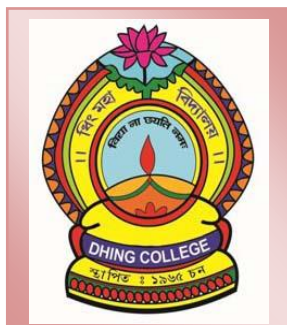

FINAL REPORT OF UGC MAJOR RESEARCH PROJECT



ENTITLED:

“SCREENING GROWTH RESPONSE AND BIOREMEDIATION POTENTIALITY OF CERTAIN CYANOBACTERIAL SPECIES TO INDUSTRIAL EFFLUENTS AND THEIR MOLECULAR CHARACTERIZATION”.

PROJECT NO: F. NO.42-968/2013(SR) DATED 14 MAR, 2013

SUBMITTED TO:
UNIVERSITY GRANTS COMMISSION: NEW DELHI

Submitted By:
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PRINCIPAL INVESTIGATOR
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DHING: NAGAON: ASSAM

Final Report Assessment/Evaluation Certificate
(Two Members Expert Committee not belonging to the institute of principal Investigator)
(to be submitted with the final report)

It is certified that the final report of Major Research Project entitled "*Screening growth response and bioremediation potential of certain cyanobacterial species to industrial effluents and their molecular characterization*" by **Dr. Manoj Kumar Saikia** has been assessed by the following members/ referee/ experts for the final submission of the report to the UGC, New Delhi under the scheme of Major Research Project.

Comments/Suggestion of the Expert

Expert 1: The problem is very interesting and useful. There is a scope of further extension of the work.

Expert 2: I would like to mention that the work of Dr Saikia may be regarded as important contribution. I am pleasure to recommend the report.

Name & signature of the experts with date:

<u>Name of Expert</u>	<u>University/college</u>	<u>Signature</u>
1. Prof. (Retd.) S. Kalita	Gauhati University	Kalita Mobile - 9435148264
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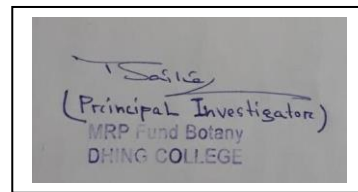
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(Registrar/Principal)

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Dhing College**

CERTIFICATE

I *Dr. Manoj Kumar Saikia* declare that the work presented in this report is original and carried throughout independently by me and my project fellow *Mrs. R. Baruah* during the complete tenure of major research project of U.G.C., New Delhi.

Date:23/06/2017



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ACKNOWLEDGEMENT

At the very outset , I am thankful to the **University Grants Commission (UGC)**, New Delhi, for the award of project and financial assistance to pursue the research work in the Department of Botany, Dhing College College, Nagaon, Assam.

I also convey my sincere thanks to the Principal Dr S.K. Dutta to provide basic infrastructure facilities in the Department of Botany . I would like to thank Mrs. Anju Deka Bora, HOD, Dr. S.K. Nath and .P.C.Sarmah, Associate Professor of Botany department for their valuable suggestions and help during my project period.

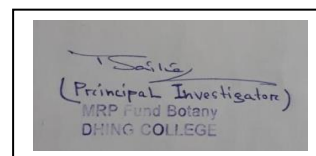
I like to extend my thanks to Professor (Retd) Dr.S. Kalita , Gauhati University for proving necessary technical support , cooperation and help during my research work.

I acknowledge my sincere thank to my friend Dr .Ramesh Nath , Associate Professor of Zoology for his valuable guidance in compilation of data analysis.

My special thanks go to the research personnels of biotech hub Dhing College for their inspiration and help during my project work.

My sincere thanks also goes to my all other friends who had helped me in their various capacities at various stages of this work.

Lastly I am indebted to my family members for their moral support and blessing.



(Dr Manoj Kr. Saikia)

PREFACE

- In the present work, the “**Screening growth response and bioremediation potentiality of certain cyanobacterial species to industrial effluents and their molecular characterization**” have been presented with particular efforts to find out the growth response of *Cyanobacteria* treated with industrial effluents and their role in bioremediation .
- **CHAPTER-I** Deals with a general introduction about Cyanobacteria, their role, importance of study, review of work done etc.
 - 1.1 Introduction
 - 1.2 Origin of Research Problem
 - **1.3** Interdisciplinary relevance
 - **1.4** Review of Research and Development in the Subject
 - **1.5** Significance of the study in the light of knowledge and social importance
- **CHAPTER-II** Discusses a review of literatures done in the concerned field.
- **CHAPTER-III** Deals with description of the study sites and maps
 - Nagaon Paper Mill (NPM), Namrup Fertilizer plant and Digboi Oil refinery
- **CHAPTER-IV** Contains details about Aims and Objectives, Methodology adopted for this particular research work.
- **CHAPTER-VI** highlights the experimental observations, results, Photoplates, recommendation and
- **BIBLIOGRAPHY.**

CHAPTER-I

1: GENERAL INTRODUCTION

1.1 INTRODUCTION: Industrialization is considered as cornerstone of development strategies due to its significant contribution to the economic growth and human welfare. It is a yardstick for placing countries in the League of Nations and index of its political stature (*FEPA, 1991*). It has brought about prosperity, comfort, health and wealth of mankind through industrial revolution and rapid utilization of natural resources, but also threatened the ecological security of the globe with consequent damage to ecosystem by generating huge waste and pollutants as by product of industrial development. Generally Industries turn out solid, liquid and gaseous effluents in its operation. Both the solid and liquid waste are the major source of water pollution in the present day (*Bachewar and Mehta, 2000; Ogedengbe and Akinbile, 2004*). Every day about two million tons of sewage, industrial wastes and agricultural wastes are discharged in to world's water (*UN-WWAP, 2003*). It is estimated that industry alone is responsible for dumping of 300-400 million tons of heavy metals, solvents, toxic sludge and other waste into water each year (*UNEP, 2010*). It has been reported that majority of industrial effluents have a hazard effect on water quality, habit quality and complex effect on flowing waters (*Ethan et al., 2003*).

The importance of water bodies cannot be overemphasized as it forms greater percentage of all biomass. Natural water maintains a wide variety of aquatic life which maintains a dynamic equilibrium with the environment which is maintained by the relative stability of temperature, chemical composition of water, the presence of sufficient dissolved oxygen and essentially neutral P^H. The physico-chemical parameters and nutrients in water play significant role in the distributional patterns and species composition of phytoplankton, their growth and density on which zooplankton and other higher consumers depends for their existence (*Mahar et al., 2000*). Significant changes of these parameters and excessive deposition of chemical nutrients into natural waters impairs the water quality as well as endangers aquatic life (*Mallin and Cahoon, 2007*). For organism level response of pollutants to aquatic organism it may range from inhibitory to stimulatory depending on nature of pollutants and type of organism exposed. The organism level responses of aquatic biota to environmental contaminants are often determined in controlled laboratory exposures or toxicity test. The ecosystem

level effect may be in the structure and function of communities.

Bioremediation is a cost effective and efficient method of decontamination that has become increasingly popular now-a-days to reduce environmental pollution. The effluents from residential and industrial discharge constitute a major source of water pollution. The industrial effluents were discharged into open drains which finally joins the rivers (**Kumari et al, 2006**). Wastewater discharge of industries are major issues of water pollution, contributing to oxygen demand and nutrient loading of the water bodies promoting toxic destabilized aquatic ecosystem (**Morrison et al, 2001; DWAF and WRC, 1995**). Wastewater is any type of water that has been negatively affected in quality by anthropogenic influence. There are mainly two kinds of wastewater i.e. municipal wastewater and industrial wastewater. Different industries produce various kinds of industrial wastewater based on their own specific combination of pollutants. Fertilizer industry, pulp & paper, oil industry is one of the major water consuming industries responsible for water and soil pollution of considerable magnitude (**Sunderamoorthy et al, 2001**). New technologies are being proposed to access the treatment of waste water.

Cyanobacteria i.e Algae form one of the components in new technology for waste water treatment. Bioremediation has been well studied over the past 40 years (**Wang, 2011**). Considerable research efforts have been devoted to the development of algal biosorbents to remediate pollutants, particularly heavy metals (**Hubbe, 2011**). Cyanobacteria are important bioremediation agents, and are already being used in wastewater treatment. They are considered as a most primitive photosynthetic prokaryotes which are supposed to have appeared on this planet during the Pre-Cambrian period (**Ash and Jenkins, 2006**). Possibly, these are first photosynthetic microorganisms which persisted over a period of 2-3 billion years, performing an important role in evolution of higher forms. Cyanobacteria are a unique assemblage of organisms which occupy a vast array of habitats (**Abd Allah, 2006 and Haande et al, 2010**). Cyanobacteria are very susceptible to sudden physical and chemical alterations of light, salinity, temperature and nutrient composition (**Boomiathan, 2005 and Semyalo, 2009**).

The application of *Cyanobacteria* showed immense potential in waste *water and industrial effluents treatment, bioremediation of aquatic and terrestrial habitats. Chemical industries, biofertilizers, food, feed .fuel* etc. Cyanobacteria, also known as blue green algae are colonizing microorganisms that are found throughout the world.

These organisms are extraordinarily well adapted to a wide range of environmental conditions. *Cyanobacteria* have a great deal of potential as bioindicator and bioremediation of aquatic habitats either as *wild type, mutant or genetically engineered* forms. More over their viability and metabolic activity are not affected by decrease in levels of the biodegradable pollutants that may break down. Besides *Cyanobacteria* have shown to be highly effective as accumulators and degraders of different kinds of environmental pollutants of different industrial origin. The study of physical, chemical and biological characteristics of industrial effluents are so vast that each industrial and waste habitat requires a separate study.

There is an urgent need to screen and develop efficient *Cyanobacteria* (alga) for the bioremediation of waste water. Keeping this fact the research work has been undertaken to assess and evaluate cyanobacterial potential for bioremediation. During the recent past, studies on cyanobacteria have emphasized their important role in ecosystem. They grow at any place and in any environment where moisture and sunlight are available. However, specific algae grow in specific environment and therefore their distribution pattern, ecology, periodicity, qualitative and quantitative occurrences differ widely. The abundance and composition of *Cyanobacterial* algal population in surface waters of ponds and lakes have been discussed in many works and a conflicting general impression exists. It is said that they flourish well either in nutrient rich and warm water or at times in water with apparently low nutrient concentrations, subjected to higher temperature and bright light conditions (**Ganapati, 1940; Philipose, 1960; Munawar, 1970; Fogg, 1975**). A thorough knowledge of the physical, chemical and biological characteristics of an industrial waste is a preliminary and essential requirement for any attempt at chemical and / or biological treatment of the waste. Hence, the present study was aimed to assess the physico-chemical characteristics of effluents and cyanobacterial diversity around three different industrial establishment such as paper mill, oil and fertilizer mills in Assam.

Cyanobacteria, also known as blue green algae comprise a unique group of organisms with worldwide distribution. These are considered as algae because of their microscopic morphology, pigmentation and oxygen evolving photosynthesis. They are by far the largest group of photosynthetic prokaryotic as judged by their widespread occurrence, frequency, abundance and morphological diversity. *Cyanobacteria* have long been recognized as having enormous potential for use in biotechnology, especially in

agriculture, and now slowly drift is towards their use in wastewater treatment, because of the following reasons:-

- Cyanobacterial growth does not require energy rich compounds like other non photosynthetic microorganisms.
- Cyanobacteria have simple growth requirements which use water as a source of reluctant. This character gives them an edge over other photosynthetic bacteria.
- Many cyanobacteria combine photosynthesis and nitrogen fixation. This is another advantage over other eukaryotic photosynthetic organisms.
- They are environmental friendly and do not cause toxicity to other biotic.
- Separation of cyanobacterial biomass is much easier than other microbial biomass due to their size.

1.2 Origin of Research Problems: *Cyanobacteria* are considered as the most primitive photosynthetic prokaryotes which are supposed to have appeared on this planet during precambium period (*Ash and Jenkins, 2006*). Possibly these are the first photosynthetic microorganism which persisted over a period of 2 to 3 billion years. *Cyanobacteria* occupy and predominate a vast array of habitats as a result of several general characteristics: some belonging to bacteria and others unique to higher plants (*Abd Ahhah, 2006*). Cyanobacteria are very susceptible to sudden physical and chemical alterations of light, salinity, temperature and nutrient composition (*Boomiathan, 2005; Semyalo, 2009*). The application of *Cyanobacteria* showed immense potential in **waste water and industrial effluents treatment, bioremediation of aquatic and terrestrial habitats, chemical industries, biofertilizers, food feed fuel** etc. In addition to these physico-chemical parameters such as P^H , *carbondioxide, organic matters, alkalinity, nitrates and phosphates* are factors important in determination the distribution of Cyanobacteria (*Podda et al, 2000*). *Cyanobacteria* have a great deal of potential as bioindicator and bioremediation of aquatic habitats either as **wild type, mutant or genetically engineered** forms. More over their viability and metabolic activity are not affected by decrease in levels of the biodegradable pollutants that may break down. Besides *Cyanobacteria* have shown to be highly effective as accumulates and degraders of different kinds of environmental pollutants of different industrial origin. However, the study of physical, chemical and biological characteristics of industrial effluents are so

vast that each waste habitat requires a separate study.

The basic purpose of the present study is to evaluate the potentiality of few *Cyanobacteria* to be isolated from different contaminated waste effluents and contaminated sites near to **Oil, Paper and Fertilizer** factories of Assam. In Assam 3 (three) big industry like Oil. Paper and Fertilizer factories are in operation. A number of research works have been done in Assam on systematics and distributional pattern of *Cyanobacteria* in polluted water bodies. But a few works has been done in treating/determining the growth response of these *Cyanobacteria particularly* to Oil, Paper and Fertilizer effluents in laboratory condition. More over the toxicity of these effluents were demonstrated many times in literature mostly on fish population, but little literature is available on *Cyanobacterial* species. Considering the above the present study was undertaken.

1.3 Interdisciplinary relevance: The study of bioremediation potentiality of *Cyanobacteria* in waste water habitat has an interdisciplinary approach to pollution control. Bioremediation have been used worldwide as efficient and low cost method for remediating pollution of industrial origin by converting dissolved nutrients in to biomass. (**Lincoln et al, 1996**) and for biotreatment or removal of organic waste as economic and low maintenance remediation technology for contaminated system.

1.4 Review of Research and Development in the Subject: However the review of beneficial application of *Cyanobacteria* as stated above in remediation of contaminated waters, either natural aquatic environments or industrial effluents is still not optimally manipulated. Algae possess the ability to take up toxic heavy metals from the environment, resulting in higher concentrations than those in the surrounding water (**Megharaja et al. 2003**). For example *Cyanobacterial* species such as *Oscillatoria salina*, *Plectonema terebrans*, *Aphanocapsa* sp. and *Synechococcus* sp, *Oscillatoria chlorina*, *Scenedesmus quadricauda* sp have been successfully used in bioremediation of pulp mill effluents and oil spills in different parts of the world (**Raghukumar et al, 2001, Cohen, 2002 , Saikia et al, 2012**). Removal of Cadmium using filamentous *Cyanobacteria Anabaena flos-aquae* west. From tannery effluent shows that cyanobacteria can remove upto 75% Cd (**Kannan et. al, 2012**). Application of biosorbents / biomass from various microbial sources, moss, aquatic plants and leaf-based adsorbents was reported by various investigators (**Chang et al. 1997; Niu et al.**

1993; King et al. 2007) with the aim of finding more efficient and cost-effective metal-removal biosorbent. Among them, microalgae have proved to possess high metal binding capacities (Schiewer et al. 2000) due to the presence of polysaccharides, proteins or lipid on the surface of their cell walls containing some functional groups such as amino, hydroxyl, carboxyl and sulphate, which can act as binding sites for metals (Yu et al. 1999). Arbib et al. (2013) studied the algal growth rate and nutrient removal rate of the species *Chlorella stigmatophora* and *Scenedesmus obliquus* in urban wastewater at different nitrogen and phosphorus ratios, ranging from 1:1 to 35:1. It was reported in this study that nitrogen to phosphorus ratios ranging between 9 and 13 achieved best biomass productivity. Renuka et al. (2013) used *Calothrix* sp to remove nitrates and phosphates from sewage wastewater. Mohamed et. al (1994) reported that *Scenedesmus* sp. is very common species that is available in fresh water bodies and plays a significant role in purifying eutrophic waters. Colak and Kaya (1988) reported removal of nitrogen (50.2%) and phosphorus (85.7%) in industrial wastewater treatment and elimination of phosphorus (97.8%) in domestic wastewater treated by Cyanobacterial alga.

1.5 Significance of the study in the light of knowledge and social importance. The application of bioremediation of industrial effluents has great significance in Assam. In Assam three large industrial establishments like pulp & paper (Nagaon *Paper Mill*), Oil (Digboi *Oil Refinery*) and fertilizer (Namrup *Fertilizer Factory*) are already in operation. Large number effluents are daily emitting from industrial sites and these were polluting surrounding land and nearby water body. Different pollution control measures were taken by mill authority to control pollution load by adopting various physical and chemical means. But still not successful. So it assumed that if bioremediation technology is applied then it may control pollution at site. For this reason is necessary to identify some tolerant species of cyanobacterial alga from industrial site, specific to pollutant types and bring them to laboratory for culture and treatment with effluents types, For this reason *Cyanobacterial* species is selected, because Cyanobacteria are first hand indicator of pollution. Cyanobacterial taxa tend to have high dispersal and growth rates and relatively short generation times than other aquatic organisms (USEPA 2002). Furthermore, they are easy to collect and identify by experienced taxonomist (Plafkin et al, 1989) and identifiable on spot (Dell'Uomo, 1991). The application of bioremediation has social relevance too. Because it is cost effective & environmental friendly.

CHAPTER –II

2. REVIEW OF LITERATURE

Bioremediation is a newer approach directed towards the treatment of decontamination. Bioremediation primarily deals with the strategies that can employ to clean up the contaminants biologically. Removal and recovery of heavy metals from wastewater is important for environmental protection and human health. Bioaccumulation process is known as an active mode of metal accumulation by living cells which depends on the metabolic activity of the cell (Volesky, 1990; Wase and Foster, 1997). Microalgae are not unique in their bioremoval capabilities while they offer advantages over other biological materials in some conceptual bioremoval process schemes. Microalgae strains purposefully cultivated and processed for specific bioremoval applications and have the potential to provide significant improvements in dealing with the world-wide problems in metal pollution (Edward and Benemann, 1993). It is reported that biosorption of heavy metals by certain types of non-living biomass is a highly cost-effective new alternative for the decontamination of metal-containing effluents (Kratohvil and Volesky, 1998). Biosorption of heavy metals from algae can be effective process for the removal and recovery of heavy metals ions from aqueous solution (Kaewsarn, 2002). *Chlorella vulgaris* and *Scenedesmus dimorphus* is highly efficient for ammonia and phosphorous removal during biotreatment of secondary effluents from an agro industrial wastewater of a dairy industry and pig farming. These microalgae were isolated from wastewater stabilization pond. Both these microalgae removed phosphorous from the wastewater to the same extent (Luz Estela Gonzalez, 1997). Dead dried *Chlorella vulgaris* was studied in terms of its performance in binding divalent copper, cadmium, and lead ions from their aqueous or 50% v/v methanol, ethanol, and acetone solutions. The percentage uptake of cadmium ions exhibited a general decrease with decrease in dielectric constant values, while that of copper and lead ions showed a general decrease with increase in donor numbers (Al-Qunaibit, 2009) Algae have received increasing attention for heavy metal removal and recovery due to their good performance, low cost and large available quantities (Wang and Chen 2008). *S. incrassatulus* was also able to remove all the tested metals to some extent (25-78%), but bivalent metals were not removed as efficiently as reported in batch cultures, probably due to the high pH values there recorded. Chromium (VI) was more efficiently removed in continuous cultures than in batch culture, because the uptake of chromate could be favoured by actively growing algae (Peña-Castro et al 2004). Micro-algae can be used for tertiary treatment of wastewater due to their capacity to assimilate nutrients. The pH increase which is mediated by the growing algae also includes

phosphorous precipitation and ammonia stripping to the air, and may in addition act disinfecting on the wastewater (**Karin Larsdotter, 2006**). Algae have been proven efficient biological vectors for heavy metal uptake. Biosorption potential of two strains *Spirogyra sp.* and *Spirulina sp.* has been studied under different initial metal concentrations (**Mane and Bhosle 2012**). The use of live and dead *Spirulina sp.* For sorption of metals like Cr³⁺, Ni²⁺, Cu²⁺ and Cr⁶⁺ in form of Cr₂O₃ *Spirulina sp.* treated with different metal ions have been employed to understand the sorption mechanism. It is hoped that live *Spirulina sp.* will be a strong candidate for management of industrial wastewater (**Doshi et al 2007**). **Huijuan Meng (2011)** reported the biodegradation rates of linear alkyl benzene sulfonate LAS by *Spirulina platensis* increased with Zn (II) and reached the maximum when Zn (II) was 4 mg/L. The joint toxicity test showed that the combined effect of LAS and Zn (II) was Synergistic. LAS can enhance the biosorption of Zn (II), and reciprocally, Zn (II) can enhance LAS biodegradation. **Bindiya et al (2012)** observed the Bioaccumulation of Cadmium in Blue Green Algae *Spirulina (Arthrospira) Indica*. **Deng et al (2007)** reported that *Cladophora fascicularis* green alga is highly efficient for the biosorption of copper (II) from aqueous solution. Biosorption is the effective method for the removal of heavy metal ions from wastewater. Results are presented showing the sorption of Pb (II) from solutions by biomass of commonly available, filamentous green algae *Spirogyra sp* (**Gupta and Rastogi 2008**). **Khalaf (2008)** observed the Biosorption of reactive dye from textile wastewater by non-viable biomass of *Aspergillus niger* and *Spirogyra sp.* **Monteiro et al (2009)** observed that strains of the *Scenedesmus obliquus* microalga tested have proven effective in removing a toxic heavy metal from aqueous solutions, hence supporting their choice for bioremediation strategies of industrial effluents. It was proven, for the first time, that such a wild microalgae can uptake and adsorb Zn very efficiently, which unfolds a particularly good potential for bioremediation. Its performance is far better than similar (reference) species, especially near neutrality, and even following heat-treatment (**Monteiro, 2011**). **Kaushik et al (2008)** reports on chromium (VI) tolerance of two cyanobacterial strains *Nostoc linckia* and *Nostoc spongiaeforme* isolated from salt affected soils using uni-algal and bi-algal systems. It was observed that the effectiveness of cyanobacterial concern because they colour and diminish the quality of water bodies into which they are released. The effectiveness of *Oscillatoria* was employed for the bioremediation of textile effluents (**Abraham and Nanda, 2010**). **Prado et al (2010)** observed the rate of biosorption of cadmium and copper ions by nonliving biomass of the brown macroalga *Sargassum sinicola* under saline conditions. They see that presence of salt did not significantly affect the rate of biosorption and there is an antagonistic effect on biosorption when both these metals are present in the solution. Among the several

microalgae used to treat effluents *Chlorella* is often found from the various types of waste water for the treatment of the water (Karlander and Krauss, 1996). Raposo *et al* (2010) analyzed the capacity of *Chlorella vulgaris* and the autochthonous flora of the effluents to remove some of the compounds present in the effluents. Cecal *et al* (2012) deals with a study of the biosorption of UO_2^{2+} ions on two green algae: *Chlorella vulgaris* and *Dunaliella salina*. By kinetic investigations it was found that the biosorption process was greater for *Chlorella vulgaris* than for *Dunaliella salina*. Kannan (2011) seen the detoxification capacity of a variety of microbes especially cyanobacteria. They collected the effluents from tannery industry and added to the cyanobacterial growth medium in various proportions. The photosynthetic pigments and nitrogen status of *Anabena flos-aquae* were analysed before and after the treatment with effluents. It showed that *Anabena flos-aquae* can serve as the potential bioremedial organism for industrial pollution. Biodegradation and biosorption capacity of some potential cyanobacterial species: *Oscillatoria* sp., *Synechococcus* sp., *Nodularia* sp., *Nostoc* sp. and *Cyanothece* sp. Dominated the effluents and mixed cultures showed varying sensitivity. Contaminants were removed by all the species either as individuals or in mixtures (Dubey *et al*, 2011). Lee and Chang (2011) observed the biosorption capacity from aqueous solutions of the green algae species, *Spirogyra* and *Cladophora*, for lead (Pb (II)) and copper (Cu (II)). In comparing the analysis of the Langmuir and Freundlich isotherm models, the adsorption of Pb (II) and Cu (II) by these two types of biosorbents showed a better fit with the Langmuir isotherm model. Miranda *et al* (2012) observed two species of cyanobacteria, *Oscillatoria laete-virens* (Crouan & Crouan) Gomont and *Oscillatoria trichoides* Szafer which were isolated from a polluted environment and it is studied for their Cr^{6+} removal efficiency from aqueous solutions. Bioaccumulation is the effective method for removal of heavy metal ions from wastewaters. Bioremediation, the use of algal to extract, sequester and or detoxify heavy metals and other pollutants. They use filamentous alga of *Pithophora* sp. for the removal of cadmium, chromium and lead from industrial wastewater (Brahmbhatt *et al* 2012). Mercury is higher in *Dunaliella* alga as compared to those of cadmium and plumb. This is vivid that *Dunaliella* is highly tolerant to the ascending concentration of heavy metals and their absorption in aquatic media. This approves the usage of *Dunaliella* as useful equipment for the elimination of heavy metals environment (Imani *et al* 2011). Gao and Yan (2012) observed the Response of *Chara globularis* and *Hydrodictyon reticulatum* to lead pollution: their survival, bioaccumulation, and defense they observed that *H. reticulatum* exhibited higher tolerance to Pb pollution than *C. globularis*. Some workers determine the feasibility of using algae growing in wastewater lagoons to absorb residual heavy metals for subsequent complete removal by algae-

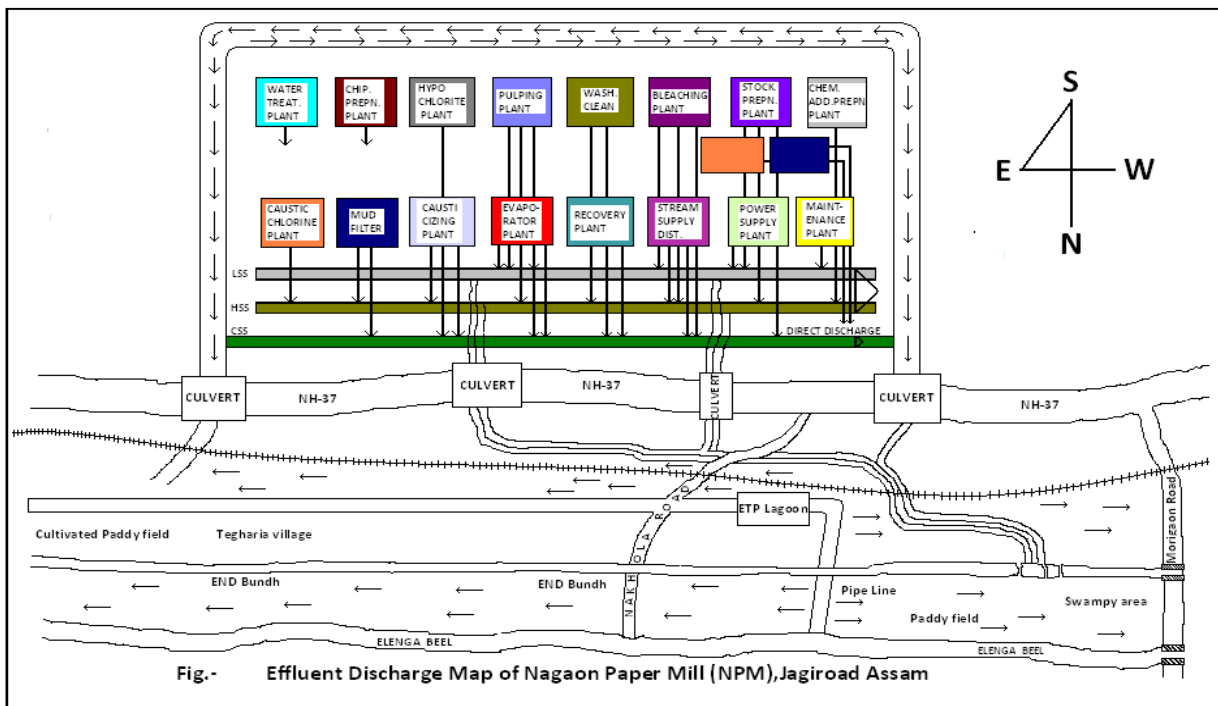
intermittent sand filtration system and they found that this technique is very helpful in removal of certain heavy metals from wastewater (**Daniel et al, 1979**). The major problem in utilization of microorganisms in any industrial or waste water treatment is harvesting of the biomass. This is solved by the strategy of immobilization. Immobilization technique is essential not only in waste water treatment but also in various industries (**Prakasham and Ramakrishna 1998**). One of the main interests for microalgae in biotechnology is focussed on their use for heavy metals removal from effluents and waste water (**Mallick, 2002**). Some research has been done dealing with the immobilization of microalgae for different purposes: morphology studies, the production of fine chemicals, energy production, wastewater treatment etc. Immobilization strains of microalgae is been used for sewage treatment. Efficiency of depuration was highest when a fluidized bed and *Chlorella vulgaris* were used (**Travieso, 1992**). Use of immobilized algae in wastewater treatment and heavy metal removal processes efficient and offer significant advantages in bioreactors (**Hameed and Ebrahim, 2007**). **Tam et al, 1994** used *chlorella vulgaris* cells immobilized in alginate beads for removing N and P from wastewater and they achieved significant reductions in wastewater ammonia and phosphate. *Spirulina platensis*, a cyanobacterium of economic important was studied for the tolerance to cadmium. The Biosorption studies showed that the algae had a great potential for adsorbing the heavy metal on to the cell. The immobilized cell of *Spirulina platensis* was able to be more effective in absorbing the metal to the cell (**Murugesan et al, 2008**). The process of biosorption of trivalent chromium (Cr³⁺) by live culture of *Spirulina platensis* and the sorption potential by the dried biomass, in both free and immobilized states have been investigated for simulated chrome liquor in the concentration range of 100–4500 ppm. Cyanobacteria have the potential to be used for bioremediation of various contaminants like heavy metals, crude oil, pesticides, phenanthrene, naphthalene, phenols, and xenobiotics [92–94]. Some species of cyanobacteria possess the characteristics to decontaminate specific pollutants. Cyanobacterial species that can have the potential for bioremediation. Cyanobacterial species could be used to degrade certain textile dyes, as dyes are complex contaminants that are difficult to degrade using conventional biological treatments. Cyanobacterial species including *Anabaena flosaquae* UTCC64, *Phormidium autumnale* UTEX1580, and *Synechococcus* sp. PCC7942 were assessed for their ability to degrade the textile dyes such as indigo, Remazol Brilliant Blue R (RBBR), and black sulfur. These cyanobacteria species showed the degradation of the dyes present in a textile effluent; thus, they have the potential to be used in tertiary treatment of wastewater effluents generated from textile industries. Interestingly, *Phormidium autumnale* UTEX1580 showed complete degradation of the indigo dye [**Dellamatrice, P.M.; Silva-Stenico, M.E.; de Moraes,**

L.A.B.; Fiore, M.F.; Monteiro 2017]. In another study, cyanobacterial cultures of *Gloeocapsa pleurocapsoides* and *Phormidium ceylanicum* were used to decolorize cyclic azo dyes. These cyanobacterial species showed more than 80% decolorization of Acid Red 97 and FF Sky Blue dyes after 26 days. However, *Chroococcus minutus* species showed 55% decolorization of Amido Black 10B [**Parikh, A.; Madamwar, D,2005**]. Other species of cyanobacteria like *Spirulina* can completely remove nitrogen from wastewaters [**Chevalier, P.; Proulx, D.; Lessard, P.; Vincent,W.F.; De La Noüe, J. 2000**]. *Phormidium tenue* can decolorize wastewater up to 60–90% [**Nagasathya, A.; Thajuddin, N, 2008**]. Cyanobacteria could play a prominent role in decontaminating oil substances from wastewaters and help in the degradation of hydrocarbons [**Cohen, Y. ,2002**]. Cyanobacteria possess an excellent ability to adapt to different environments. Due to their increased adaptability and ability to grow at a faster rate from one mode to another, cyanobacteria can be effectively used for the remediation of polluted environments. Both the physiological and genetic adaptation paved the way for cyanobacteria survival under complex environmental pollution conditions [**Cepoi, L.; Don, tu, N.; Salaru, V,2016**]. This adaptability to varying environments is due to mixotrophic growth, an approach used for the biodegradation of persistent organic pollutants (POPs). Cyanobacterial species, such as *Anabaena flosaquae* strain 4054, can degrade the endocrine-disrupting pollutants, like phthalate esters [**Babu, B.; Wu, J.-T,2010.**]. Another common cyanobacterium, *Anabaena azotica*, can effectively decompose the organochlorine pesticide -hexachlorocyclohexane (lindane) [**Zhang, H.; Hu, C.; Jia, X.; Xu, Y.; Wu, C.; Chen, L.; Wang, F,2012**]. *Environments* 2020, 7, 13 9 of 17 Cyanobacterial species are also potential candidates for removing contaminants from soil and water, thereby contributing to establish a sustainable agricultural practice. For example, the presence of *Anabaena* sp., *Lyngya* sp., *Spirulina* sp., *Nostoc* sp., or *Microcystis* sp. can help remove the glyphosate herbicide effect from the agricultural fields. Furthermore, the potential for bioremediation can be further improved by applying genetic engineering tools that can help develop a cost-effective and environmentally friendly remediation approach with the increased production of valuable biomass [**Kuritz, T.;Wolk, C.P,1995**]. Another challenging issue in the agriculture domain is desertification that can be reduced by applying cyanobacterial inoculums. Cyanobacteria can also help combat the desertification in association with bacteria, algae, mosses, lichens, or fungi, which form the biological soil crusts in distinct geographical regions [**Rossi, F.; Li, H.; Liu, Y.; De Philippis, R, 2017**]. Therefore, cyanobacteria can be used to sort out numerous environmental concerns.

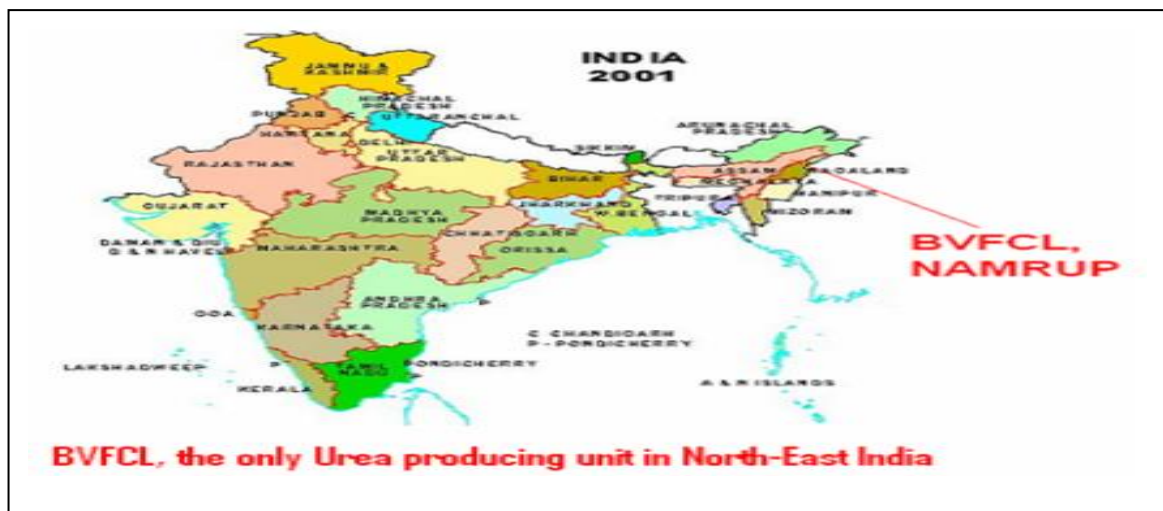
CHAPTER-III

3.1. DESCRIPTION OF THE STUDY AREA AND A BRIEF PROFILE OF NAGAON PAPER MILL (NPM), NAMRUP FERTILIZER PLANT AND DIGBOI OIL REFINARY:

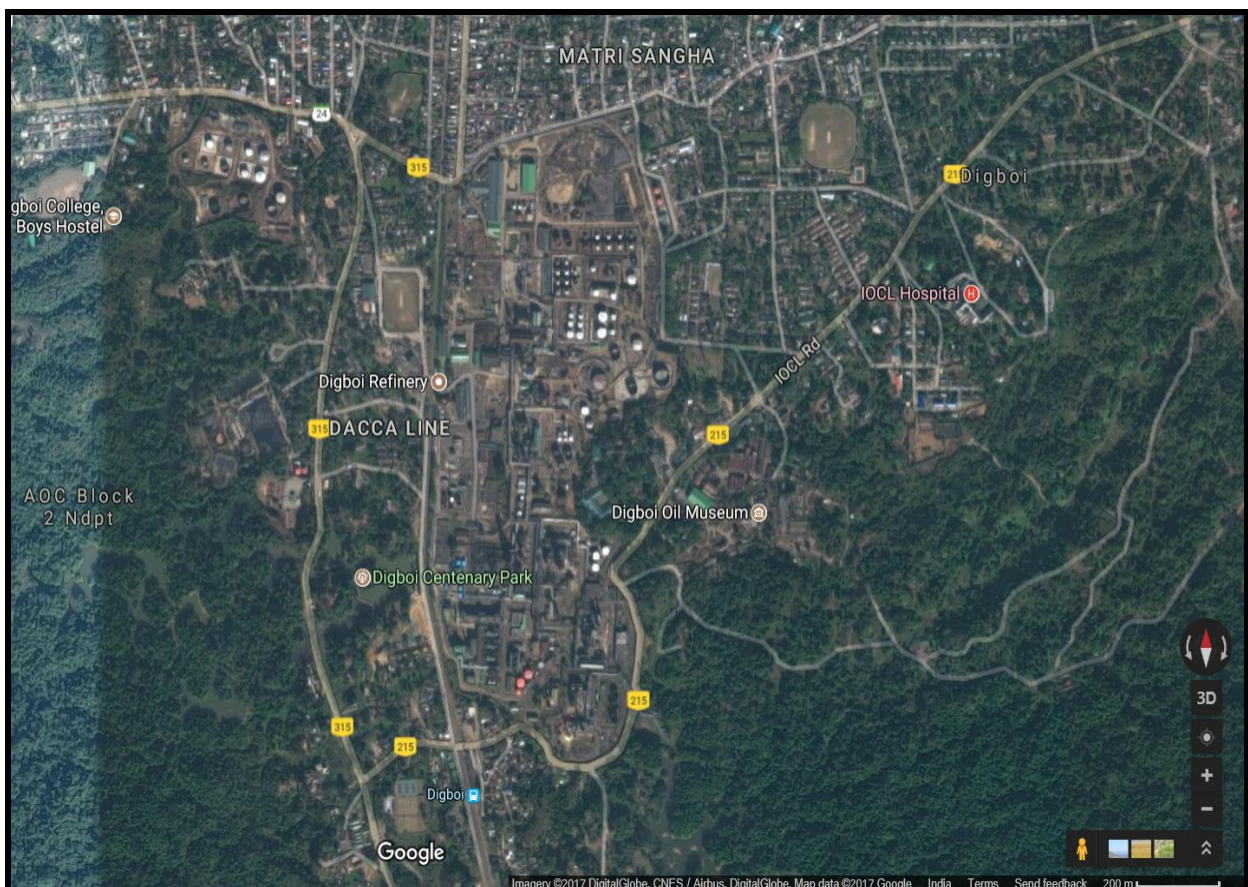
3.2 Nagaon Paper Mill (NPM) is located at Jagiroad in the Eastern part of Morigaon District of Assam. It is situated almost in the midway between Guwahati in the west and Nagaon in the east at a distance of 60 km and 65 km respectively, on the national Highway 37. The NPM covers an area of 240 hectares of land and 3km away from Jagiroad Railway Station. Morigaon a newly created district of Assam (c.f. Assam gazette notification No. GAG (B) 370/87/102 dated 29th sept1989 w.e.f 1st Oct 1989.).The district covers an area of 143150 hector. The geographical boundary comprises Nagaon district in the East, Darrang district in the North, Karbi -Anglong and a part of Nagaon and Kamrup district in the South and Kamrup district in the West. Morigaon district has 101642 hector gross cropped area and 81092 hector net sown area. Morigaon district have 12 agriculture circles. The Hindustan Paper Corporation Limited (HPC) was created on May 29, 1970. Initially HPC was entrusted with the management of sick paper mill in Karnataka and construction of a paper mill Nagaland and other News print mill in Kerala. In 1978, two large projects in Assam were added to its operationalized. Hindustan Paper Corporation Limited (HPCL) with corporate Head Quarter in Kolkata. The Hindustan Paper Corporation Limited (HPC) was created on May 29, 1970. Initially HPC was entrusted with the management of sick paper mill in Karnataka and construction of a paper mill Nagaland and other News print mill in Kerala. In 1978, two large projects in Assam were added to its operationalized. Hindustan Paper Corporation Limited (HPCL) with corporate Head Quarter in Kolkata. The Nagaon Paper Mill uses approximately 90,000 m³ of water daily and major portion of it discharged as effluents at the rate of 2100m³/h through High Solid Stream (HSS), Low Solid Stream (LSS), and Clear Water System (CWS).The Effluents Discharge Map of NPM have been shown in Figure- 1 and Effluent Treatment Plant (ETP) Map at Figure- 2. The major concerns from these effluents are: 1] Pulping liquor containing VOCS (terpenes, alcohol, phenol, methanol, and acetone). 2] Bleaching effluents containing chlorinated hydrocarbons like dioxins and furans, chloroform and other chlorinated compounds.Nonyl phenol ethoxylates (NPE) - a suspected endocrine disrupter. 3] Discharge color due to lignin.



3.2 The Namrup Fertilizer Complex, renamed as Brahmaputra Valley Fertilizer Corporation Limited after bi-furcation from erstwhile Hindustan Fertilizer Corporation Limited w.e.f. 1st April 2002 located on the bank of the river *Dilli* in the south-western border of Dibrugarh District in Assam. It is the first factory of its kind in India to use associated natural gas as basic raw material for producing nitrogenous fertilizer. The factory is located at latitude and longitude are $27^{\circ}10'N$ & $95^{\circ}21'E$ respectively and is situated at an elevation of 123 meters above Mean Sea Level. The Climatic condition of the study area is rain fed. The average rainfall of this area is 220.65mm (yearly). The temperature ranges from $30.6^{\circ}c$ in summer to $12.3^{\circ}c$ in winter. The average relative humidity is 83%. The Humidity during Monsoon is 100% and during winter is appx. 60%.



3.3 The Digboi Refinery was set up at Digboi in 1901 by Assam Oil Company Ltd. The Indian Oil Corporation Ltd (IOC) took over the refinery and marketing management of Assam Oil Company Ltd. with effect from 1981 and created a separate division. This division has both refinery and marketing operations. The refinery at Digboi had an installed capacity 0.50 MMTPA (million metric tones per annum). Digboi is the place in India where for the first time crude oil was explored in Asia during late 19th century and Digboi refinery is the oldest petroleum refinery in the subcontinent established in the year 1901. The refinery has been regularly discharging hazardous chemicals such as *oil, hydrocarbon, phenol* etc. to a natural stream that created a stress condition for growth of aquatic flora and fauna including phytoplankton. A little work has so far been done to understand the effect of petroleum refinery effluent on fresh water algal community in the region (**Singh and Gaur 1988, Baruah et al. 2009**). The present investigation was planned to undertake a study on diversity, distribution and abundance of Cyan bacterial community of effluent receiving stream of Digboi oil refinery (Assam).



CHAPTER-IV

4.1 AIMS & OBJECTIVES:

The present work has been designed to screen the growth response, diversity and bioremediation potential of Cyanobacterial population from the nearby areas and water bodies of Nagaon paper Mill, Digboi Refinery and Namrup Fertilizer Plant. This was accomplished by conducting field studies and laboratory analysis. Both field and laboratory investigations were analyzed to elucidate the effect of these factories effluents on Cyanobacteria .

The specific objectives of the present study which include:

- *To isolate naturally occurring Cyanobacterial species from the nearby areas of industrial establishment like :*
 - *Pulp and paper industry locate in Nagaon (NPM)*
 - *Oil refinery located in Digboi*
 - *and fertilizer factory located in Namrup (Assam)*
- *To study their diversity and distribution according to pollution load of different origin.*
- *To isolate naturally occurring cyanobacterial species having capacity for bioremediation of pollutants.*
- *To determining growth response of few isolated and culturally grown species of Cyan bacteria to different concentration of effluents in laboratory.*
- *To correlative effluents types and species exposed*
- *Molecular Characterization*

CHAPTER -V

5.1 Methodology Adopted:

- **Collection, Preservation and Enumeration of Cyanobacteria:** The Cyanobacterial samples were collected as per standard methods (APHA, 1989). The numerical counting per microscopic field following Lackey's (1938) drop count method was made and the total population of cyanobacterial forms was calculated. All organisms were represented numerically as units per mL. While, counting the colonial and filamentous forms were brought down to a common size factor by- (a) Using 10 μm as one unit for filamentous type like *Oscillatoria*, *Anabaena* etc. (b) One colony as one unit:- In case of old colonies of considerable size like *Anacystis* etc. The colonies were teased and broken down into pieces of smaller units; each unit was then counted as one unit.
- **Identification:** After collection and preservation, identified under microscope up to species level. The identification will be done as per Desikachay, 1959; Prescott, 1951; Smith, 1950 and up to-date journals and published literature and hand will consulted.
- **Analysis of physico-chemical parameters:** All the parameters of present investigation will be determined by following methodology of APHA (1989), Trivedy and Goel (1986), Goltzman et al.(1978), Greenberg et al.(1985). Chemical used will be mostly of analytical grade. The final results for all the parameters will be estimated quantitatively in the laboratory were expressed as mean of three samples.
- **Methods for Cyanobacterial Culture:** The bioassays of Cyanobacterial species will be conducted based on bacteriological techniques (Stein, 1973; Gopinathan, 1982; APHA, 1980; Ward and Parish, 1982).
- **Isolation of Pure cultures:** The isolation methods will be based on bacteriological techniques (Stein, 1973; Gopinathan, 1982). The cyanobacterial colony will be examined under light microscope and identified with the help of description given by Fritsch (1935) and Prescott (1982). Latter, the colonies will taken for serial dilution separately. Finally uni -cyanobacterial culture will be obtained in pure form.
- **Culture Media and Culture Condition:**For maintaining and raising pure cultures of the isolates, different types of culture media like (Miquel, 1980 ; ward and Parish, 1982; BG-11) will be tried for culturing . Among these Ward and Parish medium and BG-11 (Stainer et al., 1971) will be maintained particularly for cyanobacteria.

Growth Experiments: The waste water to be selected for use in the present study will be the pulp and paper mill effluent, oil effluents, fertilizer effluents. . Soon after collection and proper preservation, effluents were analyzed for various parameters as per methodologies of APHA. In order to determine their effect on Cyanobacteria initially a range finding test will be conducted. Different concentrations of effluents and a control without effluents supplemented with basal nutrient medium will be used for conducting range finding tests. Equal quantity of cyanobacterial cells will be inoculated in each flask and will incubate for 96 hours. The growth of test species in each flask will be compared with that of the control flask. The concentration selected for range finding test were used for conducting definitive test (APHA, 1980, Ward and parish, 1982). The experimental series will be arranged as Basal medium BM+0% effluents served as control, BM+ 20%, BM+40%, BM+60%, BM+80% and 100% effluent for all the test algae. P^H adjusted as 7.5 (Cyanobacteria) respectively before autoclaving. All the cultures will be incubated in a culture room at 28± 2°C and exposed to a continuous fluorescent lamp assembly of 5x40 watts. Flasks were shaken once per day to prevent clumping of cells. Each experiment will run for 21 days to allow good growth but without causing nutrient shortage. The experiments will be carried out in triplicate and data will be plotted for the means of the result. Growth in terms of Chlorophyll a, Carotene and Biomass contents will be studied over a period of 21 days or until declining phase.

Measurement of growth: Spectrophotometric analysis of pigment extract (Parsons et al., 1984) at different wavelengths (480, 510, 630, 647 and 664) in UV-VIS Spectrophotometer Model-117 on three days interval of time for a period of 21 days. The Chlorophyll a content will be calculated by the formula given by Parsons et al., (1984) and expressed as µg Chl. a/ ml. A] **Chlorophyll a** = $11.85E_{664} - 1.54E_{647} - 0.08E_{630}$. B] **Chlorophyll b** = $21.03E_{647} - 5.43E_{664} - 2.66E_{630}$ C] **Carotenoids** = $7.6 E_{480} - 1.49 E_{510}$. The absorbance of culture suspension on 21 days was considered as final yield. For estimation of algal biomass cultures were harvested on 21st day at in an oven and dried at 105 °C until constant weight was obtained. The final weight of the biomass of the samples were measured by subtracting the initial weight and expressed as mg/ml. The determination of DNA of test organisms

Data analysis: Data will be analysed using the Karl Pearson's co-efficient of correlation by using SPSS 9.0 windows. ANOVA test will be conducted between Concentrations and days to observe significant difference of cyanobacterial growth. Both the biological and physico-chemical parameters will be analyzed statistically for meaningful evaluation of physico-chemical parameters corresponding to diversity and cyanobacterial growth.

Photomicrograph: The Photomicrographs of Cyanobacterial species to be isolated from study sites will be taken by Olympus digital camera. The species will be visualized under trinocular microscope model CH 20i for identification and preservation.

CHAPTER -VI

OBSERVATIONS:

Table 1: Pollution index of algal genera (Palmer, 1969)

GENERA	POLLUTION INDEX	GENERA	POLLUTION INDEX
<i>Anacystis(Microcystis)</i>	1	<i>Micractinium</i>	1
<i>Ankistrodesmus</i>	2	<i>Navicula</i>	3
<i>Chlamydomonas</i>	4	<i>Nitzschia</i>	3
<i>Chlorella</i>	3	<i>Oscillatoria</i>	4
<i>Cyclotella</i>	1	<i>Pandorina</i>	1
<i>Chlosterium</i>	1	<i>Phacus</i>	2
<i>Euglena</i>	5	<i>Phormidium</i>	1
<i>Gomphonema</i>	1	<i>Scenedesmus</i>	4
<i>Lepocinclis</i>	1	<i>Stigeoclonium</i>	2
<i>Melosira</i>	1	<i>Synedra</i>	2

Table 2: Pollution index of algal species (Palmer 1969)

ALGAL SPECIES	POLLUTION INDEX	GENERA	POLLUTION INDEX
<i>Ankistrodesmus falcatus</i>	3	<i>Stigeoclonium tenue</i>	3
<i>Arthospria jenneri</i>	2	<i>Synedra ulna</i>	3
<i>Cyclotella meneghiniana</i>	2	<i>Nitzschia palea</i>	5
<i>Chlorella vulgaris</i>	2	<i>Oscillatoria chlorina</i>	2
<i>Euglena viridis</i>	6	<i>Oscillatoria limosa</i>	4
<i>Nevicula cryptocephala</i>	1	<i>Oscillatoria princeps</i>	1
<i>Euglena gracilis</i>	1	<i>Oscillatoria putrida</i>	1
<i>Gomphonema pervulum</i>	1	<i>Oscillatoria tunuis</i>	4
<i>Nitzschia acicularis</i>	1	<i>Pandorina morum</i>	3
<i>Melosira varians</i>	2	<i>Scenedesmus quadricauda</i>	4

Table 3: Pollution index Scale

Pollution Index	Pollution Load
<15	Very light organic pollution
15-20	Organic pollution
>20	High organic pollution

In the present study the algae particularly the Cyanobacteria (Blue Green algae) as per list of Palmer (1969) are shown in Table 4 & 5 according to decreasing trend of pollution at different sample sites (S1,S2 & S3) nearby the area of Pulp & Paper, Oil and Fertilizer industry. Out of 60 most pollution tolerant genera of Cyanobacteria 22, 20 & 17 are found in sample station S1, S2 & S3 of Pulp & paper industry; 20, 21 & 17 are found in oil industry and 21, 19 & 16 are found in the sampling sites of Fertilizer industry. Similarly out of 80 most pollution tolerant species 18, 20 & 16 are found in sample station S1, S2 & S3 of Pulp & Paper industry; 19, 17 & 16 are found near oil industry and in case of fertilizer industry these numbers are 18, 16 & 14.

The cyanobacterian flora of all three sampling stations was subjected to Plamer Pollution Index (1969) for rating pollution status. Out of 20 pollution index genera of algae 03, 03 & 02 and out of 20 pollution index species 07, 05 & 03 were recorded at station S1, S2 & S3 of Paper & Pulp industry. 03, 02 & 01 genera and 05, 04 & 03 species were found in the sampling station S1, S2 & S3 of oil industry whereas these values for fertilizer industry were 03, 02 & 01 genera and 04, 03 & 01 species.

The results and the total score of each sample stations according to industry wise are given in Table 4. The results reveal that the pollution load of each sample site gradually decreases in downstream. The high organic pollution was recorded in nearby areas of each industry and low organic pollution was recorded which are away from the source. The total score of these stations were more than 20 indicate high organic pollution. The study also reveals that the value of pollution index genera is always higher than the species (**Shaji and Patel, 1991**). In Palmer pollution index of cyanophycian genera *Occillatoria* was considered more tolerant than other genera of cyanobacteria. Thus the application of PPI indices shows a relationship to the status of pollution as well as evaluating the pollution load by using pollution tolerant genera and species. The study also reveals that among algae cyanophycian

algae are most suitable for bioremediation purposes since they are abundantly found in polluted sites (Saikia et. al. 2017).

Table: 4. Palmer Pollution Tolerant Genera of Cyanobacteria found in nearby areas of Digboi oil industry of Assam

Sl No	Algal Genera	PPI	PPI index at different sampling location		
			Site 1	Site 2	Site 3
1	<i>Oscillatoria</i>	4	+	+4	-
2	<i>Phormidium</i>	3	+3	-	+3
3	<i>Anabaena</i>	4	+4	+4	-
4	<i>Spirullina</i>	2	+2	-	+2
5	<i>Chroococcus</i>	-	-	-	-
6	<i>Chlorella</i>	4	+4	+	-
7	<i>Cosmerium</i>	3	+	+3	-
8	<i>Spirogyara</i>	1	+1	-	+1
9	<i>Ulothrix</i>	2	+	+2	+2
10	<i>Closterium</i>	1	+1	+1	-
11	<i>Navicula</i>	2	+2	+	+2
12	<i>Syndra</i>	1	+1	-	+1
13	<i>Pinnularia</i>	-	-	-	-
14	<i>Euglena</i>	3	+	+3	-
15	<i>Fischerella</i>	1	-	+	+1
16	<i>Phacus</i>	3	-	-	+3
17	<i>Gloeocapsa</i>	1	-	-	+1
18	<i>Nostoc</i>	2	-	+2	+
19	<i>Leibleinia</i>	1	+1	-	+1
20	<i>Pseudocapsa</i>	1	+1	+1	+
21	<i>Siphononema</i>	1	+1	-	+1
No. of Pollution Tolerant Genera			14	10	14
No. of Pollution Index Genera			11	7	11
Pollution Index for Algal Genera			21	20	18

Table: 5. Palmer Pollution Tolerant Genera of Cyanobacteria found in nearby areas of Pulp & Paper industry of Assam

SI No	Algal Genera	PPI	PPI index at different sampling location		
			Site 1	Site 2	Site 3
1	<i>Oscillatoria</i>	2	+2	+2	+
2	<i>Phormidium</i>	1	+1	+1	+
3	<i>Anabaena</i>	4	+4	-	+4
4	<i>Spirullina</i>	3	-	+	+3
5	<i>Chroococcus</i>	1	+1	+1	-
6	<i>Chlorella</i>	4	+4	+4	-
7	<i>Cosmerium</i>	2	-	+2	+2
8	<i>Spirogyara</i>	3	+3	-	+3
9	<i>Ulothrix</i>	1	+1	+1	+
10	<i>Closterium</i>	-	-	-	-
11	<i>Navicula</i>	2	+2	+2	+
12	<i>Syndra</i>	2	+2	-	+2
13	<i>Pinnularia</i>	1	+1	+1	+
14	<i>Euglena</i>	4	+	+4	-
15	<i>Fischerella</i>	-	-	-	-
16	<i>Phacus</i>	-	-	-	-
17	<i>Gloeocapsa</i>	1	+1	+1	-
18	<i>Nostoc</i>	3	+	-	+3
19	<i>Leibleinia</i>	-	-	-	-
20	<i>Pseudocapsa</i>	-	-	-	-
21	<i>Siphononema</i>	1	+1	+1	+1
No. of Pollution Tolerant Genera			12	10	12
No. of Pollution Index Genera			10	9	7
Pollution Index for Algal Genera			23	20	18

Table 6: Plamer Pollution Tolerant species of Cyanobacteria found in nearby areas of Namrup Fertilizer Factory, Assam

Sl. No	Algal Species	PPI			
			Site 1	Site 2	Site 3
1	<i>Oscillatoria chlorine</i>	4	+4	+4	+
2	<i>Oscillatoria princeps</i>	4	+4	+4	-
3	<i>Oscillatoria tenuis</i>	4	+	-	+4
4	<i>Oscillatoria amoena</i>	4	+4	+4	+
5	<i>Phormidium lucidum</i>	3	-	-	+3
6	<i>Anabaena naviculoides</i>	4	+4	-	+4
7	<i>Spirulina maior</i>	3	-	+3	-
8	<i>Chroococcus rufescens</i>	1	+1	+1	+1
9	<i>Chlorella vulgaris</i>	4	-	+4	+4
10	<i>Cosmerium undulatum</i>	2	+2	-	+
11	<i>Spirogyra porticalis</i>	3	+3	-	+3
12	<i>Navicula tripuncpata</i>	2	+	+	+2
13	<i>Syndra ulna</i>	2	+	+2	-
14	<i>Pinnularia aljustrelica</i>	3	+	+3	+
15	<i>Euglena acus</i>	4	+4	+	-
16	<i>Fischerella thermalis</i>	2	+	+	+2
17	<i>Phacus pleuronectes</i>	3	+3	+3	+
18	<i>Gloeocapsa atrata</i>	1	+	+	+1
19	<i>Nostoc commune</i>	2	+	+2	-
20	<i>Siphononema polonicum</i>	1	+1	+1	+
No. of Pollution Tolerant Species			17	15	15
No. of Pollution Index Species			11	11	9
Pollution Index for Algal Species			30	27	24

Table 7: Plamer Pollution Tolerant species of Cyanobacteria found in nearby areas of oil industry of Assam

Sl No	Algal Genera	PPI	PPI index at different sampling location		
			Site 1	Site 2	Site 3
1	<i>Oscillatoria chlorine</i>	4	+4	-	-
2	<i>Oscillatoria princeps</i>		-	+4	+4
3	<i>Oscillatoria tenuis</i>		-	-	+4
4	<i>Oscillatoria amoena</i>		+4	-	-
5	<i>Phormidium lucidum</i>	3	+	+3	-
6	<i>Anabaena naviculoides</i>	4	+4	+4	-
7	<i>Spirulina maior</i>	2	+2	-	+2
8	<i>Chlorella vulgaris</i>	4	+4	-	+4
9	<i>Cosmerium undulatum</i>	3	+3	+3	-
10	<i>Spirogyara porticalis</i>	1	-	+1	+1
11	<i>Ulothrix aequalis</i>	2	-	+2	+
12	<i>Closterium acerosum</i>	1	+1	+1	+1
13	<i>Navicula tripuncpata</i>	2	-	-	+2
14	<i>Syndra ulna</i>	1	+1	+1	+1
15	<i>Euglena acus</i>	3	+3	-	+3
16	<i>Fischerella thermalis</i>	1	+1	-	+1
17	<i>Phacus pleuronectes</i>	3	+	-	+3
18	<i>Gloeocapsa atrata</i>	1	+1	-	+1
19	<i>Nostoc commune</i>	2	+	+2	-
20	<i>Leibleinia baculum</i>	1	+1	-	+1
21	<i>Pseudocapsa dubia</i>	1	-	+	-
22	<i>Siphononema polonicum</i>	1	+	+	+1
No. of Pollution Tolerant Genera			16	11	15
No. of Pollution Index Genera			11	8	14
Pollution Index for Algal Genera			25	17	29

Table 8: Plamer Pollution Tolerant species of Cyanobacteria found in nearby areas of Pulp & Paper industry of Assam

Sl No	Algal Genera	PPI	PPI index at different sampling location		
			Site 1	Site 2	Site 3
1	<i>Oscillatoria chlorine</i>	2	+	+	+2
2	<i>Oscillatoria princeps</i>		+2	+2	+
3	<i>Oscillatoria tenuis</i>		+	+2	+2
4	<i>Oscillatoria amoena</i>		+2	-	-
5	<i>Phormidium lucidum</i>	1	+	-	+1
6	<i>Anabaena naviculoides</i>	4	+4	-	+4
7	<i>Spirulina maior</i>	3	+3	+3	+
8	<i>Chroococcus rufescens</i>	1	+	+1	+
9	<i>Chlorella vulgaris</i>	4	+	+4	+
10	<i>Cosmerium undulatum</i>	2	+2	-	-
11	<i>Spirogyra porticalis</i>	3	+3	+	-
12	<i>Ulothrix aequalis</i>	1	-	-	+
13	<i>Navicula tripuncpata</i>	2	+2	+	-
14	<i>Syndra ulna</i>	2	-	-	+2
15	<i>Pinnularia aljustrelica</i>	1	+	+	+1
16	<i>Euglena acus</i>	4	+4	-	+
17	<i>Gloeocapsa atrata</i>	1	+	+	+1
18	<i>Nostoc commune</i>	3	-	-	+3
19	<i>Siphononema polonicum</i>	1	+	+1	+
No. of Pollution Tolerant Genera			16	11	15
No. of Pollution Index Genera			8	6	8
Pollution Index for Algal Genera			22	13	16

Table 9: PPI (in average) of Different Genera of Cyanobacteria at different Industrial sites

	Fertilizer	Oil	Pulp & Paper
No. of Pollution Tolerant Genera	9.3	12.6	11.3
No. of Pollution Index Genera	6	9.6	8.6
Pollution Index for Algal Genera	16	19.6	20.3

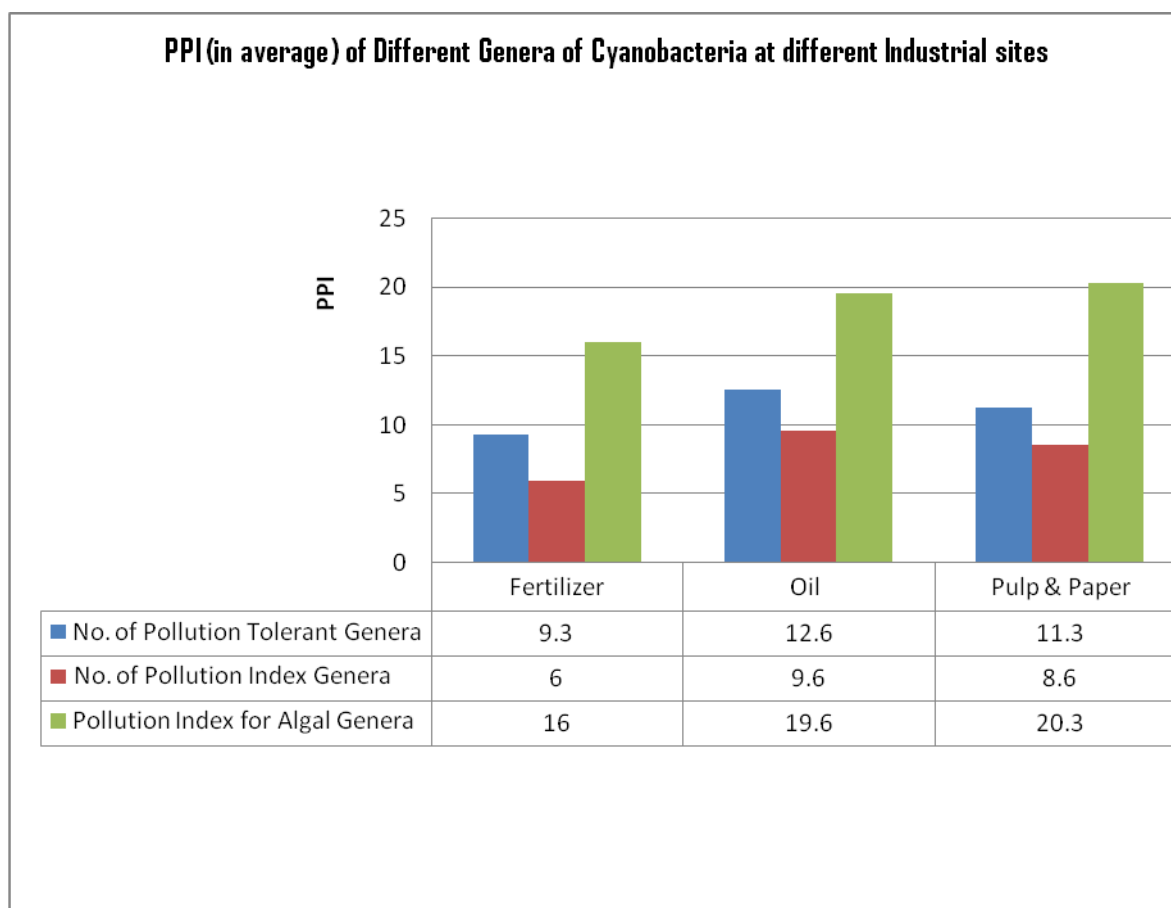


Table 10: PPI (in average) of Different Species of Cyanobacteria of different Industrial sites

	Pulp & Paper	Oil	Fertilizer
No. of Pollution Tolerant Species	15.6	14	14
No. of Pollution Index Species	10.3	11	7.3
Pollution Index for Algal Species	27	23.6	17

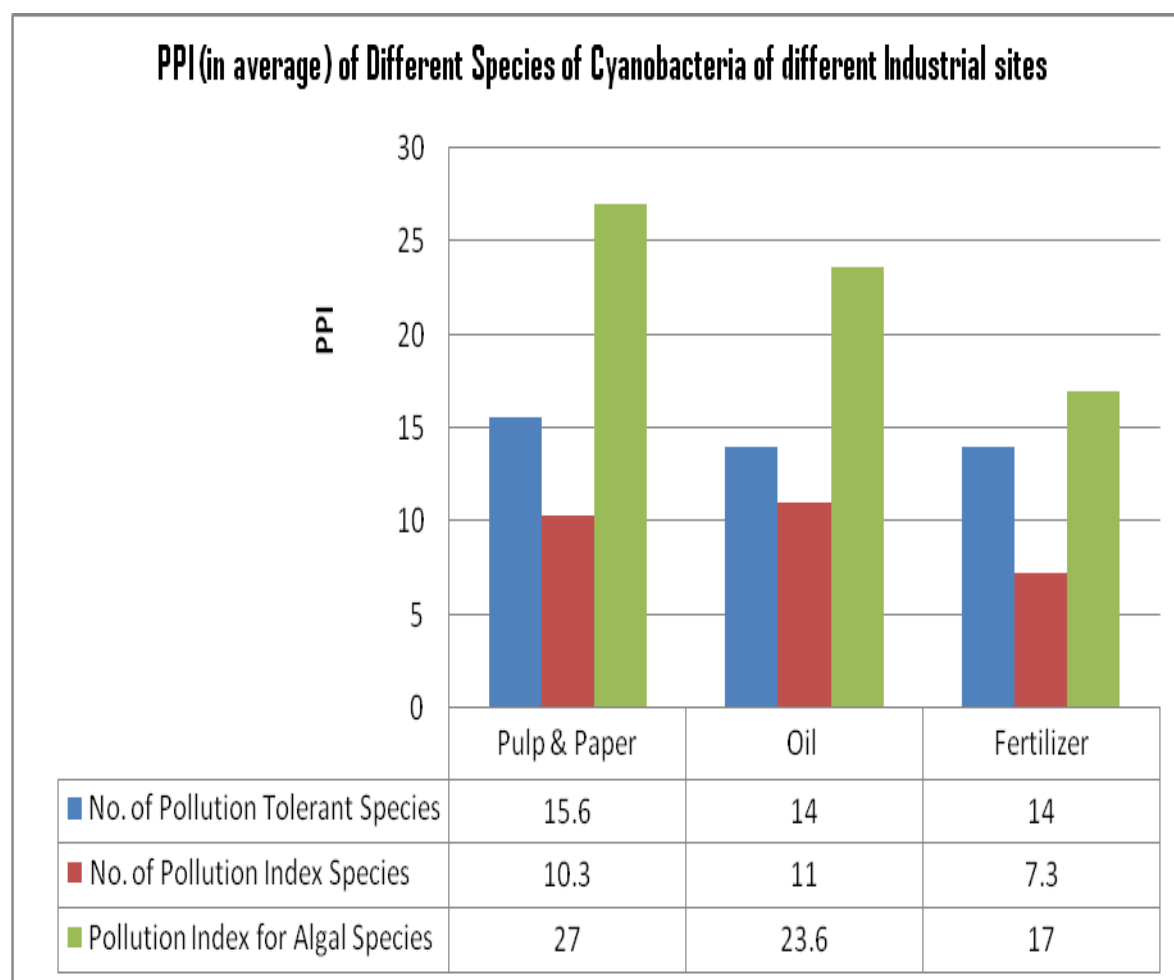


Table 11: Chemical characteristics of pulp & paper, oil and fertilizer effluents employed for algal bioassay and abatement studies.

Parameters	Value of Chemical characteristics used for bioassay		
	Pulp & Paper Industry	Oil Industry	Fertilizer Industry
Temperature	32	31	29
PH	8.9	8.2	10
Conductivity (mmho/cm)	155	148	122
Total suspended Solids (TSS) mg/L	4.05	3.9	3.7
Total Dissolved Solids (TDS) mg/L	1369	856	1048
Total Solids (TS)	1788	1100	1235
Turbidity (NTU)	124	89	150
Dissolve Oxygen (DO) (mg/mL)	0.62	2.2	1.9
Biological Oxygen Demand (BOD) mg/mL	418.00	310	28
Chemical Oxygen Demand (COD) mg.mL	594.00	215	140
Alkalinity, mg/mL	363	290	300
Total Nitrogen (TN), PPM	4.60	5.6	8.03
Total Phosphorous (TP) PPM	1.14	0.89	0.66
Sulphate, PPM	186	156	124
Total Hardness (PPM)	637	537	750

Fig:1: Graphical representation of different chemical parameters under study.

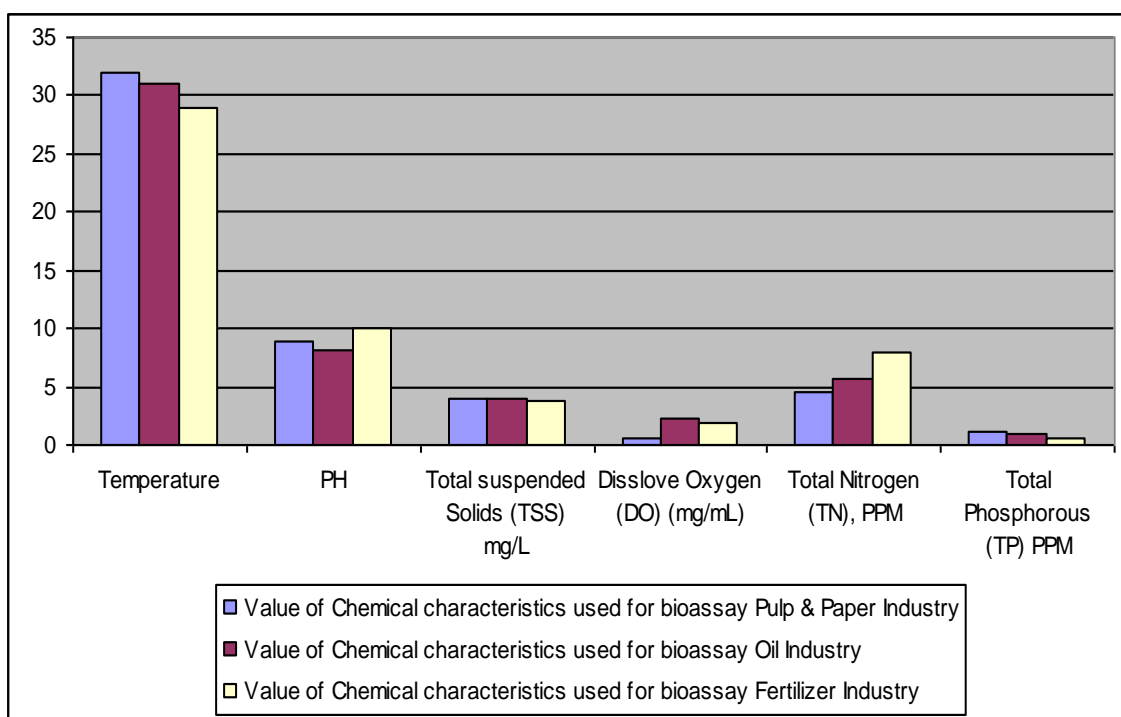
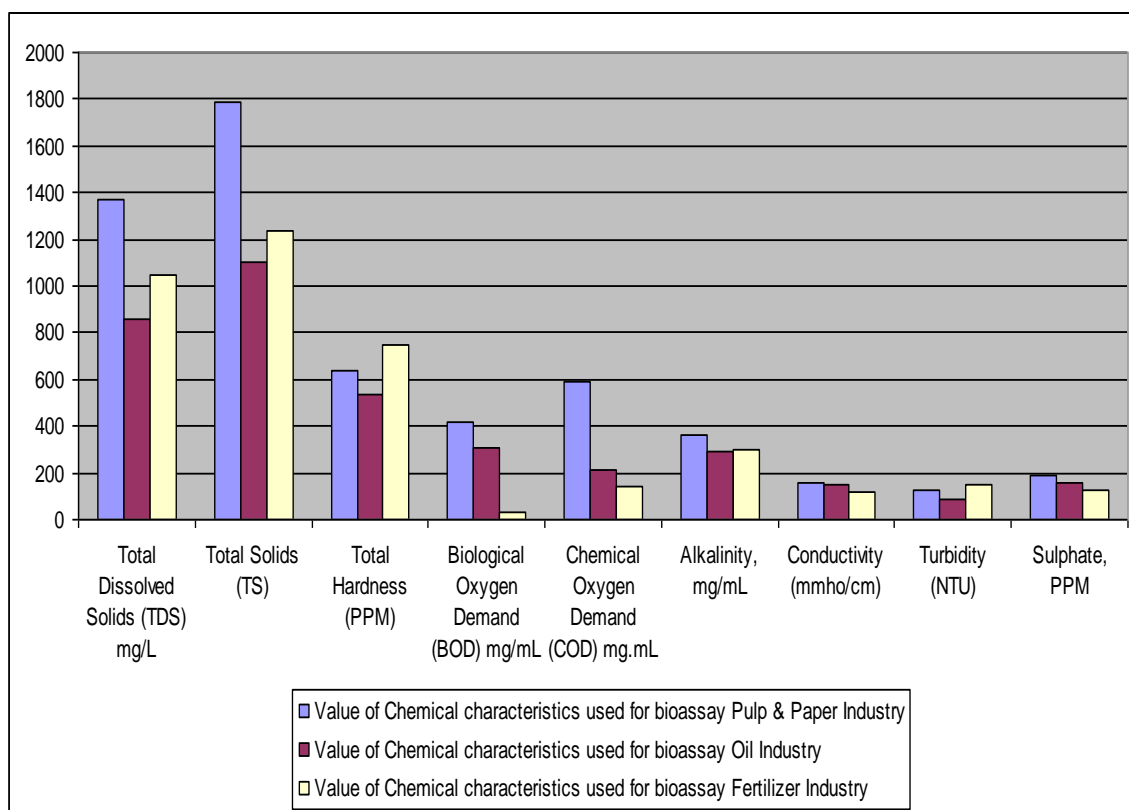


Fig:2: Graphical representation of different chemical parameters under study



○ **GROWTH RESPONSE**

The growth response of *Oscillatoria chlorina* Kuetz. Ex. Gomont, and *Anabaena sphaerica* Bornet Ex Born treated in culture media supplemented with different concentrations of Paper Mill effluent, Oil effluent and fertilizer effluent has been shown below, while their percentage stimulation (+) or inhibition (-) of growth in terms of Chlorophyll a, Carotene and Biomass content over the control at 21 (twenty one) days optimum growth phase period has been recorded. The individual growth response of algae treated at various concentrations of effluent over the control has been discussed below and given in the respective figures . The formula used to calculate the percentage (%) stimulation (+) or inhibition (-) of growth of algal species is given below. The formula used to calculate % stimulation and % inhibition over the control is adopted from Saikia et al., 2010 as given below:

$$I_i = \frac{\mu_c - \mu_i}{\mu_c} \times 100 \dots \dots \dots (1)$$

Where,

I_i = Inhibition (-) or Stimulation (+) of toxicant concentration

μ_c =Growth rate in control

μ_i = Growth rate in concentration

- The growth response of *Oscillatoria chlorine* Kutz ex. Gomont in basal medium supplemented with different percentage of pulp & paper, oil and fertilizer effluents at 665nm O.D has been shown in table 2A 2B & 2C .
- The growth response of *Anabaena sphaerica* Bornet Ex Born in basal medium supplemented with different percentage of pulp & paper, oil and fertilizer effluents at 665nm O.D has been shown in table 2D 2E & 2F .

Table 12: % Stimulation (+) and Inhibition (-) of chlorophyll 'a' & biomass content of *Oscillatoria chlorina* after 21 days optimal growth period treated with Pulp & paper mill effluent.

Effluent Conc. (%)	<i>Oscillatoria chlorina</i> Kuetz. Ex. Gomont					
	Chlorophyll a (mg/ml)			Biomass (mg/ml)		
	Mean	% (+)	% (-)	Mean	% (+)	% (-)
BM+0	1.51	-	-	1014.00	-	-
BM+20	2.02	+30.11	-	1260.00	+22.14	-
BM+40	1.53	+58.24	-	1439.00	+40.70	-
BM+60	2.73	+79.25	-	1698.00	+65.00	-
BM+80	2.92	+80.52	-	1766.00	+70.73	-
100%	1.06	-	-31.81	0758.00	-	-23.18

Table: 13. % Stimulation (+) and Inhibition (-) of chlorophyll 'a' & biomass content of *Oscillatoria chlorina* after 21 days optimal growth period treated with Oil industry effluent

Effluent Conc. (%)	<i>Oscillatoria chlorina</i> Kuetz. Ex. Gomont					
	Chlorophyll a (mg/ml)			Biomass (mg/ml)		
	Mean	% (+)	% (-)	Mean	% (+)	% (-)
BM+0	1.55	-	-	1024.00	-	-
BM+20	2.22	+29.11	-	1280.00	+21.14	-
BM+40	1.58	+51.24	-	1439.00	+40.70	-
BM+60	2.72	+63.25	-	1648.00	+65.00	-
BM+80	2.92		-25.81	1666.00	+65.73	-
100%	1.07	-	-35.81	0658.00	-	-20.18

Table: 14. % Stimulation (+) and Inhibition (-) of chlorophyll 'a' & biomass content of *Oscillatoria chlorina* after 21 days optimal growth period treated with Fertilizer industry effluent.

Effluent Conc. (%)	<i>Oscillatoria chlorina</i> Kuetz. Ex. Gomont					
	Chlorophyll a (mg/ml)			Biomass (mg/ml)		
	Mean	% (+)	% (-)	Mean	% (+)	% (-)
BM+0	1.55	-	-	1024.00	-	-
BM+20	2.22	+29.11	-	1280.00	+20.50	-
BM+40	1.58	+56.00	-	1439.00	+35.80	-
BM+60	2.72	+70.25	-	1648.00	+60.20	-
BM+80	2.92		-20.81	1666.00	+67.40	-
100%	1.07	-	-40.81	0658.00	-	-22.40

Table 15: Growth and Percent removal of COD (Average of three replications) by *Oscillatoria chlorine* treated with different industrial effluents

Treatment (Days)	Pulp & Paper Industry		Oil Industry		Fertilizer Industry	
	Growth (Dry wt., mg/100 ml)	% removal of COD	Growth (Dry wt., mg/100 ml)	% removal of COD	Growth (Dry wt., mg/100 ml)	% removal of COD
0	0.1	0.0	0.1	0	0.1	0
3	0.24	52	0.22	42	0.20	38
6	0.42	72	0.35	65	0.33	60
9	0.52	80	0.49	70	0.45	63
12	0.75	86	0.68	76	0.60	62

Fig3: Growth & Percent removal of COD by *O. chlorina* treated with different industrial effluents.

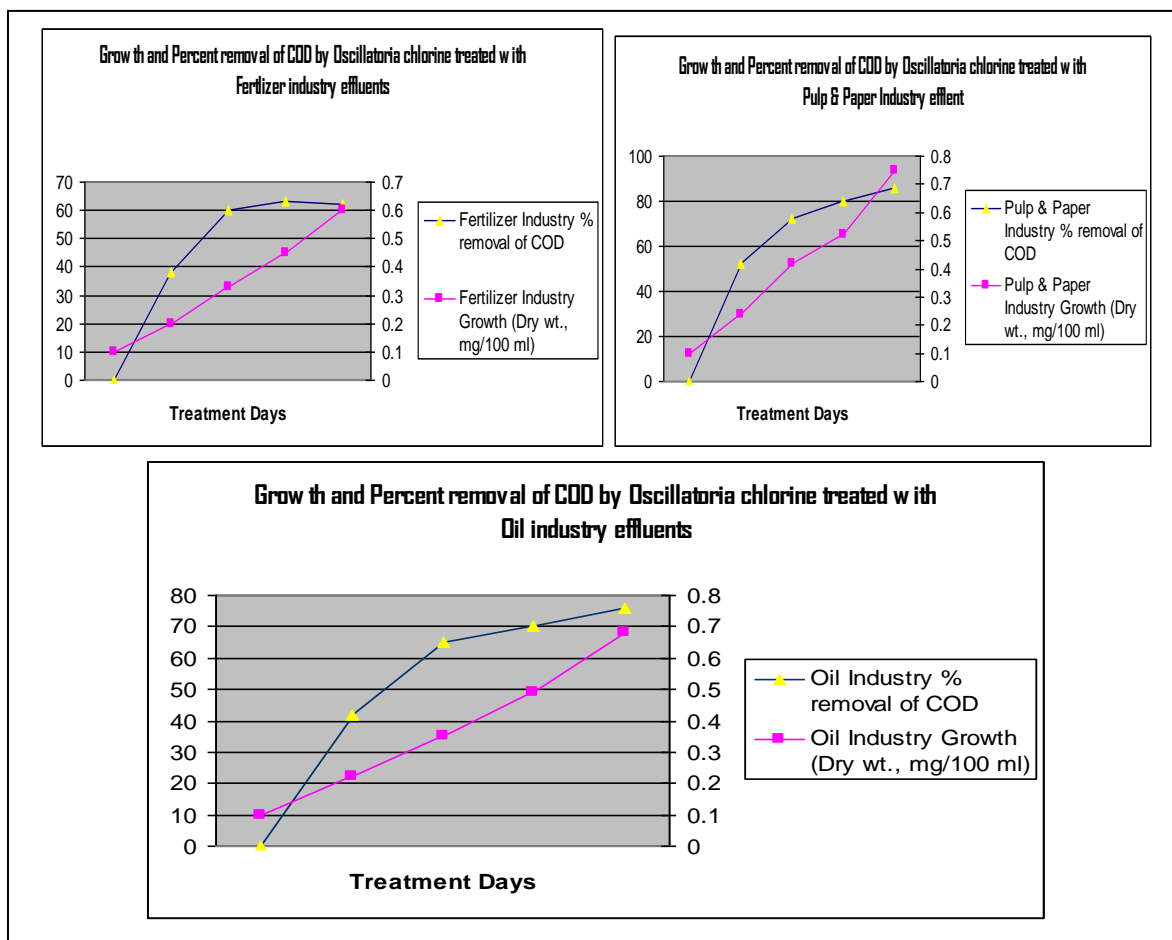


Table 16: % Stimulation (+) and Inhibition (-) of chlorophyll 'a' & biomass content of *Anabaena sphaerica* Bornet Ex Born after 21 days optimal growth period treated with Pulp & paper mill effluent.

Effluent Conc. (%)	<i>Anabaena Sphaerica</i> Bornet Ex Born					
	Chlorophyll a (mg/ml)			Biomass (mg/ml)		
	Mean	% (+)	% (-)	Mean	% (+)	% (-)
BM+0	1.45	-	-	998.00	-	-
BM+20	1.98	+28.25	-	1068.00	+18.00	-
BM+40	1.90	+48.00	-	1235.00	+32.60	-
BM+60	2.56	+65.25	-	1500.00	+55.00	-
BM+80	3.00	+68.00	-	1650.00	+67.67	-
100%	1.02	-	-34.00	685.00	-	-15.25

Table 17: % Stimulation (+) and Inhibition (-) of chlorophyll 'a' & biomass content of *Anabaena sphaerica* Bornet Ex Born after 21 days optimal growth period treated with Oil refinery effluent.

Effluent Conc. (%)	<i>Anabaena sphaerica</i> Bornet Ex Born					
	Chlorophyll a (mg/ml)			Biomass (mg/ml)		
	Mean	% (+)	% (-)	Mean	% (+)	% (-)
BM+0	1.33	-	-	788.00	-	-
BM+20	1.54	+26.24	-	1100.00	+18.00	-
BM+40	1.96	+48.00	-	1339.00	+38.00	-
BM+60	2.35	+58.39	-	1485.00	+59.00	-
BM+80	2.62		-24.00	1500.00	+59.59	-
100%	1.02	-	-32.25	0641.00	-	-17.00

Table 18: % Stimulation (+) and Inhibition (-) of chlorophyll 'a' & biomass content of *Anabaena sphaerica* Bornet Ex Born after 21 days optimal growth period treated with Fertilizer Effluent.

Effluent Conc. (%)	<i>Oscillatoria chlorina</i> Kuetz. Ex. Gomont					
	Chlorophyll a (mg/ml)			Biomass (mg/ml)		
	Mean	% (+)	% (-)	Mean	% (+)	% (-)
BM+0	0.99	-	-	1100.00	-	-
BM+20	1.95	+20.00	-	1154.00	+20.50	-
BM+40	2.00	+62.50	-	1255.00	+35.80	-
BM+60	2.63	+68.25	-	1422.00	+60.20	-
BM+80	2.72	+68.25	-	1502.00	+67.40	-
100%	1.22	-	-28.00	0956.00	-	-18.00

Table 19: Growth and Percent removal of COD (Average of three replications) by *Anabaena sphaerica* Bornet Ex Born treated with different industrial effluents.

Treatment (Days)	Pulp & Paper Industry		Oil Industry		Fertilizer Industry	
	Growth (Dry wt., mg/100 ml)	% removal of COD	Growth (Dry wt., mg/100 ml)	% removal of COD	Growth (Dry wt., mg/100 ml)	% removal of COD
0	0.19	0.0	0.14	0	0.20	0
3	0.28	35	0.18	37	0.25	25
6	0.35	52	0.25	55	0.45	48
9	0.50	68	0.54	79	0.59	62
12	0.68	68	0.71	82	0.75	57

Fig4: Growth & Percent removal of COD by *Anabaena* treated with different industrial effluents.

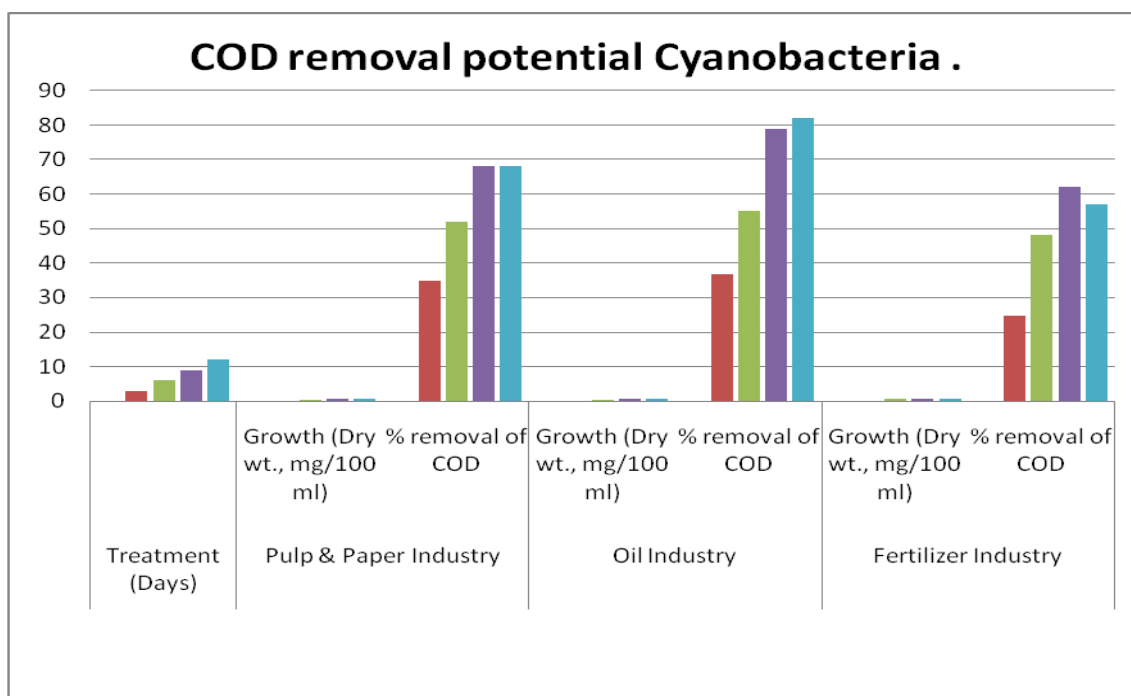
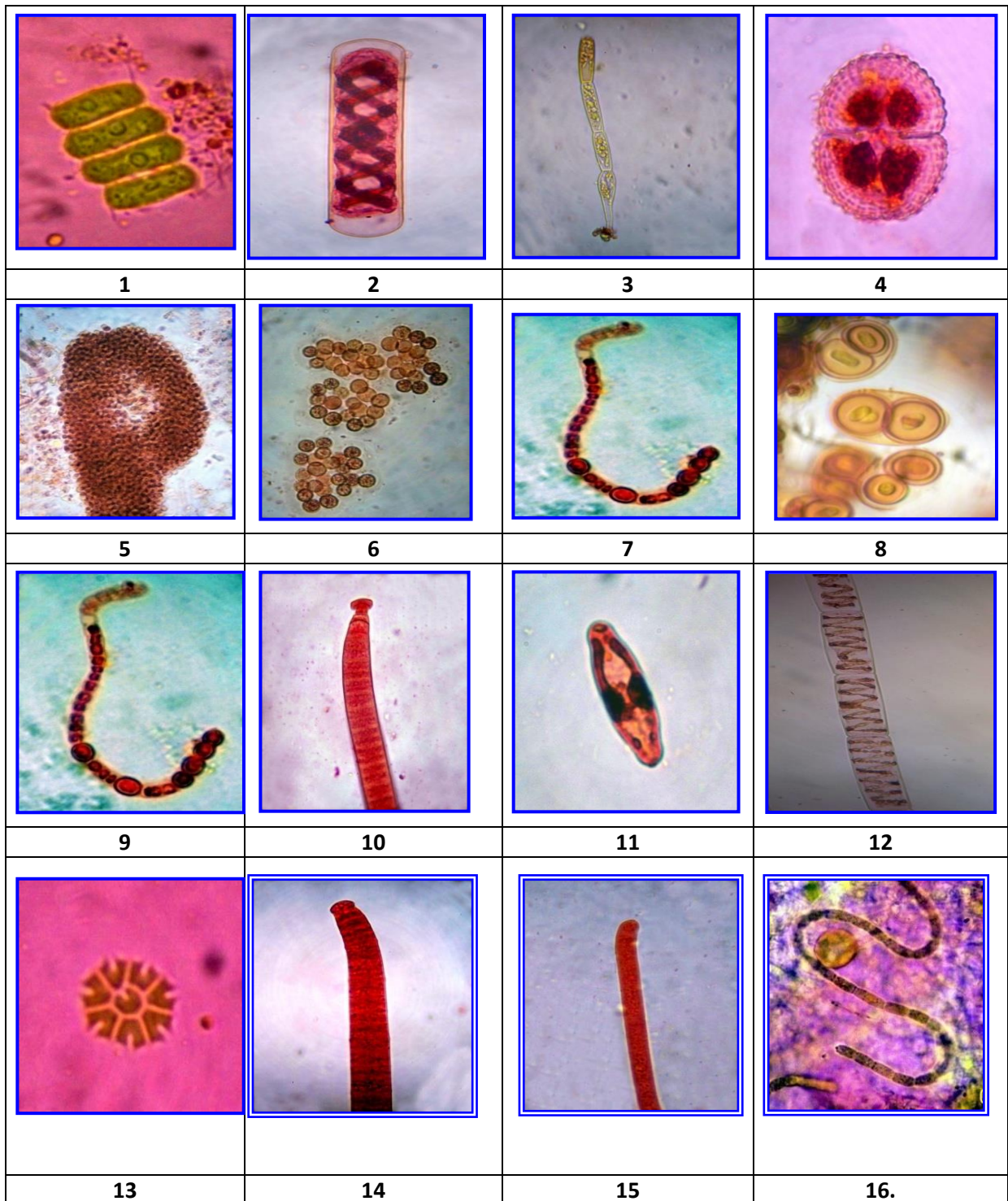
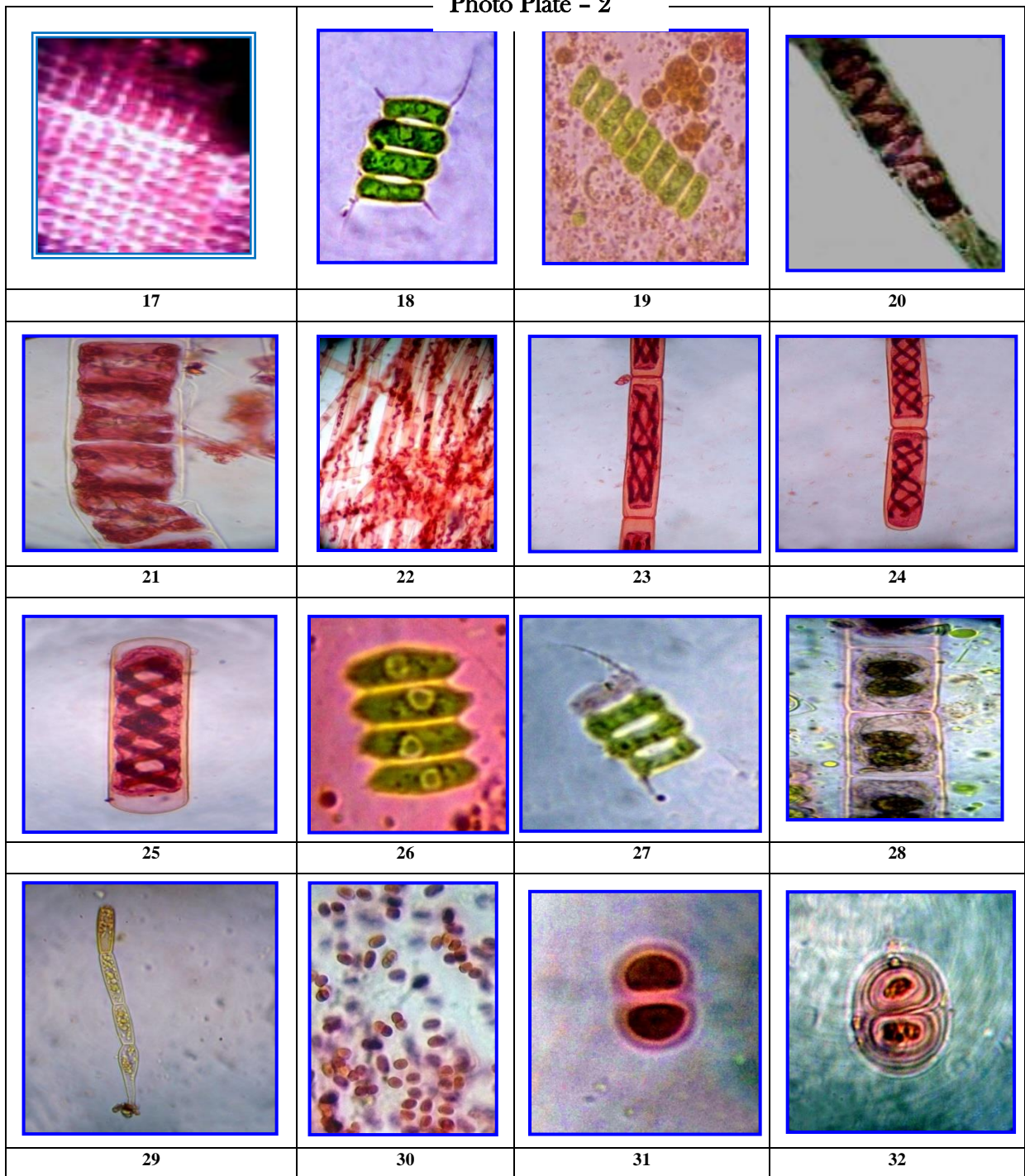


Photo Plate - 1



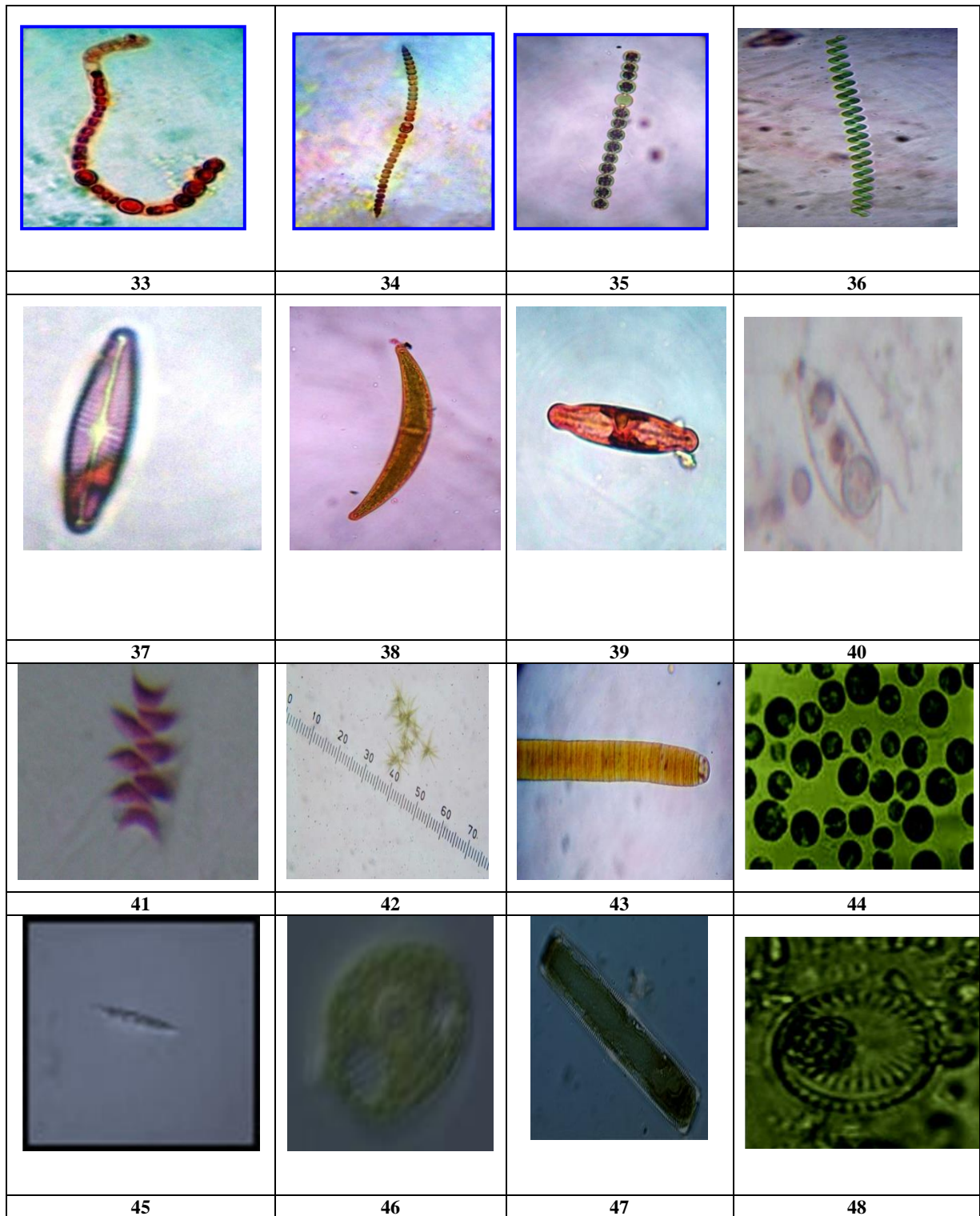
1. *Scendesmus quadricauda* Var. *westii* Smith 2 *Spirogyra neglecta* (Hassall) Kuetz. 3 *Ulothrix limnetica* Lemn 4 *Cosmarium botrytis* Menegh 5. *Microcystis robusta* (Clark.) Nygaard 6. *Microcystis lamelliformis* Holsinger 7 *Nostoc ellipso sporum* (Desm.) Rabenh..8. *Gloeocapsa decorticans* (A.Br.) Richter 9. *Anabaena sphaerica* Bornet Ex Born. et Flah 10. *Oscillatoria amoena* (Kütz.) Gomont 11. *Navicula andium* Frenguelli 12. *Spirogyra reticuliana* Randhawa 13. *Pediatrum tetras* (Her.) Ralfs.14. *Oscillatoria princeps* Gomont 15. *Oscillatoria chlorina* Kuetz 16. *Phormidium tenue* Gomont

Photo Plate - 2

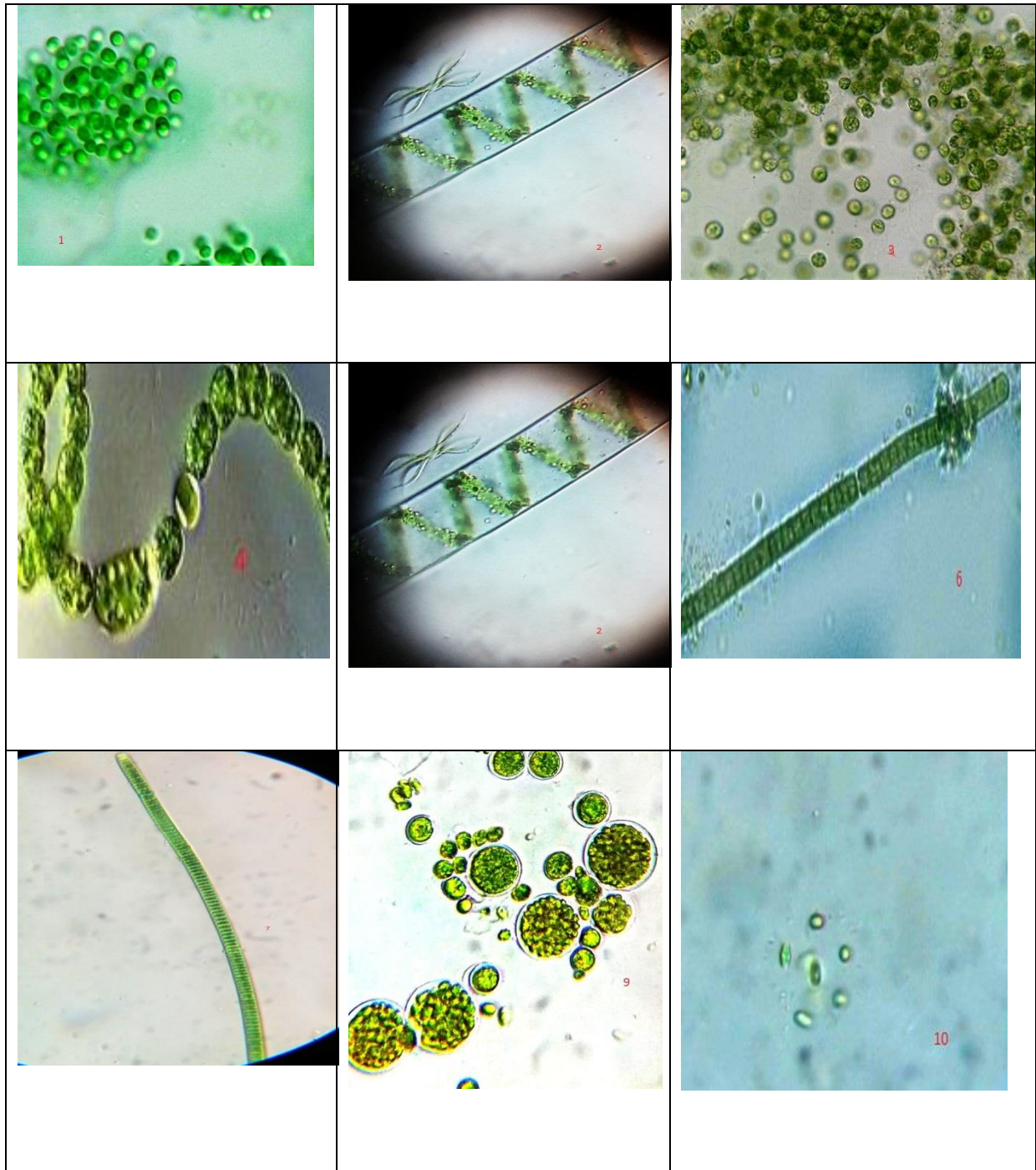


17. *Merismopedia punctata* Meyen 18. *Scenedesmus maculosus* Hortab 19. *Scenedesmus bijugatus* var. *parvus* Smith 20. *Spirogyra veleta* Norstedt 21. *Spirogyra sprecina* Rabenhorst 22. *Spirogyra skujae* Randhawa 23. *Spirogyra decimina* Kuetz 24. *Spirogyra fuellebornei* Schmidle 25. *Spirogyra neglecta* (Hassall)Kuetz 26. *Scenedesmus platydiscus* (Smith) Chodat 27. *Scenedesmus armatus* (Chodat) Smith 28. *Zygnemopsis jogensis* Iyenger 29.) *Ulothrix limnetica* Lemn 30. *Aphanothece microscopic* 31. *Chroococcus tenax* (Kirchn.) Hieron 32. *Gloeothece samoensis* Wille.

Photo Plate -3



33. *Nostoc ellipsosporum* (Desm.) Rabenh. Ex Born. et Flah 34. *Anabaena naviculoides* Fritsch 35. *Anabaena sphaerica* Bornet . 36. *Spirulina* sp. 37. *Navicula gracilis* Her. 38. *Closterium parvulum* 39. *Navicula* sp 40. *Chroococals* 41. *Scenedesmus* sp. 42. *Ankistrodesmus falcatus* Rafls 43. *Oscillatoria tenuis* 44. *Chlorella vulgaris* Beijer 45. *Euglena acus* 46. *Phacus* 47. *Pinnularia* sp. 48. *Cyclotella* sp.



PHOTOPLATE-IV : 1. *Aphanothece microspora*, 2. *Dactylococcopsis fascicularis*, 3. *Microcystis aeruginosa*, 4. *Nostoc sp.* 5. *Lyngbya sp.* 6. *Oscillatoria nigroviridis*, 7. *Oscillatoria sancta* 9. *Chlorococcum humicola*, 10. *Dictyosphaerium ehrengianum*



PHOTO PLATE-V : 11. *Pediastrum duplex*, 12. *Pediastrum simplex*, 13. *Microspora sp.* 14. *Scenedesmus hunanensis*, 15. *Scenedesmus quadricauda*, 16. *Scenedesmus armatus*, 17. *Coelastrum sphaericum*. 18. *Ankistrodesmus sp.* 19. *Ankistrodesmus falcatus*

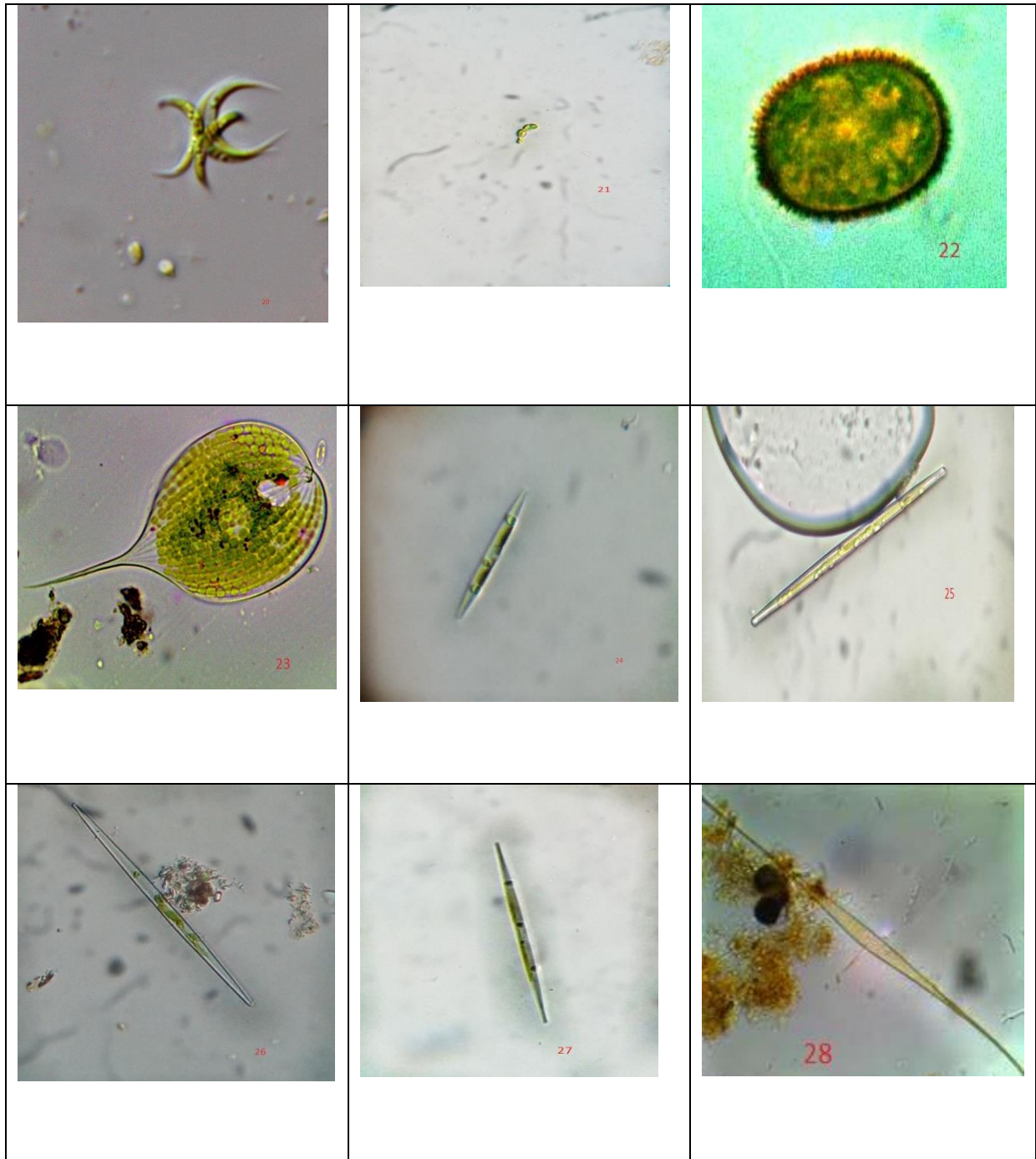


PHOTO PLATE VI: 20. *Selenestrum gracile*, 21. *Euglena elastic*, 22. *Trachelomonas planctonia*, 23. *Phacis sp.*, 24. *Nitzschia palea*, 25. *Nitzschia grandersheimiensis*, 26. *Nitzschia bacata*, 27. *Nitzschia speculum*, 28. *Nitzschia closterium*

Summary of Findings:

In the present study the algae particularly the Cyanobacteria (Blue Green algae) as per list of Palmer (1969) are shown in Table 4 & 5 according to decreasing trend of pollution at different sample sites (S1,S2 & S3) nearby the area of Pulp & Paper, Oil and Fertilizer industry. Out of 60 most pollution tolerant genera of Cyanobacteria 22, 20 & 17 are found in sample station S1, S2 & S3 of Pulp & paper industry; 20, 21 & 17 are found in oil industry and 21, 19 & 16 are found in the sampling sites of Fertilizer industry. Similarly out of 80 most pollution tolerant species 18, 20 & 16 are found in sample station S1, S2 & S3 of Pulp & Paper industry; 19, 17 & 16 are found near oil industry and in case of fertilizer industry these numbers are 18, 16 & 14.

The cyanobacterial flora of all three sampling stations around each industrial establishment was subjected to Plamer Pollution Index (1969) for rating pollution status. Out of 20 pollution index genera of algae 03, 03 & 02 and out of 20 pollution index species 07, 05 & 03 were recorded at station S1, S2 & S3 of Paper & Pulp industry. 03, 02 & 01 genera and 05, 04 & 03 species were found in the sampling station S1, S2 & S3 of oil industry whereas these values for fertilizer industry were recorded 03, 02 & 01 genera and 04, 03 & 01 species.

The results and the total score of each sample stations according to industry wise are given in **Table 4**. The results reveal that the pollution load of each sample site gradually decreases in downstream. The high organic pollution was recorded in nearby areas of each industry and low organic pollution was recorded away from the source. The total score of these stations were more than 20 indicate high organic pollution. The study also reveals that the value of pollution index genera is always higher than the species (**Shaji and Patel, 1991**). In Palmer pollution index of cyanophycian genera *Occillatoria* was considered more tolerant than other genera of cyanobacteria. Next to *O.Chlorina* *Anabaena* species occupy and important position in regards to tolerant level. Thus the application of PPI indices shows a relationship to the status of pollution as well as evaluating the pollution load by using pollution tolerant genera and species. The study also reveals that among algae cyanophycian algae are most suitable for bioremediation purposes since they are abundantly found in polluted sites when studied (**Saikia et. al. 2017**). An attempt was also made to use a few cyanobacterial spesces namely *O. Chlorina* and *Anabaena* species for assessment, evaluation and abatement of such effluents.

The result of physico-chemical parameters reflects the polluted nature of the effluents as well as of the water body of nearby sampling sites around each industrial establishment specially at S-1, S-2 and S-3 sampling stations. The nature of effluents was found to be alkaline and contained objectionable amount of DO, BOD, COD, ammoniacal nitrogen, nitrate nitrogen, sulphate, chloride, hardness and total alkalinity. From the result it is clearly evident that the physico-chemical status of Nagaon Paper Mill specially at station S-1 was being greatly affected by the effluents discharged from the Refinery. There was a gradual decreasing trend of pollution load among S1>S2>S3. Similarly decreasing trend of pollution was also recorded in case Oil and fertilizer factory from the source of origin. Significant positive correlation was found in between the effluents outlet and station S-1 for some of the important parameters such as conductivity, TDS, Ph, DO, BOD, COD, nitrate nitrogen, chloride, total hardness etc, responsible for pollution. The decreasing trend in the pollution load may be attributed partly to the dilution factor and partly to the effective utilization of the effluents by micro organisms including some tolerant species indicate that during monsoon season minimum pollution load in comparison to other three seasons.

The algal flora of the study sites were subjected these influencing physico-chemical factors also exhibited distinct zonation during the period of investigation. The effluent exhibited characteristic algal flora, Cyanobacteria, particularly members of the family Oscillatoriaceae & Nostocaceae was found to be most dominant group in the effluents in all sample sites around each industrial establishment. A horizontal variations of algal forms were observed at different sampling stations. The high pollution load at the nearby sampling sites in each establishment did not permit large number of algal species to thrive and only a few species of Cyanobacteria were encountered which obviously had profound tolerance to organic pollution. To assess the pollution status Palmer's Pollution Index (Palmer, 1969) was used. The total score of the stations greater than the value of 20 indicating high organic pollution. The trend of decreasing organic pollution towards downstream was observed and supported by physico-chemical findings.

Laboratory based bioassay experiments revealed that Cyanobacterial alga was more tolerant than the other green alga. The higher concentration of effluents was stimulatory for the growth of Cyanobacterial alga while inhibiting the growth of other alga. It was observed that it can significantly reduce the level of pollution in term of COD removal (**Table-15 & 19**).

-
- **In regards to Paper mill effluents**, there was a gradual stimulation of growth in terms of chlorophyll content of *O. chlorina* while treated with increasing paper mill effluent. 30.11 % stimulation in growth was observed at 20% effluent, 58.24% stimulation in growth was observed at 40%, 79.25% stimulation in growth at 60%, 80.52% stimulation in growth at 80% effluent concentration which is the highest stimulation (+). At 100% effluent concentration the growth declined upto -31.81%. Regarding biomass content the maximum stimulation 70.73% was recorded at 80% effluent concentration. At 100% effluent concentration the inhibition started upto -23.18%.

Similarly there was a gradual stimulation of growth in terms of chlorophyll content of *Anabaena sphaerica* Bornet while treated with increasing paper mill effluent. 28.25 % stimulation in growth was observed at 20% effluent, 48.00% stimulation in growth was observed at 40%, 65.25% stimulation in growth at 60%, 68.00% stimulation in growth at 80% effluent concentration which is the highest stimulation (+). At 100% effluent concentration the growth declined up to -34.00%. Regarding biomass content the maximum stimulation 67.67% was recorded at 80% effluent concentration. At 100% effluent concentration the inhibition started up to (-) 15.25 %.

- **In regards to oil industry effluent** the maximum stimulation (+) in terms of chlorophyll content of *O. Chlorina* (63.25%) was recorded at 60% effluent concentration and maximum inhibition (-) (35.81%) was recorded at 100 %. As regards to biomass the maximum stimulation (65.73%) was recorded at 80% effluent concentration. The inhibition(-) of *O. chlorina* in terms of biomass content was maximum (20.18%) at 100% effluent concentration.

Similarly the maximum stimulation (+) in terms of chlorophyll content of *Anabaena sphaerica* Bornet (58.39%) was recorded at 60% effluent concentration and maximum inhibition (-) (32.25%) was recorded at 100 %. As regards to biomass the maximum stimulation (59.59%) was recorded at 80% effluent concentration. The inhibition(-) of *Anabaena* in terms of biomass content was maximum (18.00%) at 100% effluent concentration.

- **In regards to fertilizer industry effluent** the maximum stimulation (+) in terms of chlorophyll content of *O. Chlorina* (70.25%) was recorded at 60% effluent concentration and maximum inhibition (-) (40.81%) was recorded at 100 %. As regards to biomass content the maximum stimulation (67.40%) was recorded at 80% effluent concentration. The inhibition of *O. chlorina* in terms of biomass content was maximum (22.40%) at 100% effluent concentration.

Similarly the maximum stimulation (+) in terms of chlorophyll content of *Anabaena sphaerica* Bornet (68.25%) was recorded at 80% effluent concentration and maximum inhibition (-) (28.00%) was recorded at 100 %. As regards to biomass the maximum stimulation (67.40%) was recorded at 80% effluent concentration. The

inhibition(-) of *Anabaena* in terms of biomass content was maximum (18.00%) at 100% effluent concentration.

- **For abatement studies** the growth of *O. chlorina* and percent removal of COD was plotted in Fig:3. The figure clearly indicated that the growth and COD removal showed liner correlation. Highest of 86% COD removal against 0.75 mg/100ml growth of test alga was observed in Pulp and Paper industry effluent in 12 days incubation period. In case of Oil industry effluent it was observed 76% COD removal and 0.68 mg/100ml respectively with a treatment for 12 days. Whereas in case of fertilizer industry the highest value was observed at 12 days of treatment which was 62% removal of COD against 0.62 mg/100ml.

Similarly in case of *Anabaena Spherica Bornet* the percent removal of COD was plotted in Fig:4. The figure clearly indicated that the growth and COD removal showed liner correlation. Highest of 68% COD removal against 0.68 mg/100ml growth of test alga was observed in Pulp and Paper industry effluent in 12 days incubation period. In case of Oil industry effluent it was observed 82% COD removal against 0.71 mg/100ml respectively with a treatment for 12 days. Whereas in case of fertilizer industry the highest value was observed at 12 days of treatment which was 57% removal of COD against 0.75 mg/100ml.

- The effect of effluents on the test alga was highly species specific. The *O. chlorina*, a cyanobacteria can tolerate higher percentage of PME effluent as compared to oil and fertilizer effluent. The enhancement of growth of *O. chlorina* in effluents also explains the results of field observation that the abundance is much higher in the nearby areas of PME than that of other two industries concerned. The growth inhibition of *O. chlorina* in oil effluent is mainly due to the presence of hydrocarbon and degraded products of oil (*Soto. et. al 1975 b*).
- The *O. chlorina* Kuetz & *Anabaena Spherica Bornet*, a pollution indicator species has immense value for future pollution abatement programme. In the present investigation, the both alga was used to test the capacity to remove the level of COD (**Fig 3 & 4 Table 15&19**). It was found that both can significantly reduce the level of COD. This study exhibit that certain cyanobacteria can be used for bioremediation. Also for assessment, evaluation and abatement of pollutants. This finding is in close with the observation of Rana and Palaria (1991).

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Impact of Pollution on Diversity of Cyanobacteria in Nearby Areas of Pulp, Oil and Fertilizer Factories of Assam (India)

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Abstract

Diversity study of Algal community particularly Cyanobacteria in terms of influential effluents released by pulp and paper, oil and fertilizer factory of Assam was conducted with a view to find out its impact on distributional pattern of Cyanobacteria according to increasing and decreasing trend of pollution load from the source. Palmer applied for rating the genus and species of Cyanobacteria. The results revealed that palmer's pollution tolerant index for algal genera and species was highest in paper mill area followed by oil and fertilizer. Similarly the number of pollution tolerant genera and species of Cyanobacteria was recorded highest in S1 and lowest in S3 which signify the higher pollution load in nearby area and lowest in the areas situated at long distance from the sources.

Keywords: Cyanobacteria; Palmer (1969); Pollution indicator; Genus; Species

Introduction

Every process on the earth needs water; industries consume a lot too. Industries, during the processing or manufacturing of products, generate waste materials and useless by products. Most of these waste materials ultimately goes to water without treatment or partially treated [1]. One of the major environmental problems in our water bodies is the disposal of untreated or partially treated effluent and sewage, as these discharges may result in deterioration of water quality. The pulp and paper industry, oil industry and fertilizer industries are among the major source of water pollution. Of the variety of organisms inhabiting water bodies, algae are one of the most suitable organisms for water quality assessment owing to their small size and quick reaction to pollutants [2]. Widespread distribution of algal flora all over the world as compared to others is an important feature for their adaptation in water quality assessment [3]. Many workers had studied the diversity of Cyanobacteria in polluted habitats to name a few are Subramaniyam et al. Srivastava and Singh, Sudhakar et al. [4-6].

A large number of water pollution indices have been developed in recent times which can provide the numerical information about the physical and chemical nature of environment. Among these the algal indices are easiest and provide quickest response to situations as biological indicator of pollution. Palmer [1] developed and designed two indices based on algal data found in organically polluted water. He prepare a list of 60 most tolerant genera and 80 species in order of decreasing order which popularly known as Palmer's Algal Genus Index (PPI-G) (Table 1) and Palmer's Algal Species Index (PPI-S) (Table 2). In

Table 1: Pollution Index of algal genera [1]. Table with 4 columns: Genera, Pollution Index, Genera, Pollution Index. Rows include Anacystis (Microcystis), Ankistrodesmus, Chlamydomonas, Chlorella, Cyclotella, Chlosterium, Euglena, Gomphonema, Lepocincis, Melosira, Microactinium, Navicula, Nitzschia, Oscillatoria, Pandorina, Phacus, Phormidium, Scenedesmus, Stigeoclonium, and Synedra.

Table 1: Pollution Index of algal genera [1].

Table 2: Pollution Index of algal species [1]. Table with 4 columns: Algal Species, Pollution Index, Genera, Pollution Index. Rows include Ankistrodesmus falcatus, Arthrospira jennneri, Cyclotella meneghiniana, Chlorella vulgaris, Euglena viridis, Nitzschia acicularis, Melosira varians, Stigeoclonium tenue, Synedra ulina, Nitzschia palea, Oscillatoria chlorina, Oscillatoria limosa, Oscillatoria princeps, Oscillatoria putrida, Oscillatoria lunata, Pandorina morum, and Scenedesmus quadricauda.

Table 2: Pollution Index of algal species [1].

the present study these two indexes was applied. The algae found and identified in the sample sites of three different factories were given and assigned mark separately for genus and species depending on relative tolerance from the list table noted below. The total score obtained from assigned species from each genus and species from each station. Palmer [1] developed a pollution index scale which is given in the Table 3.

Results and Discussions

In the present study the algae particularly the Cyanobacteria (Blue Green algae) as per list of Palmer [1] are shown in Table 4. According to decreasing trend of pollution at different sample sites (S1, S2 and S3) nearby the area of pulp and paper, oil and fertilizer industry. Out of 60 most pollution tolerant genera of Cyanobacteria 22, 20 and 17 are found in sample station S1, S2 and S3 of pulp and paper industry; 20, 21 and 17 are found in oil industry and 21, 19 and 16 are found in the sampling

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Received August 30, 2017; Accepted January 12, 2018; Published January 19, 2018

Citation: Saikia MK, Baruah R, Kafaki M (2018) Impact of Pollution on Diversity of Cyanobacteria in Nearby Areas of Pulp, Oil and Fertilizer Factories of Assam (India). J Pollut Eff Cont 6: 209. doi: 10.4176/2375-4397.1000209

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Indexed, Abstracted and Cited: ISRA Journal Impact Factor, International Impact Factor Services (IIFS), Directory of Research Journals Indexing (DRJI), International Institute of Organized Research and Scientific Indexing Services

World Journal of Biology and Medical Sciences

Published by Society for Advancement of Science*

ISSN 2349-0063 (Online/Electronic)

Volume 1, Issue- 3, 15-20, July - September 2014



WJBMS 1/3/06/2014

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A Double Blind Peer Reviewed Journal

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RESEARCH PAPER

Received: 12/06/2014

Revised: 31/07/2014

Accepted: 01/08/2014

A Study on Impact of Effluents of Paper Mill on Plants with Special Reference to Chlorophyll Content

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ABSTRACT

Pollution is a product of the activity of man. The pollutants contaminate air, water and soil. Field studies reveal that pollutants, generated at urban industrial locations, may disperse 50-100 km distance or more from the point of origin. Extensive field survey laboratory investigation has been carried out to study the effect of pollutants in and around industrial complexes of Nagaon Paper Mill (NPM) at Nagaon District of Assam. It gave an idea about the possible affect of the effluent released from the mill on the growth of the nearby plants. The left samples which were collected are subjected to chlorophyll estimation, done in the Lab of Biotech Hub, Dhing College, Nagaon, Assam. The amounts of chlorophyll a, b and carotenoids in terms of mg/g fresh leaves were calculated. The highest reduction of chlorophyll content is observed in tree is *Ficus infectoria* Roxb (1.63) and lowest in *Grewia asiatica* (4.22). In case of herb species it is highest in *Ipomoea batatas* (1.710 and lowest in *Phyllanthus niruri* (4.48).

Key words: Industrial effluents, Chlorophyll, Carotenoids, *Ficus infectoria* Roxb, *Ipomoea batatas*, *Phyllanthus niruri* and *Grewia asiatica*.