacae*

Genus: *Euglena*

Species: *acus, acus* var. r*igida, deses, proxima, spirogyra*

***Euglena* species**

**Plate - 31h**

Cells are mostly free-swimming, rarely creeping, fusiform, cylindrical, or ovate, usually round in cross section but rarely slightly flattened. The posterior end is either rounded or produced, sometimes extending into a fine point or caudus, the

anterior end usually narrowed and sometimes conspicuously two-lipped. The periplast is either firm, giving the cell a rigid shape, or soft and pliable, the cell are metabolic and constantly changing shape in its movements. When firm, the periplast is decorated with fine spiral striations or rows of granules. A gullet and a reservoir are in the anterior end from which arises a single flagellum of variable length. The chloroplasts are variable, either numerous ovoid discs, a few ribbon-like bands, or, rarely, star-shaped plates, sometimes with pyrenoids, which are embedded in the chloroplast and protrude from either side. Chlorophyll is sometimes masked by an abundance of brick-red or blood-red haematochrome, usually only temporarily present and incident to intense illumination. The food reserve is paramylon body in the form of a few large or numerous small rods, plats, rings, or discs. A pond often appears brick-red because of the production of haematochrome in the cells when exposed to intense light.

***Euglena acus*** **Ehrenberg 1838**

**Plate - 31e, f**

Cells are elongate spindle-shaped, produced posteriorly into a long, fine tapering point, narrowed and truncate at the anterior end. The membrane is indistinctly spirally striated and chloroplasts are numerous disc-like. The paramylon bodies are two to several long rods. It is almost at once identifiable by the narrow and very long, rigid cell. Cells are 114.79 µm long and 12.56 µm in diameter.

Prescott, G.W. 1951, p. 390, pl. 85, Fig. 28.

Collection No: DLS201289, Accession No: 02015

***Euglena acus*** **var.** ***rigida*** **Huebner 1886**

**Plate - 31d**

Cells are rigid spindle-shaped but narrow and elongate, tapering abruptly posteriorly into a sharply pointed tail-piece. The paramylon bodies are in the form of two long rods (rarely more numerous small rods) and chloroplasts are

numerous, plate-like and ovoid bodies, sometimes showing a spiral arrangement within the cell. Cells are 123.75 µm long and 10.81 µm in diameter.

Prescott, G.W. 1951, p. 391, pl. 85, Fig. 27.

Collection No: DLS201290, Accession No: 02016

***Euglena deses* Ehrenberg 1835**

**Plate - 31c**

Cells are highly metabolic, twisting and turning continuously. The cells are elongate-fusiform or subcylindric, posteriorly tapering rather abruptly to a short, blunt tip and membrane is finely striated. The chloroplasts are numerous, disc like and paramylon bodies are several to many rods of various length. Cells are 102.79 µm long and 12.10 µm in diameter.

Prescott, G.W. 1951, p. 392, pl. 85, Fig. 20.

Collection No: DLS201294, Accession No: 02020

***Euglena proxima* Dangeard 1902**

**Plate - 31a**

Cells are metabolic, fusiform, narrowed posteriorly to a blunt tip and periplast is spirally striated. The chloroplasts are numerous, irregularly shaped discs and paramylon bodies are also numerous small rods scattered throughout the cell. Cells are 66.12 µm long and 17.92 µm in diameter.

Prescott, G.W. 1951, p. 394, pl. 85, Fig. 25.

Collection No: DLS201295, Accession No: 02021

***Euglena spirogyra* Ehrenberg 1838**

**Plate - 31b**

Cells are somewhat metabolic, elongate-cylindric and twisted, narrowed posteriorly and extended into a sharp, bent tail-piece. The periplast is brownish, spirally striated with alternating rows of large and small shining granules. The chloroplasts are numerous disc like and paramylon bodies are flattened rings, one

anterior and one posterior to the central nucleus. Cells are 173.07 μm long and 19.49 μm in diameter.

Prescott, G.W. 1951, p. 394, pl. 86, Fig. 15.

Collection No: DLS201294, Accession No: 02020

### 4.2.4 *Bacillariophyceae*

The members belonging to this class are popularly known as diatoms. The plants are basically unicellular and in some cases become pseudo filamentous or aggregated into colonies. The cell wall of diatoms is impregnated with silica. The diatoms cell is also known as "Frustule" and the classification of diatoms is based on the pattern of ornamentation on the wall of the frustule. The cells have either bilateral or radial symmetry. The frustules are composed of two halves, "Epitheca" and "Hypotheca" and the connecting girdle bands. The valve surface has several types of markings. Punctae are regularly or irregularly arranged to form striae. Costae are elongated thickening of the frustule wall due to heavy deposition of silica. Valves of some diatoms have an opening or fissure along the apical axis called raphe. The presence of raphe or its absence on the walls of diatoms has been one of the features in the identification of diatoms and distinguishing the different genera.

**Genus*: Amphora* Ehrenberg, 1840**

Order: *Thalassiophysales*
Family: *Catenulaceae*
Genus: *Amphora*
Species: *coffeoeformis* var. *africana*

***Amphora* species Ehrenberg**

**Plate - 36b**

Cells are usually sessile with concave faces attached in girdle view and broadly elliptic in outline, with truncate ends. The girdles are usually separated by several punctate or striate intercalary bands. The valves are lunate, longitudinally

asymmetric and transversely striate. The axial field is strongly excentric, nearer the concave side of the valve and raphe is gibbous with its central nodule close to the concave margin. Cells are 33.53 µm long and 12.19 µm in diameter.

Tiffany, L.H. and Britton, M.E. 1952, p. 274.

Gupta, R.K. 2005, p. 178, pl. 54, Fig. 10.

Collection No: DLS201219, Accession No: 001945

***Amphora coffeoeformis*** **Agardh var.** ***africana*** **Fritzsch and Rich**

**Plate - 36a**

Valves are arcuate on the dorsal margin and straight on the ventral margin. The ends are pronouncedly capitate and slightly bent outwards. The dorsal side bears slightly divergent punctate striae and the ventral side is structure less. Cells are 38.70 µm long, 12.01 µm in diameter and striae are 17-19 in 10 µm.

Venkataraman, G. 1939, p. 334, 342, Fig. 105.

Collection No: DLS201223, Accession No: 001949

**Genus*: Achnanthes* Bory**

Order: *Achnanthales*

Family: *Achnanthaceae*

Genus: *Achnanthes*

Species: *coarctata* var. *parallela*

***Achnanthes coarctata*** **var.** ***parallela*** **Venkat**

**Plate - 36c**

Valves are occasionally free-floating and solitary usually attached by gelatinous stalks or united in bundles forming ribbon-like colonies, rarely into filaments. The girdle view is rectangular or curved longitudinally particularly at centre. Valves are linear with broad rounded ends and margin is almost parallel in the middle, raphe is straight, axial area broad. Cells are distinctly punctate and raidial on the raphe valve. Cells are 42.57 µm long, 9.88 µm broad and striae are 10-12 in 10 µm.

Kant, S. and Gupta, P. 1998, p. 150, pl. 170, 77, 126, 128, Figs. 7, 5, 5, 6.

Collection No: DLS201217, Accession No: 001943

### Genus*: Caloneis* Cleve 1894

Order: *Naviculales*
Family: *Naviculaceae*
Genus: *Caloneis*
Species: *silicula*

***Caloneis silicula*** **Ehrenberg Cleve**

**Plate - 39g**

Cells are solitary, free floating or attached on submerged aquatics. In girdle view, are somewhat rectangular and intercalary bands are absent. In valve view, symmetrical to the apical and transverse axis, usually with convex sides sometimes have marginal folds. The central nodules are distinct straight or slightly bent unilaterally and terminal fissures usually distinct, straight or curved. Valves are linear and biconstricted with apices rounded. Axial area is narrow, broadening to a transverse fascia with lunate thickenings on either side of the central area. The raphe is lateral and arched slightly. The striae are radiate to parallel. A fine longitudinal line is present. Cells are 83.15 µm long and 18.47 µm broad.

http://www.algaebase.org/search/genus/detail/

Collection No: DLS201210, Accession No: 001936

### Genus*: Gamphonema C*. A. Agardh, 1824

Order: *Cymbellales*
Family: *Gomphonemataceae*
Genus: *Gamphonema*
Species: *acuminatum, constrictum, truncatum, telegraphicum*

***Gamphonema acuminatum* Ehrenberg**

**Plate - 37c**

Cells are usually epiphytic on the ends of dichotomously branched gelatinous stalks, sometimes sessile, sometimes solitary and free-floating. Valves are generally cuneate, expanded near the apex and less so medianly, with cuneate and acute apex and with evidently attenuated base. The axial area is linear, narrow and central area is large, often indefinitely limited with an isolated dot. Cells are 44.77 µm long, 11.37 µm in diameter and striations are 10-13 in 10 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 272, pl. 72, Figs. 830.
Collection No: DLS201214, Accession No: 001940

***Gamphonema constrictum* Ehrenberg**

**Plate - 37b**

Cells are longer than broad, valves are clavate, constricted below the broad rounded apical pole, with attenuated basal pole. The axial area is narrow and central area is broad and irregularly defined with a dot on one side. Cells are 54.03 µm long, 7.85 µm in diameter and transverse striations are 10-12 in 10 µm,

Tiffany, L.H. and Britton, M.E. 1952, p. 271, pl. 72, Fig. 839.
Collection No: DLS201208, Accession No: 001934

***Gomphonema truncatum* Ehrenberg 1832**

**Plate - 37d**

Valves are clavate with the center tumid, a constriction at the head pole and then the apex is capitate, broadly rounded and the foot pole is rounded. Axial area is straight, expanded on either side of the axial area to form a “bow-tie” shaped central area. A single, rounded external stigma opening is present in the central area. The raphe is lateral and undulate with external proximal ends dilated and distal ends curved onto the mantle in the direction opposite the stigma. Striae are radiate, indistinctly punctuate. Cells are 37.77 µm long, 15.52 µm in diameter and striae are 10-12 in 10 µm.

www.http://research.calacademy.org/research/diatoms/names/index.asp

Collection No: DLS201209, Accession No: 01935

***Gomphonema telegraphicum* Kutzing**

**Plate - 37a**

Frustules are cuneate with apices slightly wide, truncate, base is acute and stipe is long with 2-3 valves at the end. Cells are 38.97 µm long, 9.14 µm in diameter and striae are 8-10 in 10 µm area.

Das, S.K. and Adhikary, S.P. 2012, p. 160-182, pl. 3, Fig. 79.
Collection No: DLS201217, Accession No: 001943

**Genus*: Synedra* Ehrenberg, 1830**

Order: *Fragilariales*
Family: *Fragillariaceae*

Genus: *Synedra*
Species: *capitata*, *delicatissima, dorsiventralis, ulna*, *ulna* var. *contracta, ulna* var. *amphirhynchus, tenera*

***Synedra capitata* Ehrenberg**

**Plate - 38d**

Cells are much elongated, solitary and broadly linear in girdle view. The valve is conspicuously linear with nearly parallel edges and cuneate ends. The pseudorpahe is narrowly linear, central area is usually not evident. Cells are 247.27 µm long, 12.80 µm broad and striations are 8-11 in 10 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 236, pl. 63, Fig. 722.

Collection No: DLS201256, Accession No: 001982

***Synedra delicatissima* W. Smith**

**Plate - 38f**

Frustule is linear, narrow, elongated, solitary and straight. The middle area is slightly wider and attenuated towards the apices to form rostrate end. The striations are not clearly visible in fresh material. Cells are 130.49 µm long and 5.08 µm in diameter.

Das, S.K. and Adhikary, S.P. 2012, p. 160-182, pl. 2, Fig. 59.
Collection No: DLS201262, Accession No: 001988

***Synedra dorsiventralis* Mueller**

**Plate - 38c**

Cells are solitary and valves are irregularly elliptic with narrowed but broadly obtuse ends (some-times nearly rostrate). The pseudorpahe is narrow, linear and central area is conspicuous, usually excentric. Cells are 81.18 µm long, 6.56 µm in diameter and striae are 13 - 17 in 10 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 236, pl. 63, Fig. 712.

Collection No: DLS201222, Accession No: 001948

***Synedra ulna* (Nitzsch) Ehrenberg**

**Plate - 38b**

Cells are linear in girdle view with widened extremities and solitary. The valves are linear to linear-lanceolate, gradually narrowed toward the ends, with broadly rounded poles. The pseudoraphe is narrowly linear with central area varying and often absent. Cells are197.63 µm long, 9.60 µm in diameter and strations are 8-12 (mostly 10) in 10 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 237, pl. 63, Fig. 713.

Collection No: DLS201272, Accession No: 001998

***Synedra ulna* (Nitzsch) Ehr. var. *contracta* Qstr**

**Plate - 38a**

Valves are solitary, linear with concave margins and wedge-shaped attenuated rostrate ends. The axial area is narrow, linear gradually widening towards centre. The striae are thick, lineate, parallel in the middle but strongly radiate towards apices. Cells are 93.00 µm long, 4.43 µm in diameter and striae are 10 in 10 µm.

Dwivedi, S. 2001, p. 237, pl. 63, Fig. 713.

Collection No: DLS201289, Accession No: 02015

***Synedra ulna* (Nitzsch) Ehrenberg var. *amphirhynchus* (Ehrenberg) Grunow.**
**Synonym *Synedra amphirhynchus* Ehrenberg**

**Plate - 38e**

Valves are slender, linear, straight and at the end narrow and suddenly constricted to form capitate end. The striation is distinct, parallel, absent at the middle and many times longer than broad. Cells are 86.62 µm long, 6.28 µm in diameter and striations 9-12 in 10 µm area.

Das, S.K. and Adhikary, S.P. 2012, p. 160-182, pl. 2, Fig. 61.

Collection No: DLS201250, Accession No: 001976

***Synedra tenera* W. Smith**

**Plate - 39a**

Valves are linear, narrowly lanceolate with rounded ends. The pseudorpahe is thin, narrow, formed by the union of axial and central area and central area is absent. Cells are 129.94 µm long, 3.60 µm diameter and striae are 8-10 in 10 µm area.

Prasad, B.N. and Srivastava, M.N. 1992, p. 169, pl. 24, Fig. 22.

Das, S.K. and Adhikary, S.P. 2012, p.160-182, pl. 2, Fig. 60.

Collection No: DLS201283, Accession No: 002009

**Genus: *Cymbella* C. A. Agardh, 1830**

Order: *Cymbellales*
Family: *Cymbellaceae*
Genus: *Cymbella*
Species: *affinis*, *aspera*, *lanceolata, parva, tumida*

***Cymbella affinis* Kuetzing**

**Plate - 40f**

Cells are solitary and free-floating or attached at the ends of gelatinous stalks or confined within little branched gelatinous tubes with parallel sides in girdle view

and smooth girdles, without intercalary bands. Valves are quite asymmetric, semilanceolate to semi elliptic, convex dorsally, concave to straight ventrally, with rostrate, rounded poles. The raphe is excentric, undulate toward the central nodule. The axial area is narrow and slightly medianly widened. Cells are 37.48 µm long, 16.07 µm in diameter and transverse striations are 9-12 in 10 µm.

Tiffany, L.H. and Britton, M.E. 1952. p. 279, pl. 73, Fig. 856.

Collection No: DLS201288, Accession No: 02014

***Cymbella aspera* (Ehrenberg) Cleve**

**Plate - 40i**

Valves are asymmetric, semilanceolate, dorsally convex and ventrally straight with a slight median expansion and poles are broadly rounded. The raphe is excentric somewhat curved and widened between the central nodule and the polar nodules. The axial area is very broad, sharply defined, with little central expansion without isolate dots. Cells are 112.39 µm long, 28.72 µm in diameter and 7-9 striae are in 10 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 279, pl. 73, Fig. 858.

Collection No: DLS201285, Accession No: 02011

***Cymbella lanceolata* (Ehrenberg) Van Heurck**

**Plate - 49g**

Valves are quite asymmetric, naviculoid, dorsally convex, ventrally concave with a median expansion. The raphe is excentric, narrow and medianly curved. The axial area is narrow, with slight median expansion and no isolated dots. Cells are 148.23 µm long, 26.97 µm in diameter and 9-16 striae are in 10 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 280, pl. 74, Fig. 872

Collection No: DLS201257, Accession No: 001983

***Cymbella parva* (W. M. Smith) Cleve**

**Plate - 40e**

Valves are semi-lanceolate, convex in dorsal side and slightly concave in ventral side with gradually attenuated ends, raphe exocentric, axial area narrow and without isolated dots. Cells are 86.72 µm long, 15.98 µm in diameter and striae are 10-12 in 10 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 279, pl. 74, Fig. 874.

Collection No: DLS201201, Accession No: 001926

***Cymbella tumida* (Brebisson) Van Heurck**

**Plate - 40h**

Valves are asymmetric and curved, broadly naviculoid, with rostrate poles, convex dorsal sides and straight or slightly convex ventral sides having a median expansion. The raphe is excentric and axial area narrow with central area large, round, with a ventrally placed prominent isolated dot. Cells are 80.35 µm long, 23.55µm in diameter and striations are 8-10 striae in 10 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 278, pl. 74, Fig. 860.

Collection No: DLS201218, Accession No: 001944

**Genus: *Cyclotella* Kuetzing, 1834**

Order: *Thalassiosirales*

Family: *Stephanodiscaceae*

Genus: *Cyclotella*

Species: *meneghiniana*

***Cyclotella meneghiniana* Kuetz**

**Plate - 36d**

Cells are solitary or colonial within a gelatinous envelope, small, circular, rectangular in girdle view, in valve view discoid, elliptical and radially symmetrical, central zone smooth. The ornamentation of valve is in two

concentric regions, outer zone is radially striate or punctate and inner zone is smooth or irregularly and finely punctate. Cells are 12.47 µm in diameter and costae are 7-8 in 10 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 218, pl. 58, Fig. 60.

Collection No: DLS201220, Accession No: 001946

**Genus: *Eunotia* Ehrenberg, 1837**

Order: *Eunotiales*
Family: *Eunotiaceae*
Genus: *Eunotia*
Species: *monodon*, *parallela*

***Eunotia monodon* Ehrenberg**

**Plate - 37e**

Cells are free-floating or epiphytic, solitary or united valve to valve into chains. Frustules in girdle view are broadly rectangular and in valve view are linear, slightly arched, convex on dorsal side, gradually narrowing towards ends, ventral margin is slightly concave. The ends are slightly constricted on the dorsal side, rounded. The raphe is thin, distinct and striae are coarse lineate, somewhat more closely placed near apices. Cells are 41.56 µm long, 10.99 µm in diameter and striae are 12-13 in 10 µm.

Prasad, B.N. and Misra, P.K. 1992 II, p. 201, pl. 26, Fig. 3.

Tiffany, L.H. and Britton, M.E. 1952, p. 240, pl. 64, Fig. 732.

Collection No: DLS201217, Accession No: 001943

***Eunotia parallela* Her**

**Plate - 37f**

Valves are long almost straight with parallel margins and having slight inflation near the rounded ends and terminal nodules and raphe are small, distinct. The striae are coarse, lineate, distinct, parallel, distantly placed in the middle

becoming comparatively dense towards apices. Cells are 77.02 µm long, 7.11µm in diameter and striae are 10-15 in 10 µm.

Prasad, B.N. and Srivastava, M.N. 1992 I, p. 183, pl. 25, Fig. 2.

Collection No: DLS201293, Accession No: 02019

**Genus: *Cocconeis* Ehrenberg, 1835; Grunow, 1868**

Order: *Achnanthales*
Family: *Cocconeidaceae*
Genus: *Cocconeis*
Species: *placentula*

***Cocconeis placentula*** **Ehrenberg**

**Plate - 36f**

Cells are solitary, epiphytic upon submerged aquatics and especially upon slow-growing filamentous algae. The septa are incomplete and intercalary bands are absent. Cells flat or slightly curved; Valves small elliptical, valve with raphe not seen, rapheless valve bears distinctly punctate, radially arranged striae, pseudo-raphe distinct, narrow and linear. Cells are 36.94 µm long, 27.71 µm in diameter and 23-25 striae are in 10 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 241, pl. 64, Figs. 734,735.

Collection No: DLS201255, Accession No: 001981

**Genus: *Fragilaria* Lyngbye, 1819; Rabenhorst, 1864**

Order: *Fragilariales*
Family: *Fragilariaceae*
Genus: *Fragilaria*
Species: *biceps*, *crotonensis, capucina, intermedia* var. *robusta*

***Fragilaria biceps*** **(Kutzing) Lange-Bertalot: Basionym** ***Synedra biceps*** **Kutzing 1865**

**Plate - 40b**

Frustules in girdle view are linear, rectangular, valves are linear with parallel sides, gradually tapering ends, striae coarse and distinct, ends slightly capitate, striae absent in one side of the middle region and therefore with a unilateral central area. Cells are 136.31 µm long and 4.06 µm broad.

Das, S.K., Bhakta, S. and Adhikary, S.P. 2010, p. 352, pl. 4, Fig. 101.

Collection No: DLS201250, Accession No: 02014

***Fragilaria crotonensis*** **Kitton**

**Plate - 40c**

Cells are united medianly into ribbon-like bands, ends often touching. The valves are narrowly linear, somewhat widened medianly and slightly enlarged at the poles. The pseudorpahe is narrow with a rectangular central area. Cells are 42.04 µm long, 5.53 µm in diameter and 15-18 striae are in 10 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 232, pl. 62, Fig. 703.

Collection No: DLS201203, Accession No: 001928

***Fragilaria capucina*** **Desmazieres**

**Plate - 40d**

Synonym *Fragilaria capucina* var. *lanceolata* Grunow, *Fragilaria capucina* f. *lanceolata* (Grunow in Van Heurck) Hustedt, *Fragilaria capucina* f. *lanceolata* (Grunow) Skabichevshii.

Valve are linear, united laterally forming free floating ribbon shaped colonies, rectangular elongated cells, bilaterally symmetrical and pseudorapahe present. Cells are 62.80 µm long and 9.97 µm in diameter and striae are 10-12 in 10 µm.

Das, S.K. and Adhikary, S.P. 2012, p. 160-182, pl. 2, Fig. 53.

Collection No: DLS201250, Accession No: 001976

***Fragilaria intermedia* Grun. var. *robusta* Venkataraman**

**Plate - 40a**

Frustules in girdle view are linear, rectangular, united together to form long bands. The valves are linear with parallel sides with gradually tapering ends, ends slightly capitates. The striae are coarse and distinct and on one side absent in the middle region and therefore with a unilateral central area. Cells are 136.96 µm long and 9.42 µm in diameter.

Venkataraman, G. 139, p. 306, Figs. 27, 42.

Dwivedi, S. 2001, p. 160, pl. 23, Fig. 1.

Collection No: DLS201221, Accession No: 001947

**Genus: *Hantzschia* Grunow, 1880**

Order: *Bacillariales*
Family: *Bacillariaceae*
Genus: *Hantzschia*

***Hantzschia* species Grunow**

**Plate - 36e**

Valves are solitary, free floating or attached on submerged aquatics and in girdle view are elongate, rectangular somewhat attenuated, in valve view are dorsiventral symmetrical on the trans apical axis but symmetrical in apical axis, linear to linear-lanceolate with margin usually convex, ventral margin concave, biaccurate or straight. The ends are usually produced rostrate to rostrate capitate and raphe of each valve in a keel near the ventral margin so that both keels on the same side of the longitudinal axis. Sometimes raphe is with carinal dots opening towards the interior of the cells and keel is punctate with short marginal or extended as transverse-costae lines. The valve surface is striated. Cells are 125.42 µm long and 15.98 µm in diameter.

Tiffany, L.H. and Britton, M.E. 1952, p. 288.

Collection No: DLS201241, Accession No: 001967

**Genus: *Nitzschia* Hassall, 1845**

Order: *Bacillariales*

Family: *Bacillariaceae*

Genus: *Nitzschia*

Species: *acicularis*

***Nitzschia acicularis* (Kutzing) W. Smith 1835** **Plate - 39h**

Cells are solitary, free-floating, isopolar, bilaterally symmetrical. Cells sometimes densely clustered in simple or unbranched gelatinous tubes and show elongate-rectangular or sigmoid girdle view. Cells usually lie in valve view and isolated valves always in valve view. Valves are bilaterally symmetrical elongate with a linear to linear-lanceolate body. Transverse striae and Raphe system are invisible in the light microscope. In a frustule, the raphe systems of the two valves lie on opposite sides (nitazschioid symmetry). Cells are 61.78 µm long and 4.16 µm in diameter.

Smith, W. 1853, p. 43.

Collection No: DLS201294, Accession No: 02020

**Genus: *Rhopalodia* Mueller, 1895**

Order: *Rhopalodiales*

Family: *Rhopalodiaceae*

Genus: *Rhopalodia*

Species: *gibba*

***Rhopalodia gibba* (Ehr.) O. Mueller**

**Plate - 39d**

Frustules are usually solitary and free-floating or in groups, linear with medianly inflated sides narrowing towards the broadly rounded ends, valve is linear, dorsal side arcuate and ventral is straight but bent at the ends, ends are acute. Cells are 189.78 µm long, 23.37 µm in diameter and striae are 7 in 10 µm.

Dwivedi, S. 2001, p. 173, pl. 22, Fig. 14.

Tiffany, L.H. and Britton, M.E. 1952, p. 283, pl. 75, Fig. 884.

Collection No: DLS201281, Accession No: 02007

## Genus: *Navicula* Bory, 1822; emend. Cleve, 1894

Order: *Naviculales*

Family: *Naviculaceae*

Genus: *Navicula*

Species: *cryptocephaloides, radiosa, viridis*

***Navicula* species Bory**

**Plate - 39f**

Cells are generally solitary and free-floating, sometimes aggregated into irregularly radiating clusters, rectangular in girdle view with smooth girdles and without intercalary bands. The valves are elongate usually attenuated toward capitate, rounded or rostrate poles. The axial field is narrow with distinct, straight raphe and polar and central expansions and small nodules. The transverse striations are sometimes somewhat medianly radial.

***Navicula cryptocephaloides* Hustedt**

**Plate - 39b**

Valves are linear-lanceolate with somewhat abruptly constricted produced rounded ends. The raphe is thin and straight with terminal fissures curved. The axial area is narrow, linear central area is fairly wide, rounded or slightly quadrate. Cells are 39.53 µm long, 9.05 µm in diameter and striae are 12-14 in 10 µm.

Kant, S. and Gupta, P. 1998, p. 159, pl. 123, Fig. 6.

Collection No: DLS201274, Accession No: 02000

***Navicula radiosa* Kuetz**

**Plate - 39c**

Valves are linear, lanceolata, gradually tapering to more or less pointed ends. The transverse striations are coarse, lineate and radial. The central area is broad and

elliptical. Cells are 75.45 µm long, 12.10 µm in diameter and striae are 10-12 in 10 µm.

Tiffany, L.H. and Britton, M.E. 1952, p. 255, pl. 67, Fig. 780.
Collection No: DLS201272, Accession No: 001998

***Navicula viridis* Kutzing**

**Plate - 39e**

Frustules are linear oblong, rectangular in valve view and slightly attenuated towards the apex. The apices are rotundatus. Cells are 44.05 µm long, 11.08 µm in diameter and striae are 8-12 in 10 µm.

Das, S.K., Bhakta, S. and Adhikary, S.P. 2010, p. 354, pl. 4, Fig. 113.
Collection No: DLS201203, Accession No: 001928

### 4.2.5 *Rhodophyceae*

The Rhodophyceae or red algae are multicellular organisms ranging from simple branched to macroscopic thalli. Vegetative cells are uninucleate as well as multinucleate with peripheral distribution of cytoplasm. Chromatophores may be single bearing single pyrenoid without starch sheath.

**Genus: *Glaucosphaera* Korsh.**

Order: *Glaucosphaerales*
Family: *Glaucosphaeraceae*
Genus: *Glaucosphaera*
Species: *vacuolata*

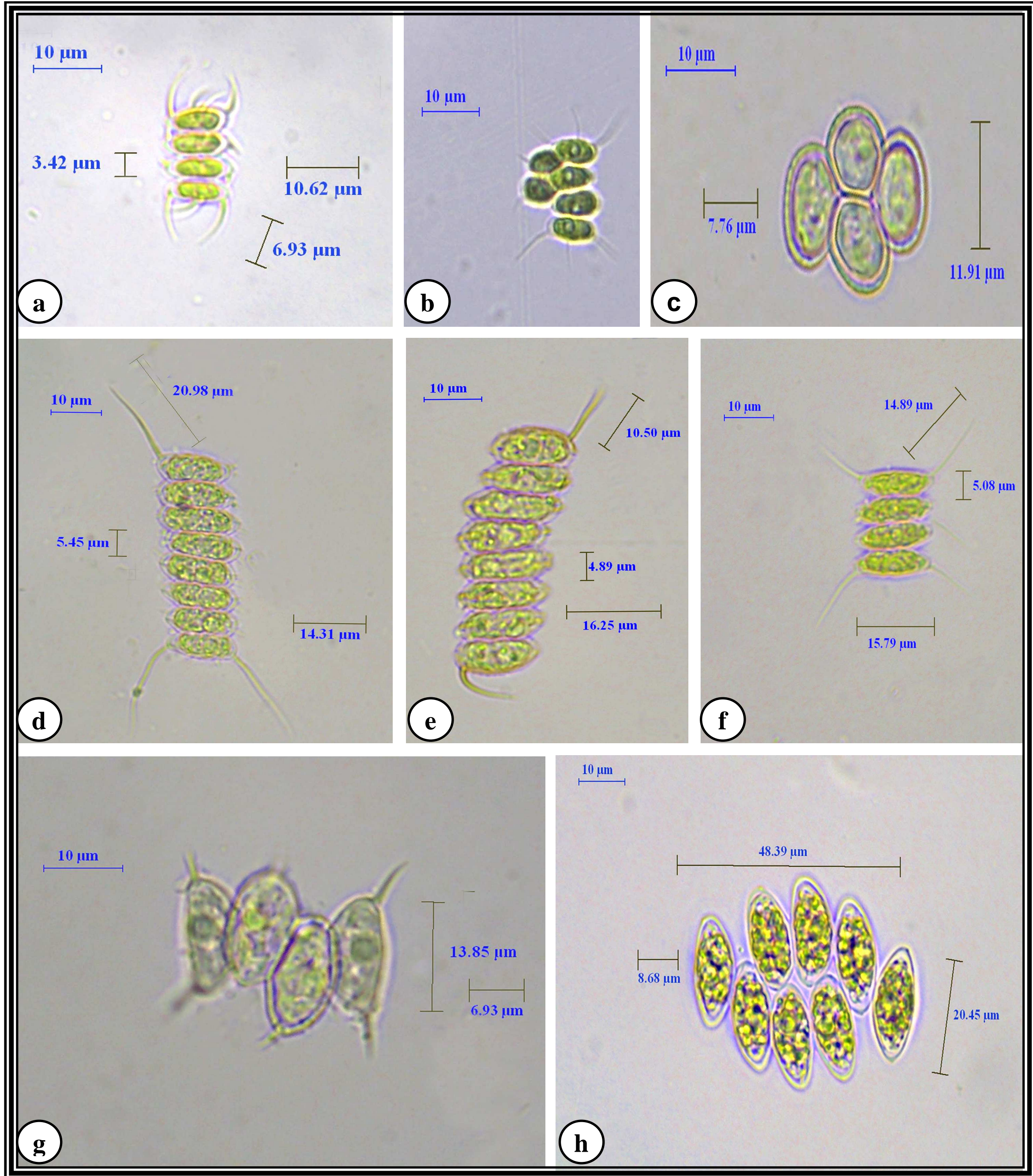
***Glaucosphaera vacuolata* Korsh.**

**Plate - 23f**

Cells are solitary with numerous contractile vacuoles. Cells are uninucleate with numerous pseudocilia present. The multiplication of cell is by vegetative division of the cell. Cells are 23.73 µm in diameter.

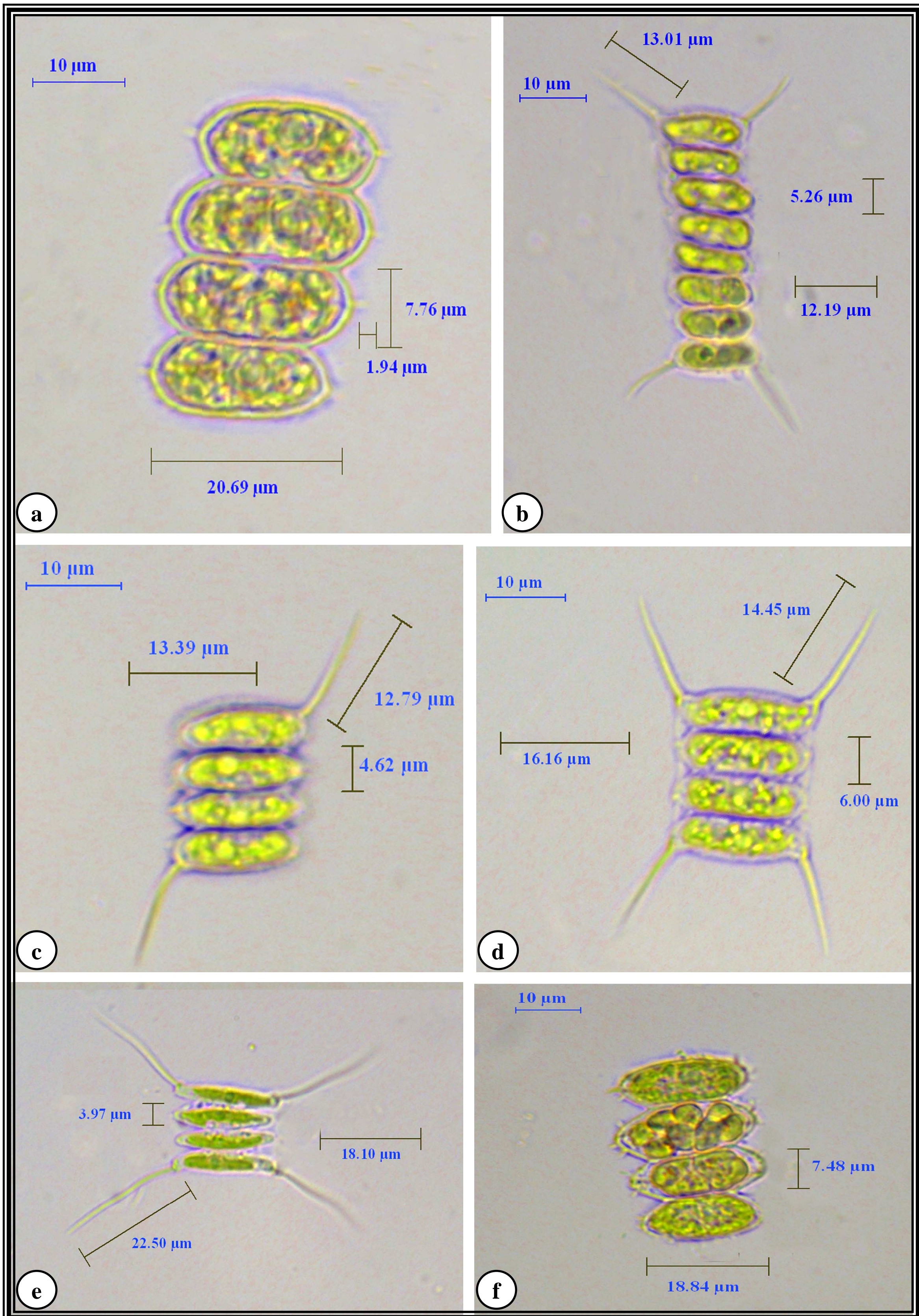
Kant, S. and Gupta, P. 1998, p. 76, pl. 91 Fig. 9.
Collection No: DLS201286, Accession No: 02012

# PLATE 2



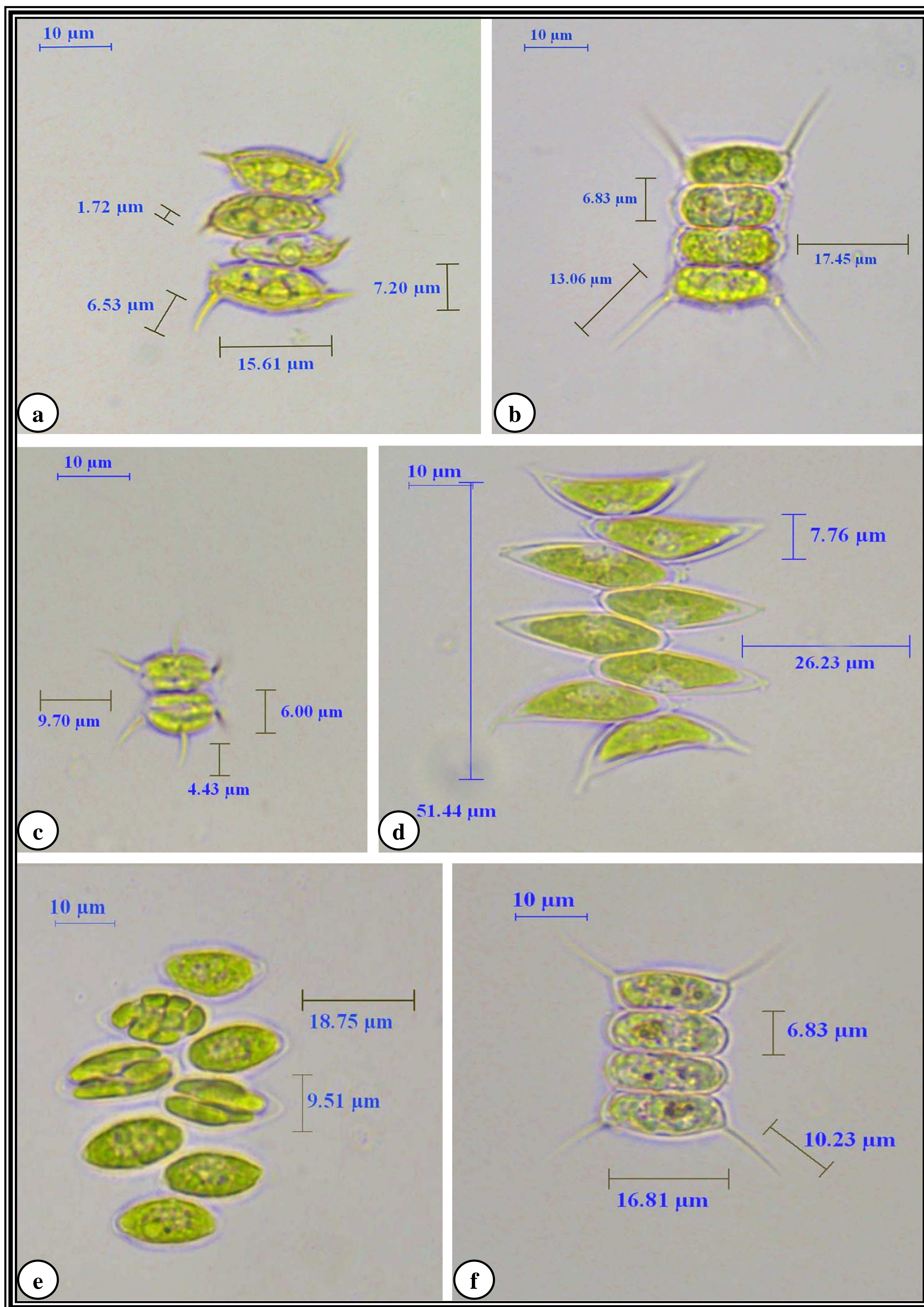

**a:** *Scenedesmus abundans* var. *longicauda*, **b:** *Scenedesmus abundans,* **c:** *Scenedesmus cumbricus*, **d:** *Scenedesmus perforatus*, **e:** *Scenedesmus armatus* var. *boglariensis* f. *bicaudatus,* **f:** *Scenedesmus quadricauda* var. *longispina*, **g:** *Scenedesmus denticulatus*, **h:** *Scenedesmus bijugatus* var. *graevenitzii*

## PLATE 3



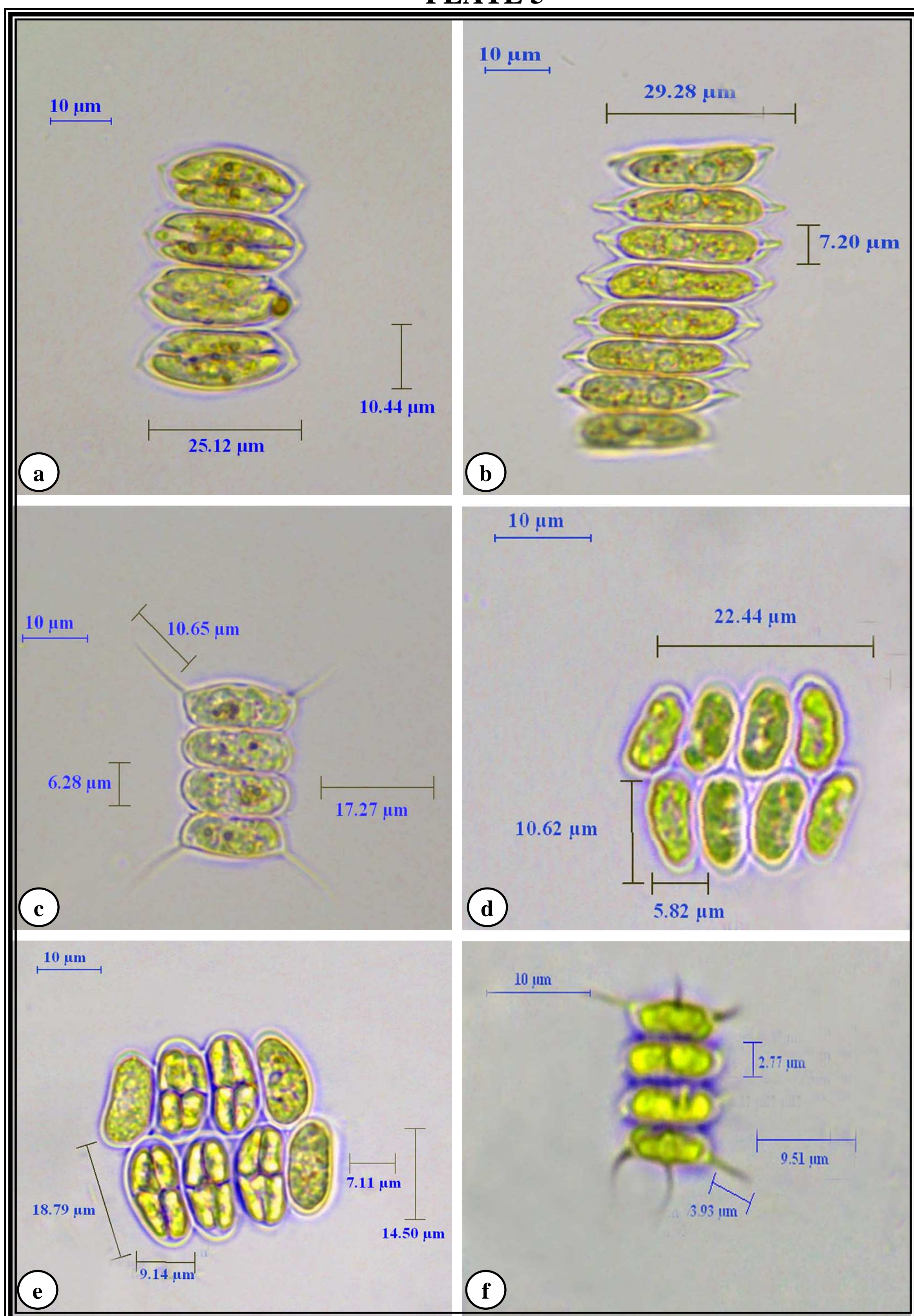

**a:** *Scenedesmus denticulatus* var. *australis*, **b:** *Scenedesmus quadricauda*, **c:** *Scenedesmus armatus* var. *bicaudatus*, **d:** *Scenedesmus quadricauda* var. *westii*, **e:** *Scenedesmus opoliensis*, **f:** *Scenedesmus serratus*

## PLATE 4



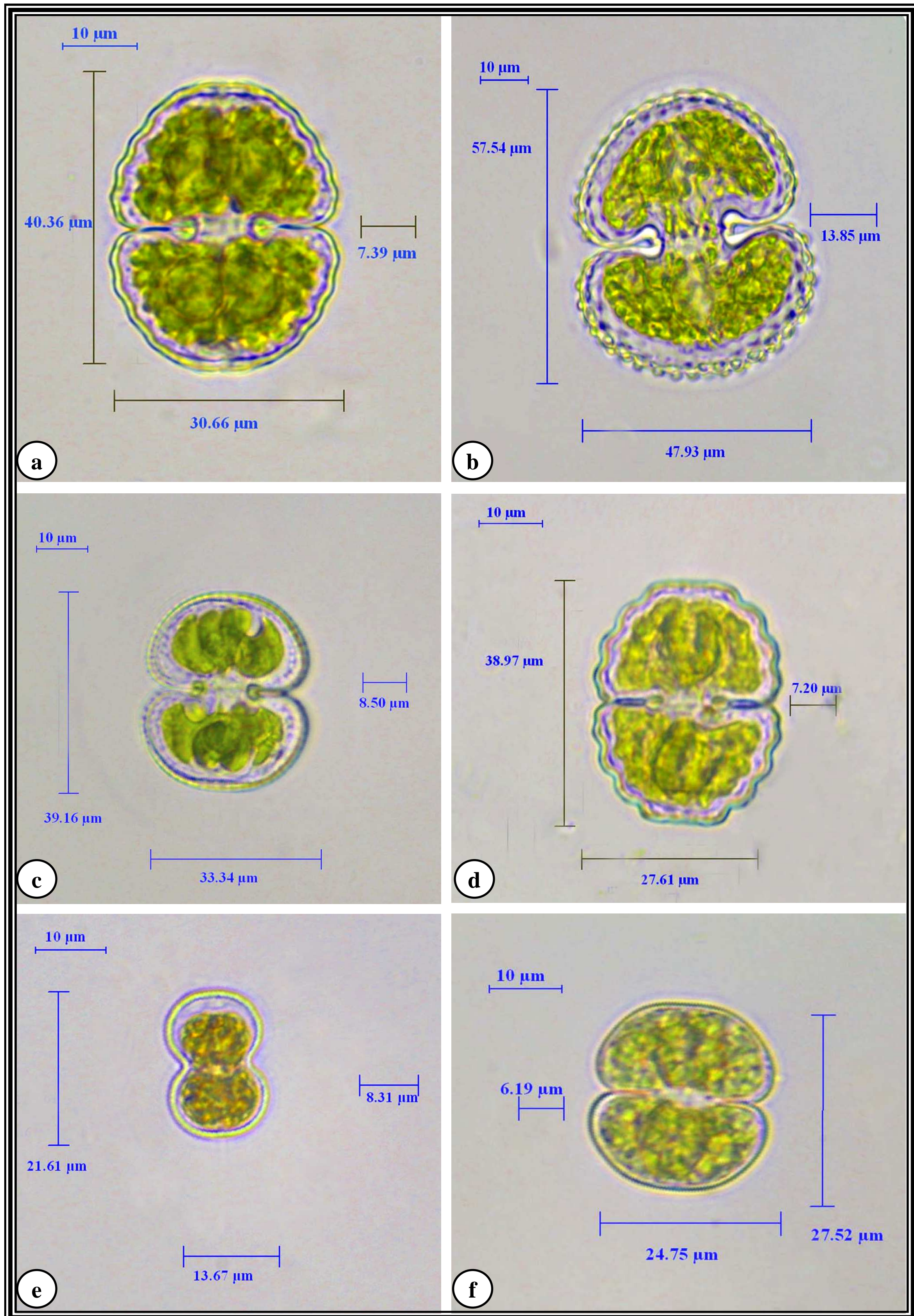

**a:** *Scenedesmus armatus* var. *dispar*, **b** and **f:** *Scenedesmus quadricauda*, **c:** *Scenedesmus abundans,* **d:** *Scenedesmus dimorphus*, **e:** *Scenedesmus bijugatus* var. *graevenitzii*

# PLATE 5



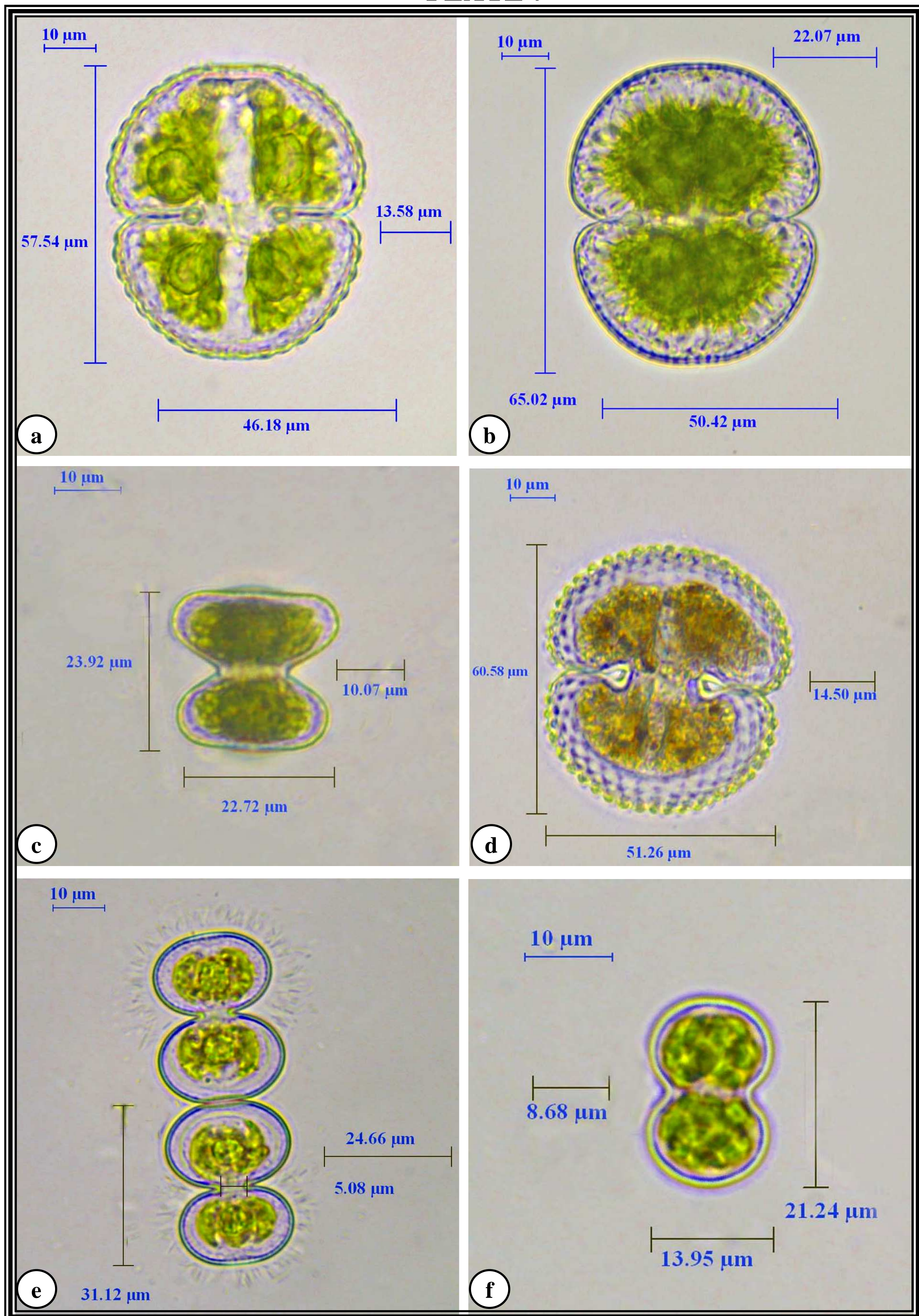

**a:** *Scenedesmus acutiformis*, **b:** *Scenedesmus obliquus*, **c:** *Scenedesmus quadricauda* var. *quadrispina*, **d and e**: *Scenedesmus arcuatus*, **f:** *Scenedesmus abundans*

# PLATE 6



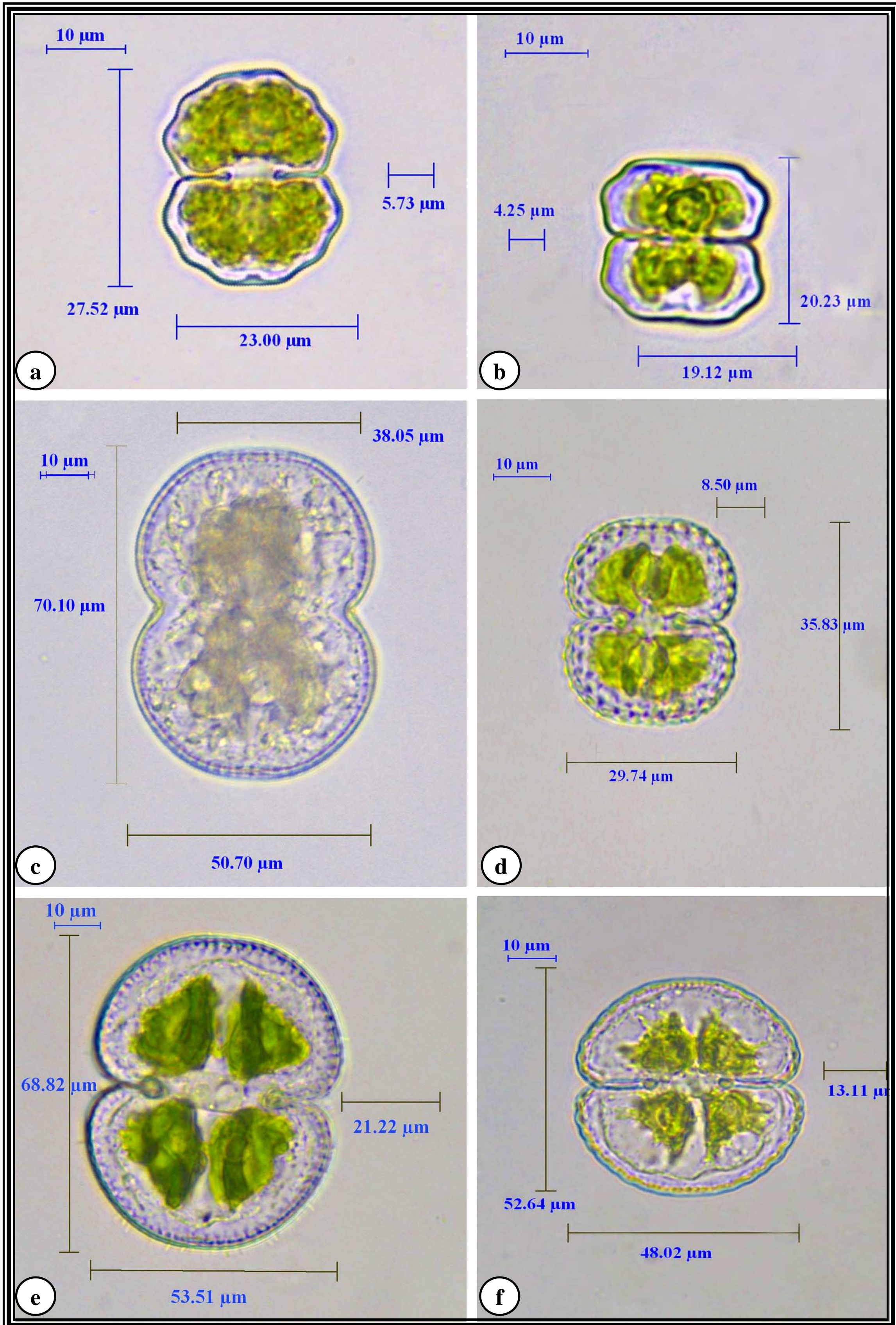

**a:** *Cosmarium subundulattum*, **b:** *Cosmarium reniforme*, **c:** *Cosmarium subtumidum*, **d:** *Cosmarium subimpressulum*, **e:** *Cosmarium moniliforme*, **f:** *Cosmarium laeve*

# PLATE 7



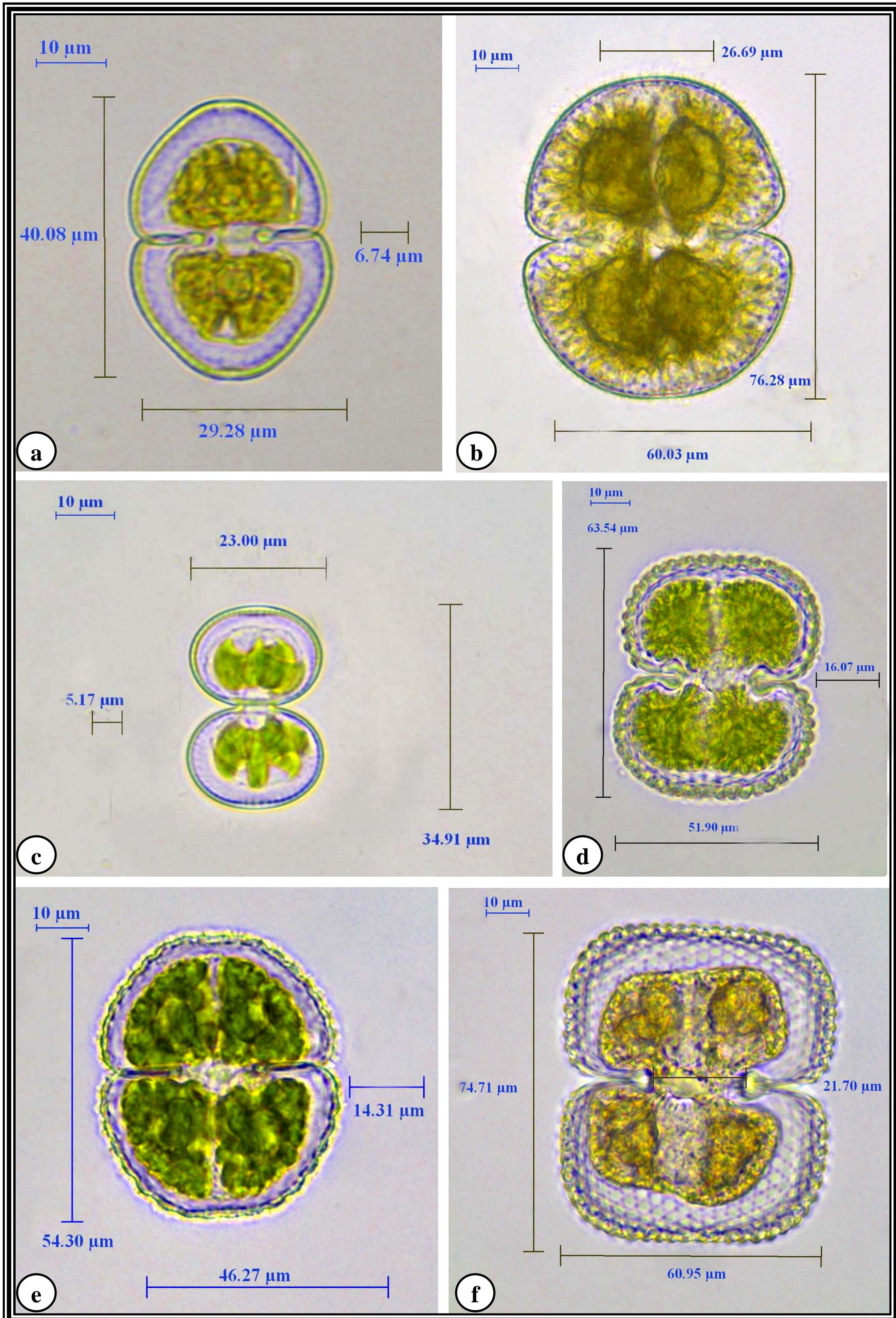

**a:** *Cosmarium botrytis*, **b:** *Cosmarium pachydermum* var. *minus*, **c:** *Cosmarium perfissum*, **d:** *Cosmarium pardalis*, **e:** *Cosmarium contractum* f. *jacobsenii*, **f:** *Cosmarium moniliforme*

## PLATE 8



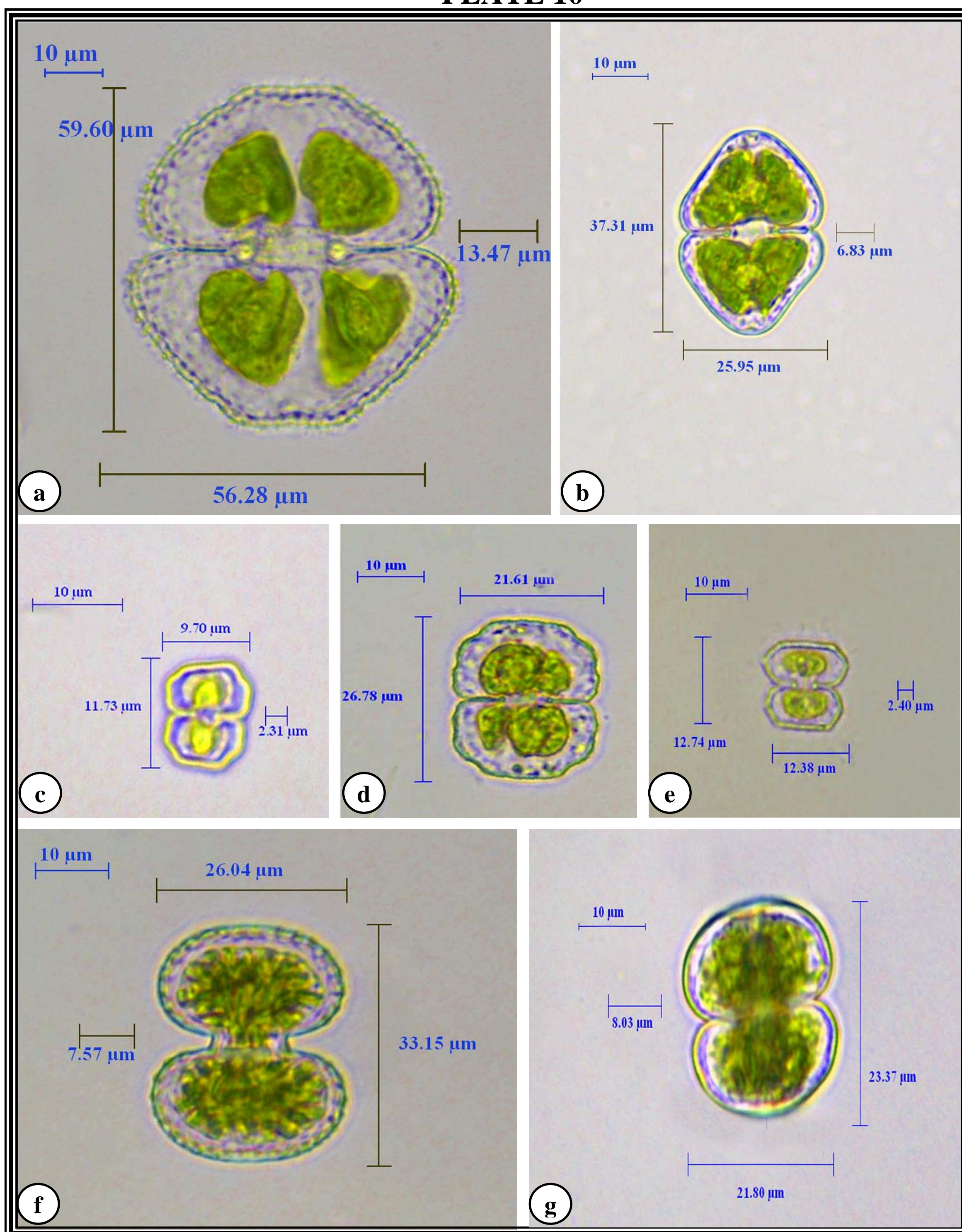

**a:** *Cosmarium subgranatum* var. *borgei*, **b:** *Cosmarium polygonum*, **c:** *Cosmarium connatum*, **d:** *Cosmarium punctulatum*, **e:** *Cosmarium lundellii*, **f:** *Cosmarium botrytis*

## PLATE 9



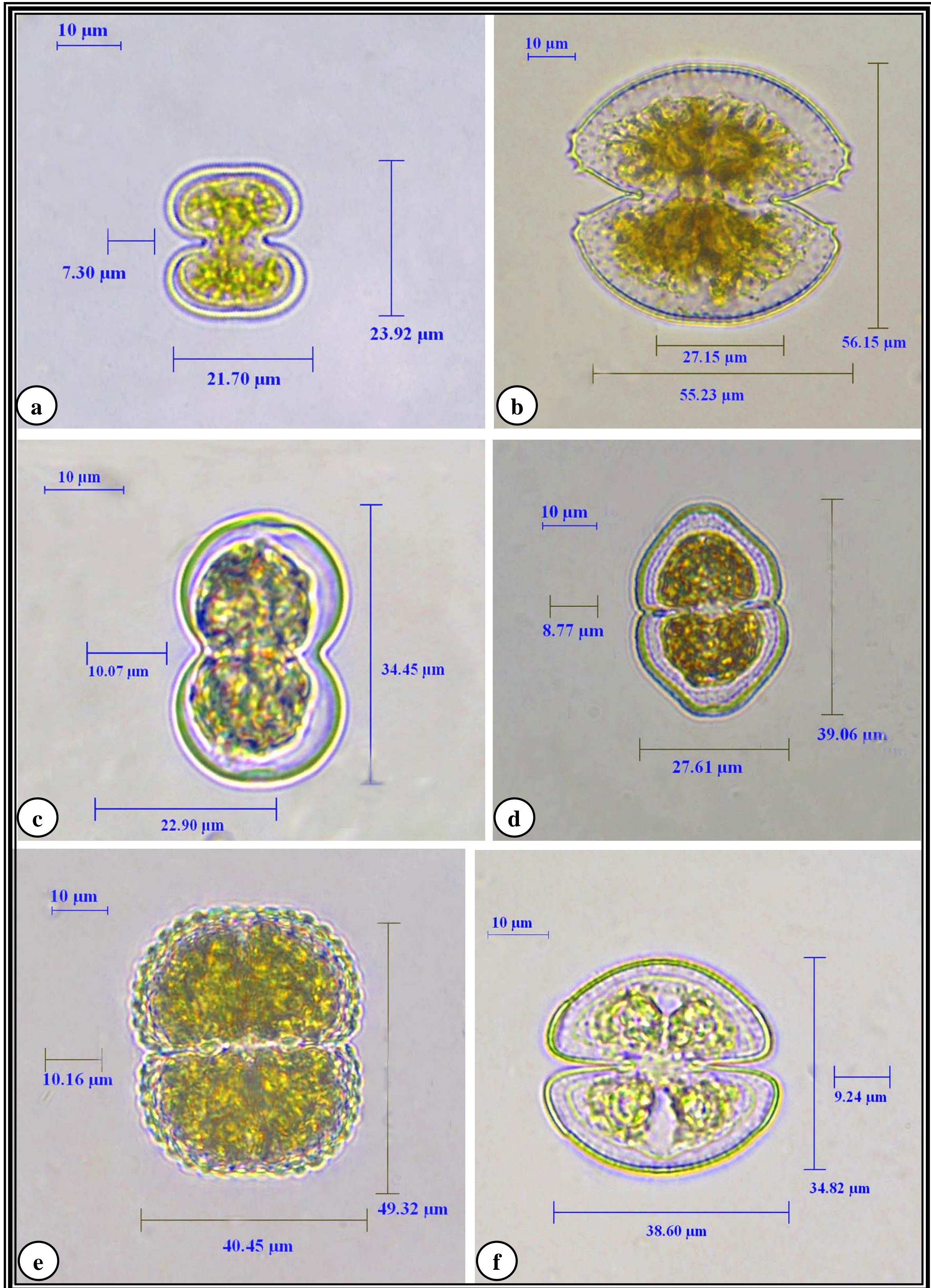

**a:** *Cosmarium granatum*, **b:** *Cosmarium capense*, **c:** *Cosmarium contractum* f. *jacobsenii*, **d:** *Cosmarium margaritatum*, **e:** *Cosmarium botrytis* var. *mediolaeve*, **f:** *Cosmarium quadrum*

## PLATE 10



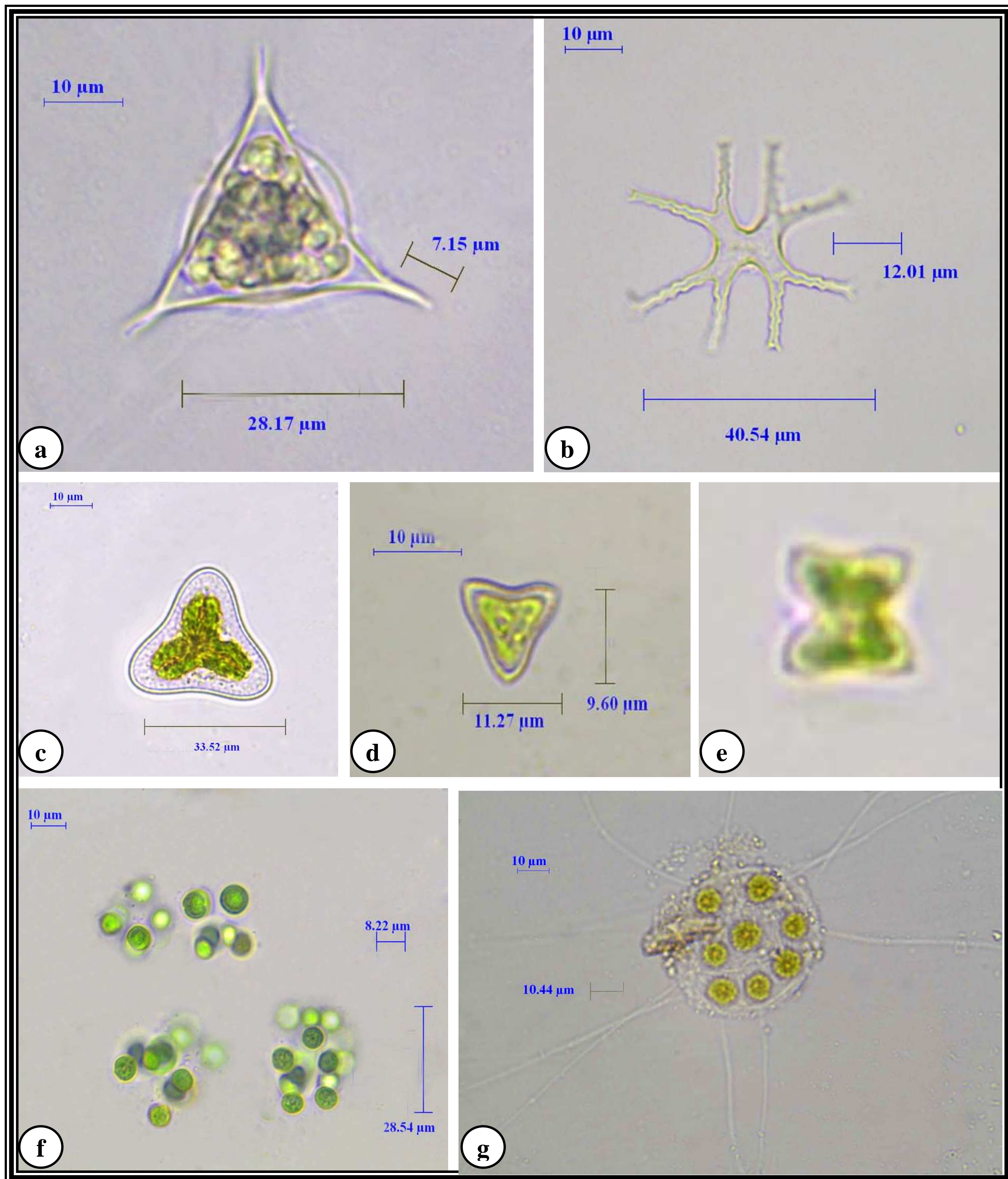

**a:** *Cosmarium turpinii*, **b:** *Cosmarium pseudogranatum*, **c:** *Cosmarium regnellii* var. *minimum*, **d:** *Cosmarium vermae*, **e:** *Cosmarium pygmaeum*, **f:** *Cosmarium portianum*, **g:** *Cosmarium phaseolus* var. *omphalum*

## PLATE 11



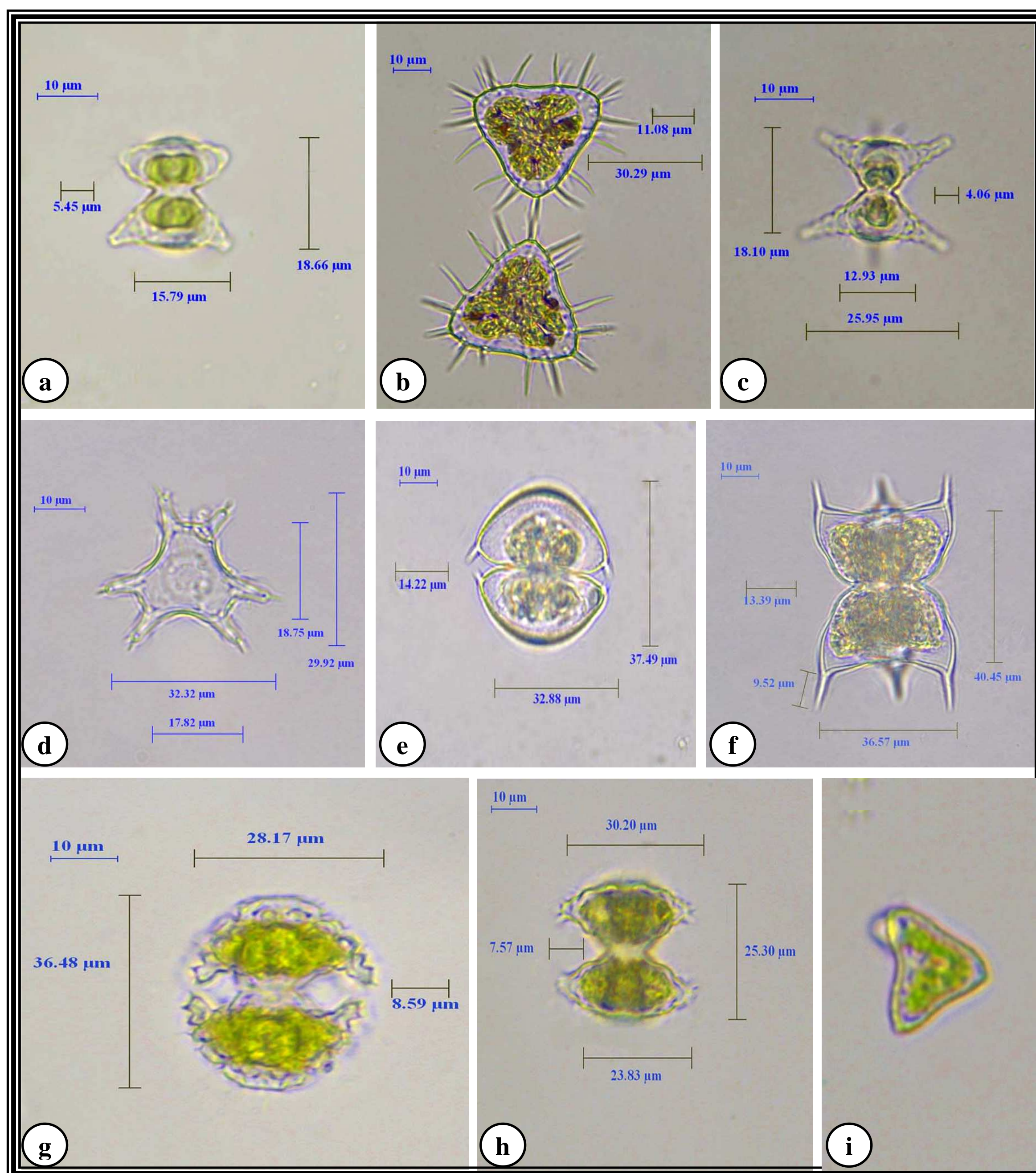

**a:** *Cosmarium perfissum*, **b:** *Cosmarium auriculatum*, **c:** *Cosmarium moniliforme*, **d:** *Cosmarium retusiforme*, **e:** *Cosmarium pseudobroomei*, **f:** *Cosmarium candianum* var. *depressum*

# PLATE 12



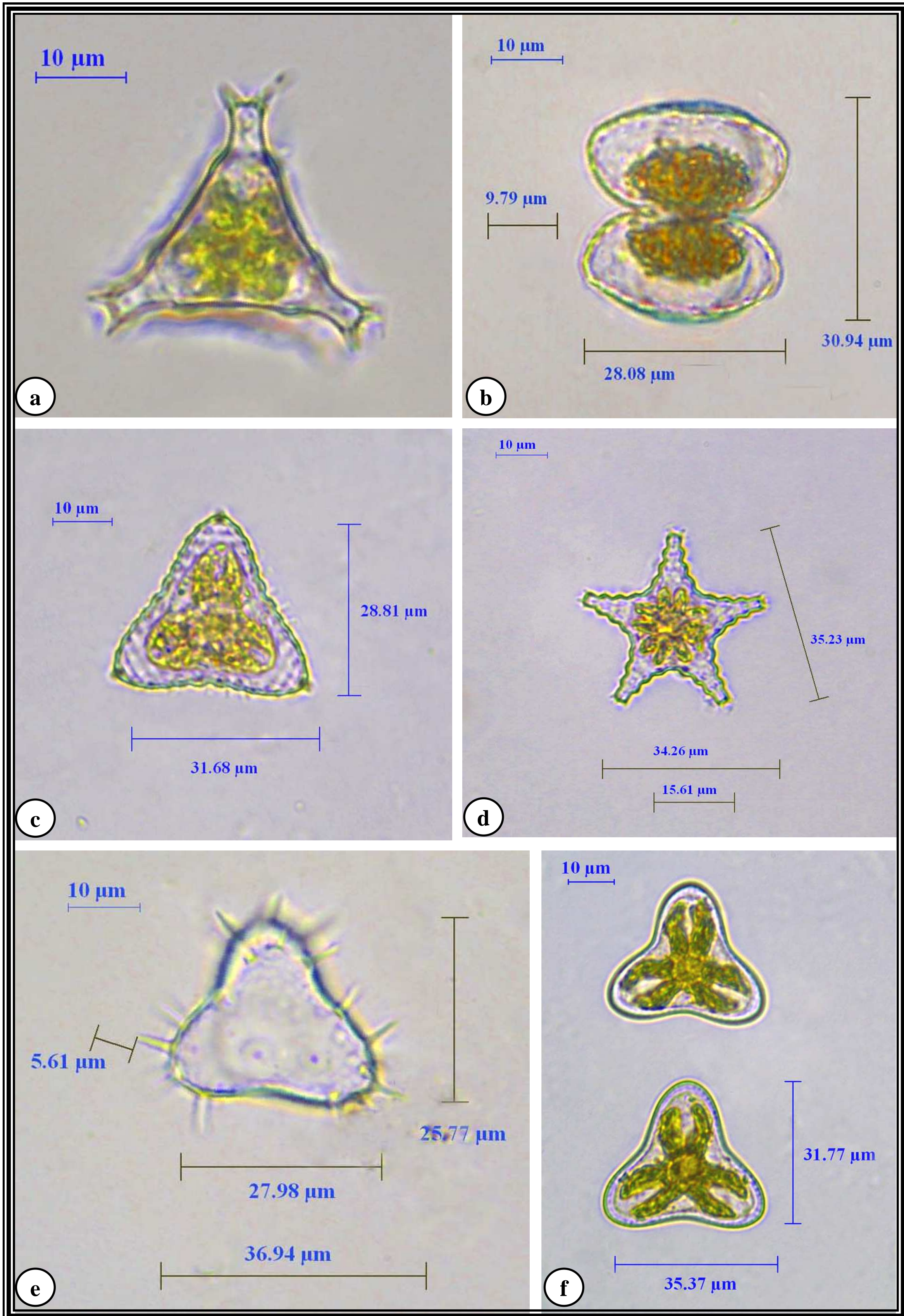

**a**: *Tetraedron trigonum*, **b:** *Tetraedron gracile*, **c:** *Tetraedron tumidulum*, **d:** *Tetraedron muticum*, **e:** *Tetraedron minimum* f. *apiculatum*, **f:** *Tetraspora lubrica*, **g:** *Tetraspora lamellose*

## PLATE 13



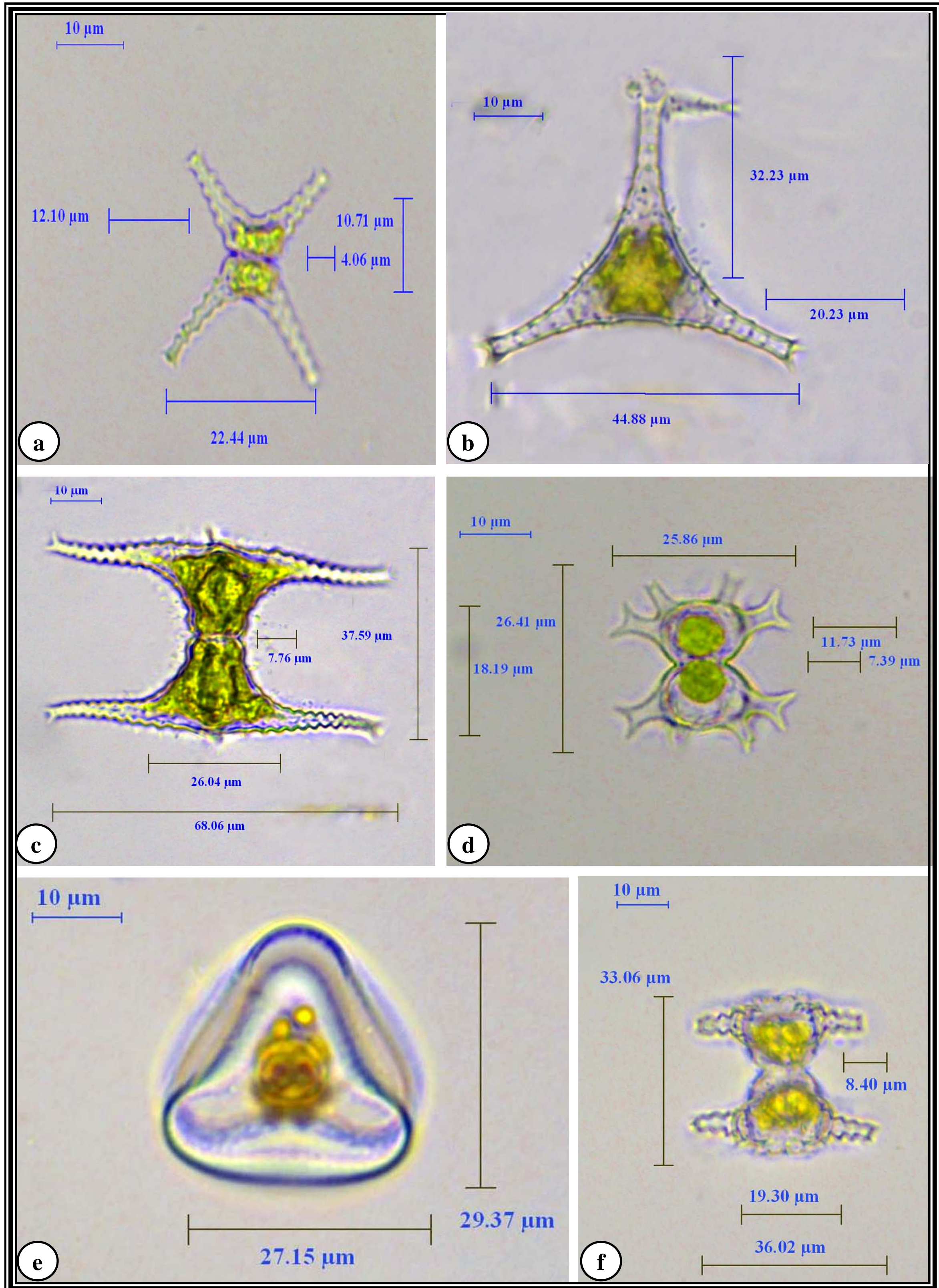

**a:** *Staurastrum kalapanii*, **b:** *Staurstrum setigerum*, **c:** *Staurastrum tetracerum*, **d:** *Staurastrum gemelliparum*, **e:** *Staurastrum dickiei* var. *circulare*, **f:** *Staurodesmus* sp., **g:** *Staurastrum rhynchoceps*, **h:** *Staurastrum hexacerum*, **i:** *Staurastrum cuspidatum*

**PLATE 14**



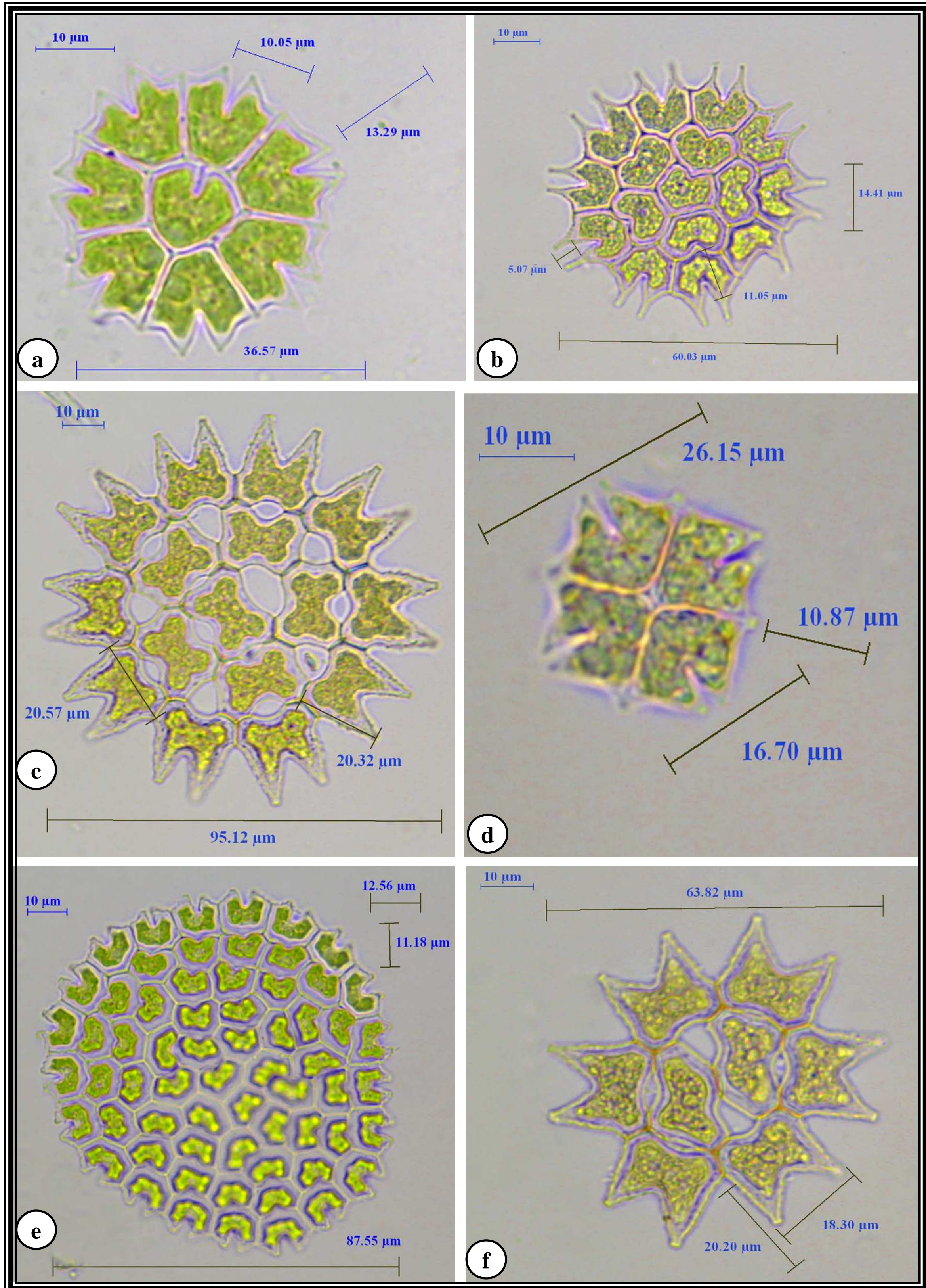

**a:** *Staurastrum* sp., **b:** *Staurastrum punctulatum*, **c:** *Staurastrum granulosum*, **d:** *Staurastrum arachne* var. *sumatranum*, **e:** *Staurastrum gladiosum*, **f:** *Staurastrum retusum*

**PLATE 15**



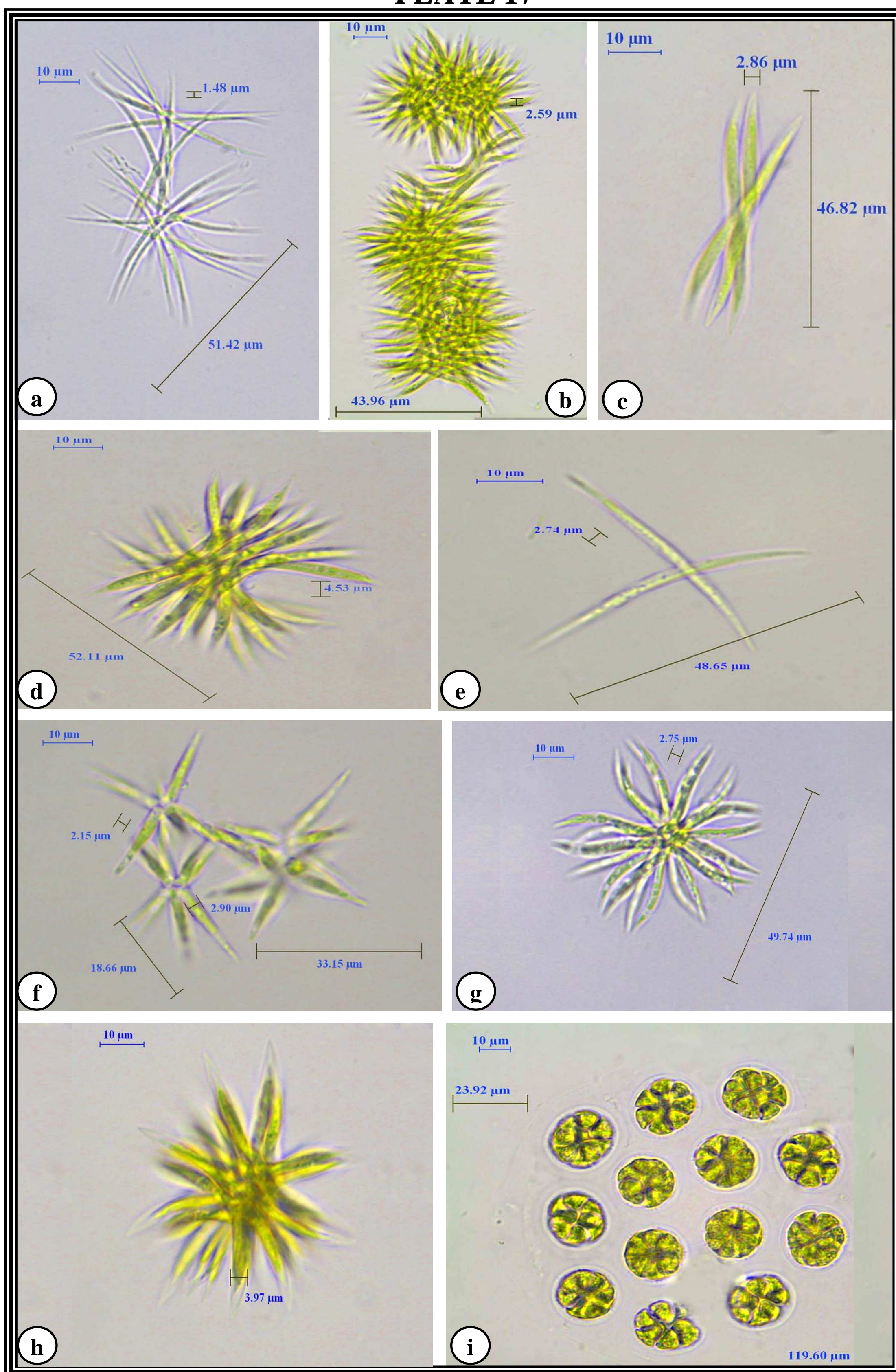

**a:** *Staturastrum pseudotetracerum*, **b:** *Staurastrum gracile*, **c:** *Staurastrum longibrachiatum* var. *intermedium*, **d:** *Staurastrum furcatum*, **e:** *Staurastrum pachyrhynchum*, **f:** *Staurastrum gracile* f. *iyengar*

## PLATE 16



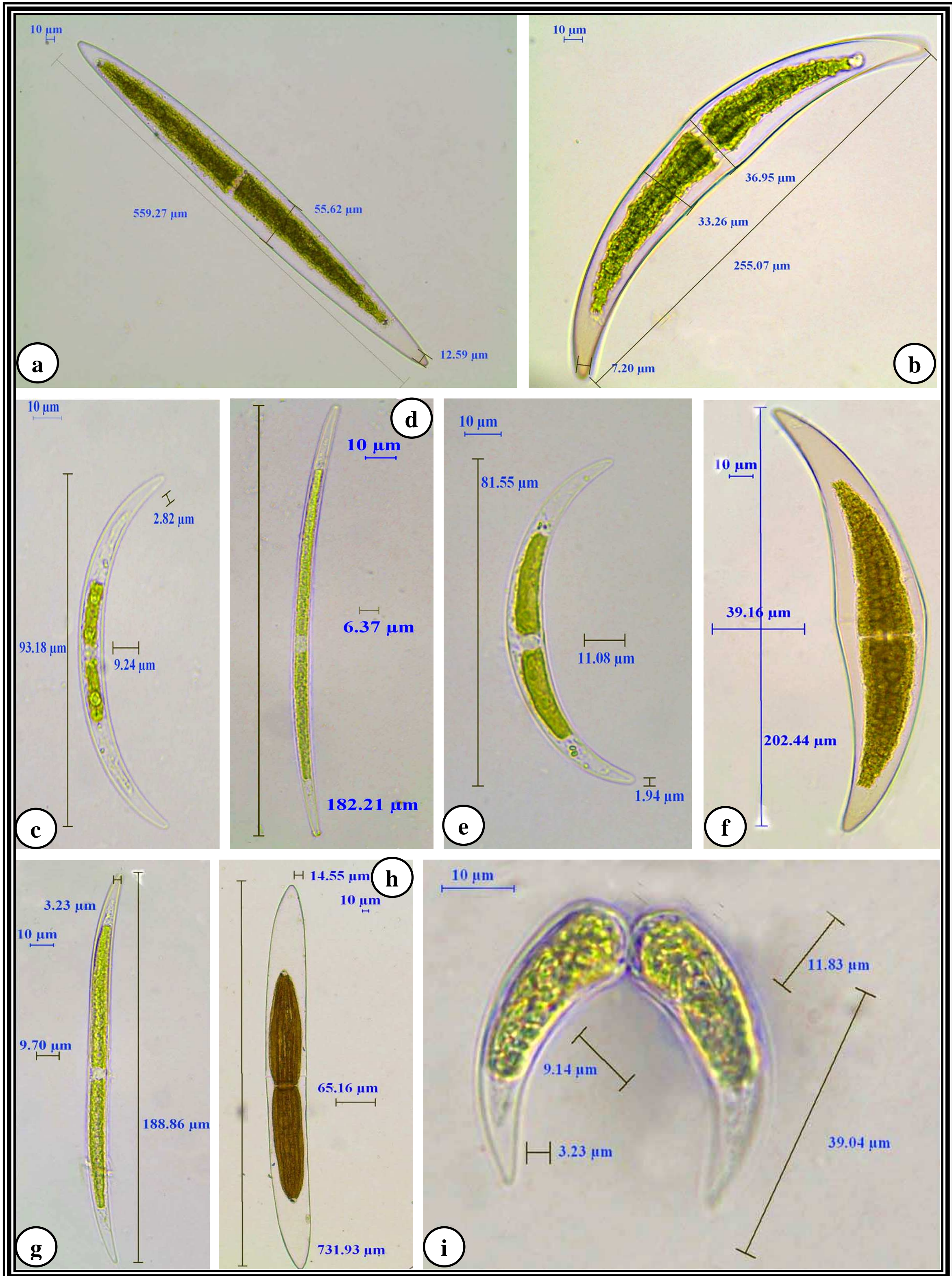

**a:** *Pediastrum tetras* var. *tetraedron*, **b:** *Pediastrum boryanum* var. *longicorne*, **c:** *Pediastrum duplex* var. *reticulatum* X 400, **d:** *Pediastrum tetras*, **e:** *Pediastrum angulosum*, **f:** *Pediastrum duplex* var. *clathratum*

# PLATE 17



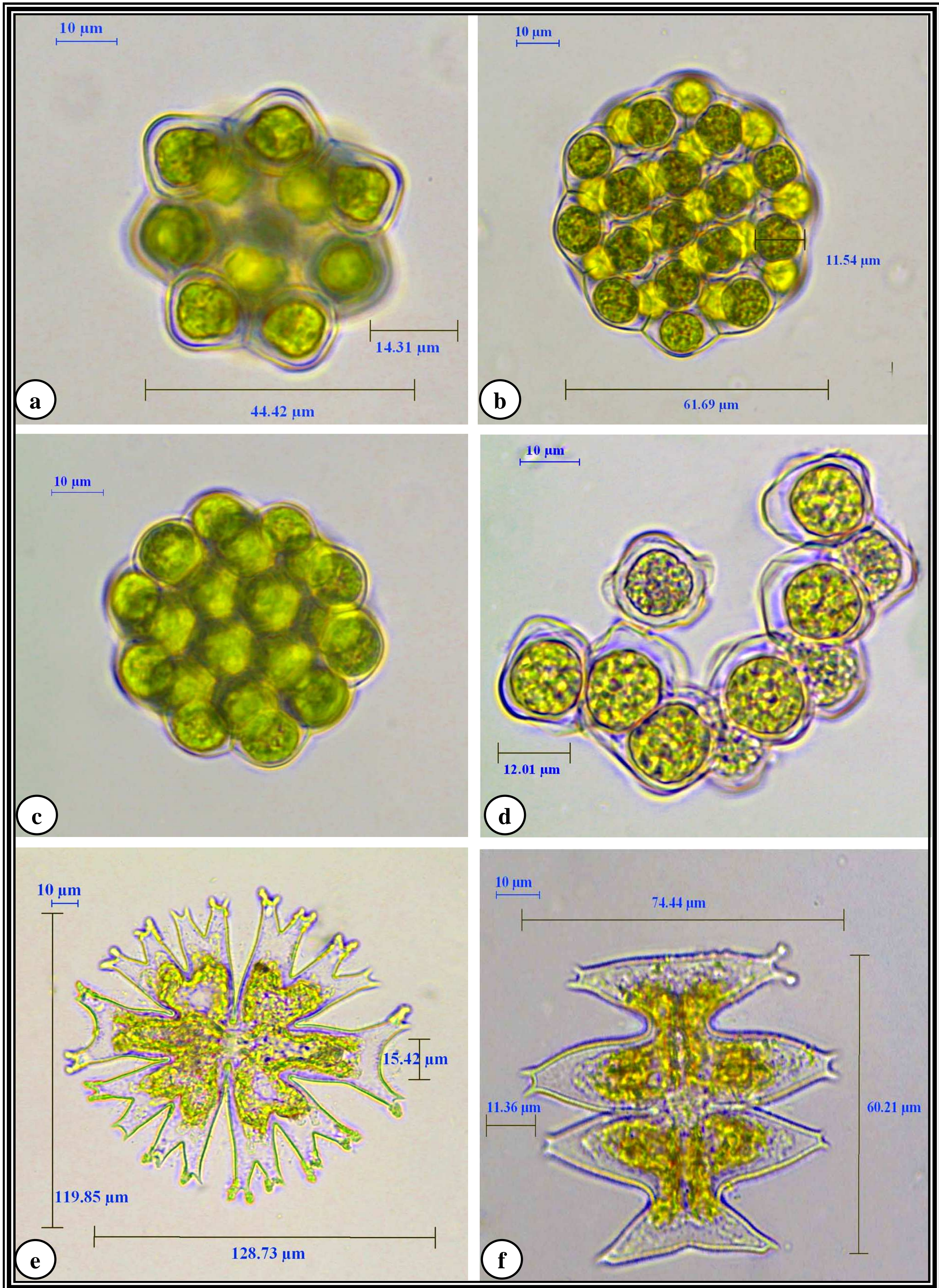

**a:** *Ankistrodesmus falcatus* var. *radiatus*, **b**: *Ankistrodesmus spiralis*, **c:** *Ankistrodesmus falcatus* var. *spirilliformis*, **d** and **e:** *Ankistrodesmus falcatus*, **f:** *Actinastrum hantzschii*, **g:** *Actinastrum lagerh*, **h:** *Actinastrum hantzschii* var. *fluviatile*, **i:** *Asterococcus limneticus*

# PLATE 18



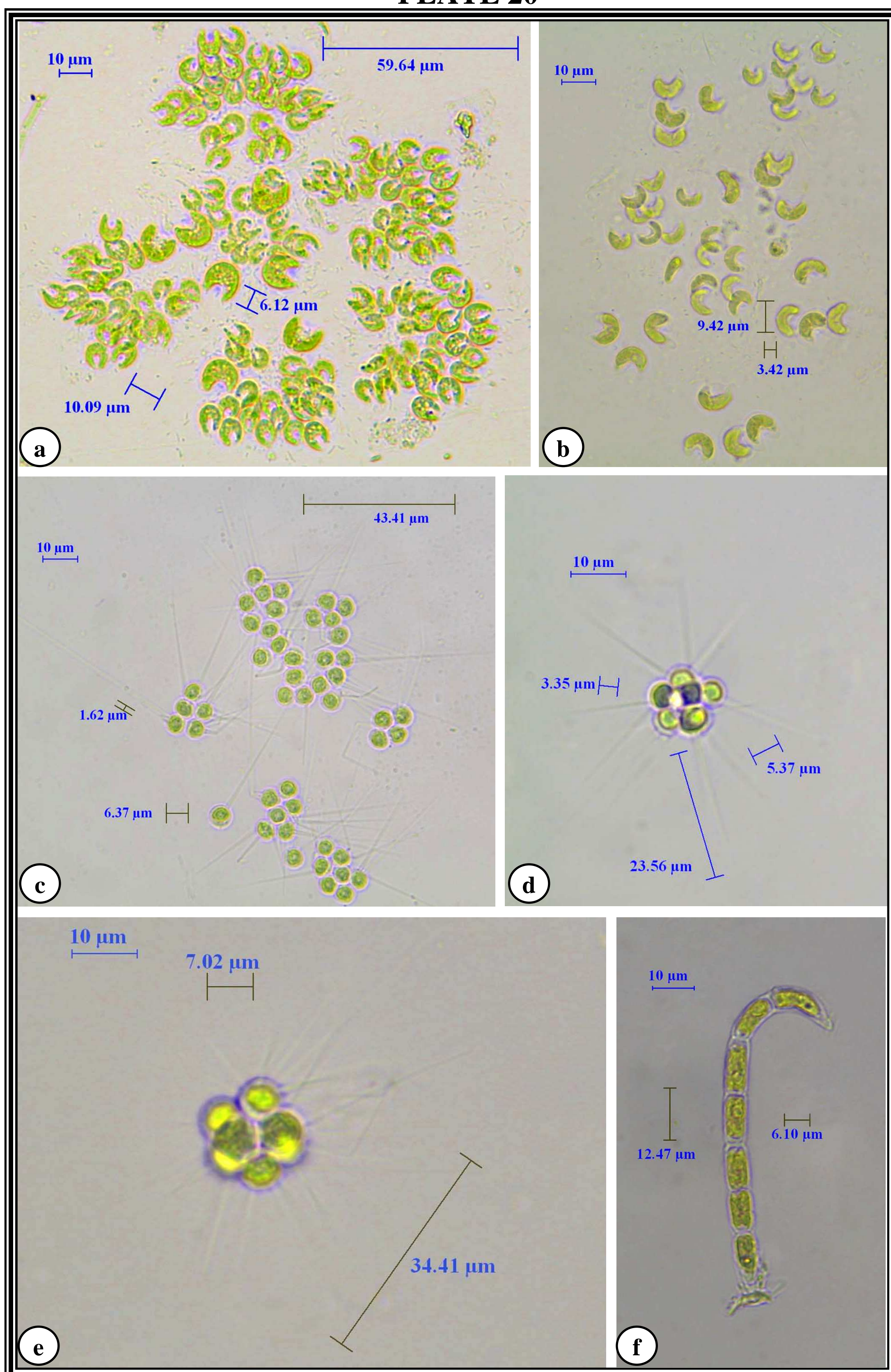

**a:** *Closterium punctulatum* X 200, **b:** *Closterium moniliferum* X 400**,** **c:** *Closterium parvulum*, **d:** *Closterium gracile*, **e:** *Closterium venus*, **f:** *Closterium ehrenbergii*, **g:** *Closterium acutum*, **h:** *Closterium braunii* X 100, **i:** *Closterium lunula* f. *biconvexum*

# PLATE 19



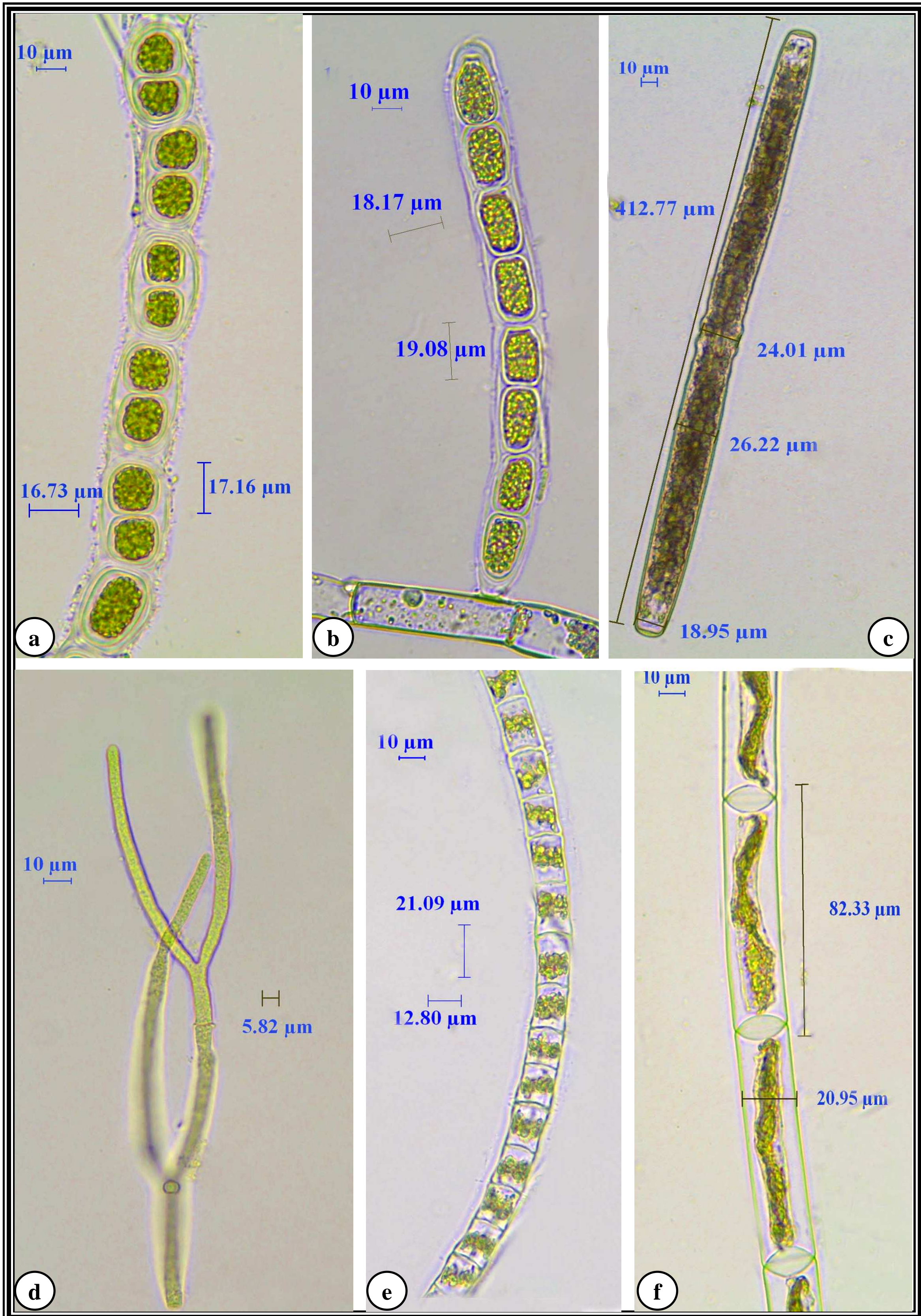

**a** and **d:** *Coelastrum sphaericum*, **b** and **c:** *Coelastrum microporum*, **e:** *Micrasterias radians* X 400, **f:** *Micrasterias pinnatifida* X 400

## PLATE 20



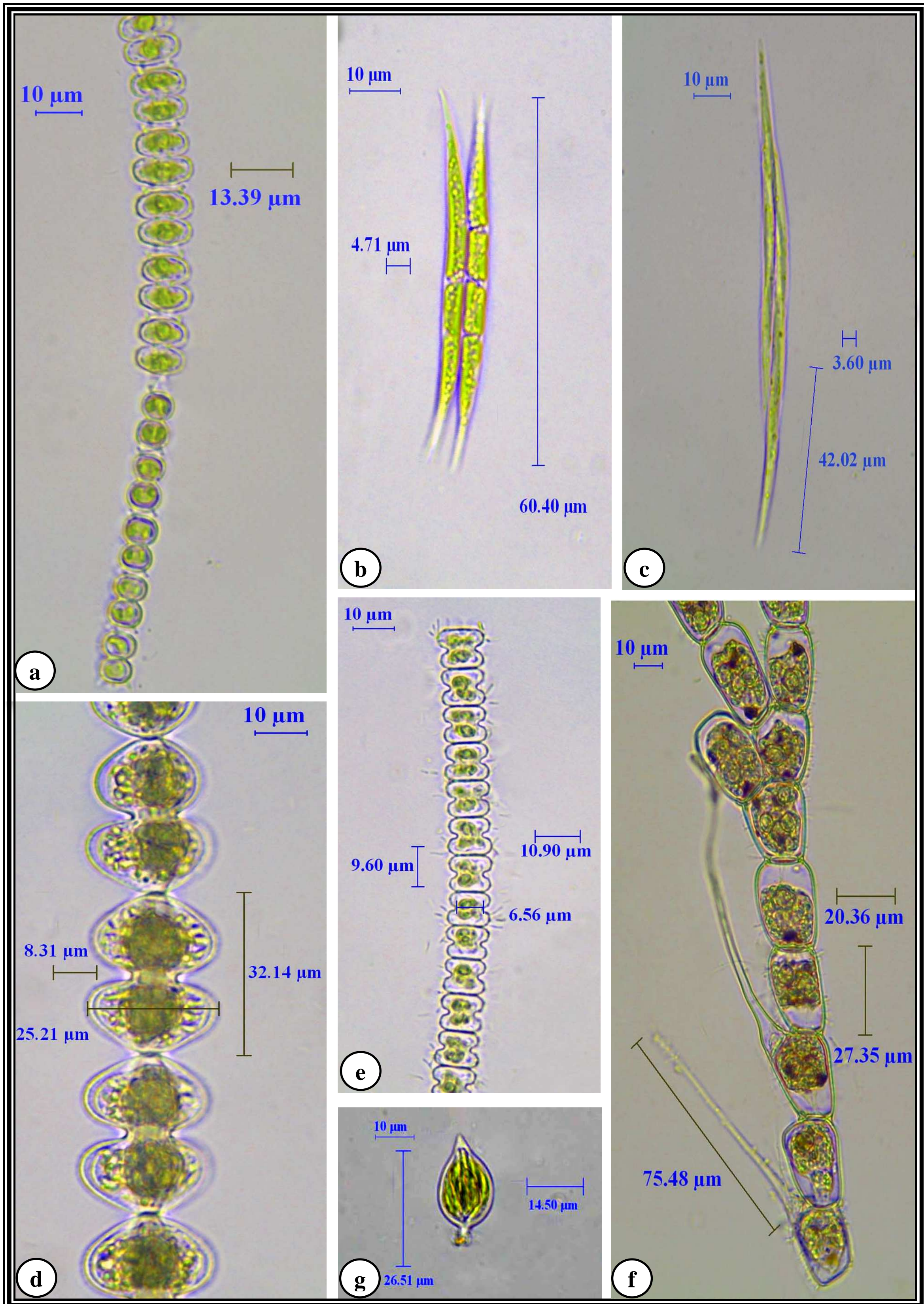

**a:** *Kirchneriella lunaris* X 400, **b:** *Kirchneriella concorta* var. *elegans*, **c:** *Micractinium pusillum*, **d** and **e:** *Micractinium pusillum* var. *elegans*, **f:** *Uronema confervicolum*

## PLATE 21



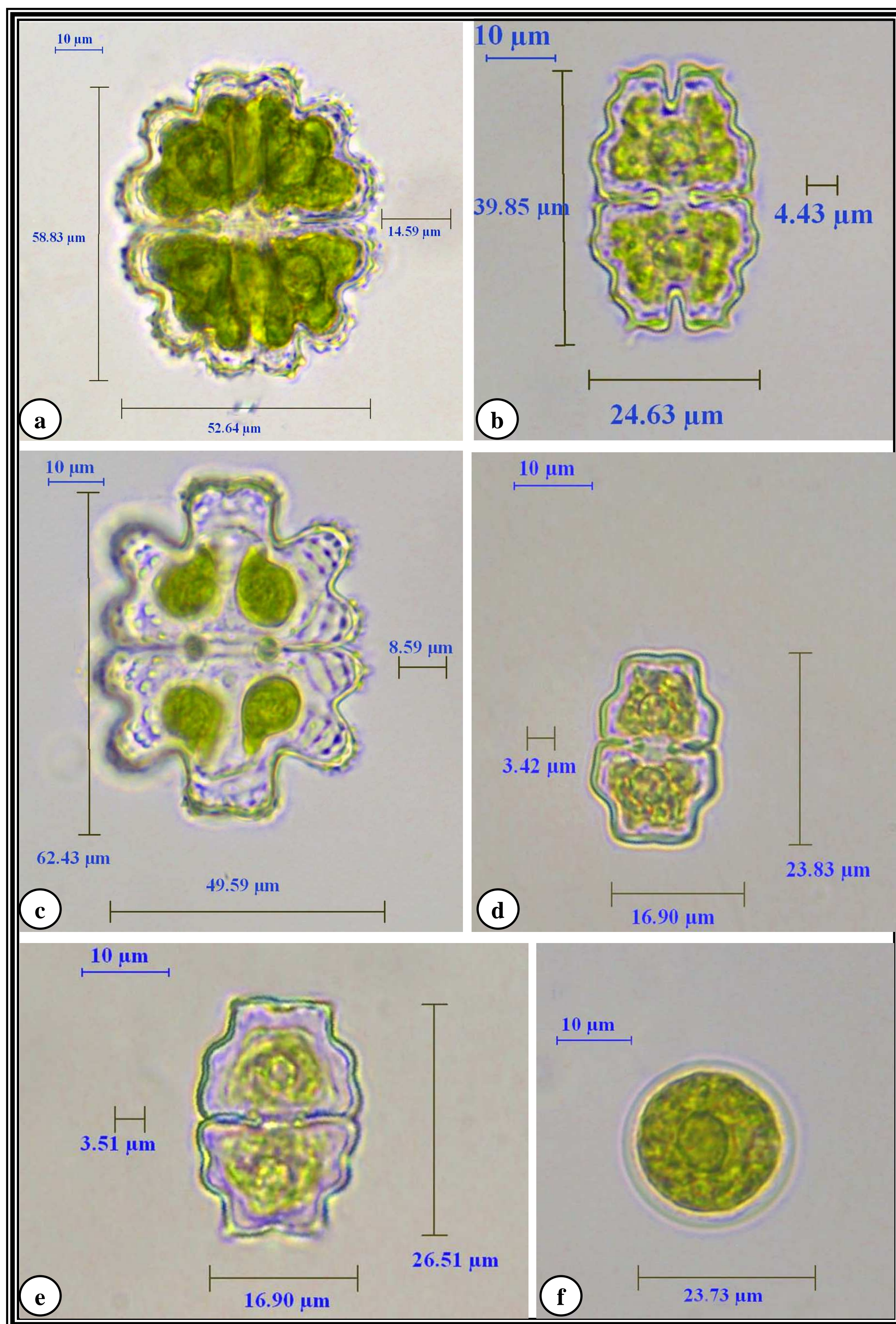

**a:** *Cylindrocapsa conferta* X 400, **b:** *Cylindrocapsa geminella* var. *minor*, **c:** *Pleurotaenium ehrenbergii* X 100, **d:** *Dichotomosiphon tuberosus* X 400, **e:** *Ulothrix aequalis* X 400, **f:** *Mougeotia sphaerocarpa* X 400

# PLATE 22



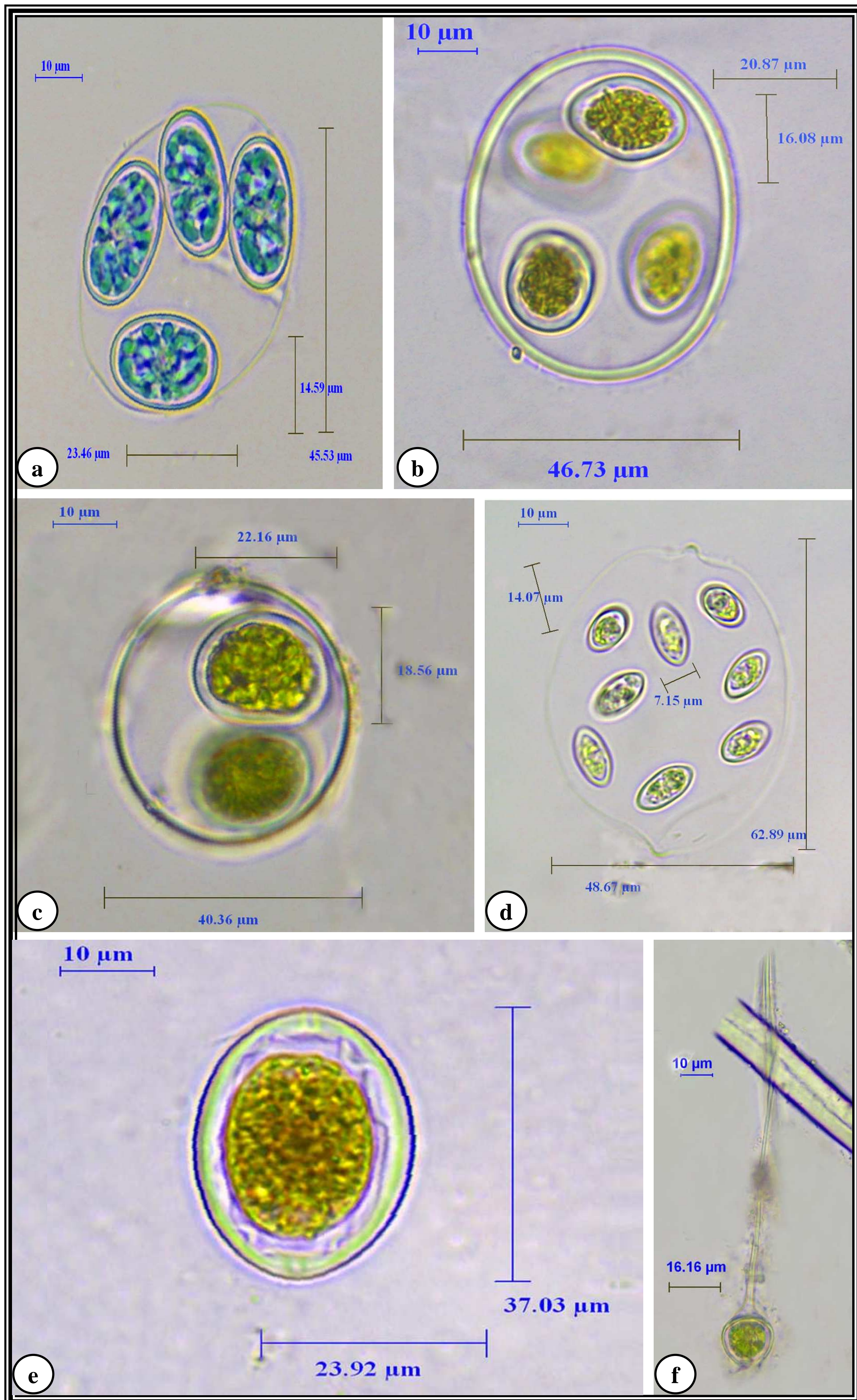

**a:** *Sphaerozosma filliforme*, **b:** *Dactylococcopsis acicularis*, **c:** *Monoraphidium griffithii*, **d:** *Spondylosium nitens* var. *triangular* f. *javanicum*, **e:** *Spondylosium planum*, **f:** *Bulbochaete mirabilis* X 400, **g:** *Characium acuminatum*

**PLATE 23**



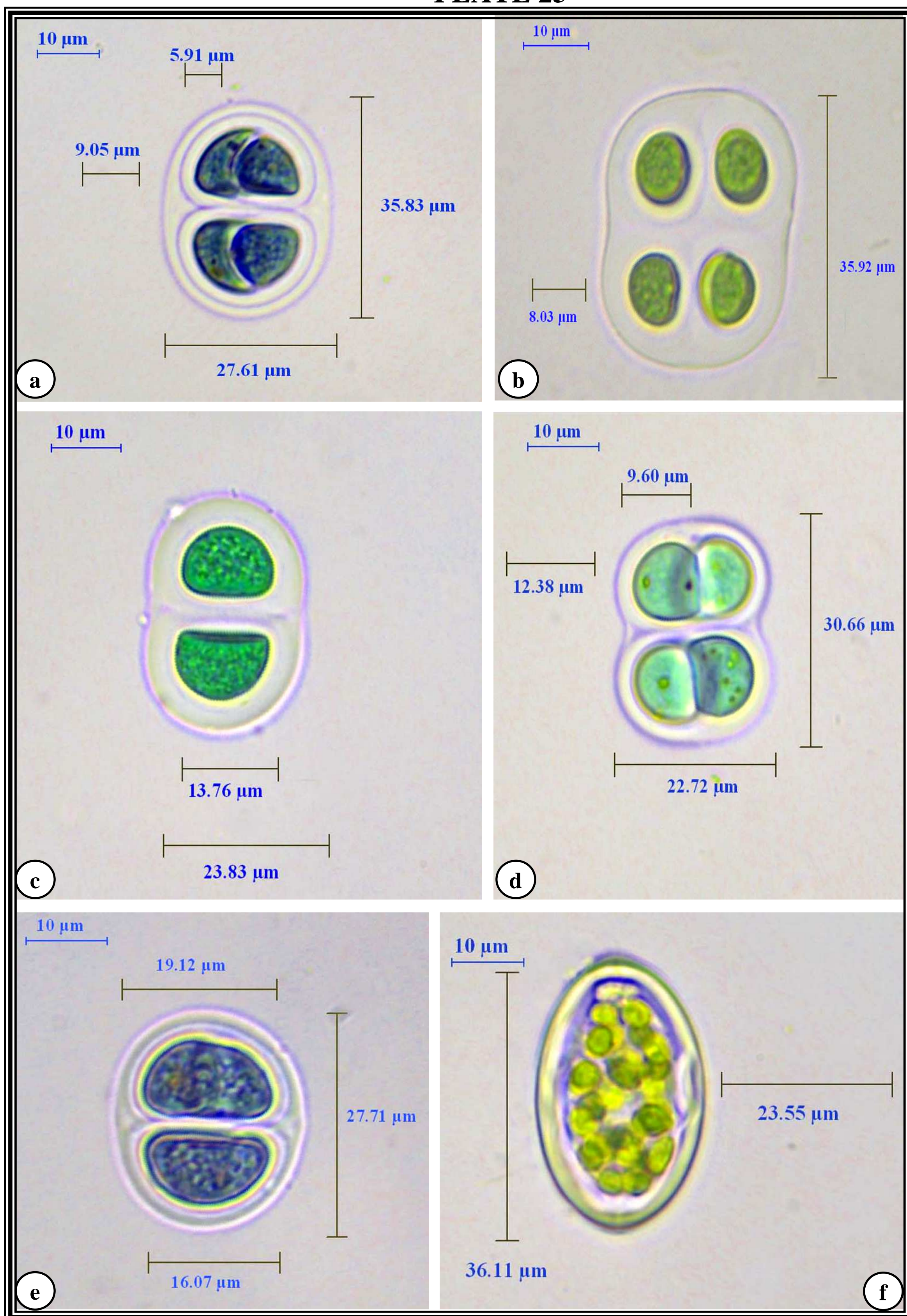

**a:** *Euastrum spinulosum*, **b:** *Euastrum denticulatum* var. *rectangulare*, **c:** *Euastrum interminus* var. *burmense*, **d:** *Euastrum sublobatum* var. *sumatranum*, **e:** *Euastrum insulare*, **f:** *Glaucosphaera vacuolata*

## PLATE 24



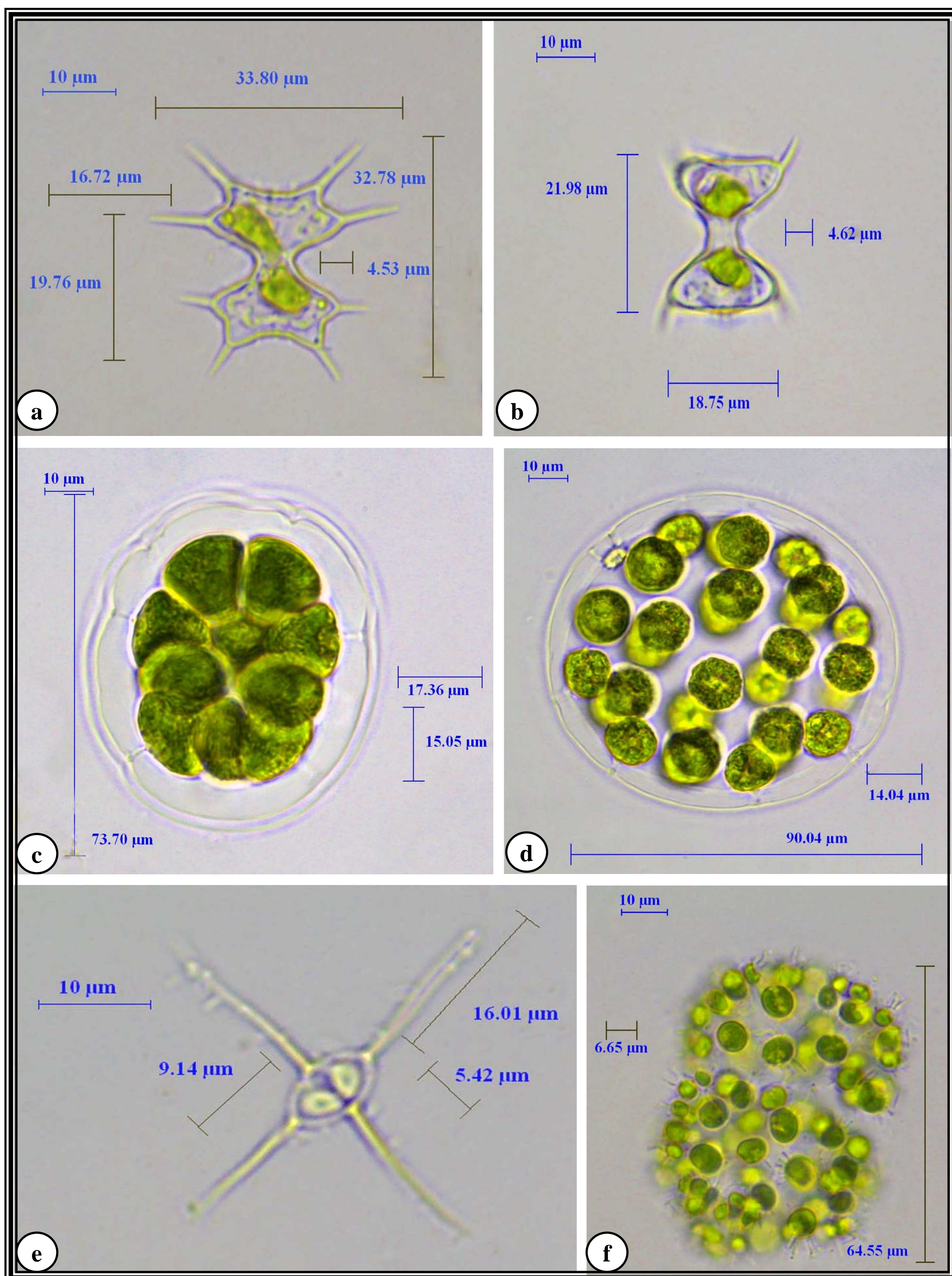

**a** and **b:** *Oocystis elliptica*, **c:** *Oocystis gigas*, **d:** *Oocystis lacustris*, **e:** *Oocystis solitaria*, **f:** *Chaetosphaeridium* sp.

## PLATE 25



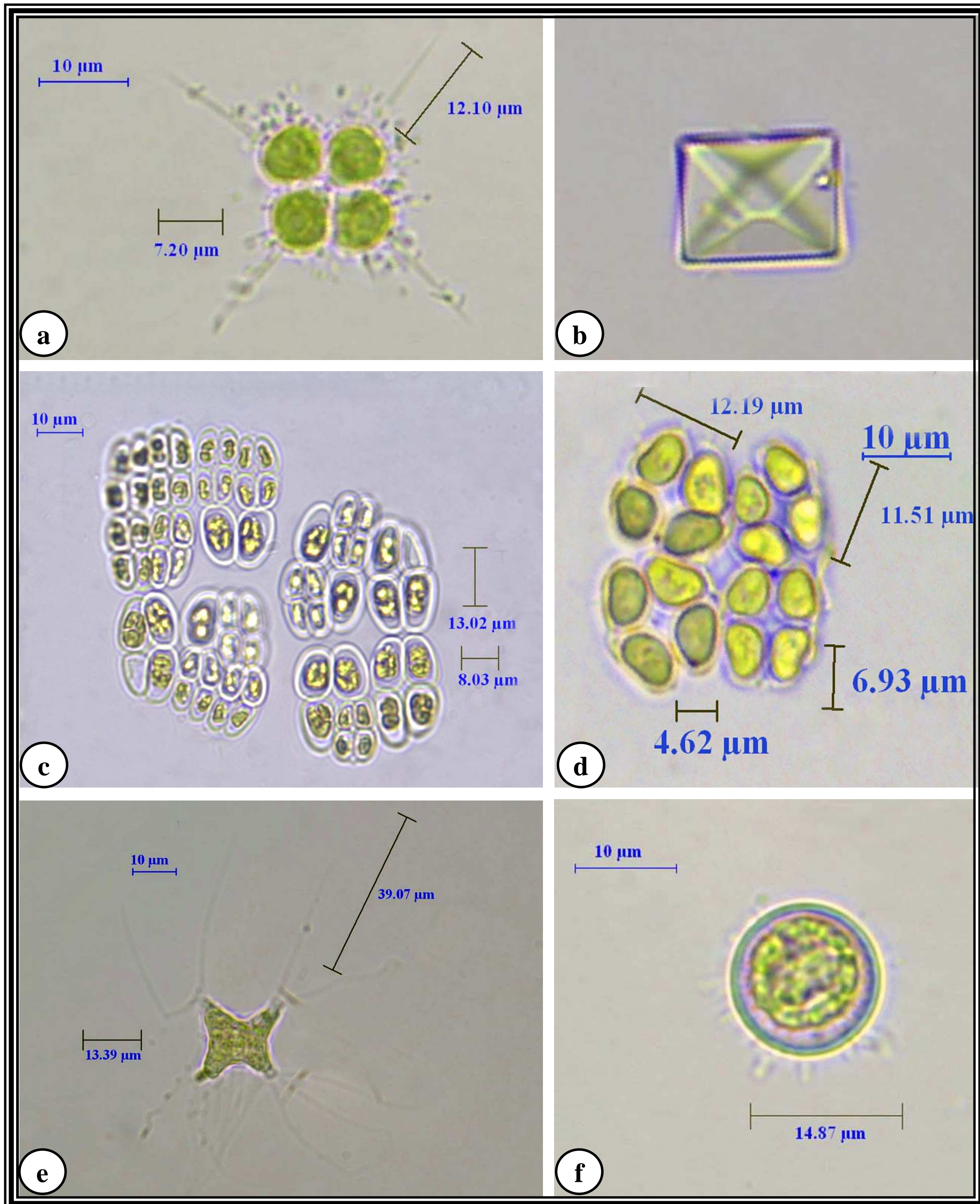

**a** and **e:** *Chroococcus turgidus* var. *maximus*, **b:** *Chroococcus schizodermaticus*, **c:** *Chroococcus tenax*, **d:** *Chroococcus minutes*, **f:** *Palmellococcus saccharophilus*

# PLATE 26



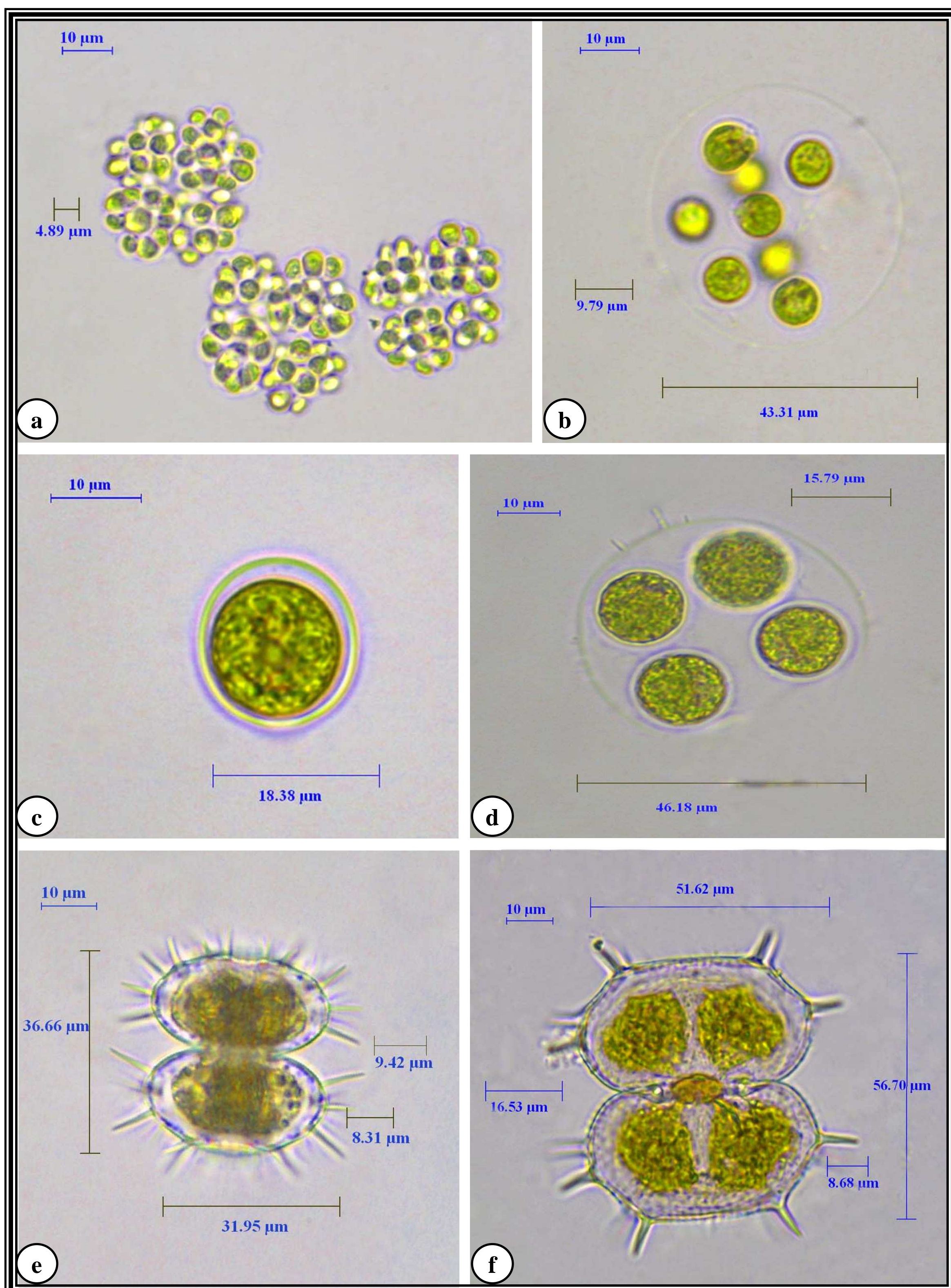

**a:** *Arthrodesmus octocornis*, **b:** *Staurodesmus cuspidatus*, **c:** *Pandorina morum* X 400, **d:** *Eudorina elegans*, **e:** *Lagerheimia wratislawiensis*, **f:** *Gonium pectorale*

## PLATE 27



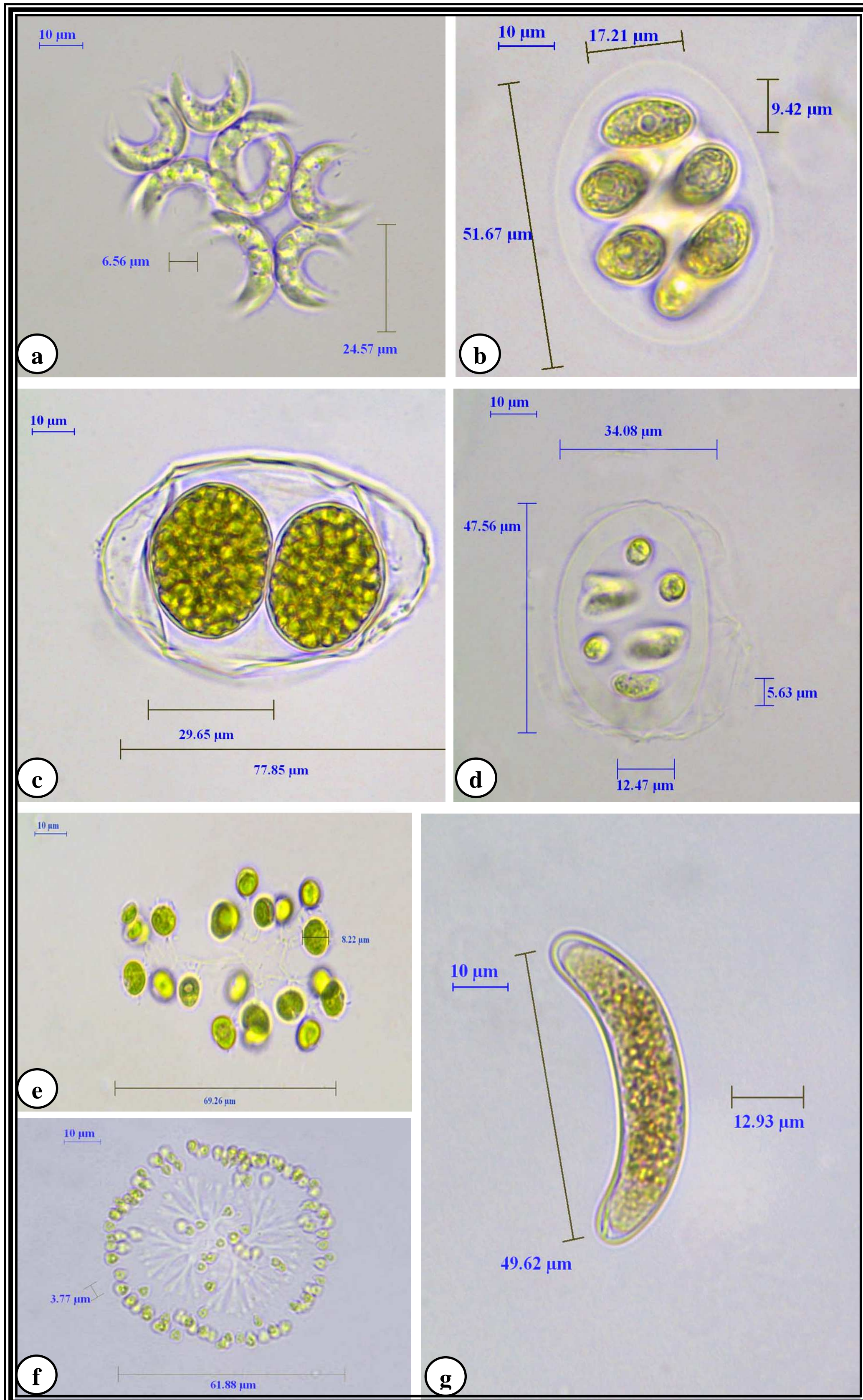

**a:** *Tetrastrum heteracanthum*, **b:** *Crucigenia tetrapedia*, **c:** *Crucigenia rectangularis*, **d:** *Crucigenia crucifera*, **e:** *Polyedriopsis spinulosa*, **f:** *Trochiscia aciculifera*

## PLATE 28



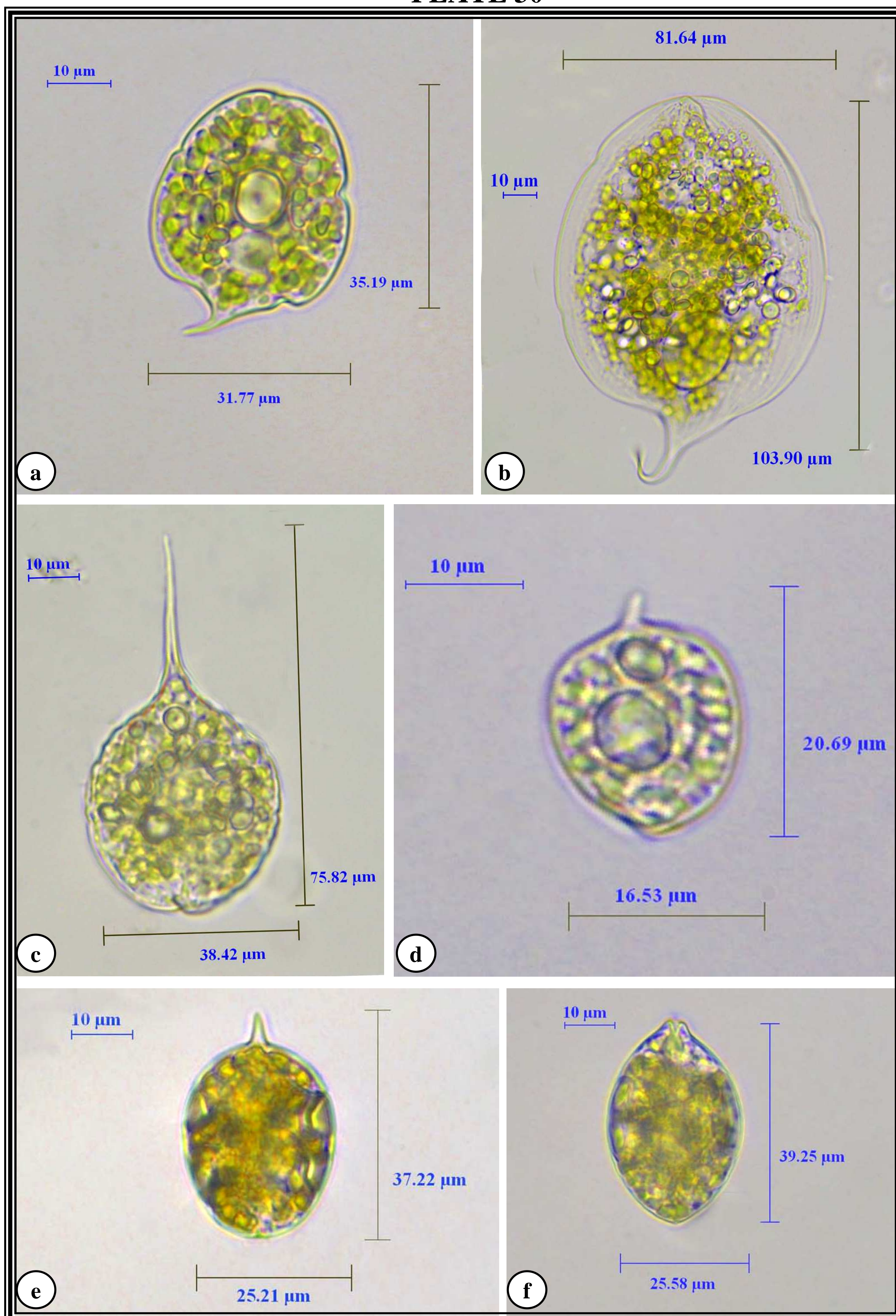

**a:** *Coenococcus planctonicus*, **b**: *Coenococcus polycoccus*, **c** and **d:** *Planktosphaeria gelatinosa*, **e:** *Xanthidium acanthophorum*, **f:** *Xanthidium antilopaeum* var. *polymazum*

## PLATE 29



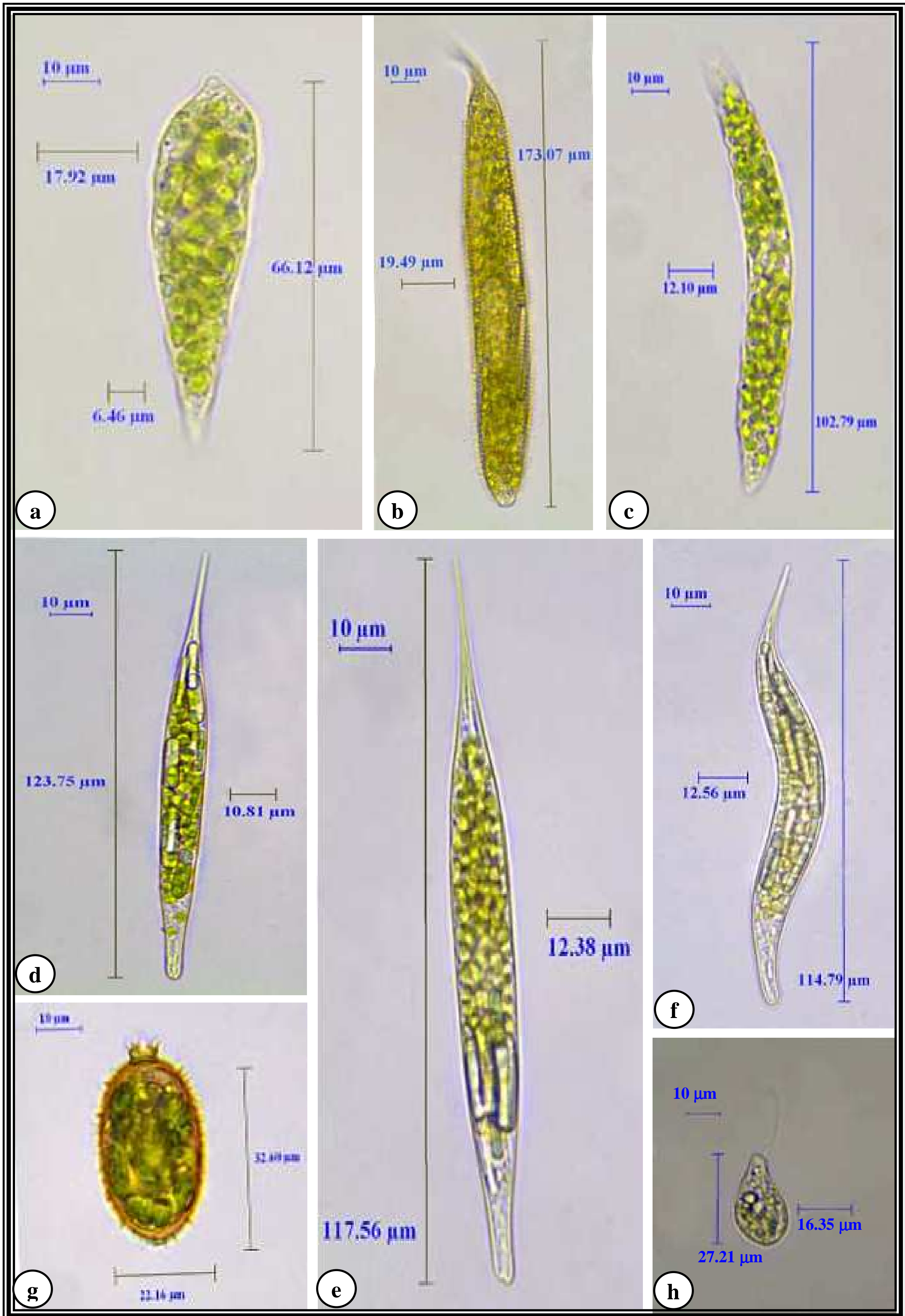

**a:** *Selenastrum gracile*, **b:** *Nephrocytium agardhianum*, **c:** *Gloeotaenium loitlesbergerianum*, **d:** *Nephrocytium lunatum* X 400, **e** and **g**: *Dictyosphaerium pulchellum*, **f:** *Dictyosphaerium reniforme* f. *major*

## PLATE 30



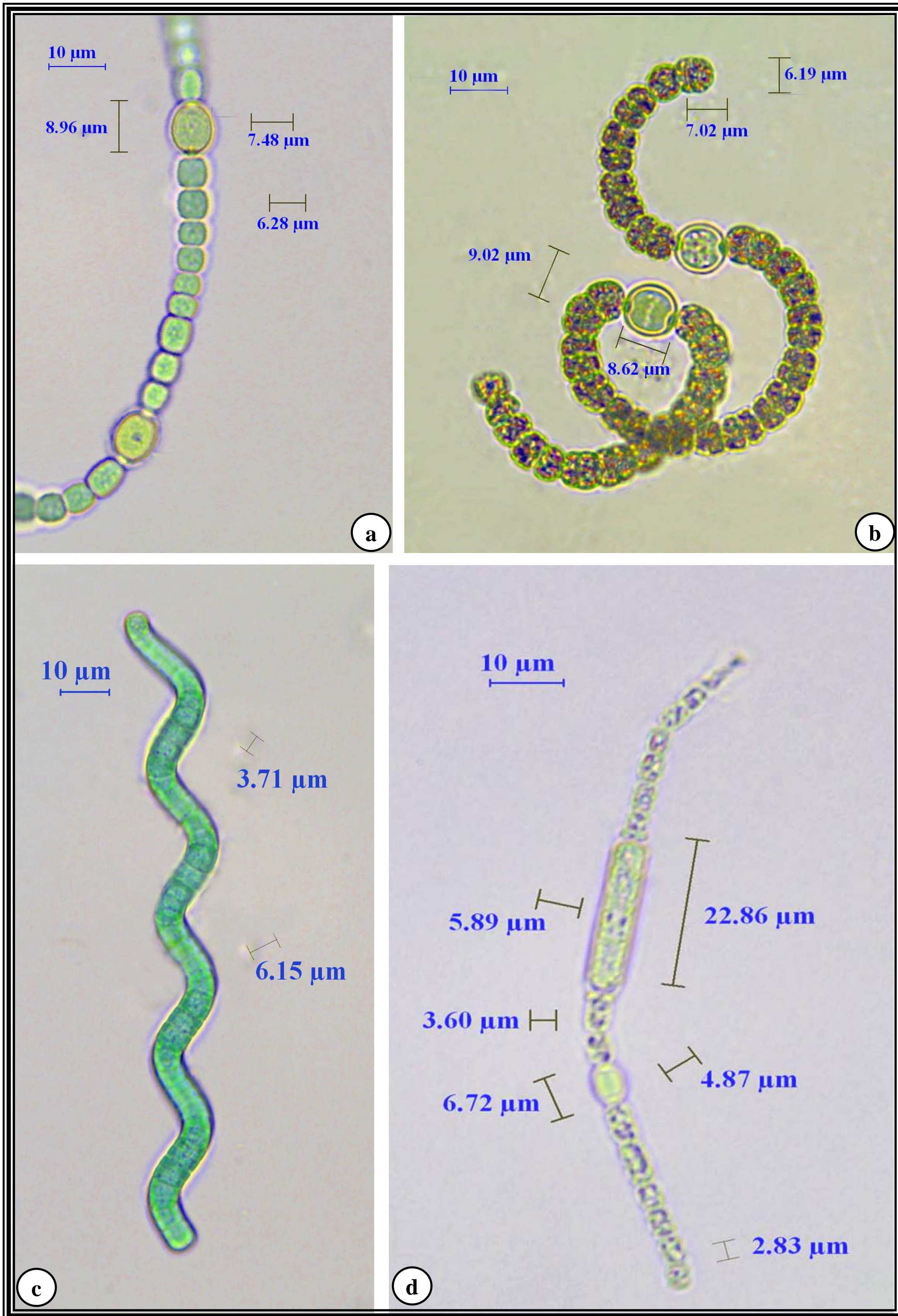

**a:** *Phacus anacoelus*, **b:** *Phacus anacoelus* var. *undulata*, **c:** *Phacus longicauda*, **d:** *Phacus acuminatus*, **e:** *Lepocinclis fusiformis*, **f:** *Lepocinclis fusiformis* var. *minor*

## PLATE 31



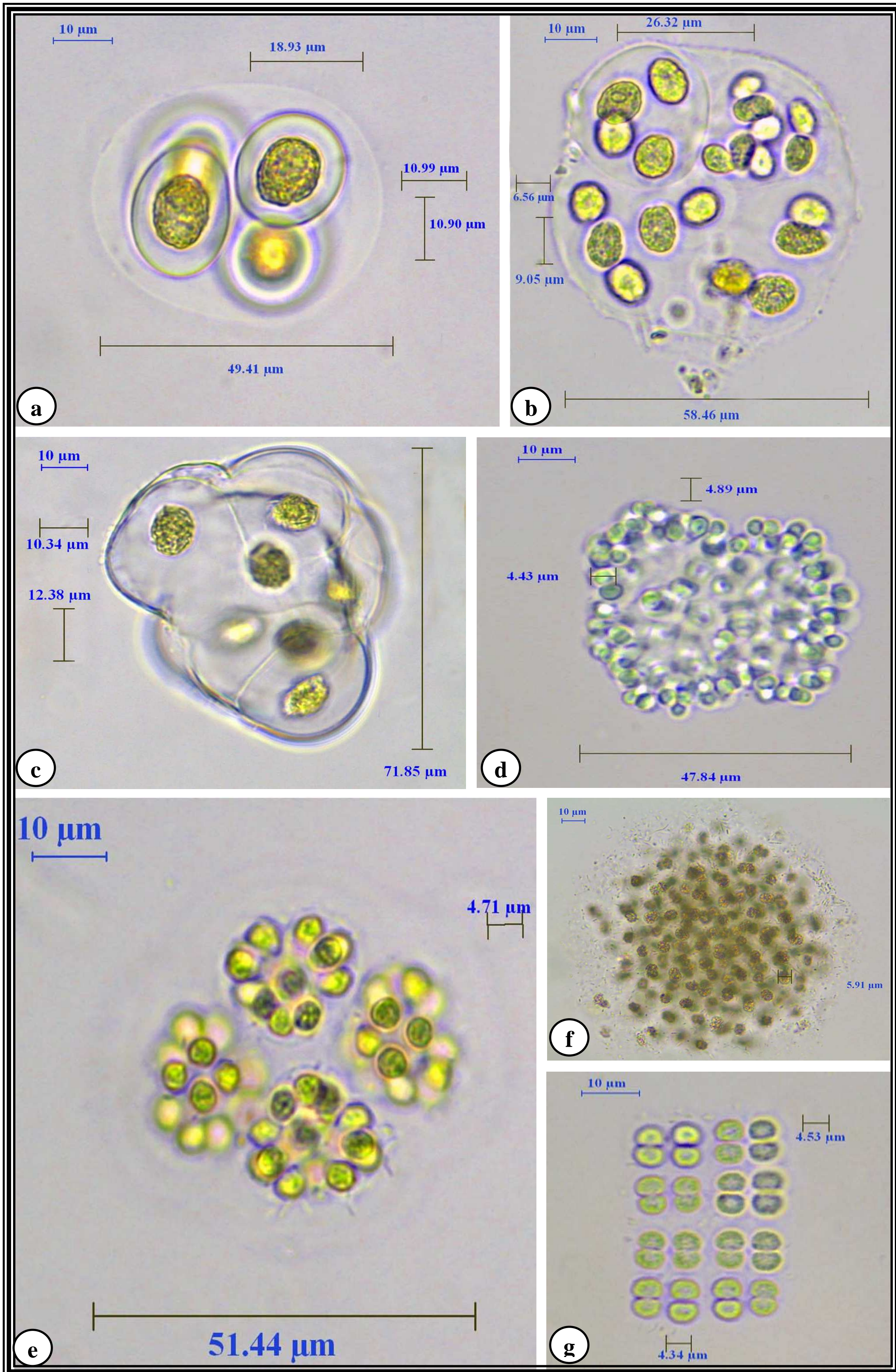

**a:** *Euglena proxima*, **b:** *Euglena spirogyra*, **c:** *Euglena deses*, **d:** *Euglena acus* var. *rigida*, **e** and **f:** *Euglena acus*, **g:** *Trachelomonas hispida* var. *coronata*, **h:** *Euglena* sp.

## PLATE 32



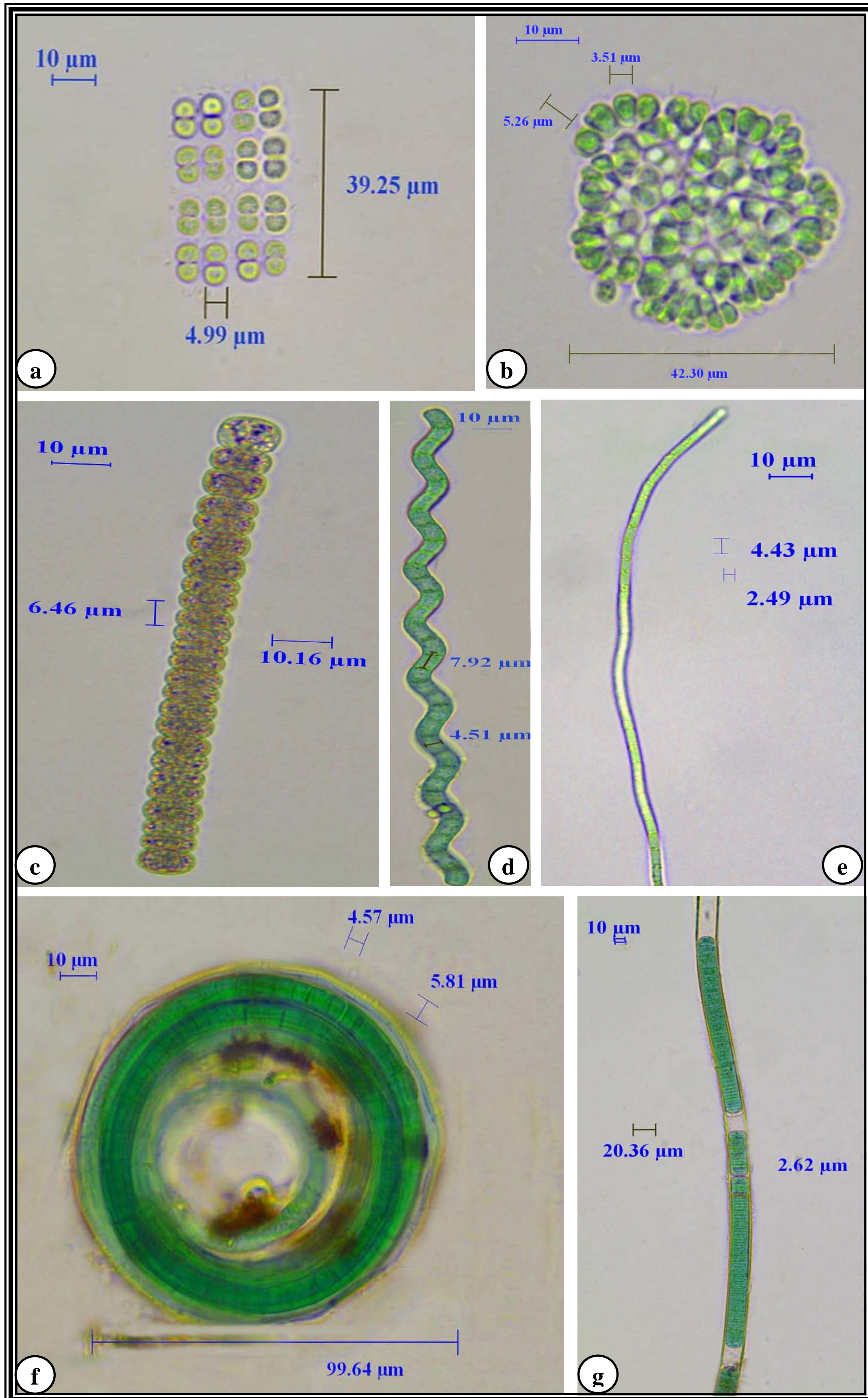

**a:** *Anabaena doliolum*, **b:** *Anabaena circinalis*, **c:** *Arthrospira jenneri*, **d:** *Aphanizomenon flos-aquae*

## PLATE 33



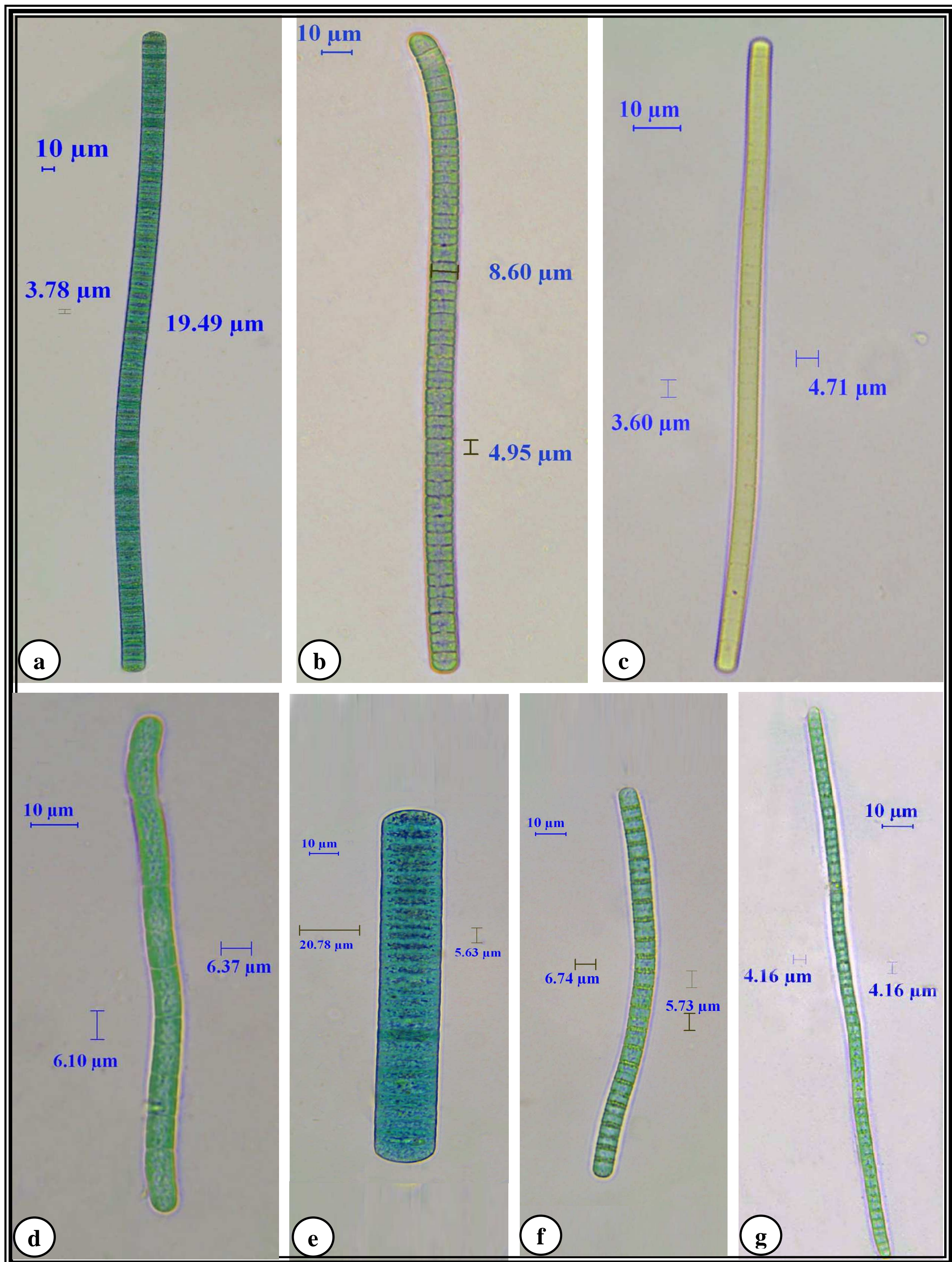

**a:** *Gloeothece samoensis* var. *major*, **b:** *Gloeothece rupestris*, **c:** *Gloeocystis ampla*, **d:** *Gomphosphaeria naegeliana*, **e:** *Gomphosphaeria* sp., **f:** *Microcystis aeruginosa*, **g:** *Merismopedia glauca*

## PLATE 34



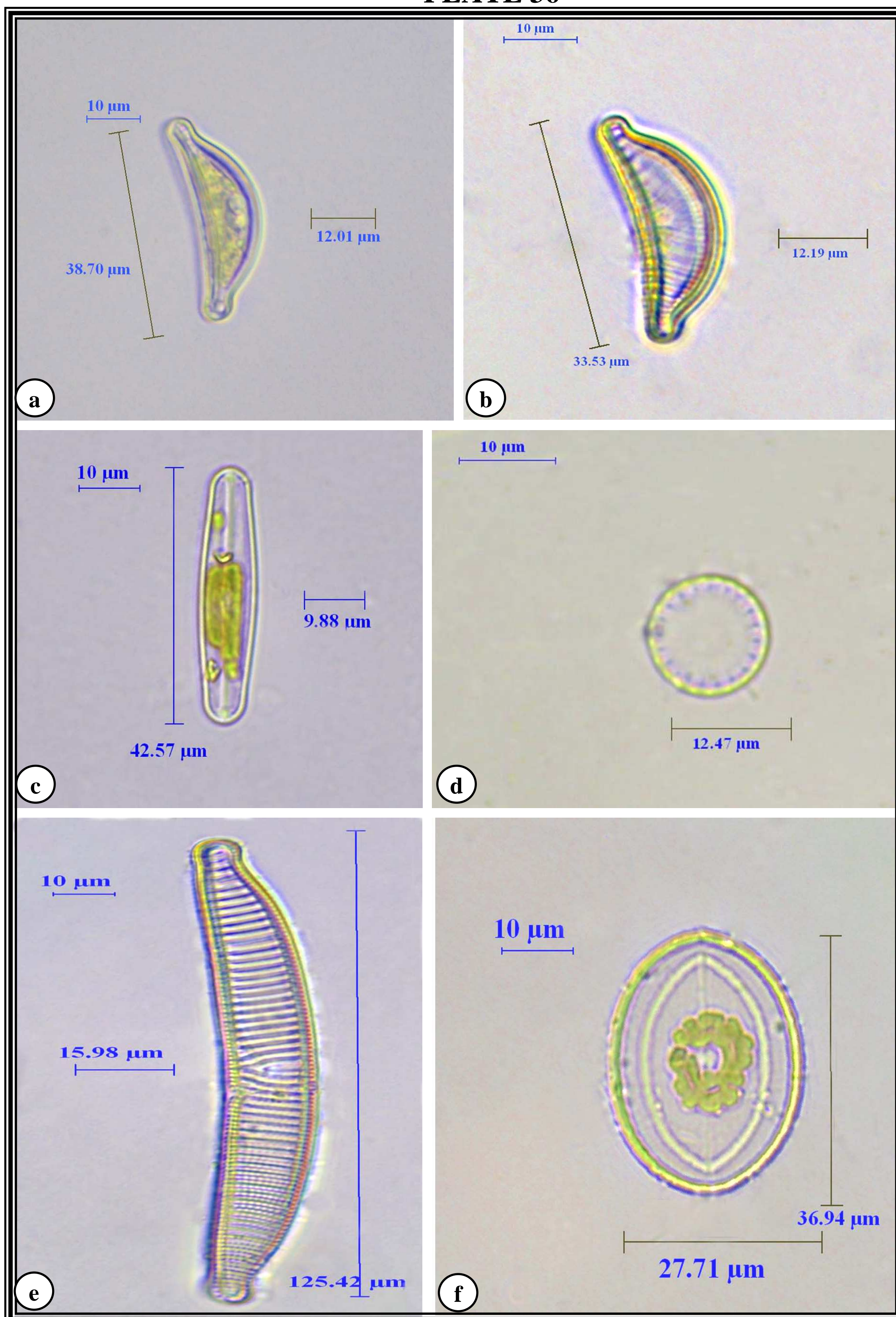

**a:** *Merismopedia glauca*, **b:** *Coelosphaerium collinsii*, **c:** *Trichodesmium lacustre*, **d:** *Spirulina meneghiniana* X 400, **e:** *Phormidium purpurascens*, **f:** *Lyngbya contorta* X 400, **g:** *Lyngbya birgei* X 200

# PLATE 35



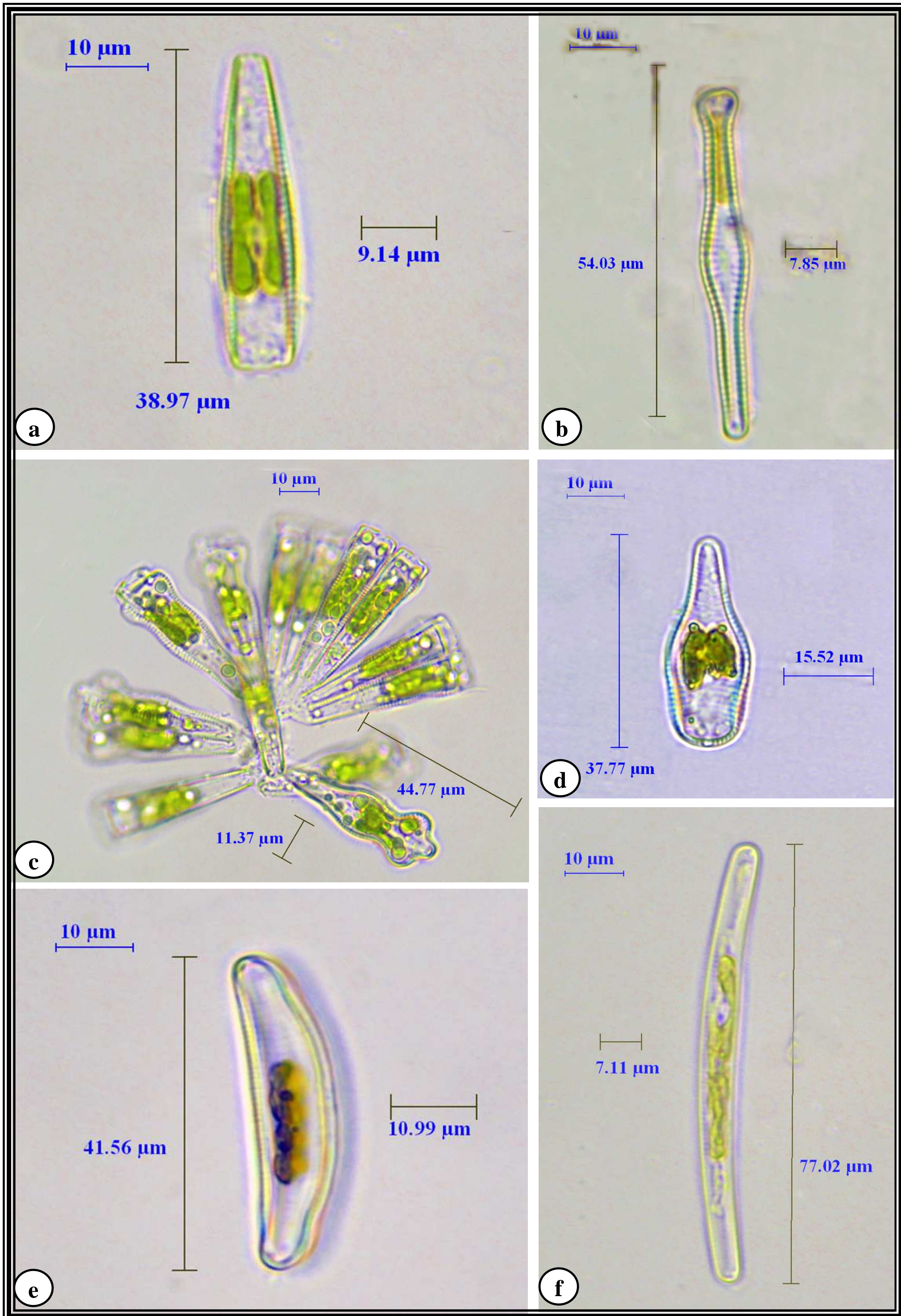

**a:** *Oscillatoria curviceps* X 200, **b:** *Oscillatoria chalybea* X 400, **c:** *Oscillatoria granulate*, **d:** *Oscillatoria chlorina*, **e:** *Oscillatoria limosa*, **f:** *Oscillatoria irrigua*, **g:** *Oscillatoria formosa*

## PLATE 36



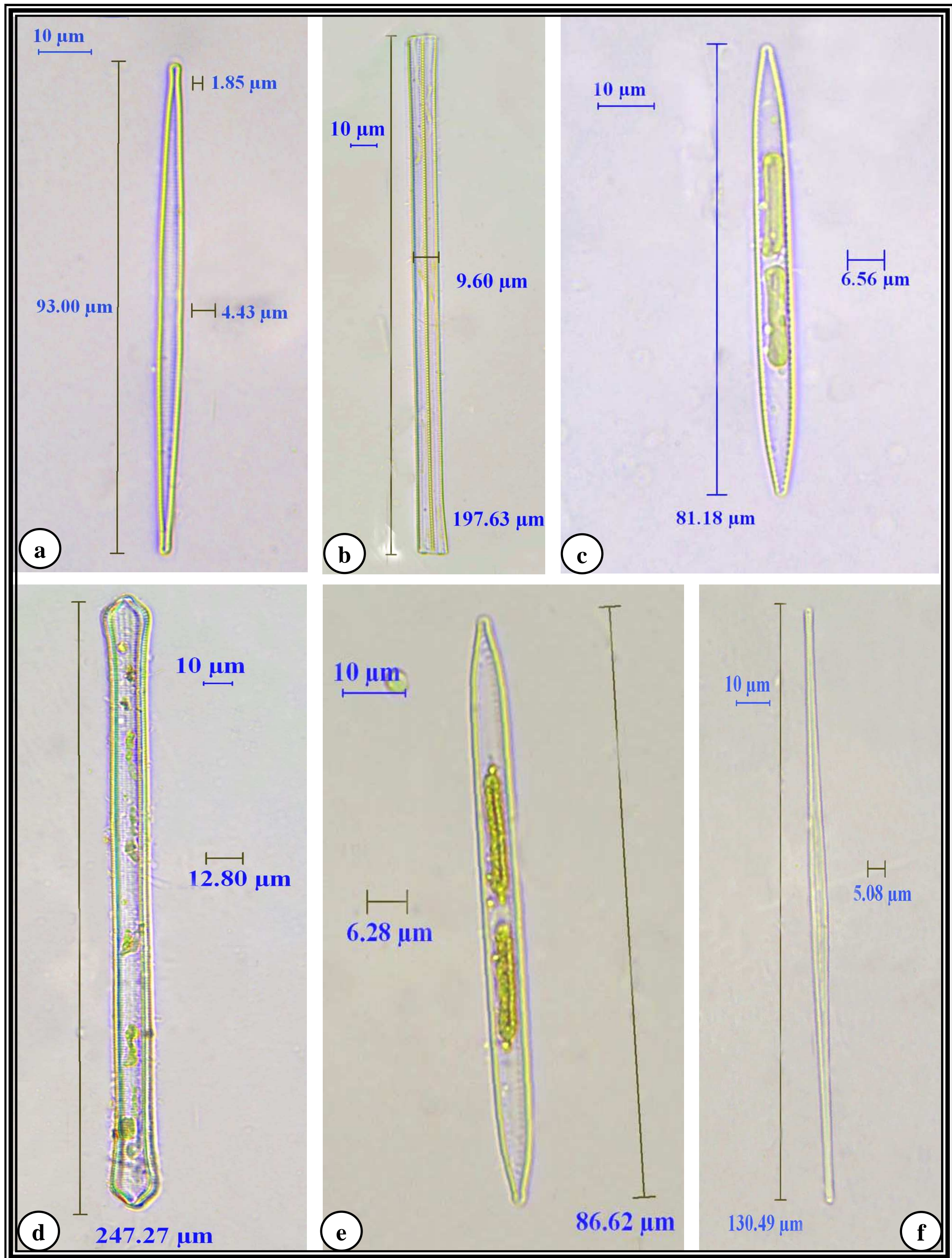

**a:** *Amphora coffeoeformis* var. *africana*, **b:** *Amphora* sp., **c:** *Achnanthes coarctata* var. *parallela*, **d:** *Cyclotella meneghiniana*, **e:** *Hantzschia* sp., **f:** *Cocconeis placentula*

# PLATE 37



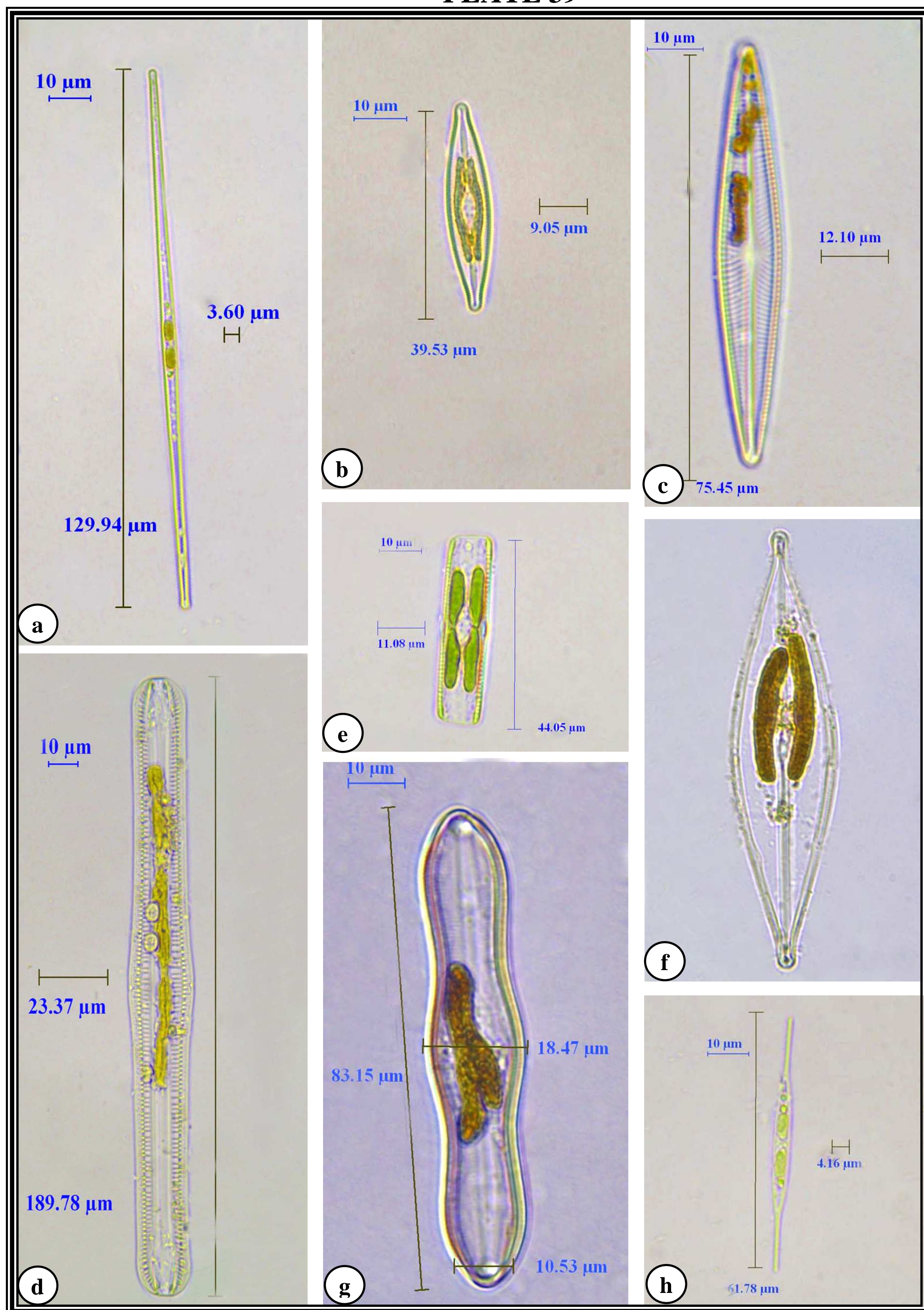

**a:** *Gamphonema telegraphicum*, **b:** *Gamphonema constrictum*, **c:** *Gamphonema acuminatum*, **d:** *Gamphonema truncatum*, **e:** *Eunotia monodon*, **f:** *Eunotia parallela*

# PLATE 38



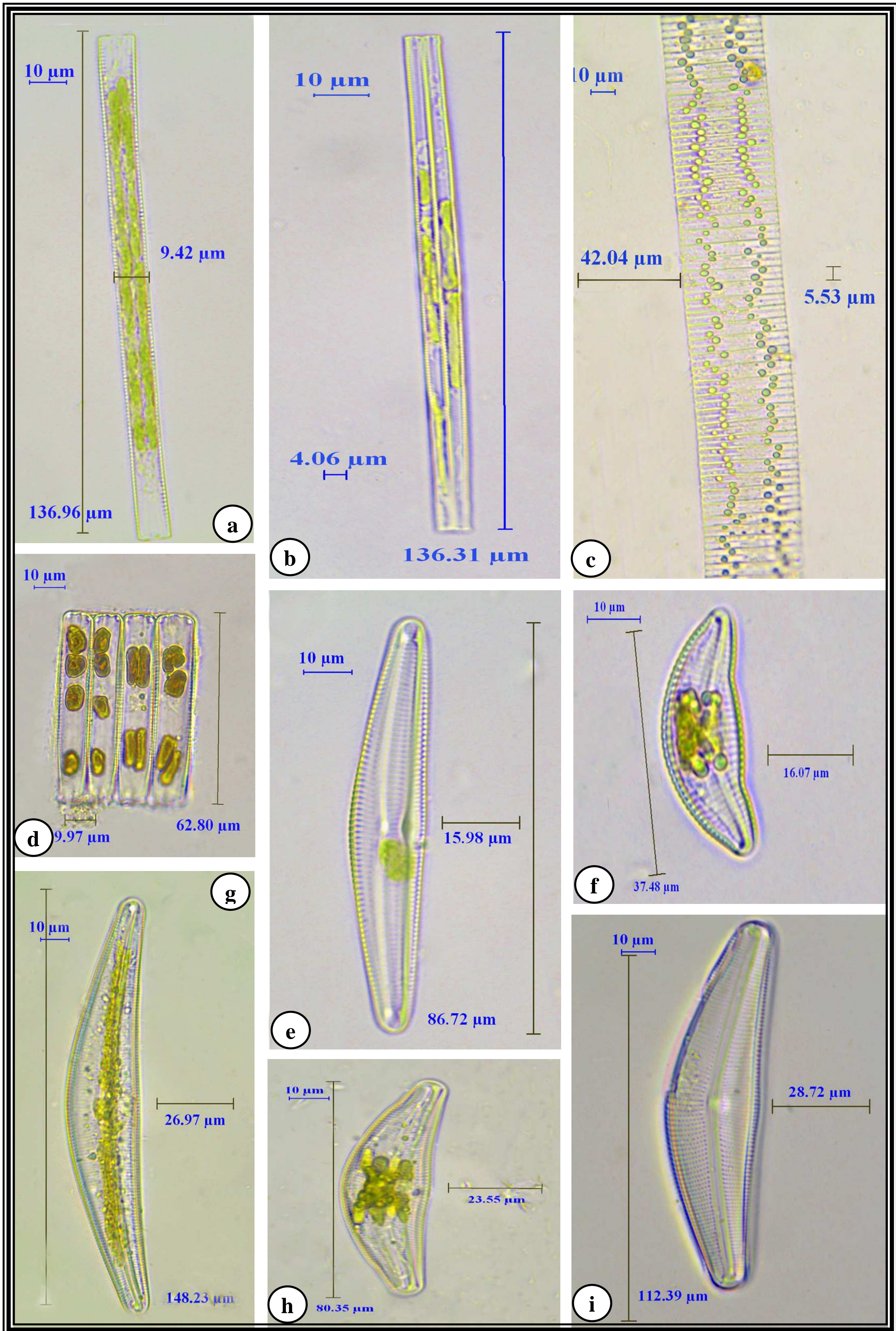

**a:** *Synedra ulna* var. *contracta*, **b:** *Synedra ulna*, **c:** *Synedra dorsiventralis*, **d:** *Synedra capitata* X 400, **e:** *Synedra ulna* var. *amphirhynchus*, **f:** *Synedra delicatissima*

# PLATE 39



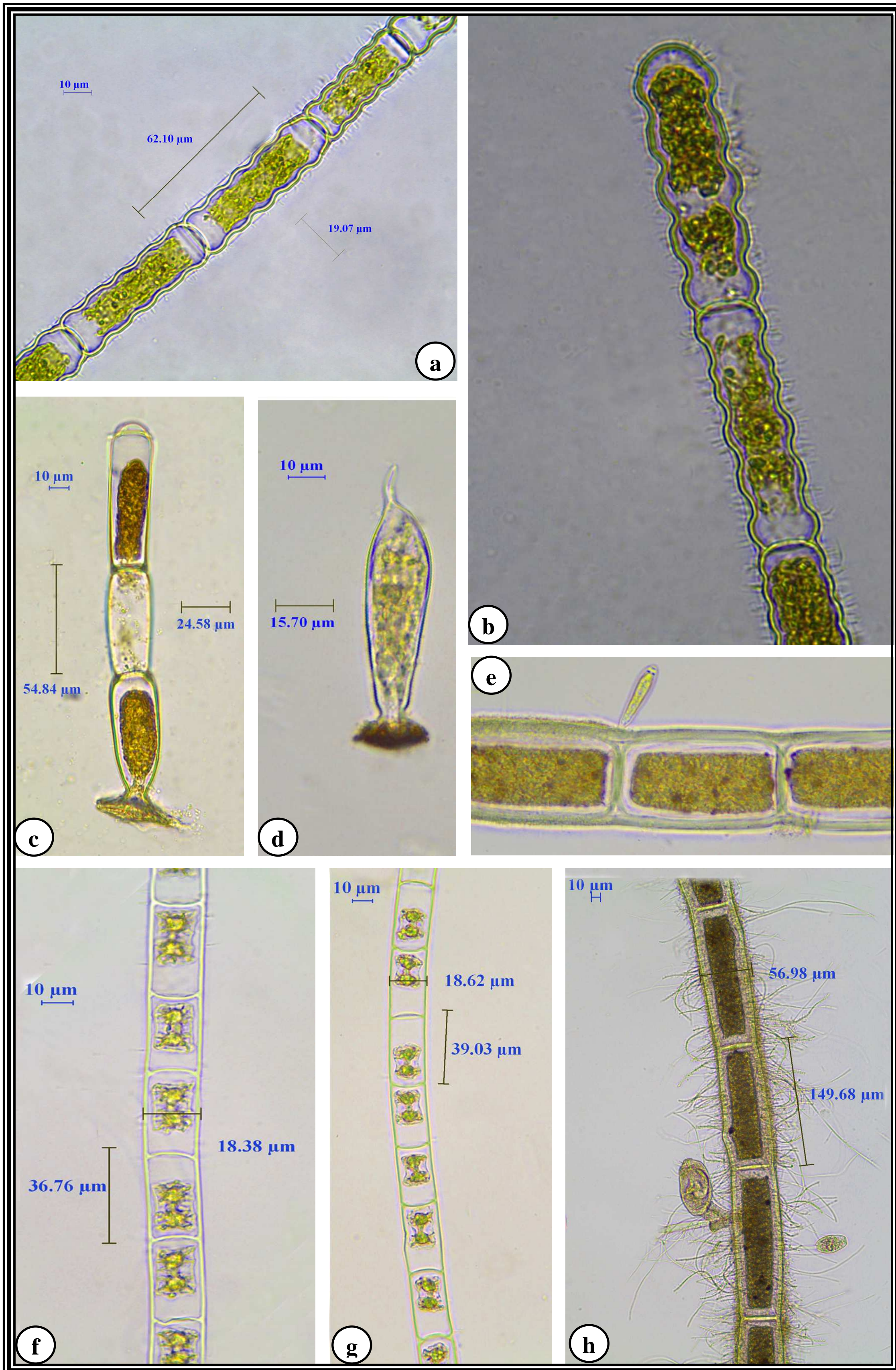

**a:** *Synedra tenera*, **b:** *Navicula cryptocephaloides*, **c:** *Navicula radiosa*, **d:** *Rhopalodia gibba*, **e:** *Navicula viridis*, **f:** *Navicula* sp., **g:** *Caloneis silicula,* **h:** *Nitzschia acicularis*

# PLATE 40



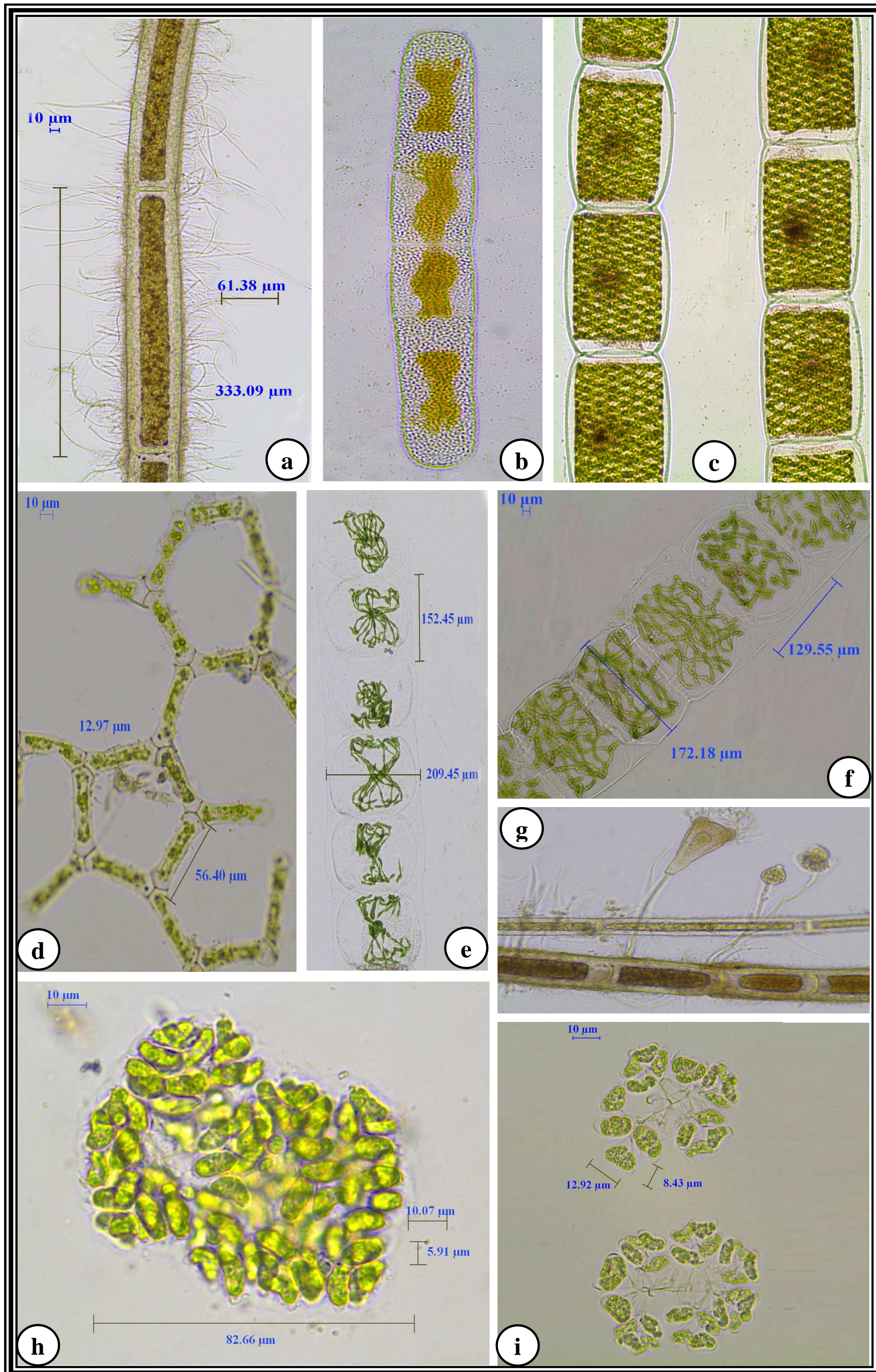

**a:** *Fragilaria intermedia* var. *robusta*, **b:** *Fragilaria biceps*, **c:** *Fragilaria crotonensis* X 400, **d:** *Fragilaria capucina*, **e:** *Cymbella parva*, **f:** *Cymbella affinis*, **g:** *Cymbella lanceolata*, **h:** *Cymbella tumida*, **i:** *Cymbella aspera*

## PLATE 41



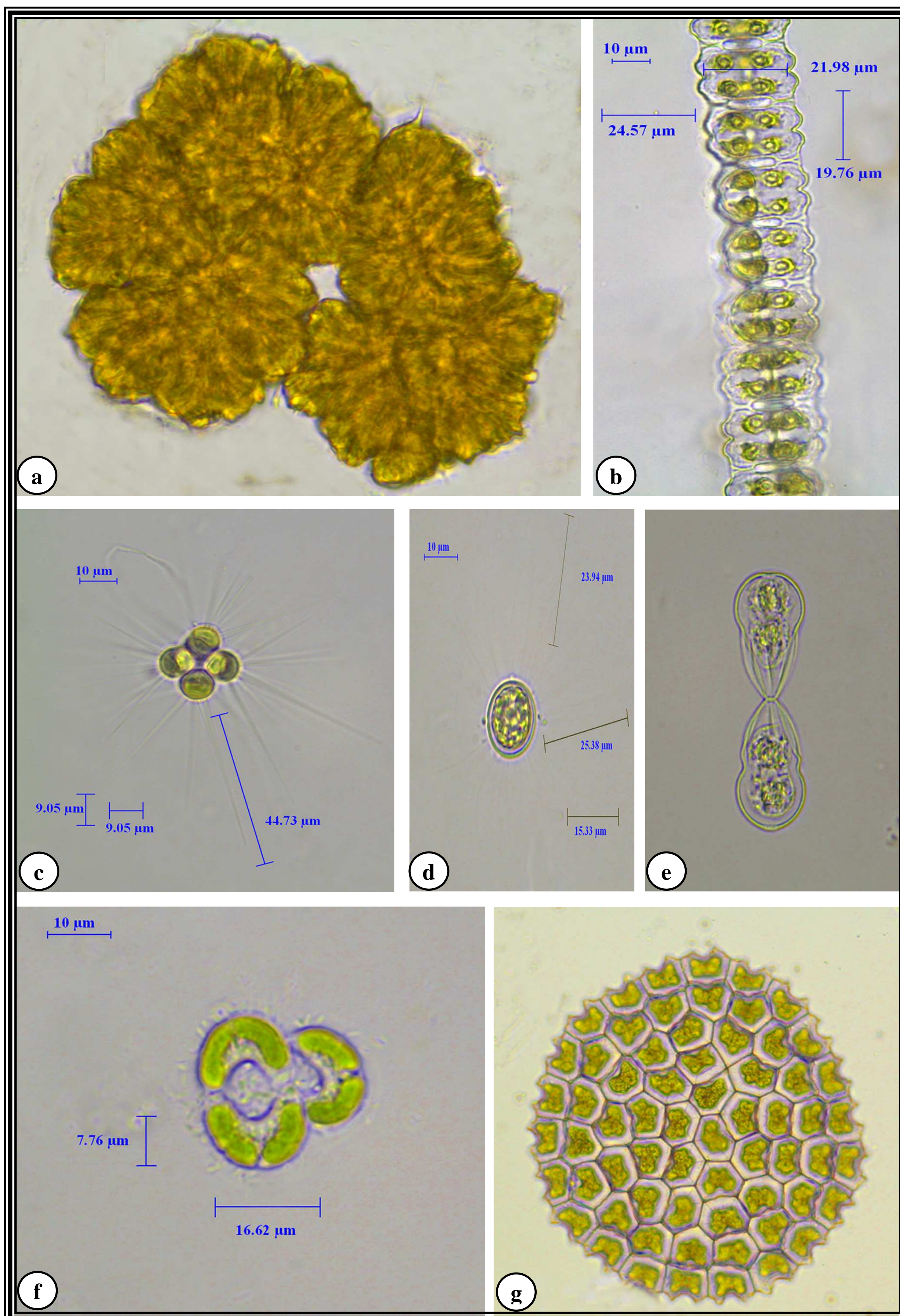

**a** and **b:** *Oedeogonium undulatum* f. *senegalense*, **c:** *Oedeogonium* sp. X 100, **d:** *Oedeogonium intermedium*, **e:** *Oedeogonium* sp. on *Rhizoclonium* sp., **f:** *Zygnema collinisanum*, **g:** *Zygnema* sp. X 400, **h:** *Rhizoclonium heiroglyphicum* X 200

# PLATE 42




**a:** *Rhizoclonium heiroglyphicum* X 100, **b:** *Penium margaritaceum*, **c** and **f:** *Spirogyra* sp. X 200, **d:** *Hydrodictyon reticulatum* X 200, **e:** *Spirogyra* sp. X 100, **g:** Epiphytic *Gamphonema* sp. on *Rhizoclonium* sp. X 200, **h** and **i**: *Dimorphococcus lunatus*

# PLATE 43




**a:** *Botryococcus braunii*, **b:** *Desmidium bengalicum*, **c** and **d:** *Golenkinia radiata*, **e:** Unidentified, **f:** *Tetrallantos lagerheimii*, **g:** *Pediastrum duplex* X 400

### 4.3 Screening of algal isolates for lipid content

#### 4.3.1 Nile red technique for the preliminary screening of lipids

Thirteen microalgal species were successfully purified and maintained at Algology Laboratory, NBRI-Lucknow for further studies. Twelve of them were green algae and one belonged to cyanobacteria (Plate 44). Among the green algae majority belonged to genus *Scenedesmus.* All the thirteen microalgae were subjected to neutral lipid observations qualitatively (to confirm the presence of lipids) using fluorescent microscope for the preliminary screening as shown in the Plate 45. After staining the algal samples with Nile red, strong fluorescence emissions (yellow gold) were detected in almost all the green algae and no fluorescent signal was found in case of blue green algae *Aphanothece microscopica.* In particular *S. dimorphus, S. quadricauda, Chlorella and Chlamydomonas* sp. yielded strong fluorescence emissions and no fluorescence were detected in case of blue green algae.

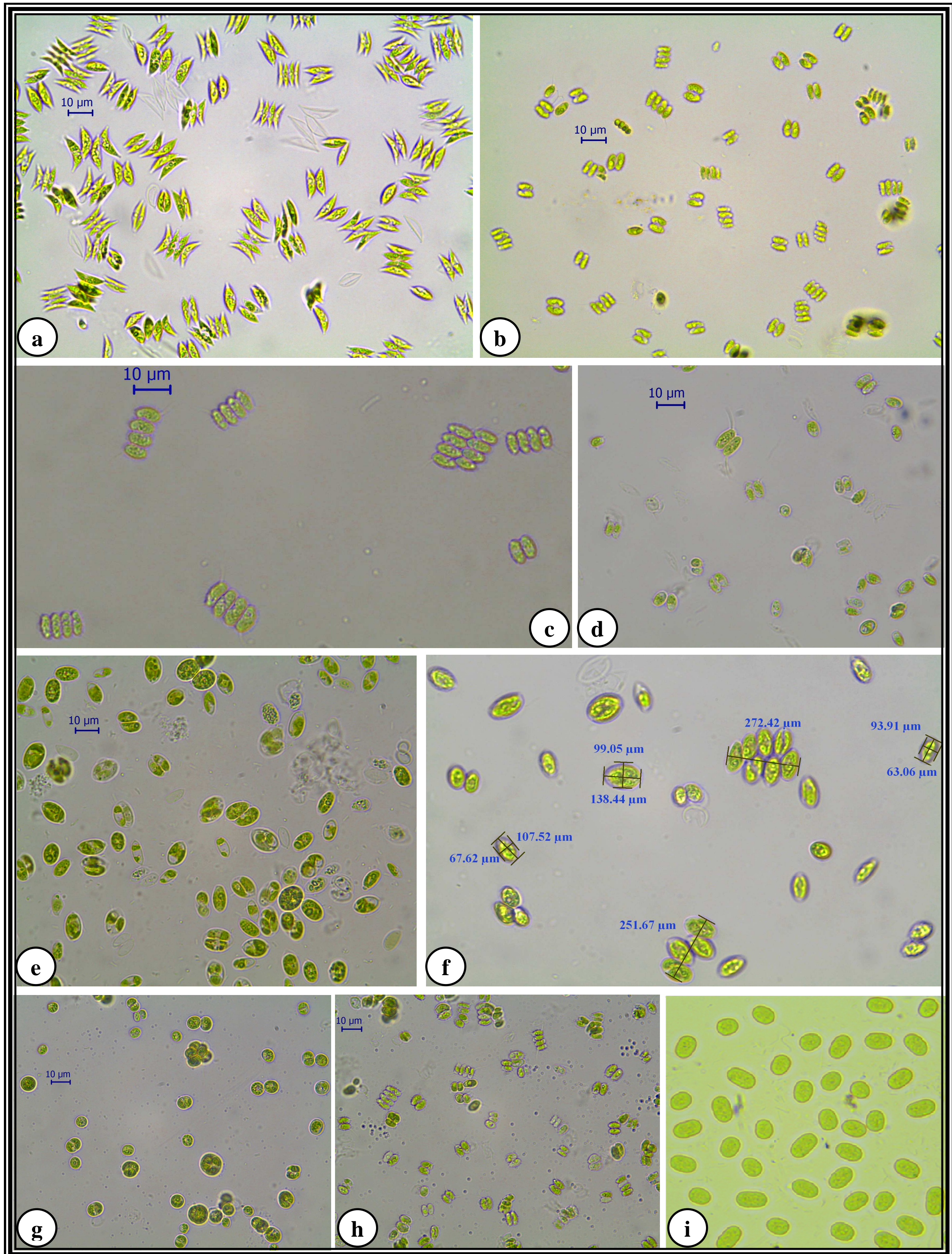
#### 4.3.2 Quantification of total lipids by gravimetric method

The lipid contents of different microalgal species isolated from the six different sites of Dal Lake are presented in the Table 12. The percentage of oil yields of these microalgal isolates were expressed in terms of dry biomass by slight modifications of Folch method (1957). The percentage of oil content of all microalgal isolates ranged from 4.63-30.99 per cent. The results showed that two green algae (*S. dimorphus* and *S. quadricauda*) were found to accumulate the highest amount of lipids (30.99 and 28.61% respectively) compared to other microalgal isolates. The other oleaferous microalgae with decreasing order of oil per cent are *S. armatus* var. *major* (23.96%), *Chlamydomonas* sp. (22.61%), *S. armatus* (21.54%), *Chlorella* sp. (19.76%). The lowest oil content was observed in *Aphanothece microscopica* (4.63%).

**Table-12 : Percentage lipid yields in different microalgal isolates**

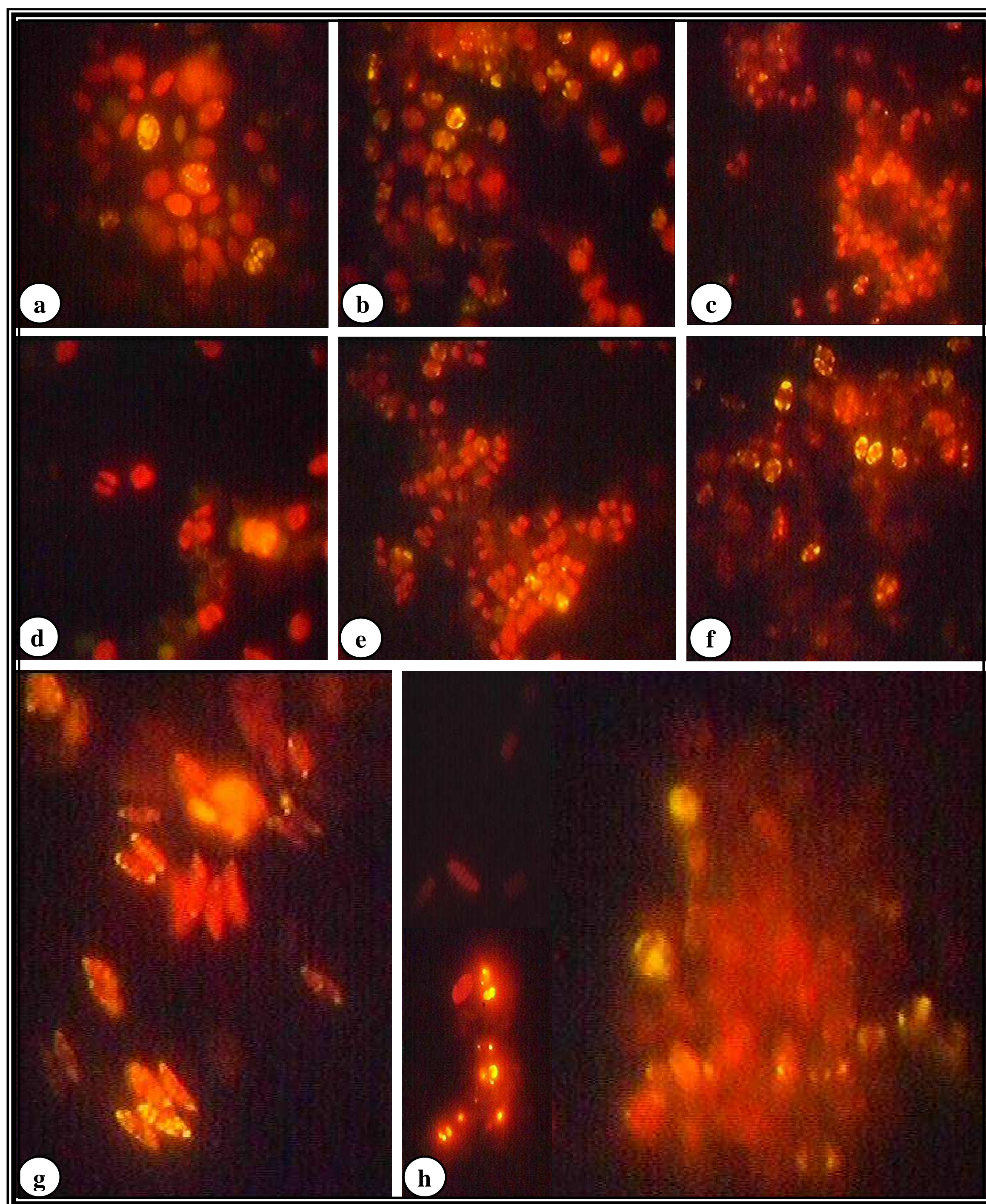
| S. No. | Name of Microalgae | Collection Site from Dal Lake | Algal biomass (mg) | Algal oil (mg) | Oil (%) |
|---|---|---|---|---|---|
| 01 | *Scenedesmus bijugatus* | DLS-I | 38.1 | 4.7 | 12.33 |
| 02 | *S. armatus* | DLS-III | 115.8 | 24.95 | 21.54 |
| 03 | *S. armatus* var. *bicaudatus* | DLS-VI | 119.4 | 9.4 | 7.87 |
| 04 | *S. bijugatus* | DLS-V | 39.7 | 7.1 | 17.88 |
| 05 | *S. dimorphus* | DLS-III | 51.3 | 15.9 | 30.99 |
| 06 | *Chlamydomonas* sp. | DLS-II | 87.1 | 19.7 | 22.61 |
| 07 | *S. armatus* var. *major* | DLS-IV | 104.8 | 11.4 | 10.87 |
| 08 | S. *armatus* var. *major* | DLS-I | 142.3 | 18.4 | 12.93 |
| 09 | *S. armatus* var. *major* | DLS-III | 120.3 | 22.75 | 18.91 |
| 10 | *S. armatus* var. *major* | DLS-II | 108.9 | 26.1 | 23.96 |
| 11 | *S. quadricauda* | DLS-V | 30.4 | 8.7 | 28.61 |
| 12 | *Chlorella* sp. | DLS-V | 50.1 | 9.9 | 19.76 |
| 13 | *Aphanothece microscopica* | DLS-VI | 49.7 | 2.3 | 4.63 |

# PLATE 44




Pure cultures of different microalgae:
**a:** *Scenedesmus dimorphus*, **b:** *Scenedesmus quadricauda*, **c:** *Scenedesmus armatus*, **d:** *Scenedesmus armatus* var. *bicaudatus*, **e:** *Chlamydomonas* sp., **f:** *Scenedesmus bijugatus*, **g:** *Chlorella* sp., **h:** *Scenedesmus armatus* var. *major*, **i:** *Aphanotheceae microscopica*

# PLATE 45




Nile red fluorescent yellow gold emissions by different microalgae showing presence of lipids:-
**a:** *Chlamydomonas* sp., **b:** *Chlorella* sp., **c:** *Scenedesmus armatus* var. *major*, **d:** *Scenedesmus armatus* var. *bicaudatus*, **e:** *Scenedesmus armatus*, **f:** *Scenedesmus bijugatus*, **g:** *Scenedesmus dimorphus*, **h:** *Scenedesmus quadricauda*

### 4.4 Growth measurement of two promising microalgae by spectrophotometric method

The maximum absorbance was inspected by scanning a culture sample between 400 and 1100 nm and the highest absorbance peak value obtained at 680 nm was then used to measure the optical density as shown in the Figs. 6 and 7. Therefore, growth of both the species was read in this wavelength. The specific data pertaining to the growth measurements of two tested microalgae *S. dimorphus* and *S. quadricauda* is shown in Fig. 8. During the whole experiment, the measurements of the OD values at 680 nm were done in triplicates and the mean ± standard error (±SE) was calculated and OD values in tabular form were converted into growth curve using GraphPad Prism 5 statistical software. The plot clearly shows distinct phases of a typical growth curve of two microalgae where the growth reached a stationary phase on $14^{th}$ day of incubation and during the investigations it was found that both the species thrive well in the BBM media. During all the phases of growth curve it has been observed that *S. quadricauda* is slightly fast growing as compared to *S. dimorphus.* Initially the cultures showed gradual growth rate and from the $4^{th}$ day onwards, both the species had significant increase in total number of cells. As evident by the growth curve, both the species show lag phase of 5 days and on $6^{th}$ day both the cultures showed signs of exponential phase. During the stationary phase, maximum growth was found in *S. quadricauda* with OD of 3.37 compared with initial reading of 0.084 and minimum growth was found in *S. dimorphus* with OD of 2.97 compared with initial reading of 0.045.

### 4.5 Estimation of photosynthetic pigments

The photosynthetic pigments of two algal species were determined by DMSO method using two different set of formulae provided by Arnon, 1949 and Wellburn, 1994. The comparison of pigment concentration is presented in the Table 13.

**Table-13 : Chlorophyll estimation (mg $g^{-1}$ fw) in two microalgae by using two different set of equations**

| Microalgae | (Chl-a) | (Chl-b) | Total Chl | Total Carotenoids | Total pigment |
|---|---|---|---|---|---|
| Chlorophyll concentration as estimated by Wellburn equations, 1994: | | | | | |
| *S. dimorphus* (±SD) | 11.609 (±0.131) | 4.447 (±0.042) | 16.056 (±0.173) | 3.750 (±0.109) | 19.806 (±0.064) |
| *S. quadricauda* (±SD) | 16.711 (±0.0891) | 5.447 (±0.1181) | 22.158 (±0.0297) | 4.941 (±0.0212) | 27.099 (±0.0085) |
| Chlorophyll concentration as estimated by Arnon's formulae, 1949**:** | | | | | |
| *S. dimorphus* (±SD) | 6.008 (±0.625) | 0.796 (±0.476) | 6.803 (±1.100) | 0.471 (±0.008) | 7.274 (±1.109) |
| *S. quadricauda* (±SD) | 8.015 (±0.0262) | 1.168 (±0.597) | 8.701 (±0.110) | 0.530 (±0.009) | 9.713 (±0.561) |

(±SD) = Standard deviation

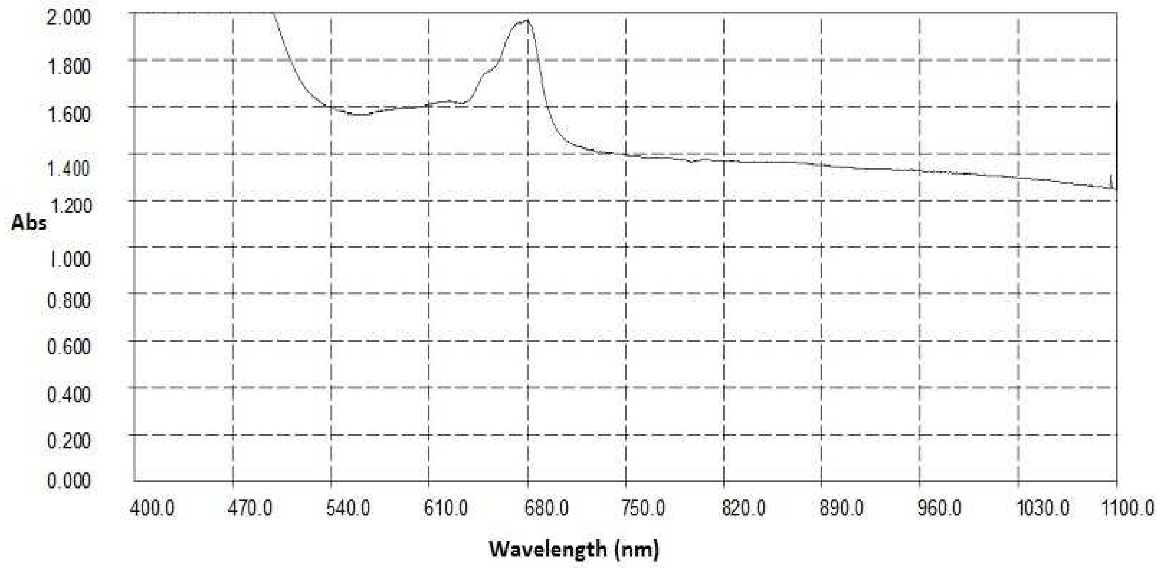



**Fig. 6 : Spectrascan of *S. quadricauda* with scan speed of 1800 nm/min screened between 400 and 1100 nm**

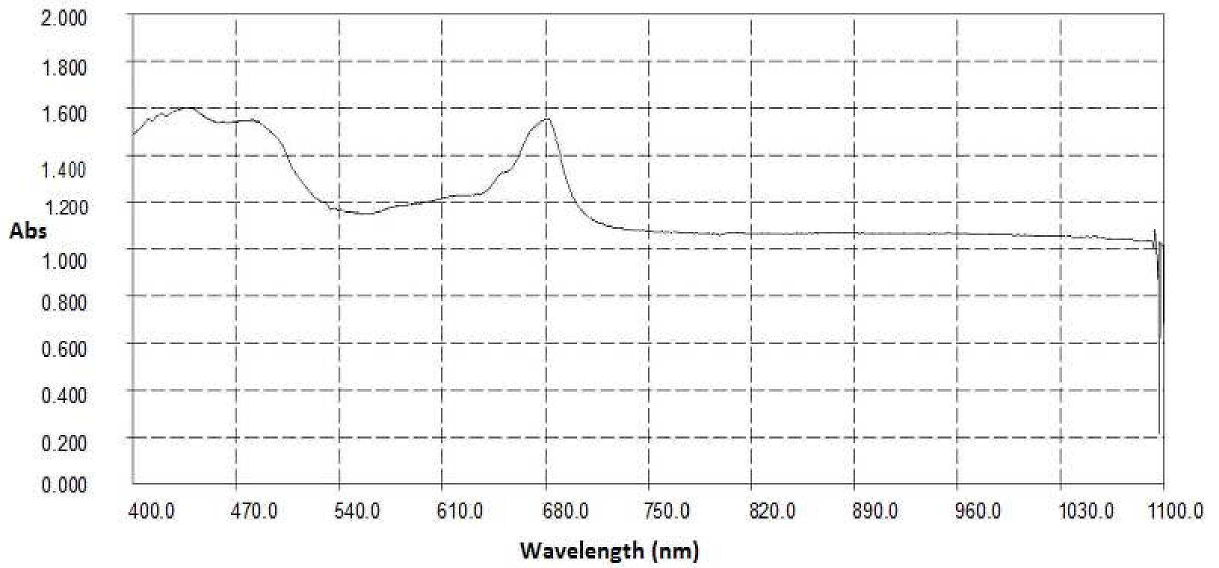



**Fig. 7 : Spectrascan of *S. dimorphus* with scan speed of 1800 nm/min screened between 400 and 1100 nm**

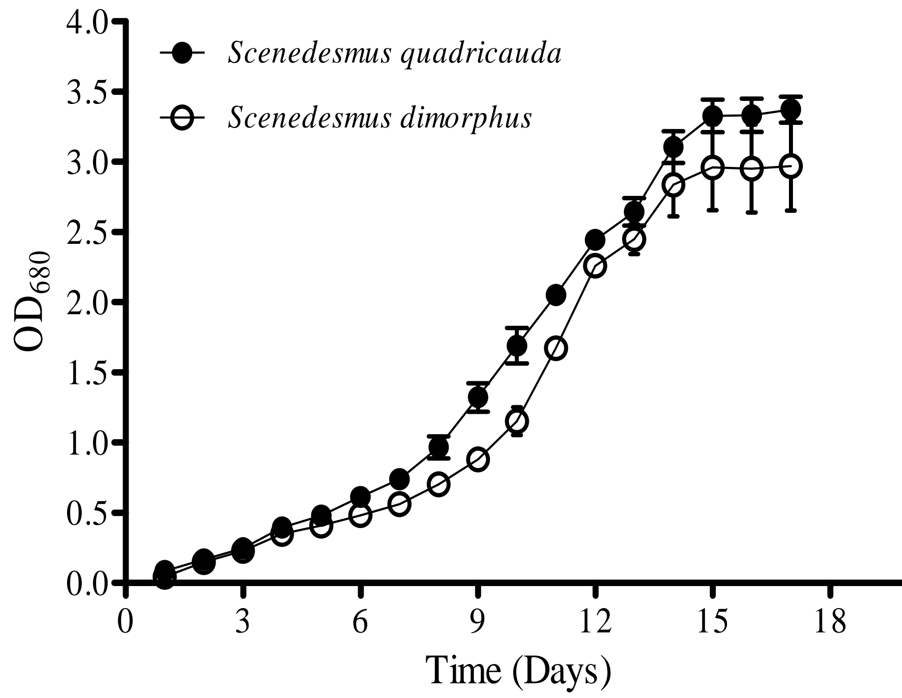



**Fig. 8 : Growth curve of two microalgae under batch mode**

By using Wellburn equations total photosynthetic pigments were maximum in *S. quadricauda* (27.099 mg $g^{-1}$) as compared to *S. dimorphus* (19.806 mg $g^{-1}$). In case of Arnon formulae, total photosynthetic pigments were also maximum in *S. quadricauda* (9.713 mg $g^{-1}$) as compared to *S. dimorphus* (7.274 mg $g^{-1}$).

The results show that by using Wellburn equations maximum chlorophyll-a (16.711 mg $g^{-1}$), chlorophyll-b (5.447 mg $g^{-1}$), total chlorophyll (22.158 mg $g^{-1}$) and total carotenoids (4.941mg $g^{-1}$) were reported in *S. quadricauda.* On the other hand minimum chlorophyll-a (11.609 mg $g^{-1}$), chlorophyll-b (4.447 mg $g^{-1}$), total chlorophyll (16.056 mg$g^{-1}$) and total carotenoids (3.750 mg $g^{-1}$) were reported in *S. dimorphus.*

Similarly, maximum chlorophyll-a (8.015 mg $g^{-1}$), chlorophyll-b (1.168 mg $g^{-1}$), total chlorophyll (8.701 mg $g^{-1}$) and total carotenoids (0.530 mg $g^{-1}$) were reported in *S. quadricauda* and minimum chlorophyll-a (6.008 mg $g^{-1}$), chlorophyll-b (0.796 mg $g^{-1}$), total chlorophyll (6.803 mg $g^{-1}$) and total carotenoids (0.471 mg $g^{-1}$) were reported in *S. dimorphus* by using Arnon formulae.

Overall in both the tested microalgal species the photosynthetic pigments were reported maximum by applying Wellburn (1994) equations, while as minimum pigments were reported by Arnon (1994) formulae as shown in Fig. 9.

### 4.6 Estimation of total protein content of two microalgal species

The data (mean values with SE) in the Fig. 10 shows the protein content (% w/w) in *S. dimorphus* and *S. quadricauda.* The results show that maximum total protein content was found in *S. quadricauda* (13.026%) and the minimum concentration was found in *S. dimorphus* (8.284%) (Table-14).

**Table-14 : Total protein content in two microalgal species**

| Microalgae | Aliquot of sample (ml) | OD $_{750nm}$ | Protein content in aliquot (μg) | Protein content (μg $ml^{-1}$) | Protein (%) | Mean Protein (%) |
|---|---|---|---|---|---|---|
| *S. dimorphus* | 0.1 | 0.048 | 24 | 240 | 7.101 | 8.284 |
| | 0.1 | 0.064 | 32 | 320 | 9.467 | |
| *S. quadricauda* | 0.1 | 0.075 | 37.5 | 375 | 12.544 | 13.026 |
| | 0.1 | 0.071 | 35.5 | 355 | 13.509 | |

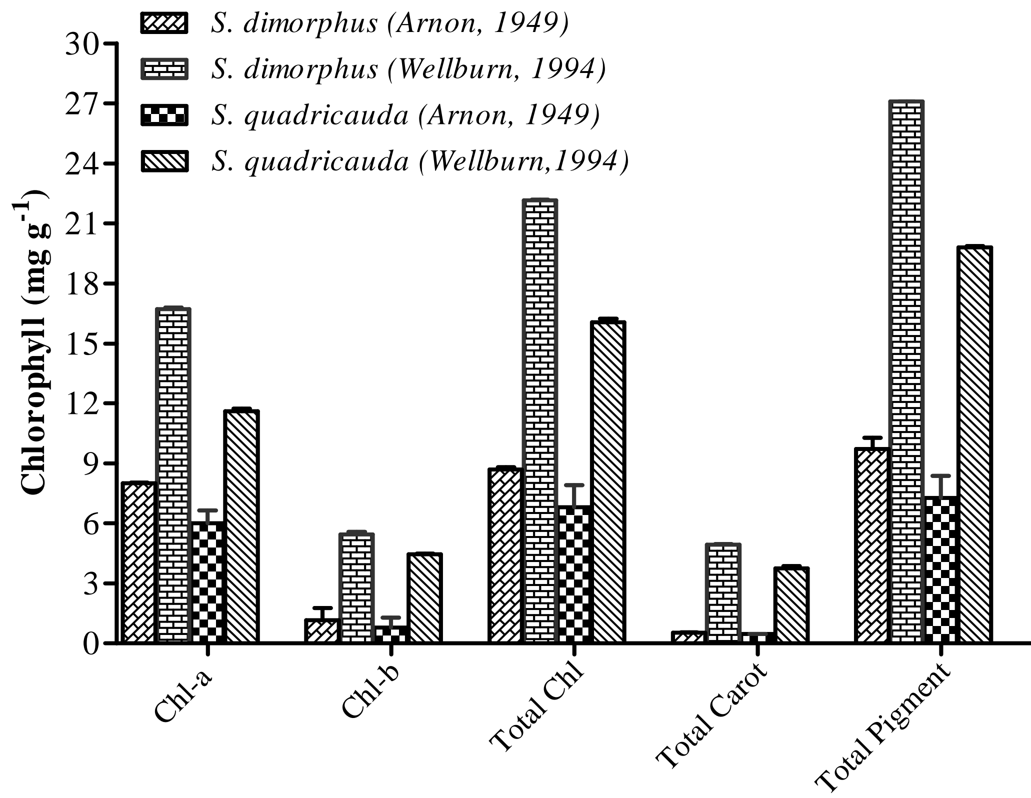



**Fig. 9 : Chlorophyll estimation in two microalgal species by using two different set of equations: Arnon, 1949 and Wellburn, 1994 equations**

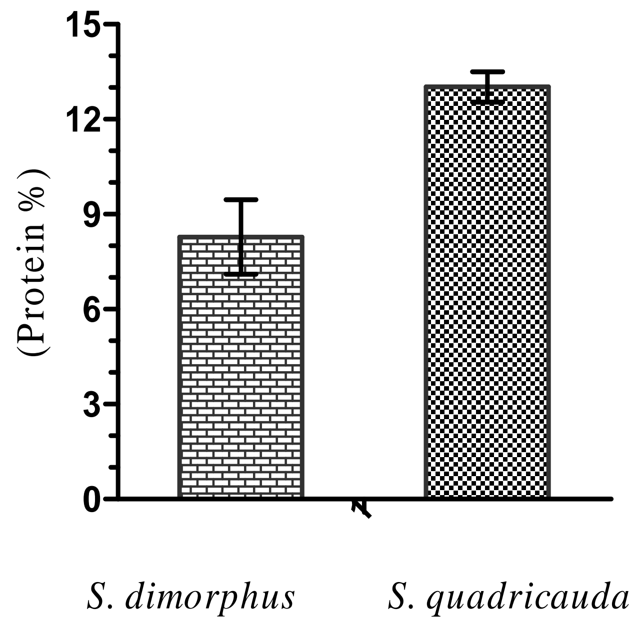



**Fig. 10 : Total protein content of two microalgal species**

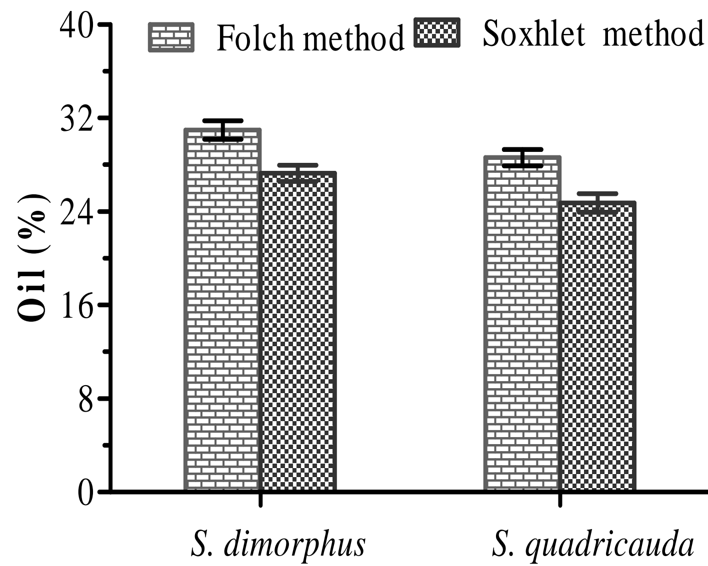



**Fig. 11 : Comparison of lipid content in two different methods using same solvent**

## 4.7 Comparison of lipid content in two different methods using same solvent

The lipid content of two promising algal species was estimated by two different methods (Folch, 1957 and Soxhlet, 1879) using same solvent Chloroform: Methanol (2:1) and the comparison mean values of oil content are presented in the Table 15. In Folch method 30.99 per cent oil content was reported from *S. dimorphus* from 51.3 mg of algal biomass while as 28.61 per cent was reported from 30.4 mg of algal biomass from the *S. quadricauda.* In Soxhlet extraction 27.29 per cent oil was reported in *S. dimorphus* from 7889.3 mg algal biomass while as 24.75 per cent oil was reported from 8856.7 mg of algal biomass in *S. quadricauda.* In both the species the maximum oil content was reported by Folch extraction method while as minimum oil content was extracted by Soxhlet method (Fig. 11).

## 4.8 Fatty acid estimation using GC and GC-MS

Fatty acid (FA) profile of two microalgal strains *S. dimorphus* and *S. quadricauda* was determined and the results are presented in the Table 16. Fatty acid composition was calculated as percentage of the total fatty acids present in these two microalgae determined from the peak areas. In the two tested microalgae the dominant fatty acids in both the species were oleic acid (18:1), palmitic acid (16:0), linoleic acid (18:2) and linolenic acid (18:3) and cis-10-heptadeceoic acid (17:1). The total amount of fatty acid methyl ester of the *S. dimorphus* was 86.2 per cent and that of *S. quadricauda* was 85.7 per cent with 13.8 per cent and 14.3 per cent hydrocarbons and unidentified respectively. Myristoleic acid and pentadecanoic acid were absent in *S. quadricauda* while as arachidic acid, gadoleic acid, eicosapentaenoic acid and heneicosanoic acid were found to be absent in both the species. The most dominant fatty acid in *S. dimorphus* was oleic acid (21.1%) followed by palmitic acid (18.9%), linoleic acid (13.1%), linolenic acid (8.9%), cis-10-heptadeceoic acid (7.1%), margaric acid (6.3%) and the fatty acids present in least concentrations were behenic acid (0.1%) and myristic acid (0.4%). Also similar trend was seen in *S. quadricauda* with most dominant fatty acid as oleic acid (26.2%) followed by palmitic acid

**Table-15 : Comparison of lipid content in two different methods using same solvent**

| Microalgae | Folch method | | | | Soxhlet method | | | |
|---|---|---|---|---|---|---|---|---|
| | Amount of culture (ml) | Dried algal biomass (mg) | Algal oil (mg) | Oil (%) | Amount of culture (ml) | Dried algal biomass (mg) | Algal oil (mg) | Oil (%) |
| *S. dimorphus* | 80 | 51.3 | 15.9 | 30.99 | 150000 | 7889.3 | 2152.9 | 27.29 |
| *S. quadricauda* | 80 | 30.4 | 8.7 | 28.61 | 150000 | 8856.7 | 2191.9 | 24.75 |

**Table-16 : Fatty acid profiles (%) of two microalgal strains**

| S. No | Fatty acid | Carbon Skeleton | *Scenedesmus dimorphus* | *Scenedesmus quadricauda* |
|---|---|---|---|---|
| 1 | Myristic acid | 14:0 | 0.4 | 0.5 |
| 2 | Myristoleic acid | 14:1 | 1.6 | - |
| 3 | Pentadecanoic acid | 15:0 | 0.9 | - |
| 4 | Palmitic acid | 16:0 | 18.9 | 17.8 |
| 5 | Palmitoleic acid | 16:1 | 2.4 | 1.5 |
| 6 | Hexadienoic acid/ Hexadecadienoic | 16:2 | 2.8 | 4.5 |
| 7 | Hexadecatrienoic | 16:3 | 2.2 | 3.6 |
| 8 | Heptadecanoic acid/ Margaric acid | 17:0 | 6.3 | 3.9 |
| 9 | Cis-10-Heptadeceoic acid | 17:1 | 7.1 | 7.1 |
| 10 | Stearic acid | 18:0 | 0.4 | 0.5 |
| 11 | Oleic acid | 18:1 | 21.1 | 26.2 |
| 12 | Linoleic acid | 18:2 | 13.1 | 13.8 |
| 13 | Linolenic acid | 18:3 | 8.9 | 6.0 |
| 14 | Arachidic acid | 20:0 | - | - |
| 15 | Gadoleic acid | 20:1 | - | - |
| 16 | Eicosapentaenoic acid | 20:5 | - | - |
| 17 | Heneicosanoic acid | 21:0 | - | - |
| 18 | Behenic acid | 22:0 | 0.1 | 0.3 |
| 19 | Lignoceric acid | 24:0 | - | - |
| 20 | *HC and UI | | 13.8 | 14.3 |

*HC and UI-Hydrocarbons and Un-identified, (-) Not detected

(17.8%), linoleic acid (13.8%), linolenic acid (6.0%), cis-10-heptadeceoic acid (7.1%) and palmitoleic acid (4.5%). The fatty acids present in least concentrations were behenic acid (0.3%) followed by myristic acid (0.5%) as shown in Fig. 12.

### 4.9 Quality parameters of microalgal oil for biodiesel production

The quality parameters of microalgal oil obtained from both the species for biodiesel production are presented in the Table 17. The degree of unsaturation (DU), cetane number (CN), iodine value (IV) and saponification value (SV) for *S. dimorphus* were 86.2, 50.46, 116.74 and 179.40 respectively. The DU, CN, IV and SV for *S. quadricauda* were 90.6, 51.04, 115.71 and 177.40 respectively. The values obtained for the concentrations of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) are also presented in the Table 17. In case of *S. dimorphus* the total amount of SFAs, MUFAs and PUFAs were 27, 32.2 and 27 per cent respectively. While as in *S. quadricauda* the total amount of SFAs, MUFAs and PUFAs were 23, 34.8 and 27.9 per cent respectively.

### 4.10 Characteristics of microalgal oil obtained from two tested microalgae

The micro algal oil obtained from *S. dimorphus* and *S. quadricauda* by Soxhlet extraction method was subjected to physico-chemical analysis and results are presented in Table 18.

The colour (physical appearance) of oil obtained from both the microalgal species was dark dull yellow green from plate XXXII when compared with the colour standards and nomenclature of Ridgway's (1912).

The dynamic viscosity of oil obtained from *S. dimorphus* and of *S. quadricauda* was found to be 0.539 Pa s and 0.673 Pa s as shown in the Table 13.

The density of oil extracted from *S. dimorphus* was 0.980 g $cc^{-1}$ and that of *S. quadricauda* was 1.014 g $cc^{-1}$.

The visual inspection test is a visual comparison method used to determine the odour of microalgal oil and its solubility in water. Both the microalgal species were insoluable in water and shows oily and fishy odour.

**Table-17 : Quality parameters of oil from two microalgae for biodiesel production**

| Microalgae | DU (%) | CN[1] | IV[2] | SV | SFAs | MUFAs | PUFAs | References |
|---|---|---|---|---|---|---|---|---|
| *S. dimorphus* | 86.2 | 50.457 | 116.745 | 179.392 | 27 | 32.2 | 27 | Present study |
| *S. quadricauda* | 90.6 | 51.039 | 115.714 | 177.352 | 23 | 34.8 | 27.9 | Present study |
| *Chlorella* species | 74.1 | 56.7 | 65 | 217.8 | - | - | - | (Francisco *et al.,* 2010) |
| Peanut | 113.1 | 53 | 97 | - | - | - | - | (Ramos *et al.,* 2009) |

DU, degree of unsaturation; CN, cetane number; IV, iodine value; SV, saponification value (mg KOH $g^{-1}$) .
[1]Minimum limit CN of European standards (EN 14214)-47
[2]Maximum limit of IV of European standards (EN 14214) – 120 g $I_2$ 100 $g^{-1}$ .

**Table-18 : Characteristics of microalgal oil obtained from two tested microalgae**

| Parameters | Values | |
|---|---|---|
| | ***S. dimorphus*** | ***S. quadricauda*** |
| Density (g $cc^{-1}$) | 0.980 | 1.014 |
| Viscosity (Pa s) | 0.539 | 0.673 |
| Physical appearance | Dark dull yellow-green | Dark dull yellow-green |
| Solubility in water | Insoluble | Insoluble |
| Odour | Oily & fishy | Oily & fishy |

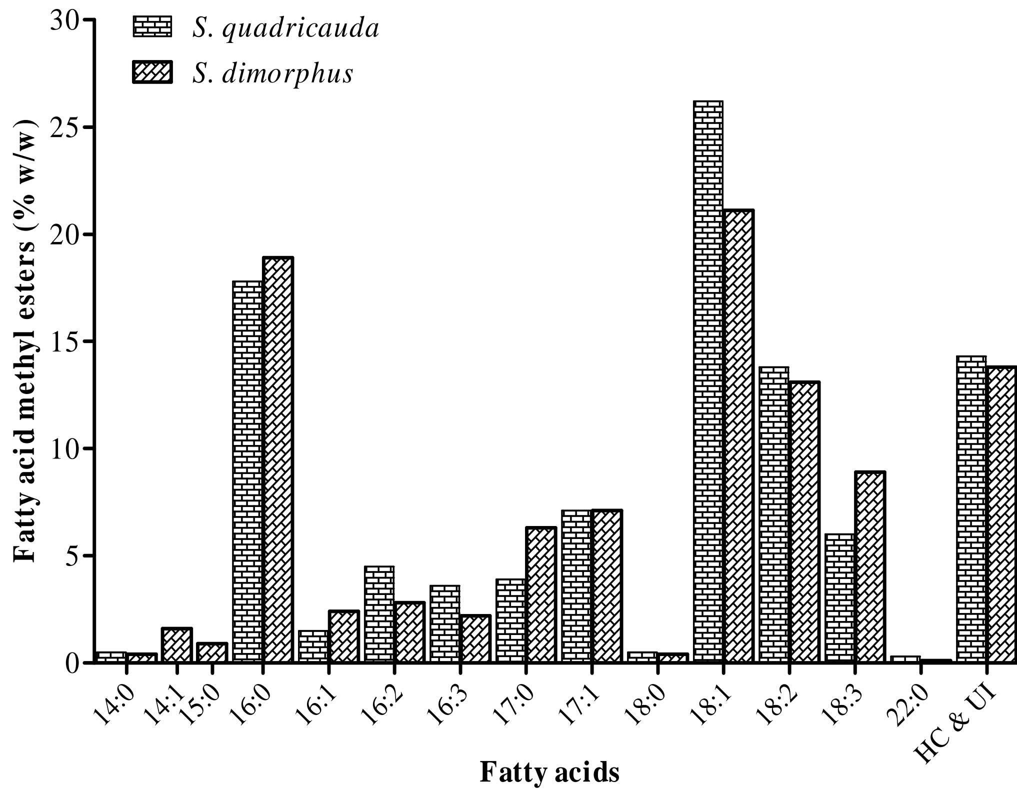



**Fig. 12 :** **Fatty acid profile of *Scenedesmus dimorphus* and *Scenedesmus quadricauda***

Chapter – 5

# DISCUSSION

## 5.1 Physico-chemical characteristics of Dal Lake water samples

### 5.1.1 Temperature

The surface water temperature of all the study sites revealed that the seasonal temperature significantly varied with maximum temperature being exhibited during summer season (24.28-25.60 $^{o}$C) followed by autumn season (20.90-21.55 $^{o}$C) and minimum temperature being exhibited during winter season (12.53-14.10 $^{o}$C) followed by spring season (16.18-16.80 $^{o}$C). The temperature recorded during the summer and autumn season was optimum for the normal growth and survival of aquatic organisms as phytoplanktons recorded during our study also showed peak growth and abundance in these two seasons. Pawar (2010) reported that water temperature influences the chemical and biological activity besides growth of aquatic organisms. However temperatures of winter and spring season were not found suitable for most of the phytoplanktons except diatoms as shown in diversity and distribution Tables 3 to 6. Boyd (1979) found that temperature plays a pivotal role for the growth and survival of aquatic organisms. Kanue *et al.* (2013) also reported that water temperature is an important factor that controls the energy relationship at different trophic levels.

### 5.1.2 pH

Natural water is usually alkaline due to the presence of high concentration of carbonates. Considerable fluctuations in pH can be observed in natural water during day, season to season and within years because of exposure to the air and other related biological activities (Mushtaq *et al.,* 2013). The data regarding the pH of the water at different sites of the lake ecosystem exhibited similar fluctuations in the pH during the different seasons of the year. The lake is characterized by highly alkaline pH during spring (7.10-8.88) as well as in the summer season (7.69-9.47). Although, during the winter season there are not

much evident changes in the pH (7.38-7.97), but in other seasons, DLS-II shows highest values of pH compared to other sites. In the present study it was reported that during summer and autumn months blue green algae *Microcystis aeruginosa* was found abundantly forming blooms at this site. The blooms absorb more carbon dioxide which in turn shifts pH to alkaline side because the pH of natural waters is highly influenced by carbonate system and carbon dioxide is one of the components of carbonate system. This alkaline range of the pH might also be attributed to the geological features of the catchment, comprising of a karewa bed, composed of calcium carbonate rock and some other salts in high proportions (Khan *et al.,* 2012).

Such an alkaline pH may also be due to the continuous dilution of the lake waters with the main Telbal stream and other sister streams which in fact are also the sources of water in Dal Lake. This is in conformity with the conclusions also drawn by Harrel (1973). The continuous inflow of waters into the different basins of the Dal Lake might increases pH to alkaline side. Over all, the pH recorded being in alkaline range indicates that the lake is well buffered throughout the study period and indicates productive nature of water body as also reported by Garg *et al.* (2010).

### 5.1.3 Chemistry of Ions

Conductivity is a measure of capacity of substance or solution to conduct electric current. Electric conductivity (EC) depends upon the quantity of dissolved salts present in water and also nutrient loading of the lakes (Gupta *et al.,* 2008). Conductivity is also closely related to lake type, being low in mountain lakes and high in valley lakes, especially in non-drainage types (Kaul, 1977).

The relatively higher electric conductivity, total dissolved solids, total hardness and total alkalinity values recorded from sites DLS-V and DLS-VI during all the seasons in the present study is attributed to the presence of nutrient load released into the Dal Lake due to various high degree of anthropogenic

activities in the catchment area such as domestic waste water, waste disposal, agricultural runoff and geological nature of drainage basin particularly at sites DLS-V (Nagin) and DLS-VI (Ranawari). Shastree *et al*. (1991) and Kanue *et al.* (2013) also reported that high level of conductivity reflects the pollution status as well as trophic levels of the lake and rivers. The results also show that the lake shows medium level of electric conductivity, total dissolved solids, total hardness and total alkalinity during spring as well as summer season. These medium values are attributed to moderate levels of various anions and cations and might be due to the self purification phenomenon of the lakes by the inflow of the waters from different streams and precipitation during these two seasons.

In most natural waters bicarbonates and some times carbonates are present in appreciable amounts. Their salts get hydrolysed in solution and produce hydroxyl ions consequently raising the pH. Reduction in the bicarbonate content of the alkalinity at the time of algal blooms (summer season) is attributed to the uptake of carbon by the bottom vegetation and the phytoplanktons which also leads to change in the calcium content of waters (Ahl, 1996). The alkalinity values led to the lake waters being included within the “hard water type” of Moyle’s (1945) lake typology.

Total hardness in water is the sum of the concentrations of alkaline earth metal cations (e.g., $Ca^{++}$, $Mg^{++}$). In most freshwaters nearly all the hardness is imparted by the calcium and magnesium ions which are in combination with bicarbonates and carbonates (temporary hardness) apart from sulphates, chlorides and nitrates. When free carbon dioxide and bicarbonates are removed from water by photosynthesis the carbonates get precipitated as marl. The determination of this parameter helps us in deciding the use pattern of water. Both calcium and magnesium are important for growth of plants thus their concentration is lower at the time of peak development in algae (Mir and Kachroo, 1982). Calcium in the form of water soluble calcium formate is utilized in the formation of sheaths of blue green algae and magnesium is responsible for the healthy pigmentation

(Fogg, 1953; Munawar, 1974). Calcium and magnesium concentration of freshwater bodies of Kashmir valley has been associated with the thick population of plankton, especially Cyanophyceae (Bhat and Pandit, 2003) and it is attributed to the predominance of lime rich rocks in the catchment area (Khan *et al.,* 2004). In our study luxuriant growth of algae was also found during summers resulting in lower concentrations of total hardness. The study also revealed that not only carbonates and bicarbonates of Ca and Mg but also their sulphates and chlorides contributed to the hardness of water in the present lake.

In general, higher ionic concentration in winter and lower in summers in the lake water is also largely governed by the dilution effect of snow melt waters during summers (Jeelani and Shah, 2006). The lower values in summer with a considerable increase in spring, autumn and winter may also be attributed to the uptake of these ions by plants, as in our study most of these sites were covered by phytoplanktons in summer. Over all, our results are in conformity with the results of Jeelani and Shah (2006) and Mushtaq *et al.* (2013). In our study the seasonal trend of electric conductivity, total alkalinity, total dissolved solids and total hardness values being higher in winter season than in summer. Similar trend was also observed by Yaqoob *et al.* (2008).

### 5.1.4 Key nutrients

Total phosphorus in freshwater comprises of soluble reactive phosphate, polyphosphate, soluble and insoluble organic phosphorus. In recent times, increased use of detergents with long chain polyphosphate compounds and the use of water bodies as receptacles for waste disposal have resulted in excessive phosphorus loading of aquatic systems, causing eutrophication. For the living organisms, phosphorus is ecologically most important element. Orthophosphates which are readily taken up by the phytoplankton often deplete rapidly becoming the first limiting nutrient. In eutrophic lakes high phosphorus content supports an increased level of primary production till nitrogen becomes limiting. This has far reaching ecological implications on the distribution and diversity of

phytoplankton (Adoni *et al.,* 1985). Phosphorus compounds are present in fertilizers and in many detergents. Consequently, they are carried into both ground and surface waters with sewage, industrial wastes and storm run-off. High concentrations of phosphorus compounds may produce a secondary problem in water bodies where algal growth is normally limited by phosphorus and in such situations the presence of additional phosphorus compounds can stimulate algal productivity and enhance eutrophication processes (Bartram and Balance, 1996). Phosphate is present in the water body in organic and inorganic forms. Various human activities including disposal of sewage, household and industrial waste generally enhances the content of phosphate in water body.

The data on the concentration of phosphates recorded at various sites in different seasons show that its levels were higher in summer and autumn season compared to other seasons with Ranawari site (DLS-VI) exhibiting highest value of 1.80 and 1.45 ppm and Char Chinar site (DLS-III) with lowest value of 0.47 and 0.38 ppm respectively in both the seasons.

Nitrate is the most oxidized form of nitrogen and is an important plant nutrient. Due to its higher mobility as compared to other vital nutrients, its concentration in freshwaters apart from autochthonous production is largely regulated by wastewater loading, agricultural runoff and ground water inputs. In a system approaching higher trophic levels the organic materials or metabolic wastes descend to deeper waters, where nitrogen which does not get lost to the sediments is remineralized to nitrates via bacterial oxidative processes by nitrifying bacteria (Adoni *et al.,* 1985).

The results on the concentration of nitrates recorded at various sites in different seasons show that maximum contents were observed in summer season with Ranawari site (DLS-VI) exhibiting highest value of 3.95 ppm. Similarly the lowest concentration of nitrates in the same season was reported in Char Chinar site (DLS-III) showing a value of 1.13 ppm. The water samples collected in the

autumn season also exhibited increased levels of nitrates ranging from 0.86 ppm at DLS-III to 2.75 ppm at DLS-VI.

The elevated concentrations of nutrients during summer, autumn and especially at Ranawari site (DLS-VI) may be attributed to the application of fertilizers in vegetable gardens, paddy fields located within and around the catchment area of lake. The use of detergents, soaps and sewage etc emanating from the houseboats, hotels, restaurants and stake holders living within and along its catchment area has resulted in appreciable amounts of nitrate and phosphate within the lake. The present observation is in complete conformity with the findings of Mushtaq *et al.* (2013). Zuber (2007) also found that phosphate build up in the Mansar Lake is due to inflow of fertilizers from the agricultural fields in the catchment area of the lake. Yaqoob *et al.* (2008) also found that in the valley lakes the problem of pollution is mainly due to addition of major plant nutrients derived from human wastes and agricultural activities etc. Vyas *et al.* (2006) and Pandit and Yousf (2002) also found that nitrate and phosphate enters the lakes through domestic wastewater, accounting for the accelerated eutrophication and the augmented concentration of phosphate and nitrate in lakes also resulted in enhanced phytoplankton productivity. In our study during the summer and autumn seasons green and blue green algae were also found in appreciable amounts as in these two seasons at all the sites nutrients were found in higher amounts. Sharma (2004) also reported that each 400 g of phosphate encourages about 350 tones of algal slime. The increase of phosphate in summers is also associated with the increase in temperature, the rising temperature increases the regeneration of phosphorus from the sediment and littoral zone. The reduction in winter and spring may be due to slower regeneration as stated by Mir and Kachroo (1982).

Silicate is the soluble reactive form of silica, which is an important structural constituent of the body of diatoms. It is often found dissolved in natural waters in considerable quantities and shows marked seasonal variations (Adoni *et al.,* 1985). Silicon is one of the most abundant element in the earth's crust and

most important element which aid the development of diatom shells. Silicon in the lake is derived from rock weathering (lithogenic) and diatoms (biogenic) (Jeelani and Shah, 2006). During the investigations it was found that silica is the most abundant ion in the lake. Seasonally highest values of silicates were recorded in summers and lowest in winters and overall Ranawari site (DLS-VI) shows elevated concentrations of silicates in all the four seasons. The highest values recorded may be due to the decomposition of aluminium silicate minerals in rocks as also reported by Mushtaq *et al.* (2013). The reduction in the silica content depends on uptake of silica by the diatoms (Mir and Kachroo, 1982) as diatoms in present study were found abundant at DLS-VI during winters especially *Gamphomena, Navicula* and *Cymbella* species. The data also shows that consumption of silicates by diatoms was maximum in winter as compared to summers. These results were similar to Sarwar (1986) who found that the silica content in the lake water gets depleted as it is taken up by the abundance of diatoms. However, Mir and Kachroo (1982) reported negligible consumption of silica by nanoplanktons in the same season.

On the basis of the physico-chemical characteristics observed in the lake it may be concluded that the environmental condition of the Himalayan Dal Lake ecosystem is deteriorating. The evidence shows that the lake water is basic in characteristics especially at DLS-VI, DLS-V and DLS-IV. The key nutrients that contribute to cultural eutrophication of water bodies such as phosphate and nitrate were high in concentrations. Due to anthropogenic activities within the lake and catchment area appreciable quantities of wastes from human settlements, houseboats, hotels, agricultural fields and orchards are being directly dumped into the lake and these events are directly responsible for the ecological disturbances of the lake ecosystem.

## 5.2 Algological studies

Algal taxonomy with diverse families is of great diagnostic importance in floristic analysis as well as basic and applied aspects of research (Medvedeva,

2001). Besides searching for some potential oleaferous algal flora of Kashmir Himalaya an attempt has also been made to develop a data base of algal flora of Kashmir Himalaya with the help of modern tools and techniques. As a part of the data base information the present investigation has been carried out from the six different sites of Dal lake ecosystem covering all the lake basins, which will help in preparing algal distributional map and identify the site specific algal species. Based on its geographical position Dal lake ecosystem also enjoys the richest and the most diverse biodiversity among the other fresh water lakes of Kashmir Himalayas and also in the Indian subcontinent. Fresh water algal floristic exploration of the lake revealed very interesting and exciting results. In the present investigation morphotaxonomic characters, diversity and distribution of fresh water algae along with the physicochemical characteristics of each site has been analysed. The study shows that a total of 91 algal genera, 217 species, 41 varieties and 8 forma have been recorded from the six sites of Dal Lake ecosystem (Table 7).

From the results it is clear that out of 217 species the maximum number of species (149) belongs to Chlorophyta with 30 varieties and 8 forma. The Chlorophycean algae of Dal Lake is best represented in summer and autumn months and lowest in the winter followed by spring season (Table 8). The dominance of Chlorophycean algae during the summer and autumn seasons could be attributed to increasing and favourable temperature besides enhancement in phosphorus and nitrate concentrations. Kant and Kachroo (1977) and Ganai *et al.* (2010) also reported a Chlorophycean peak during summer months in Kashmir Himalayan lakes. *Oedeogonium*, *Spirogyra* and *Rhizoclonium* were found in fresh waters throughout the year almost at all the sites. As summer approaches these vegetative forms reaches to the reproductive stages. Similar reproductive stages during summer months in these algae were found in the research of Dwivedi (2001). Among the Chlorophyceae, the maximum species recorded are 30 species of *Cosmarium,* 18 species of *Staurastrum,* 13 species of *Scenedesmus*, where as

the minimum and rarely recorded are *Palmellococcus saccharophilus, Oedeogonium undulatum* f. *senegalense* and *Sphaerozosma filiforme.*

Diatoms occupy a wide range of ecological niches (Brook, 1959, 1965; Moss, 1972) which probably account for their occurrence in all the studied sites. In the class Bacillariophyceae, 30 species and 5 varieties were recorded showing their peaks of standing crop during winter months. The most dominant species recorded were *Synedra, Cymbella and Gamphonema.* The diatoms showed their peaks by remaining dominant in the lake during winter season (Table 11), which could be attributed to the fact that they are able to grow under the condition of weak light and low temperatures which are less suitable for other algae. These findings are in conformity with the findings of Munawar (1974) and Ganai *et al.* (2010). In the present study the reduction in the silicates was also found during winter months which also support our results. As Mir and Kachroo (1982) also found that the reduction in the silica content depends on uptake of silica by the diatoms as these organisms were found in abundance during winters as compared to other seasons. The observations made by Nautiyal *et al.* (1996), Pareek *et al.* (2011) and Bhatnagar and Bhardwaj (2013) also showed seasonal distribution of members of Bacillariophyceae following winter>summer>monsoon trend. These results are thus in conformity with our findings regarding diatoms. Kant and Kachroo (1973) also reported peak abundance of Bacillariophyceae during winter in the Dal and Nagin Lakes.

The data also reveals that Cyanophyceae is the third largest class with 28 species and 2 varieties (Table 9). The abundance of cyanophyceae during the summer and autumn months indicates the eutrophic nature of water body (Lin, 1972). The cyanophyceae showed their peaks during summer and autumn seasons with the maximum standing crop especially at site DLS-VI. The possible reasons behind this result may be the favourable temperature, alkaline pH, low water volume and availability of nutrients which created favourable condition for better propagation of this group of phytoplankton. The dominant species of

cyanophyceae was *Oscillatoria*. Ganai and Parveen (2013) also found *Osillatoria* spp., the most abundant amongst cyanophyceae in Wular Lake in their study during March, 2007 to February, 2008. The Presence of some pollution tolerant blue green algae like *Microcystis aeruginosa* and *Oscillatoria* spp. can be used as indicator species for polluted habitats, as these species were recorded from those sites having high concentration of pollutants. These results are in accordance with the findings of Bhatnagar and Bhardwaj (2013).

Microalgae *Microcystis aeruginosa* was found abundantly at site DLS-II during the summer and autumn months indicating alarming toxic nature of water as this alga contains neuro and hepato toxins. The toxic nature of *Microcystis aeruginosa* was reported by Bishop *et al.* (1959), Al-Jassabi and Khalil (2006) and Ahmed (2009). Protenious algae *Spirulina* was common at sites of DLS-IV, DLS-VI which indicates that the algae is able to tolerate the high level of pollutants as these sites contain appreciable quantities of nutrient load (Tables 3, 4, 5 and 6). The high tolerance limit of *Spirulina platensis* in various toxicants and pollutants was also reported by Lone *et al.* (2013) and is in conformity with our results.

Euglenoid algae form a relatively large and diverse group but few species are truly planktonic (Wetzel, 1983). Among the Euglenophyta 4 genera, 9 species with 4 varieties were found in appreciable numbers at site DLS-VI during the autumn season (Table 10). Increasing temperature and accumulation of organic loads from catchment area, autothonous and allocthonus organic load, sewage, clear sun-shine, temperature may be the possible reasons for the dominance of euglenophyceae in autumn months (Munawar, 1972).

The study also shows that class Rhodophyceae was monotypic in its representation of having only one taxon namely *Glaucosphaera vacuolata* reported from Hazratbal site (DLS-IV). This is a new record reported in India and Kashmir Himalayas to the phycological studies (Table 11).

Inspite of the heavy load of pollutants from various activites (as discussed in water chemistry) the dominance of green algae is not getting diminished, although almost all the blue green algae showed their dominance at those sites receiving heavy load of pollutants. The water of this lentic fresh water body is a prime example of a natural ecosystem severely affected by pollution. These algal assemblages can be regarded as 'models' that will be of great value in providing baseline data for future monitoring and for assessing the effects of anthropogenic pollution.

### 5.3 Lipid studies of different microalgae (qualitative and quantitative)

The Nile red technique has also been previously successfully applied to a number of microalgae for the rapid determination of lipids (Cooksey *et al.,* 1987; Lee *et al.,* 1998; and Elsey *et al.,* 2007). In our study the lipid droplets of microalgae emitted yellow-gold fluorescence in the cells under fluorescent microscope and these lipid globules are the signs of adequate lipids present in the studied algae (Plate 45). Our current findings are well supported by the previous research carried out by Cooksey *et al.* (1987) and Lee *et al.* (1998) who reported the Nile red technique as useful tool for the rapid determination of lipids in algae.

The maximum lipid content was reported in *S. dimorphus* (30.99%) followed by *S. quadricauda* (28.61%) and these microalgae were isolated from the fresh water samples of sites DLS-III and DLS-V. Our results are in conformity with the results of Becker (1994) and Gouveia and Oliveira (2009) who found that *Scenedesmus dimorphus* possess 16-40 per cent of the lipid content. Also in case of *Scenedesmus quadricauda,* Mohapatra (2006) and Goswami (2011) reported 19.9 and 31 per cent lipid content. The seasonal water chemistry of these two sites also reveals that these two sites are less polluted as compared to others and the high lipid content might be due to non-toxic nature of the fresh waters and less nitrate and phosphate present in these sites. Increase in lipid content during nutrient limitations can be explained by the fact that many microalgae under stress conditions alter their lipid synthesis pathway and accumulates large amount of

lipids to sustain adverse conditions (Sharma *et al.,* 2012). Another possible explanation for this could be that, nitrogen is the essential component of protein synthesis. During nitrogen starvation, the overall growth and cell division of the cell impairs.

The data also shows that the minimum lipid content was reported in *Aphanothece microscopica*. (4.63%), *S. armatus var bicaudatus* (7.87%), *S. armatus* var. *major* (10.87%). All these microalgal species were found comparatively in the more polluted sites of our research work. The low lipid content might be due to the toxicity of the pollutants and appreciable amounts of nitrate and phosphate in these sites which might decrease their lipid content. Previous studies have also demonstrated that lipid content in some microalgae increases during different cultivation conditions such as nitrogen deprivation (Hsieh and Wu, 2009), and salt concentration (Araujo *et al.,* 2011). The lipid content of *Chlorella* spp. typically ranges between 28 and 32 per cent on dry weight basis (Chisti, 2007). However, our isolated strain yielded up to 19.76 per cent. Chisti (2007) also reported that oil content of microalgae is usually between 20 and 80 per cent. In our study, many microalgal strains exhibited high lipid content, therefore it may be possible to increase their lipid content by optimizing growth determining factors such as the control of nitrogen level, light intensity, temperature, salinity, $CO_2$ concentration and harvesting procedure.

### 5.4 Growth measurement of microalgae by Spectrophotometric method

In the present research work the two green microalge *Scenedesmus dimorphus* and *Scenedesmus quadricauda* were chosen for the study because, it was found that both the species accumulate more lipids as compared to other microalgal species. In spite of huge differences in the climatic conditions of the places where collection has been done and the place where all the experimental research work was carried out, both the microalgae showed luxurious growth in the BBM media, which reveals its flexible nature to adapt the wide range of the environmental conditions.

As shown in the Figs. 6 and 7 maximum absorbance was inspected by scanning the culture samples and the highest absorbance value was then used to calibrate the curve of algal density. Standard routines to estimate algal concentration include direct cell counts, chlorophyll content measurement and absorbance (EPA, 1994). When spectrophotometrical absorbance is the chosen method, a reading wavelength of 750 nm is usually recommended (EPA, 1994; Eaton *et al;* 1995) although values of 680 nm (Geis *et al.,* 2000) and 687 nm (Valer and Glock, 1998) have also been used. These values are correlated to the light absorbance of chlorophyll. Fig. 1 and 2 presents the pattern of light absorbance for *S. quadricauda* and *S. dimorphus* screened between 400 and 1100 nm. In both the microalgal species peaks could be observed with the highest absorbance obtained at 680 nm, representing the wavelength of maximum sensitivity to quantify these *Scenedesmus* samples. Therefore, growth of both the species was read in this wavelength.

Under suitable conditions and sufficient nutrients, microalgae can grow profusely (Chisti, 2007). Fig. 8 shows the growth curve among the examined microalgal species grown autotrophically during 17 days of batch cultures using BBM media, indicating the enhanced growth rate corresponding with incubation time. The growth curve pattern of both the microalgae showed similar patterns depicting log, lag and stationary phase. Growth rate is one important way of expressing the relative ecological success of species in adapting to its natural environment or the experimental environment imposed upon it. The growth curve of *S. quadricauda* and *S. dimorphus* was determined from parallel cultures starting from inoculums. Microalgal growth is directly affected by the availability of nutrients, temperature, light and pH (Xun *et al*., 2012). The present study revealed that cell growth started from the 1$^{st}$ day itself and reached its maximum at 14$^{th}$ day of the culture. The growth rate of *S. quadricauda* was fastest and higher as compared to *S. dimorphus.* Similar results were also observed in the culture of *Nannochloropsis salina* and *Chlorella marina by* Muthukumar *et al.*

(2012). Our results indicate that both the microalgal species are suitable for high-density culture.

### 5.5 Pigment estimation in two promising microalgae by using different equations

The result of the chlorophyll content in *S. quadricauda* and *S. dimorphus* are presented in the Table 13. In the present research work DMSO was used as a solvent as compared to acetone because DMSO is superior to acetone for the extraction of chlorophyll from green algae. Shoaf and Lium (1976) reported that DMSO is superior to acetone giving 2-60 times more chlorophyll depending on the algal species. Also, as no maceration of algal tissue is required, chances of losing chlorophyll due to filtration step (acetone method) are totally eliminated. Moreover acetone does not extract all the major pigments completely (Lichtenthaler, 1987)

The extraction of chlorophyll depends upon many factors like solvent type (Porra *et al.,* 1989), solvent impurity (Garcia and Nicolas, 1998b), tissue type and degree of maceration (Shinano *et al.,* 1996) and the equations used to calculate Chl- concentration (Wellburn, 1994; Porra, 2002).The equations used to calculate Chl- concentration might also influence the results because the absorption spectra of Chl-a and Chl-b are different in different types of equations. Wellburn (1994) has presented accurate extinction coefficients and relevant simultaneous equations for use with various solvents including DMSO (Porra, 2002). However, in the present study two different sets of equations provided by Arnon (1949) and Wellburn (1994) using DMSO as a solvent were used for calculations. The data revealed that the maximum photosynthetic pigments were estimated with Wellburn equations and minimum pigments were calculated with Arnon's formulas. Our results are in conformity with the results of Wellburn (1994) and Tait and Hik (2003). The much-quoted equations of Arnon (1949) to determine individual levels of Chl-a and Chl-b in 80 per cent acetone in water are still used by many researchers despite the fact that they are inaccurate and that particular

solvent mix has many disadvantages (Wellburn, 1994). The Arnon's equations will give inaccurate results for DMSO (Porra, 2002) and several studies have modified Arnon's equations and most importantly Wellburn (1994) provided equations that give accurate results. There are many reasons for this inaccuracy including the poor resolution of the spectrophotometers of the 1940's but the main problem is the solvent itself.

Chlorophyll content decides the photosynthetic rate of a particular strain. Changes in chlorophyll level are probably controlled as algal density and climatic factors (light and temperature). Photosynthetic efficiency of microalgae is directly related to its growth and hence biofuel production. Using the Wellburn equations in our study the Chl-a content in *S. quadricauda* was reported to be 16.711 mg $g^{-1}$, while as in *S. dimorphus* Chl a was found to be 11.609 mg $g^{-1}$. The Chl-a content was highest in both the species, as the photo-systems in tested microalgae contain Chl-a. However Chl-b also augments the overall fluorescent signal and enables green algae to perform the photosynthesis. Our results are well supported by Kong *et al.* (2011) who reported the Chlorophyll content of 27.99 mg $g^{-1}$ in *C. vulgaris* under photoautotrophic nutritional modes. Carotenoids are important components of photosynthetic apparatus of vegetative cells serving as additional pigments (Giovannoni *et al.,* 1988). They protect chlorophyll molecules against photo destruction and oxidation by molecular oxygen (Krinsky, 1979). Appreciable amounts of carotenoids were also present in both the microalgal species and the total carotenoids in *S. quadricauda* and *S. dimorphus* were reported 4.941 and 3.750 mg $g^{-1}$ respectively. The total photosynthetic pigments in *S. quadricauda* and *S. dimorphus* were 27.099 and 19.806 mg $g^{-1}$ respectively. Shaaban *et al.* (2011) also found the total chlorophyll contents of the identified algae during summer 2007 from 9.66 to 23.0 mg $L^{-1}$ respectively comparing with the total chlorophyll contents in other seasons using DMSO solvent. The Chl-values of Shaaban *et al.* (2011) support our pigment values. In our study both the microalgal species possess appreciable amounts of photosynthetic pigments which

is due to the fact and obvious due to its enormous growth as shown in its growth curve (Fig. 8).

### 5.6 Estimation of total protein content of two microalgal species

The Lowry method is one of the most accurate methods for quantifying proteins (Peterson, 1979). Intracellular protein can be determined accurately only if all of it is accessible to the reagents used in the Lowry method. The cells must therefore be pretreated to release all the cellular protein. Pretreatments typically involve disrupting the cells by physical or chemical means (Barbarino and Lourenco, 2005). Milling a cell slurry in presence of glass beads or other fine ceramic particles is known to be one of the most effective methods of releasing intracellular proteins from even the hardiest of microbial cells (Chisti and Moo-Young, 1986). However lysis buffer and ultrasonication was used in our study to lyse the cell walls and disrupt the microalgal cells to release the proteins. Chisti and Moo-Young (1986) also found that ultrasonication is another effective method of disrupting cells. Lysis buffer was used to facilitate the extraction of proteins by Lopez *et al.* (2010).

For a given algal species, the intracellular concentration of protein depends on the growth phase (Lourenco *et al.,* 1998). In our batch cultures, all the biomass samples came from stationary phase because the growth phase also has an impact on the ease of cell disruption, with rapidly growing cells likely to be less robust than slow growing or stationary-phase cells (Chisti and Moo-Young, 1986). Therefore, lysis buffer and ultrasonication is expected to be critically important for ensuring that all the intracellular protein is released from the latter type of cells.

The total protein content in *S. quadricauda* and *S. dimorphus* was 13.026 and 8.284 per cent respectively. Becker (1994) found that *S. dimorphus* possess 8-18 per cent proteins which support the results. However, in the present study the proteins reported in *S. quadricauda* were less. On the other hand Becker (1994)

reported 47 per cent proteins in the same organism. Cifferri (1983) claimed that the culture conditions such as temperature, light intensity and pH etc were known to change the protein content of the blue green algae *S. platensis* as the same case might be in our organism *S. quadricauda.*

## 5.7 Comparison of lipid content in two different methods using same solvent

The two different methods were employed for the extraction of lipids from the tested microalgae. The lipid extracted by Folch extraction method was comparatively higher than the Soxhlet method as shown in the Fig. 11. From the results it is clear that *S. dimorphus* and *S. quadricauda* yielded 30.99 and 28.61 per cent of lipids respectively by Folch method. However, in Soxhlet method the contents were 27.29 and 24.75 per cent respectively. The effectiveness of Folch extraction method may be due to the fact that it involves completely mechanical cell wall disruption step. Converti *et al.* (2009) applied different method of lipid extraction on microalgae *Nannochloropsis oculata* and *Chlorella vulgaris*. As a result of his study he concluded that, the combination of ultrasound with Folch extraction method was the most effective lipid extraction method. Shen *et al.* (2009) recovered the lipid contents of *S. dimorphus* and *C. protothecoides* through several methods with the solvent system of ethanol/hexane (1:1, V/V). By applying sonication method 21.0 per cent and by direct soxhlet extraction 6.3 per cent lipid content was recovered from *S. dimorphus*. However in case of *C. protothecoides* 10.7 per cent of lipid content was achieved by using sonication and 5.6 per cent by direct Soxhlet extraction.

In the present study ultrasonication was also used for cell wall disruption. As per our findings also during the experimental work it was found that Soxhlet extraction method was time consuming, labour intensive, requires more energy and large amount of biomass which was also studied by Bligh and Dyer (1959) and Elsey *et al.* (2007).

## 5.8 Fatty acid estimation using gas chromatography (GC) and gas chromatography - mass spectrometry (GC-MS)

The fatty acid profile of *S. dimorphus* and *S. quadricauda* grown under large scale cultivation using indigenously made photobioreactor is shown in Fig. 12. In the aspect of biodiesel, the fatty acid (FA) profile is considered to be the most important as that of the total fatty acid content. The FA profile of both the microalgae reveals the appreciable amounts of FA with carbon chain length of C16 and C18. In the two tested microalgae, oleic acid (18:1), palmitic acid (16:0), linoleic acid (18:2), linolenic acid (18:3), cis-10-heptadeceoic acid (17:1), margaric acid (17:0) were also the most dominant. It was previously reported that oleic acid, palmitic acid, stearic acid and linolenic acid were recognized as the most common fatty acids contained in biodiesel (Knothe, 2008).The properties of biodiesel are highly influenced by the FA profile of the algae. In a previous study carried out by Lee *et al.* (2010) the most commonly synthesized fatty acids have carbon chain lengths that range from C16 to C18, similar to those of higher plants. Moreover palmitic, stearic, oleic and linolenic acids were recognized as the most common fatty acids contained in biodiesel. In the present study these fatty acids were also present in the two tested microalgae. Oils with high oleic acid content have been reported to have a reasonable balance of fuel, including their ignition quality, combustion heat, oxidative stability, viscosity and lubricity, which are determined by the structure of their component fatty esters (Knothe 2008). In our study the two isolates also contain appreciable amounts of oleic acid content making it suitable feedstock for the production of good quality biodiesel. Prabakaran and Ravindran (2012) concluded that among their tested microalgal species, *Scenedesmus* sp. showed the highest oleic acid content. Also Xu *et al.* (2006) studied the fatty acid composition of algal oil from *Chlorella protothecoides* using GC analysis.

According to European standards EN 14214, for an ideal biodiesel the percentage of linolenic acid (C18:3) and polyunsaturated FA (≥4 double bond) should not increase 12 and 1 per cent respectively (Gouveia and Oliveira, 2009;

Pereira *et al.,* 2013). In our study the linolenic acid contributes 8.9 and 6.0 per cent of the FAME for *S. dimorphus and S. quadricauda*, whereas, polyunsaturated FA with ≥4 double bond were completely absent.

However, it should be considered that all these characteristics are primarily dependent on the microalgal strain (Romano *et al.,* 2000) and culture conditions employed. Techniques for the preservation of the biomass are also important (Zepka *et al.,* 2008). Over all the two tested microalgae are the best suitable for the production of good quality biodiesel.

### 5.9 Quality parameters of oil for biodiesel production

Degree of unsaturation (DU) is sum of the masses of monounsaturated and polyunsaturated fatty acid and it is one of the important properties that influence the oxidative stability of biodiesel (Francisco *et al.,* 2010). Presence of large quantities of polyunsaturated (more than one double bond) FAME negatively affects the oxidative stability of the biodiesel due to the fact that they contain reactive sites which are susceptible for free radical attack. FAME profile obtained from both the microalgal species also contains appreciable amount of saturated and monounsaturated FAMEs which shows its suitable nature to be a good biodiesel fuel. The long chain saturated and monounsaturated FAMEs are most suitable for biodiesel as they improve oxidative stability without greatly affecting cold flow properties of biodiesel (Chen *et al.,* 2012; Demirbas, 2009; Wahlen *et al.,* 2013).

Cetane number (CN) is also one of the important fuel properties of biodiesel which is highly influenced by the FA profile (Table 17). High cetane value is the indicator of better combustion, low nitrous oxide (NOx) emission, less occurrence of knocking and easier start-up of engine (Knoth, 2012 and Arias-Penarands *et al.,* 2013). Diesel fuel with large quantities of saturated and monounsaturated FAMEs have high value of CN. The minimum cetane value of ASTM D6751, European (EN 14214) and National Petroleum Agency (ANP 255)

standards are 47, 51 and 45 respectively (Francisco *et al.,* 2010). In the present study, the values of CN calculated for *S. dimorphus* and *S. quadricauda* were in accordance with the standards reported (Table 17).

Iodine value (IV) is used to determine the unsaturation of biodiesel oil, more the double bond in the fatty acid chain, higher the IV for that oil (Knoth, 2012). European biodiesel standard EN 14214 specifies the maximum limit of IV to be 120 g $I_2$ 100 $g^{-1}$, whereas, Germany's standard DIN 51606 and South African standards SANS 1935 for IV are 115 and 140 g $I_2$ 100 $g^{-1}$ respectively. Higher IV of the biodiesel oil may results in the polymerization of glycerides and deposition of lubricant in the engine (Francisco *et al.,* 2010). Study performed by Predojevic *et al.* (2012) on sunflower oil, however suggest that, higher IV does not necessarily indicate the unsuitability of oil for biodiesel. Several promising biodiesel feedstock sources such as soyabean, sunflower seed oil and linseed oil has higher IV (128, 132 and 184.5) values, which do not satisfy the European biodiesel standards (Giakoumis, 2013; Ramos *et al.,* 2009). In the present study as shown in the Table 17, the IV values of *S. dimorphus* and *S. quadricauda* shows that, oil from both the microalgae is much better biodiesel source in comparison to soybean, sunflower seed and linseed oil.

The saponification value (SV) is the measure of milligrams of potassium hydroxide (KOH) required to completely saponify one gram of oil. The SV is inversely proportional to the molecular mass of fatty acids. The main aim of calculating SV is to calculate the CN of the biodiesel. The European and Serbian biodiesel standards have not included the SV as a restricted property of biodiesel oil (Predojevic *et al.,* 2012). In the present study the SV for *S. dimorphus* and *S. quadricauda* were less as compared to the SV of *Chlorella* sp. (217.8 mg KOH $g^{-1}$) calculated by Francisco *et al.* (2010). However, these values were more or less comparable to the results of Stanley *et al.* (2010) who reported SV value of 173.56 in *Chaetoceros* species.

## 5.10 Characteristics of microalgal oil obtained from two tested microalgae

### 5.10.1 Density

Relative density is the density of the component compared to the density of water and is used to calculate the flow and viscosity properties and to judge the homogeneity of oils. The density of both the microalgal oils in our study was higher than 0.900 g $cc^{-1}$ and may not pass EN Standard 14214, which specifies the density at 15°C to be 0.860-0.900 g $cc^{-1}$. Xu *et al.* (2006) reported 0.864 kg $L^{-1}$ density of biodiesel obtained from *Chlorella protothecoides*. The higher densities of our oils indicate that transesterification step is required to minimize the density because of presence of high amounts of hydroxyl esters. Sanford *et al.* (2009) also found that the presence of high amounts of hydroxyl esters may be associated with higher density. Stanley *et al.* (2010) extracted oil from *Chaetoceros species* and found it highly dense with density of 1.305 g $ml^{-1}$. In view of their high values of density parameter they found that microalgal oil obtained from the same algae can be efficiently used for biodiesel production.

### 5.10.2 Viscosity

Viscosity is defined as the resistance to shear or flow and is highly dependent on temperature. It also describes the behavior of a liquid in motion near a solid boundary like the walls of a pipe. The presence of strong or weak interactions at the molecular level can greatly affect the way the molecules of an oil or fat slide pass each other, therefore affecting their resistance to flow (Sanford *et al.,* 2009).

In the present investigation both the microalgal oils exhibited the highest dynamic viscosities. Stanley *et al.* (2010) extracted oil from *Chaetoceros* species and also found it highly viscous. One possible reason for this observation is that these two oils contain high concentrations of hydroxy containing fatty acids that are capable of forming hydrogen bonding (Firestone, 2006). The high viscosity can increase injector fouling, cause carbon deposits on cylinder walls and exhaust

ports and cause fuel system problems such as filter plugging. The higher viscosities of our microalgal oils indicate that transesterification step is required to minimize the viscosity because of presence of high amounts of hydroxyl esters and to make this algal oil suitable for biodiesel production. Kumar *et al.* (2014) also reported the highly viscous oil (0.3 Pa s at shear rate of 500 $s^{-1}$) obtained from the consortia of algae collected from natural water bodies of Himachal Pradesh. Kerschbaum and Rinke (2004) measured dynamic viscosity of rapeseed oil at shear rate of 420.3 $s^{-1}$ from 323.15 K upto 258.15 K and found viscosity continuously increases from 23.2 mPa s up to 489 mPa s which indicates that, at the lower temperatures viscosity of algae oil becomes higher. Similar findings were reported in algal oil, 5-30 times viscous than that of Cashew nut oil, Palm Kernel and Peanut oil (Davis, 2009; Latinwo, 2010).

### 5.10.3 Colour

The Fat Analysis Committee colour method determines the colour of oils and fats by comparing them with colour codes and standards. However in our study we have used colour codes and standards of Ridgway's (1912). The dark dull yellow green colour of algal oil might be due to the presence of abundant dark fats as algal oil obtained from Soxhlet extraction was not subjected to transesterification process. Transesterification process removes the glycerol content from fatty acids of the algal oil which can result in the shifting of colour. Sanford *et al.* (2009) also carried out colour determination in biodiesel obtained from various feedstocks and he also found dark fats in the feedstocks obtained from algae. Our results of colour standards match with that of Sanford *et al.* (2009) as he also found that during the esterification reactions, all fats and oils appeared to shift in colour.

Chapter – 6

# SUMMARY AND CONCLUSION

The present study concludes that the environmental condition of the Himalayan Dal Lake is deteriorating at an accelerated rate. The evidences show that the lentic ecosystem is alkaline in nature and the problem of pollution is mainly due to addition of major nutrients especially nitrate and phosphate derived from human wastes, detergents, fertilizers, agricultural activities etc by direct drainage from nearby residential areas and from inner side human habitation particularly houseboats and floating gardens. These anthropogenic activities seem to be directly responsible for the chemical pollution of the lake as pollutants are being directly dumped into the lake. The nutrients have been chiefly responsible for an increase in organic production and the overall deterioration of water quality. In conclusion, the lake is productive and is supporting diverse and appreciable numbers of phytoplanktons especially Chlorophyta, Cyanophyta, Bacillariophyta and Euglenophyta. The Chlorophycean algae of Dal Lake were best represented in summer and autumn months and lowest in the winter followed by spring season. Bacillariophyceae showed their peaks of standing crop during winter months while as Cyanophyceae showed their peak abundance during summer and autumn seasons and in case of Euglenophyceae autumn season was favourable. A total of 91 algal genera comprising of 217 species, 41 varieties and 8 forma were identified during the study. Among the thirteen isolated microalgae, *Scenedesmus dimorphus* and *Scenedesmus quadricauda* were found to be the most promising microalgae for the production of good quality biodiesel as both the species possess fast growth and contains appreciable amounts of lipids (30.99% and 28.61%) and fatty acids (FAME 86.2% and 85.7%). The growth analysis pattern of these two robust algae in the BBM media show that both the species are fast growing and are also suitable for high-density culture. The high amount of total pigments were calculated when Wellburn equations were applied and Arnon's equations were found to be inefficient when using DMSO as a solvent in both the species of *Scenedesmus*. Folch method

was found to be more efficient as compared to Soxhlet in terms of oil extraction as in the former one there is complete mechanical disruption of *Scenedesmus* cells.The fatty acid methyl ester (FAME) profiles showed that both the species possess appreciable amounts of major FA with carbon chain length of C16 to C18 making them suitable feedstock for the production of good quality biodiesel. The quality parameters of both the microalgal oils like degree of unsaturation, cetane number, iodine value and saponification value were within the limits of international standards respectively. The physico-chemical characteristics of oil obtained from these two tested microalgae viz colour, density and viscosity were too high and did not pass the standards. The highly dense and viscous oils of both the microalgae reveal that transesterification is an important step to minimize these physico-chemical characteristics of the oil and conversion of the algal oil into biodiesel. Overall, our research programme concludes that both the species of *Scenedesmus* are the best potential isolates for producing high quality biodiesel. However, more in-depth studies needs to be carried out to ascertain the more oleaferous algae of the Dal Lake ecosystem as the lake is having tremendous biological diversity and optimization of various growth conditions of microalgae are required for the higher lipid production to yield good quality biodiesel.

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## Certificate

Certified that all the corrections/amendments as suggested by External Examiner Dr. M.R. Suseela, Senior Principal Scientist & Head, Algology Laboratory, CSIR-National Botanical Research Institute, Lucknow during Viva-Voce examination held on 30$^{th}$ of October, 2014 have been incorporated in the manuscript entitled **"Survey and Evaluation of Oleaferous Freshwater Algae of Dal Lake Ecosystem"** submitted by **Mr. Javeed Ahmad Lone (Regd. No. 2010-332-D)**.

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