TROPHIC STATE AND PHYTOPLANKTON BLOOMS OF SHALLOW TROPICAL PONDS- A CASE STUDY FROM PALLIPPURAM, KERALA

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By

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Ph.D. Thesis under the Faculty of Environmental Studies

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Certificate

This is to certify that the thesis entitled **"Trophic state and phytoplankton blooms of shallow tropical ponds- a case study from Pallippuram, Kerala"** is a bonafide record of research carried out by Ms. Dhanya S. under my guidance and supervision in partial fulfilment of the requirements for the degree of Doctor of Philosophy under the Faculty of Environmental Studies, Cochin University of Science and Technology and that no part thereof has been included for the award of any other degree. All the relevant corrections and modifications suggested by the audience during the pre-synopsis seminar and recommended by the Doctoral committee of the candidate has been incorporated in the thesis.

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Declaration

I hereby declare that the thesis entitled **"Trophic state and phytoplankton blooms of shallow tropical ponds- a case study from Pallippuram, Kerala"** is an authentic record of research work carried out by me under the guidance of Dr. Ammini Joseph, Professor, School of Environmental Studies, Cochin University of Science and Technology in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy under the Faculty of Environmental Studies, Cochin University of Science and Technology and no part of this thesis has been submitted for the award of any degree, diploma, associateship, or any other title or recognition from any University/ Institution.

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Dhanya S.

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List of Abbreviations

HAB	Harmful Algal Bloom
SAV	Submerged Aquatic Vegetation
TSI	Trophic State Index
SD	Secchi disc
TP	Total Phosphorus
TN	Total Nitrogen
Chl	Chlorophyll
EC	Electrical Conductivity
DO	Dissolved Oxygen
BOD	Biochemical Oxygen Demand
NO_2-N	Nitrite-nitrogen
NO_3-N	Nitrate-nitrogen
NH_4-N	Ammonia-nitrogen
SRP	Soluble Reactive Phosphorus
ANOVA	Analysis of Variance
PRM	Pre-monsoon
POM	Post-monsoon
PCA	Principal Component Analysis
DA	Discriminant Analysis
A.u	Arbitrary Unit
C1	Component 1
C2	Component 2
C3	Component 3
C4	Component 4

Chapter 1

INTRODUCTION AND REVIEW OF LITERATURE

Contents

1.1 Introduction

Ramsar convention- an intergovernmental treaty for the conservation and sustainable utilization of wetlands- had adopted some strategic plans to encourage the use of regional wetlands directories. The Wetlands International report also draws attention to certain wetland types for which inventory data is clearly lacking. According to the report, the priority wetlands include ponds in the category 'artificial wetlands'. According to the Ramsar definition ponds include farm ponds, stock ponds and small tanks generally below 8 ha.

Ponds are shallow bodies of standing water with muddy or silty bottom allowing light to penetrate the entire water column (Caduto, 1990). According to Das and Mukhopadhyay (2015) "Ponds are standing water bodies having a seasonal fluctuation, basically excavated or embanked in nature, formed by natural or man- made processes". The National pond survey guidelines, UK defined ponds as "water bodies between 1m^2 and 2 ha which may be permanent or seasonal, including both man-made and natural water bodies".

Natural ponds are formed by the activities of rivers, streams, glaciers etc. Most of the natural ponds are small and shallow having a depth less than half a metre (Biggs *et al.*, 1994). They are found in almost all types of landscapes ranging from mountainous regions to coastal areas (Wood *et al.*, 2003).

Based on the residence time of water, ponds are classified into two types: permanent or perennial ponds which hold water year round, and temporary or seasonal ponds which dry out during summer and will be active only during the wet seasons. Community structure in a pond ecosystem is connected with its hydro-period.

Man-made ponds are classified into six types based on their mode of construction (NAERLS, 1999).

- Earthen ponds (dugout ponds): Dugout ponds are created by excavating the soil and allowing water to fill. These excavated ponds are generally constructed in flat terrains.
- Embankment ponds: They are constructed above the ground surface with concrete walls, usually in sloping terrains by constructing a dam between two hillsides to collect and hold water from overland runoff.
- Barrage ponds: Barrage ponds are constructed by creating a wall across a river or stream in a low valley.
- Diversion ponds: Ponds maintained by supplying water through a diverted channel from a river or stream.

- Rosary ponds: A series of ponds constructed and interconnected with each other by draining water from one another, and managed as a single unit are rosary ponds.
- Parallel ponds: These are ponds located in an area having its own inlet and outlet and are commonly used for rearing fish.

From ancient time onwards ponds have been used for religious purposes like ritual practices and offerings of water, and it still prevails in every corner of India (Rees, 1997). According to the Hindu mythology and religious views, temple ponds are considered sacred, and are well protected from external disturbances even today. Some religious ponds in India are used for holy dips and immersion of idols (Pareek *et al.*, 2016). People in villages were very much dependent on the ponds for various needs such as fishing, irrigation, and other domestic purposes (Bhagyaleena and Gopalan, 2012). Other than short term services ponds perform long term ecological functions such as maintaining the natural water regime, water purification, function as an effective carbon sink, mitigation of flood etc. (Keddy *et al.*, 2009). Ponds play a potential role in rain water harvesting and ground water recharge, thereby contributing to the overall maintenance of ground water level (Bhagyaleena and Gopalan, 2012). Hence they provide sustainable solution to various water management issues (Cereghino *et al.*, 2014).

Ponds reflect local natural variations in geology, hydrology, climate and vegetation (Biggs *et al.*, 2005). Pond hydro-period is mainly determined by their size and volume (Razgour *et al.*, 2010). Ponds are an exception to the bio-geographic principle of larger areas supporting more species diversity. So regardless of their size and area, ponds have potential conservation value (Oertli *et al.*, 2002). Ponds were neglected and remained unnoticed in the scientific world for a long time since they are smaller in size compared to lakes and rivers (Williams *et al.*, 2004 and Boix *et al.*, 2012). Despite their smaller size and depth they overbid the lakes for almost all biological parameters (Teissier *et al.*, 2012). Ponds actually magnify the processes that take place in lakes with a greater amount of physico-chemical variables and nutrient load per unit volume that have a direct bearing on phytoplankton production (Wetzel, 2001 and Teissier *et al.*, 2012).

Ponds can be of many kinds but generally they are shallow enough for the rooted plants to grow even in the deepest part. The abundance of plants ensures plentiful oxygen in the day time. Temperature fluctuations in ponds are rather wide (Clegg, 1996). Pond ecosystem can be divided into four habitats. These are surface film habitat, open water habitat, bottom habitat and the littoral habitat. The surface film habitat located on the pond water surface is a frontier between air and water. Surface film residing communities include air-breathing insects and animals. They are specially adapted to live on the surface film with light weight, long legs and water repellent hairs. They walk over the surface of the pond water without breaking through. The open water habitat is inhabited by suspended and free swimming organisms such as plankton and nekton. The littoral habitat is the peripheral edges of the ponds where the rooted plants grow. Littoral vegetation is often dominated with rooted emergent plants and also with floating and submerged plants (Hoverman *et al.*, 2012). The characteristic animals in ponds are that live on the bottom and among the plants. The bottom habitat supports less number of organisms even though food is available in the form of decaying vegetation and animal remains. The deficiency of oxygen in the bottom mud floor allows only organisms that can thrive in the adverse conditions (Clegg, 1996).

Ponds are nevertheless a miniature form of lakes. It has a unique ecology. Ecology of a pond or any other ecosystem is concerned with the interactions between the living things and their abiotic environment (Mustapha and Omotosho, 2002). The abiotic frame that a pond provides for the organisms is unique for each pond. Biological production in ponds is a dynamic process involving nutrient uptake, incorporation and recycling (Knud-Hansen *et al.*, 1998). Photosynthetic plants and autotrophic bacteria are the producers of organic matter in a pond (Caduto, 1990). Photosynthetic plants include phytoplankton, rooted and floating plants. Phytoplankton are distributed throughout the water body since light penetrates almost the entire depth of the shallow ponds. When in abundance they impart a greenish colour to the water. The energy flow in a pond ecosystem starts from phytoplankton and other rooted green plants and pass through different organisms along the food chain**.** Primary consumers like zooplankton and benthos feed on the producers and are consumed by the secondary consumers like nekton. Intense predation by secondary consumers leads to minimal zooplankton grazing favouring proliferation of phytoplankton. In contrast the same pond without fish population will have active zooplankton grazing and will be marked with the dominance of macrophytes and periphyton over phytoplankton. Any ecosystem is incomplete without the decomposing organisms. Decomposers like bacteria, fungi, and flagellates-which degrade the dead remains of plants and animals -will be distributed throughout the water body; but their presence is especially found in the mud-water interface (Odum and Barrett, 2004).

1.2 Review of literature

Numerous studies were undertaken especially in the European countries on the ecology, diversity and conservation values of permanent as well as temporary ponds.

Ruggiero *et al.* (2003) studied the eutrophic ponds with macrophyte vegetation in the mountain ranges of Italy. He tried to decode the nutrient and chlorophyll patterns with the trophic condition and general ecology of the ponds. Williams *et al.* (2004) made a comparative study on the biodiversity of rivers, streams, small ditches and ponds in the agricultural landscape of British country side. Their study revealed that most of the biodiversity at the regional level was contributed by ponds. The study points out the considerable variation of species richness in individual ponds. Ditches were reported with the least biodiversity even though they were observed with some rare species.

Biggs *et al.* (2005) presented a detailed progress on the pond conservation programme from the initiative of the 'Foundation of Pond conservation' a UK based NGO. It is an assessment report on the fifteen years pond monitoring and conservation programmes in UK.

A PSYM method (Predictive System for Multimetrics) was developed by Environmental agency of England and Wales jointly for the assessment of the biological quality of still waters. The PSYM method consolidates the metrics on aquatic plants and invertebrate species to form a single representative value for the overall quality of the water body (Biggs *et al.*, 2005).

De Meester *et al.* (2005) proposed the use of ponds as model systems for large scale surveys and hypothesis testing for experiments. This will help in tackling the complexities in ecology and evolutionary biology and to resolve the key issues involved in the management of biodiversity. Scheffer *et al.* (2006) put forward the concept that smaller habitat like isolated ponds promotes more species richness than large and connected ecosystems like rivers and streams.

According to Zacharias *et al.* (2007) temporary ponds are as important as that of permanent ponds. Temporary ponds differ from permanent ponds in hydro chemical properties and biodiversity. They studied the status of Mediterranean ponds, the threats they face and envisaged necessary conservation and management strategies for conservation of temporary ponds in the region.

Cereghino *et al.* (2008) tried to sum up the scientific problems that are faced in understanding the ecology of ponds and highlighted the need for their protection. The article also deciphers the research areas that can be further investigated. Miracle *et al.* (2010) in his article consolidated main topics presented in the sessions of third biennial meeting in Spain (Valenia) with the theme ‘ Pond conservation from science to practice’ organised by European pond conservation Network (EPCN) and it covered the areas of pond ecology, conservation and management, and temporary ponds as a biodiversity hotspot. The faunal and floral communities of 51 ponds in northern England were characterised by Hassall *et al.* (2012). Each of these ponds was surveyed in 1995-1996 for invertebrates and plant communities.

The ecological role that ponds play in the developing world was discussed by Cereghino *et al.* (2014). He recognised the importance of connected networks of ponds in playing vital role in providing new climate space as a response to the global climate change. Gallego *et al.* (2014) studied the diversity of three categories of primary producers in pond ecosystem, phytoplankton, filamentous green algae and submerged macrophytes in 87 ponds in southern Spain. α, β, and δ diversity of these functional groups were studied in detail. Environmental factors contributing to the diversity were worked out with generalised additive models.

Even though reclaimed and lost to the urban encroaching, ponds still have their presence even in the middle of cities. Most of them are garden ponds. A few studies were conducted on the ecological role of these ponds in urban environment. Hassall (2014) opined that ponds are networks of habitat patches especially in the urban environment. Ponds act as refuge habitat for the threatened flora and fauna from the disturbed environment of cities.

Hill and Wood (2014) investigated the biodiversity and conservation value of macro-invertebrates in the garden ponds and field ponds of urban-rural continuum over three seasons. The study emphasised the hypothesis of lesser species diversity in garden ponds compared to that of field ponds.

A long term geo-spatial study on the disappearance of Arctic Tundra ponds over a period of 65 years were analysed by Andresen and Lougheed (2015). The study concludes that the decrease in the number and size of ponds across the Barrow Peninsula has major implications on surface energy balance, carbon exchange and adversely affect the fauna in the Arctic coastal plain.

Caria *et al.* (2015) studied the patterns of plant functional types and soil features of Mediterranean temporary ponds. Gallego *et al.* (2015) investigated on the pond typology and management issues of 87 farm ponds located at Andalusia in southern Spain. The article related the phytoplankton and macrophyte richness and composition with the environmental variables and pond management. Chumchal *et al.* (2016) studied the abundance of ponds in the Southern Great Plains of US with the help of aerial images.

Restored ponds are most appropriate for the study of successional processes on aquatic communities. Olmo *et al.* (2016) investigated the environmental and zooplankton community changes in restored ponds over four years.

Ballon *et al.* (2016) tested the effect of size and locality difference on the environmental variables of temporary ponds. The study found a weak influence of pond size on the environmental characteristics of temporary ponds. It is found that the locality has a strong influence on the physico-chemical and biological characteristics of the ponds.

National scenario

The history of construction and usage of ponds and reservoirs in India started way back at the time of Indus valley civilization. There is archaeological evidence of wells and reservoirs in Mohanjodaro and Harappa. One of the major constructions found in the Harappan civilization was 'Great bath' – a large pool in Mohanjodaro which exhibited the great hydraulic engineering skills of our forefathers. India being a country with strong culture and tradition always maintained the traditional and religious commitments intervened with the conservation and wise usage of natural resources. Water was considered sacred and they worshiped it. In ancient India every village had ponds and wells as a reliable source of water during the non- rainy season. In the Medieval period, the rulers encouraged farmers and villagers to build their own rainwater harvesting systems. Satellite pictures show there were as many as 1.2 billion such ponds in India (Kapur, 2010). State level initiatives are now being taken in India in the creation and conservation of ponds (Manoj and Padhy, 2015).

India has an estimated pond area of 0.72 million ha, a large portion of which is confined to villages (Rajagopal *et al.,* 2010). Ponds and the organisms in it was the subject of study in India even in 1960-70s. Detailed investigation on the ecology of freshwater ponds in Hyderabad were carried out by Sitaramaiah (1966), Zafar (1967), Seenayya (1971, 1972), Rao (1971, 1975), and Munawar (1974) with special emphasis on the phytoplankton. These studies mainly focussed on the hydrology and phytoplankton succession and periodicity in the ponds.

Being small in size, ponds reflect changes in the ecology and water chemistry more evidently than other water ecosystems. Hence many scientists chose ponds for the studies on the diurnal variations in aquatic environment (George, 1961 and Khan *et al.*, 1970).

The hydrology and ecology of lentic ecosystems in the higher altitude regions in the sub-tropical countries are of great interest to the scientific community. Sitaramaiah (1966) studied the diurnal cycles and the inter relationships of physiography, physico-chemical and biological characteristics of a pond in Thirumalai hills. Some of the studies on pond ecology were published from the northern most parts of India including Himalayan higher altitudes. Bisht *et al.* (2013) compared the water chemistry of an earthen pond, a cemented pond and a lake in Himalayan region of India. Hydrology and water quality status of Lahru pond in Himachal Pradesh was studied by Kumar *et al.* (2014).

Bhuiyan and Gupta (2007) inquired on nine ponds in Barak valley of Assam for their hydro-biological characteristics. The study stresses the role of rural ponds as a good source of freshwater for drinking and other domestic uses. He implies on the effective utilization of pond for aquaculture accompanied with scientific management. Chemical characterization of three ponds in Ayodhya in Faizabad was performed by Chaurasya and Pandey (2007). Toor *et al.* (2011) recommended the use of pond water for irrigation from the analysis of 78 ponds from the villages of Ludhiana district, Punjab.

Temporal variation in water quality and phytoplankton population in ponds in different parts of India was carried out vigorously in the 21st century (Rajagopal *et al.*, 2010; Nath *et al.*, 2015). Plankton dynamics of two adjacent unmanaged ponds in Howra district of west Bengal was studied by Bhanja *et al.* (2014). The paper deals with the phytoplankton composition and abundance in accordance with the physico- chemical variables of the perennial ponds. Mukhopadhyay *et al.* (2011) studied the ecology and aquatic macrophyte species turn over in relation to the limnological parameters of two ponds in Kolkata.

An urban tropical pond in Bihar at the centre of Sasaram city was studied for the zooplankton diversity in relation to the trophic status of the ponds (Kumar *et al.*, 2011). Ekhalak *et al.* (2013) investigated the physico-chemical properties and phytoplankton composition of a village pond with diversified algal flora in Surat (Gujrat).

Yadav *et al.* (2013) made an extensive study on the physico-chemical properties of Mahil pond in Jhalam district (UP). The study provides a base line data for the conservation and monitoring of the pond. The pollution status of four ponds in Tirunelveli, Tamil Nadu was reported by Selvamohan *et al*. (2014). Parithabhanu *et al.* (2014) analysed the physical and chemical properties of a perennial pond in Erode city (Tamil Nadu) to identify the suitability of fish culture.

A study by Mishra *et al.* (2014) evaluated the physico-chemical status of ponds in Varanasi city. The ponds were under deterioration due to anthropogenic influence. The study recommended an immediate restoration and management of the ponds.

Dalal and Gupta (2014) investigated the insect diversity of two temple ponds and its conservation values in Silchar, Asam. Kulkarni *et al.* (2015) documented the fauna of a small temporary pond in Pune, Maharashtra. In this study, that single pond itself recorded 125 species of aquatic fauna and twenty five species of associated fauna reconfirming the role of temporary ponds in contributing to the regional biodiversity.

Water quality of pond water from 27 villages of Chattisgarh was evaluated by Dixit *et al.* (2015). Das and Mukhopadyay (2015) investigated the distribution and status of ponds in Kopai river basin of Eastern India. The study reported 40-60% of the ponds in good condition. The rest of the ponds were in the verge of degradation due to desiltation and eutrophication. Anand *et al.* (2016) used the zooplankton population and physico-chemical properties of Lapkaman pond, Gujarat to study the deterioration of water quality due to anthropogenic activities.

Kerala scenario

In Kerala studies on the ecology and hydrology of natural and man-made ponds are limited to a few reports. Dhanya *et al.* (2012) investigated the distribution and management patterns of ponds in Pallippuram, Alappuzha dist. Literatures show some studies on aquaculture ponds such as the cost benefit analysis of fish culture ponds and their use as a mosquito control measure (Panicker *et al.*, 1992). Some studies investigated on the reusable value of unused fish culture ponds for resuming aquaculture (Saraswathy *et al.*, 2016).

Limnological studies of Thirumullavaram temple pond situated in the coastal region of Kollam district confirm the serenity of temple ponds and their utility as a drinking water source (Sulabha and Prakasam, 2006). A similar kind of study on four temple ponds of Arattukulangara, Kottayam revealed high level of organic pollution (Jose *et al.*, 2011).

Thrivikramji and Thomas (2013) investigated the sediment bound organic carbon in the ponds of Palakkad. The study pointed out the mesotrophic and eutrophic status of 99% of the ponds studied. It states the allochthonous origin of the organic matter present in the ponds. A study by Jipsa *et al.* (2013) on the water quality of two ponds in Palakkad with respect to its physico-chemical as well as biological characteristics propounded the high quality of pond water.

Physico-chemical characterisation of 37 ponds in Anthiyoor block panchayath, Thiruvananthapuram pointed out the non-potable state of the water and recommended remedies and immediate restoration (Aswathy Ashok *et al.*, 2015). A geospatial study was conducted by Smitha Asok *et al.* (2015) in Anad panchayath, Thiruvananthapuram to examine the usability of thirteen ponds. The study recommended the usage of pond water for domestic as well as agricultural uses.

A study by Sajitha and Vijayamma (2016) on fifteen ponds of Athiyannoor Panchayath in Thiruvananthapuram recommended the pond water for domestic usage in respect of water quality index values.

Even though ponds were a vital part of all residence in the villages of Kerala, now they are in the verge of degradation and facing the threat of reclamation for alternate land uses.

1.3 Significance of the study

In Kerala state, village ponds had been the sole source of drinking water along the coastal regions a few decades ago. The quality of ponds was maintained by traditional methods by the village people ensuring the water quality. When urbanization set in many of these ponds were reclaimed. The rest of the ponds were neglected as and when public water supply became accessible. In the current scenario it is high time to document the existing ponds throughout the state of Kerala, their water quality, biodiversity, potential services, and develop conservation strategies. Therefore this study attempts to document and investigate the status of ponds in a Panchayath in Alappuzha district of Kerala. The scope of the study would be

1) Regional surveys on ponds will be a source of information for management and sustainable utilisation.
2) These data can be used for developing spatial patterns of surface water quality and distribution of biological community.
3) Trophic state and phytoplankton diversity analysis can indicate chances of harmful algal bloom (HAB).
4) It can contribute to the freshwater science in general.

1.4 Objectives of the study

The present work has broad objectives

1) Documenting the ponds existing in the study area with respect to their use and management.

2) To investigate the water quality of ponds and their trophic state.

3) To analyse the phytoplankton in the ponds and study the dynamics of the algal bloom.

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Chapter 2

SURVEY OF PONDS IN PALLIPPURAM

Contents

2.1 Introduction

Surveys are intended to collect information on the characteristics of some or all units of population in an organised and methodological manner with a well-defined concept and methods and the information collected are compiled into a useful summary (Ministry of Industry Canada, 2010). It is a kind of descriptive research for acquiring primary data from target population (Glasow, 2005; Mathiyazhagan and Nandan, 2010). Surveys offer the opportunity to execute studies with various designs, including cross-sectional, repeated cross-sectional, panel, mixed designs etc., each of which is suitable for addressing particular research questions of long-standing interest. The need for surveys arises when there is no data or insufficient data.

Biological survey research reports on ponds are very few in number. Most of the surveys of this kind are undertaken by NGO’s with the legislative initiative. A country side survey of ponds was conducted in a total of 5911 km × 1 km sample squares spread across UK for a period of 29 years during 1978-2007 with the partnership of nine government funded bodies led by Natural Environment Research Council (NERC) and the Department of Environment, UK (Williams *et al.*, 2010).

Two hundred lakes in Ireland were sampled and analysed in 1997 for their hydrochemistry and acid sensitivity. The results were published by Aherne *et al.* (2002). A survey on the water storage practices and beliefs among the residents of Bonao, Dominian Republic was conducted by Holt (2009). Masser and Schonrock (2006) reported the results of an internet based survey of ponds in Texas. The pond owners were surveyed by online questionnaires on the pond characteristics, aquatic vegetation, fish, wild life and management of ponds.

Based on the survey data of Finnish lakes, Kamari *et al.* (1991) opined that the lakes in northern Finland are under threat of deterioration. Surveys were also performed to identify and enlist planktonic nuisance blooms in water bodies. Sivonen *et al.* (1990) published a survey result of his study which accounted the toxic cyanobacterial blooms over the Finnish fresh waters.

A survey study by Saenger *et al.* (2006) in Kiritimati and Washington islands of Republic of Kiribati on the physico-chemical properties of lakes and saline ponds established both intra and inter island variations in sediment characteristics. Malanda and Louzolo-kimbembe (2014) conducted a survey on the storage tanks of Brazzaville, republic of Congo. The survey documented data on the domestic water tanks, the building materials of tank and the potability of stored water. The basic environmental characteristics of 92 ponds were surveyed in Slovakia

(Novikmec *et al.*, 2016). The study established the positive relationship of pond area and their catchment area.

Central Ground Water Board of India have been conducting surveys on the groundwater sources in Kerala for many years and they have published consolidated reports on the ground water information of different districts. Central Ground Water Board (2013) published a report on the hydro-geological surveys of tribal villages of Devikulam Taluk carried out in the period of 1980- 81. The sites with scarcity of water were demarcated. The survey data were used to locate sites for bore wells. Central Ground Water Board (2013) reported 2574 ha area in Alappuzha being irrigated by ponds and tanks during 2009-10 period.

Multi-taxa survey on temporary ponds of Pune was conducted by Kulkarni *et al.* (2015). The survey reported 125 species of strictly aquatic fauna and 25 species of associated fauna. Kumar *et al.* (2015) conducted a survey of macrophyte diversity in the ponds of a village in Chhattisgarh.

This chapter describes the results of the survey undertaken in Pallippuram Panchayath in Alappuzha district of Kerala. The survey was conducted to collect information in an organized and methodological manner about characteristics of ponds in the Panchayath using a well-defined concept and to compile the information collected into a useful summary form.

The need for this survey arouse from the observation of wide spread algal blooms in the domestic ponds of the Panchayath where a large number of ponds are located in a small geographical landscape. There was no comprehensive list of these freshwater sources in the Panchayath. The lack of data on ponds arouse a need to survey and to build a data base. This data base could be utilized by the governing body for relevant policy making.

2.2 Specific objectives

1) To enumerate the ponds in each ward of the Pallippuram Panchayath.

2) To document the present state of use and management of the ponds.

3) To document the occurrence of algal blooms.

2.3 Description of the study area

Pallippuram Panchayath is an administrative entity situated in Cherthala taluk of Alappuzha District, Kerala located at $9^{0}45' 20''$N and $76^{0}21'39''$ E. It is the southern end of an island which is surrounded by the Vembanad Lake. The Panchayath has the estuary on three sides, and the northern side is contiguous with the rest of the island. The region has tropical monsoon climate with heavy rainfall during southwest monsoon (June-September) and northeast monsoon (October-December). The period from January to May is comparatively dry with low rainfall. The lowest rainfall received during 2011-14 was 1.2 mm in January 2014 and highest rainfall of 1031 mm in June 2013. The lowest temperature recorded was 21.6°C in January 2012 and highest temperature of 33.5°C in March-April 2014. The data obtained from India Meteorological Department (IMD) is presented in Figure 2.1 and Table 2.1.

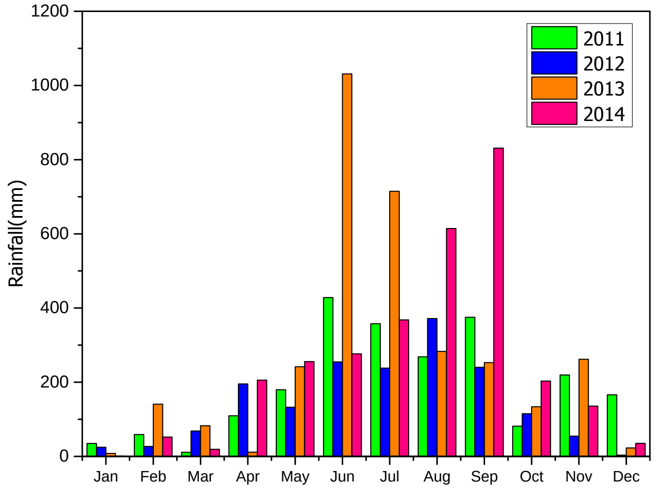


Figure 2.1: Monthly rainfall data of Alappuzha district for the period 2011-2014

Table 2.1: Monthly mean of maximum and minimum temperature in the study area for the years 2011-2014

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2011	max	31.5	31.6	32.7	32.8	32.6	30.9	29.3	29.2	29.6	31.6	31.5	32.2
	min	23.0	23.1	24.9	24.5	25.7	24.2	23.3	23.1	23.0	23.9	23.1	22.5
2012	max	31.6	32.2	32.4	33.0	32.3	30.4	30.0	29.3	30.3	31.8	31.7	32.7
	min	21.6	22.6	24.1	24.3	25.6	24.3	23.8	23.2	23.8	24.3	23.8	23.4
2013	max	32.5	32.4	33.0	33.2	32.4	28.6	28.5	29.1	29.9	30.5	31.4	31.5
	min	23.2	23.6	25.4	26.8	26.2	23.4	23.3	23.8	24.3	24.5	24.4	23.4
2014	max	32.4	32.7	33.5	33.5	32.8	31.8	30.0	29.5	30.5	31.8	31.5	31.7
	min	23.3	24.0	25.2	25.8	26.0	25.5	23.9	24.1	24.5	24.4	24.1	24.2

The Panchayath has a population of 28276 in an area of 25.53 km^2 as per census 2011 with a total number of 6910 households. It was one of the very active coir producing parts of Alappuzha district though it has lost its prominence over the years. Pallippuram was also well known for its silica-rich sandy soil, which has extensive use in glass and cement industries. Huge white sand dunes dotted with cashew trees were the typical landscape of Pallippuram. The over-exploitation of sand has altered the landscape over the years. The predominant land use includes paddy fields, coconut gardens and residential. Agriculture was traditionally the occupation of the villagers of Pallippuram; especially summer vegetable cultivation as intermittent crops in the paddy fields. Many of the paddy fields are now left fallow. The Pallippuram Grama Panchayath which is a local body of the civil administration is divided into 17 wards or administrative units (Figure 2.2).

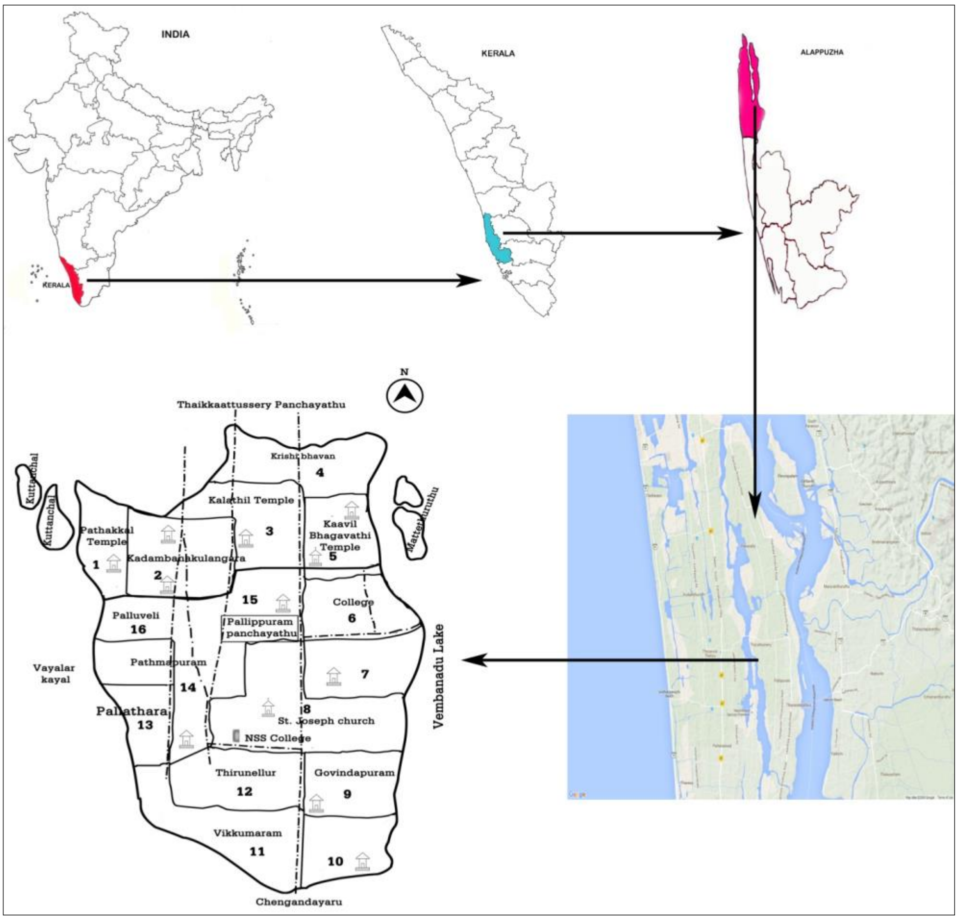


Figure 2.2: Map of Pallippuram Grama Panchayath showing 17 wards (ward no. 17 is recently formed from ward no. 15 and 16).

The drinking water source in the village was traditionally ponds. These are earth dug ponds attached to each dwelling which was maintained by the owner. Most of these domestic ponds were cleaned yearly by digging out the sediment and cleaning the peripheries, as it was the only source of water for the people. Over the years as alternate sources of clean drinking water were available, the ponds came to be neglected, and the traditional maintenance practices were discontinued. In recent years through the rural employment guarantee scheme of Government of India, the cleaning of a few ponds is taken up at two year intervals. It is in this context that a survey of those existing ponds was undertaken to provide the primary data on the state of these ponds so as to devise steps to conserve them as clean freshwater sources.

2.4 Method of survey

The survey of ponds was conducted in two phases. Phase I was conducted in January - February 2011. In this survey all the ponds in the seventeen wards were enumerated. Phase II was selective based on the observation of the Phase I survey. Phase II study was conducted immediately following Phase I, in the month of March 2011.

2.4.1 Survey of ponds- Phase I

Definition of pond by the National pond survey guidelines, UK was adopted for the survey. It states ponds as "water bodies between 1m^2 and 2 ha holding water for at least four months a year". All perennial, seasonal, private and public ponds distributed through the 17 wards of Pallippuram Panchayath were documented in the survey. The survey documented the total number of ponds in each ward, their area, pond types, type of use, transparency, proportion of shaded area, occurrence of algal blooms, macrophyte vegetation and management strategies. The physical features of the ponds and its surroundings observed were noted in the field recording sheets. Management of ponds and the type of their use were identified by interviewing the people with the help of a questionnaire. All information were collected personally by visiting the households and face to face questionnaire method by asking questions to the respondents and recording the response of the people. The format of the survey sheet is attached (Annexure I).

2.4.1.1 Procedure of pond survey

a) *Pond area*

Pond area was calculated roughly from the surface diameter of the outer boundary of ponds. Area of ponds of different shapes was calculated using the standard formula:

Circular ponds $= \pi r^2$

Rectangular ponds $= length \times breadth$

Oval shaped ponds $= \pi \times a \times b$, where a is the length of semi-major axis and b is the length of semi-minor axis.

b) *Pond types*

Ponds were classified into two major types based on their year-round water holding capacity. They are perennial and seasonal ponds.

c) *Type of use*

The type of usage of ponds was marked in the survey sheets from the response of the pond owners to the questionnaire. The ponds which are regularly used by the people were categorized under different uses: drinking, bathing, washing, irrigation, and multiple uses.

d) ***Transparency***

Transparency of water was recorded in the survey fact sheets on the basis of visual observation. In accordance with this, clarity of water was categorized arbitrarily into three groups: clear, moderately turbid and highly turbid.

e) ***Shade***

Shading over the ponds by large trees and surrounding vegetation at the peripheral region of ponds were recorded in the survey sheets. Based on the proportion of shaded area over the ponds, they were grouped arbitrarily under any of the three categories: fully shaded, partially shaded and unshaded.

f) ***Algal bloom***

The ponds with algal blooms were identified from physical observation. The algal blooms were located as greenish or blue-green turbidity or floating scum or benthic mats or metaphyton.

g) ***Aquatic vegetation***

Aquatic macrophyte vegetation was classified under floating hydrophytes, submerged and emergent vegetation. Macrophytes were recorded by walking around the perimeter of the ponds and recording the observation.

h) ***Pond management***

The owners of the ponds were interviewed to know how the ponds were maintained, the economic feasibility of managing the ponds etc.

2.4.1.2 Compilation of data

Data of the field survey recorded in the survey fact sheets were tabulated separately for each ward in excel format. The consolidated data are presented in graphical and tabular forms for effective interpretation and drawing conclusions.

2.4.2 Survey of ponds- Phase II

In the second phase of the survey, those ponds observed with algal blooms were visited and water samples were collected to identify the algal blooms. Algal blooms were observed in forty eight ponds during the first phase of survey. Water samples were collected from thirty two of these ponds and transported to laboratory in polythene containers. The rest of the sixteen ponds were seasonal and had dried up. The algal blooms were observed under microscope and identified based on the guidelines of http://www.algaebase.org. (Guiry and Guiry, 2011) and available monographs (Desikachary, 1959; Prescott, 1962; Philipose, 1967; Whitford and Schuacher, 1984). The macrophyte vegetation was collected and brought to the laboratory and identified with the available literature.

2.5 Observations of survey

2.5.1 Pond number and area

A total of 873 ponds were recorded in the Panchayath. Ward No.12 had the least number of ponds *i.e.,* 20. Ward No.3 recorded 120 ponds topping the list followed by ward No.4 with 94 ponds (Table 2.2). The area of these ponds ranged from 12 m^2 to 300 m^2. Ponds with area of 50-100 m^2 constituted 48.5 %. Ponds coming under the category of 0- 50 m^2 and

100-150 m^2 comprised 23.9 % and 18.5 % respectively (Figure 2.3). The largest ponds were those with area 250-300 m^2 which formed only 0.45% of the total and all of these were either public ponds or temple ponds.

Table 2.2: Number of ponds in 17 wards of Pallippuram Panchayath

Ward number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
No of ponds	51	48	120	94	41	57	40	27	46	60	50	20	39	32	82	41	25

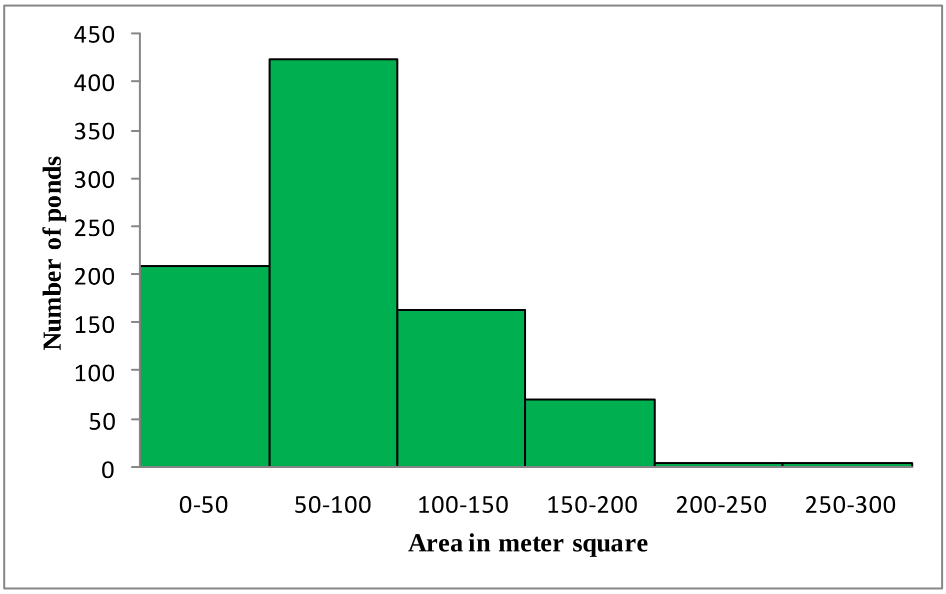


Figure 2.3: Number of ponds in Pallippuram against area of ponds

2.5.2 Pond types

Perennial ponds which hold water throughout the year accounted 59% and the remaining 41% were seasonal ponds which dry up during summer (Figure 2.4). Out of the 873 ponds of the Panchayath, six ponds are salty. They were found in Ward No.1, 10 and 11. Ward No.1 constitutes only a small area in the main land and the major portion is in a separate island in the Vembanadu Estuary. Ward No.10 and 11 is in nearest proximity to the Chenganda canal (part of Vembanadu Estuary).

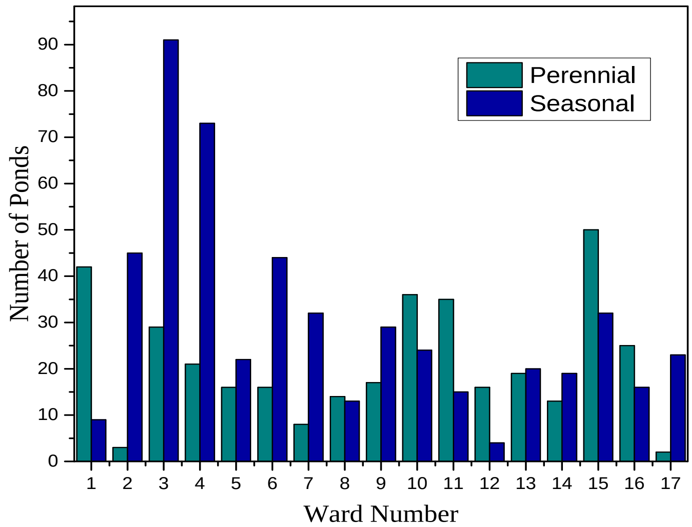


Figure 2.4: Ward wise distribution of perennial and seasonal ponds

As per the ownership there are four public ponds owned by the Panchayath, seven temple ponds, forty three ponds associated with paddy fields and unused land plots and eight hundred and nineteen domestic ponds of which thirty three are associated with sacred groves or 'Kavu' (Figure 2.5). These sacred groves are rich in endemic herbs and trees and are home to various wild species. They also help in soil and water conservation besides preserving its rich biological wealth. The ponds associated with these groves are perennial water sources. These ponds are protected from human interference and are used only for worship and temple rituals.

Figure 2.5: Sacred groves (sarpa kavu) and ponds associated with it.

2.5.3 Type of use

The ponds used for drinking, irrigation, or washing purpose constituted 34% of the total ponds. Among these, eleven ponds of ward No. 1 are used for drinking purpose. Eighty three ponds in the Panchayath are used for bathing, fifty three ponds for washing clothes, and eighty eight ponds for irrigation.

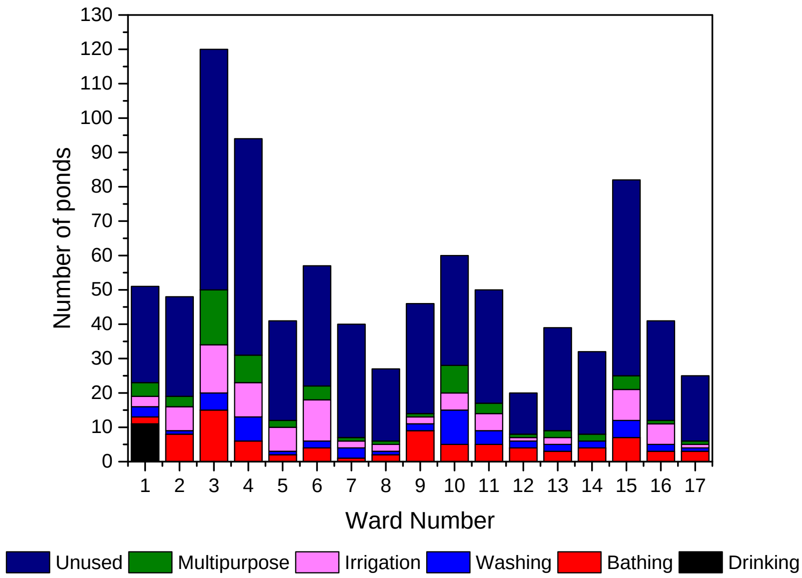


Figure 2.6: Ward wise distribution of ponds categorized under different type of use.

Sixty two ponds account for multipurpose use including bathing, washing and irrigation. The use category of ponds in each ward is given in Figure 2.6. Since the coir production by the natives has come down, only six ponds are now used for coconut husk retting. One pond in ward No. 10 was observed with prawn culture in a small scale. Two ponds, one in ward No.3 and one in Ward no. 6 were used as a source of livestock water.

Unused ponds represented 66% of total number of ponds in the Panchayath. Figure 2.7 provides the graphical distribution of the used and unused ponds in the study area. Maximum number of used ponds (46%) was observed in ward no.10 followed by ward No.1 with 45%. Highest percentage of unused ponds was observed in Ward no.7 (82.5%).

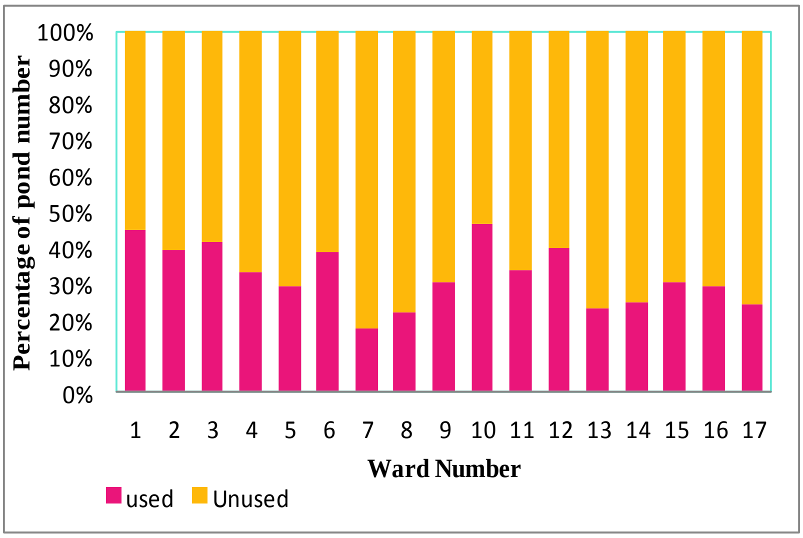


Figure 2.7: Percentage distribution of used and unused ponds in seventeen wards of the Pallippuram Panchayath

2.5.4 Transparency of water

Clarity of a water body is explained by the transparency or transmittance of light by the water. Suspended and colloidal materials such as silt, clay, organic particles, and plankton impart turbidity to water reducing the transparency. Since many of the ponds were surrounded by vegetation and had macrophyte growth, leaf litter and organic products of degradation were present.

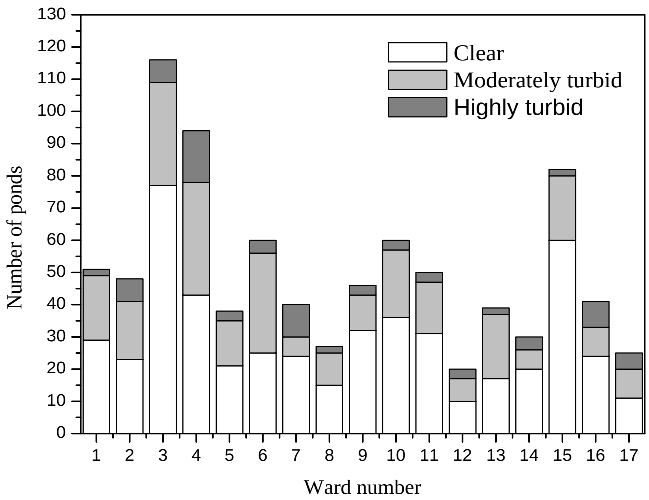


Figure 2.8: Transparency of water in ponds across seventeen wards of Pallippuram Panchayath

In this survey 10% of the ponds were found highly turbid and 33% moderately turbid. Rest of the 57% constituted clear ponds. Ward-wise distribution of ponds with respect to clarity of water is represented in the graph (Figure 2.8).

2.5.5 Shade

A variety of vegetation in and around ponds such as overhanging plants, shrub and trees causes shade over the ponds. Other than plants, buildings nearby the ponds also impart shade. The ponds were classified under three categories based on shading (fully shaded, partially shaded and unshaded).

Only 4.9% of the ponds in the Panchayath were unshaded and the rest of the major portions were either partially shaded or fully shaded (Figure 2.9).

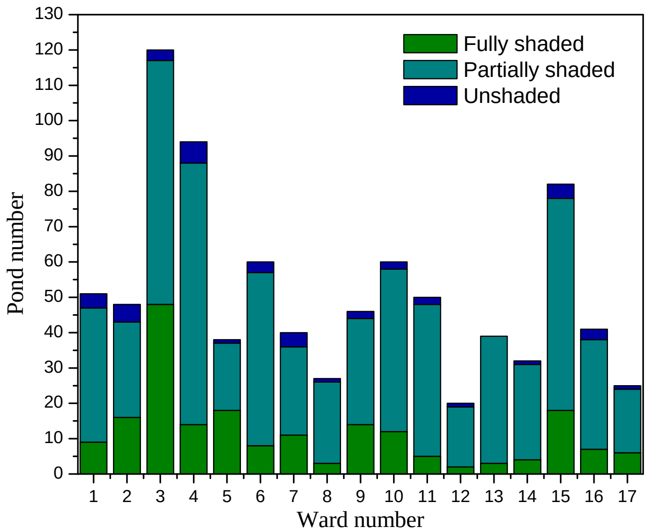


Figure 2.9: Ponds classified according to the shade condition

2.5.6 Algal bloom

Algal blooms appeared as green or blue-green turbidity, floating scum, or as thick blue-green floating mats (Figure 2.10). Algal blooms were observed in a total of 48 ponds all together from seventeen wards. Ward wise distribution of ponds having algal blooms is represented in Figure 2.11. Ward No.10 has sixty ponds; algal blooms were not observed in any of these.

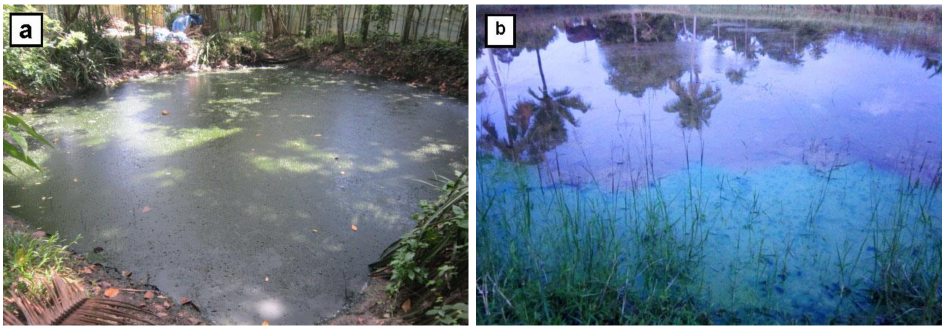


Figure 2.10: Algal bloom in ponds as a) surface scum and b) floating mat

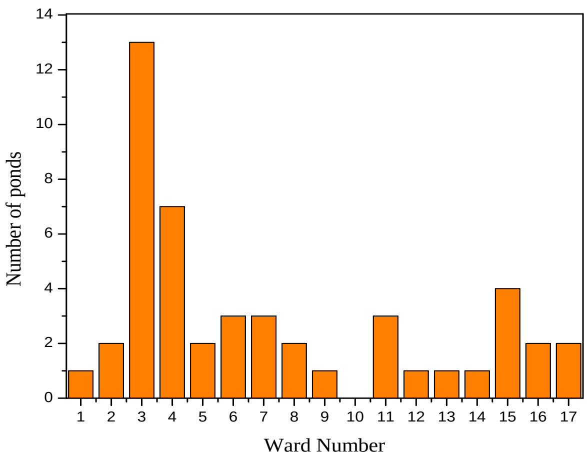


Figure 2.11: Ward wise representation of ponds with algal bloom

2.5.7 Macrophyte vegetation

Macrophyte flora in ponds comprised floating hydrophytes, emergent plants and submerged vegetation. Floating hydrophytes were present in 66.44% of the total ponds. Floating hydrophytes found in the ponds were *Lemna minor*, *Pistia stratiotes, Eichhornia crassipes, Salvinia molesta* and *Azolla pinnata*. *Pistia stratiotes* and *Salvinia molesta* occurred in all the seventeen wards (Figure 2.12). Emergent plants were *Nymphaea* sp. and *Colocasia* sp. *Nymphaea* sp. occurred in four ponds and *Colocasia* sp. in two ponds.

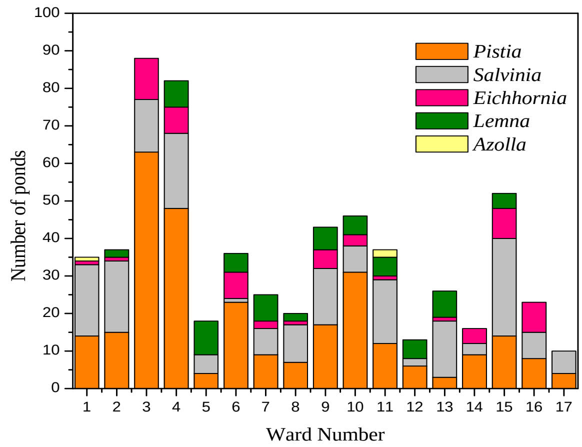


Figure 2.12: Number of ponds with floating hydrophytes and their ward wise distribution in the ponds

Submerged vegetation occurred in 19 ponds (Figure 2.13). It was represented by *Vallisneria* sp., *Hydrilla verticillata and Ceratophyllum submersum.*

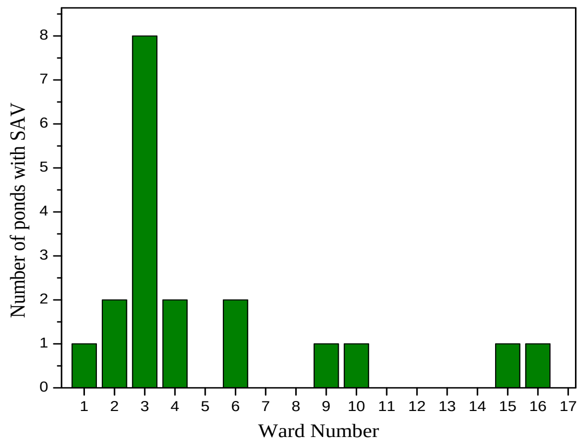


Figure 2.13: Number of ponds having SAV in each ward of Pallippuram

2.5.8 Pond management

34% of ponds in the Panchayath are under active maintenance (Figure 2.14). Management is through the yearly cleanup programme undertaken by the local self governing body for the maintenance of the ponds. 63% of the ponds coming under unused category are under great risk of reclamation and degradation. 3% of the ponds are already degraded due to natural ageing, dumping of wastes and lack of maintenance.

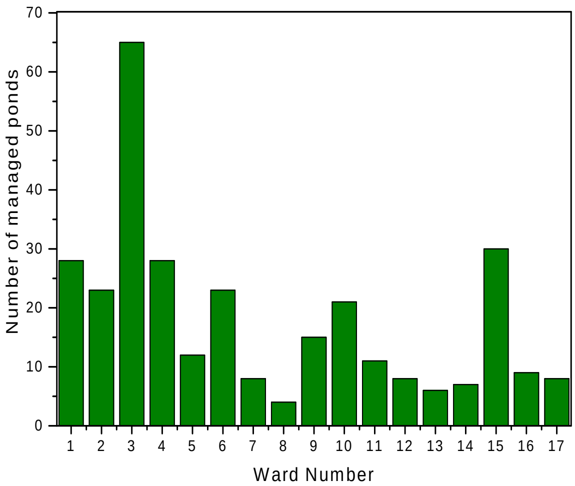


Figure 2.14: Managed ponds in 17 wards of the Panchayath

2.5.9 Bloom forming algae

Out of the thirty two ponds sampled for identification of algal bloom, six ponds had blooms of Charophyta, two had Euglenozoa and the rest of the ponds had Cyanobateria (Table 2.3). Charophyta was represented by *Spirogyra* sp., *Klebsormidium* sp., and *Mougeotia scalaris*. Blooms of *Spirogyra* sp. occurred in four ponds whereas *Klebsormidium* sp. and *M. scalaris* were present only in one pond each. *Euglena proxima* was present in two ponds. Blue-green algal bloom occurred in the rest of the twenty four ponds examined. *Oscillatoria* sps. occurred in ninteen ponds represented by three species. They were *O. princeps*, *O. subbrevis* and *O. limosa*. Blooms of *Microcystis aeruginosa* occurred in three ponds. *Coelosphaerium kuetzingianum* and *Phormidium tenue* were present in three ponds. *Anabaena* sp. was present only in a single pond. The microphotographs in original are given in page number 37-40.

Table 2.3: Occurrence of algal blooms in ponds of Pallippuram Panchayath (+ presence, - absence)

Ward No.	Pond No.	Presence (+), absence (-) of bloom										
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
3	1	-	+	-	-	-	-	-	-	-	-	-
	29	+	-	-	-	-	-	-	-	-	-	-
	38	-	-	-	-	-	-	-	+	-	-	-
	52	-	-	-	-	-	-	-	-	+	-	-
	56	-	+	-	-	-	-	-	-	-	-	-
	58	-	-	-	-	-	-	-	-	-	-	+
	65	+	-	-	-	-	-	-	-	-	-	-
	83	-	+	-	-	-	-	-	-	-	-	-
	106	-	-	-	-	-	-	-	+	-	-	-
	108	-	-	+	-	-	+	-	-	-	-	-
	112	-	+	-	-	-	-	-	-	-	-	-
	118	-	-	-	-	+	-	-	-	-	-	-
	120	-	-	-	+	-	-	-	-	-	-	-
4	10	-	-	-	-	-	-	-	-	-	+	-
	11	-	+	-	-	-	-	-	-	-	-	-
	26	-	+	-	-	-	-	-	-	-	-	-
	31	-	-	+	-	-	-	-	-	-	-	-
	33	-	+	-	-	-	-	-	-	-	-	-
	34	-	+	-	-	-	-	-	+	-	-	-
	35	-	-	-	-	-	-	+	-	-	-	-
	36	-	-	+	-	-	-	-	-	-	-	-
	49	-	+	-	-	-	-	-	-	-	-	-
	60	-	+	-	-	-	-	-	-	-	-	-
5	27	-	-	-	-	-	-	+	-	-	-	-
	39	-	+	-	-	-	-	-	-	-	-	-
6	25	-	-	-	-	-	-	-	-	-	-	+
	26	-	+	-	-	-	-	-	-	-	-	-
	27	-	-	+	-	-	-	-	-	-	-	-
	28	-	-	-	-	-	-	+	-	-	-	-
16	8	+	-	-	-	-	-	-	-	-	-	-
17	6	-	-	+	-	+	-	-	-	-	-	-
	12	+	+	-	-	-	-	-	-	-	-	-

(**1.***Spirogyra* sp. **2.** *Oscillatoria princeps* **3.** *O. subbrevis* **4.** *O. limosa* **5.***Phormidium tenue* **6.** *Anabaena* sp. **7.** *Microcystis aeruginosa* **8.** *Coelosphaerium kuetzingianum* **9.** *Mougeotia scalaris* **10.** *Klebsormidium* sp. **11.** *Euglena proxima)*

Species spectrum of bloom forming algae

1. *Microcystis aeruginosa*

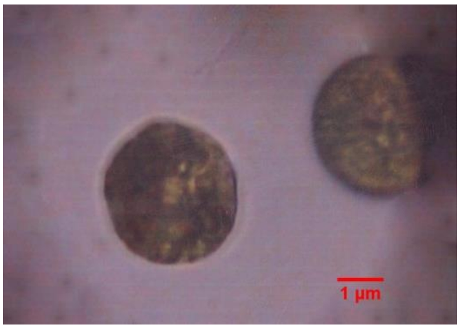
Division: Cyanobacteria

Class: Cyanophyceae

Order: Chroococcale

Family: Microcystaceae

2. *Coelosphaerium kuetzingianum*



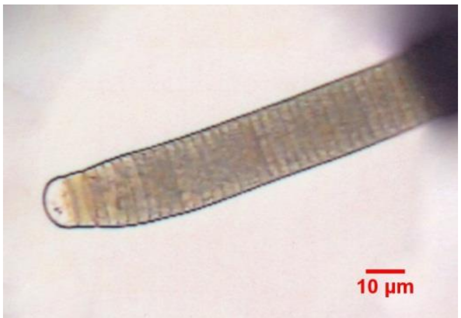
Division: Cyanophyta

Class: Cyanophyceae

Order: Chroococcales

Family: Merismopediaceae

3. *Oscillatoria princeps*



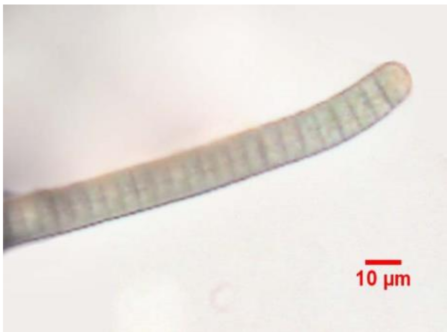
Division: Cyanobacteria

Class: Cyanophyceae

Order: Nostocales

Family: Oscillatoriaceae

4. *Oscillatoria subbrevis*



Division: Cyanobacteria

Class: Cyanophyceae

Order: Nostocales

Family: Oscillatoriaceae

5. *Oscillatoria limosa*

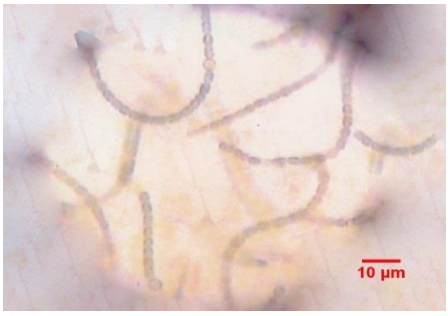
Division: Cyanobacteria

Class: Cyanophyceae

Order: Nostocales

Family: Oscillatoriaceae

6. *Anabaena* sp.



Division: Cyanobacteria

Class: Cyanophyceae

Order: Nostocales

Family: Nostocacae

7. *Phormidium tenue*

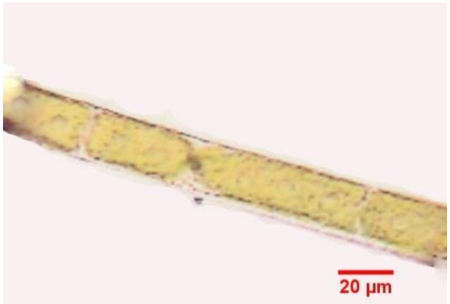
Division: Cyanobacteria

Class: Cyanophyceae

Order: Nostocales

Family: Oscillatoriaceae

8. *Mougeotia scalaris*



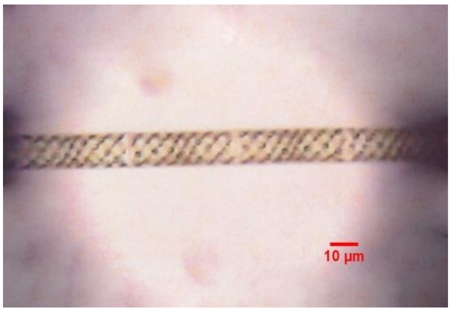
Division: Charophyta

Class: congugatophyceae

Order: Zygnematales

Family: Zygnemataceae

9. *Spirogyra* sp.



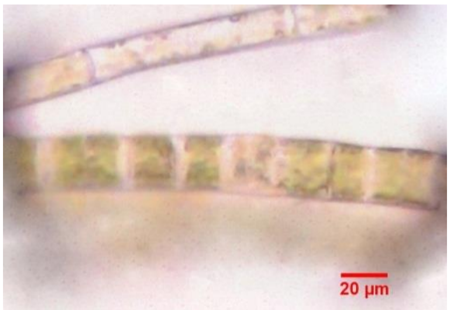
Division: Charophyta

Class: congugatophyceae

Order: Zygnematales

Family: Zygnemataceae

10. *Klebsormidium* sp.



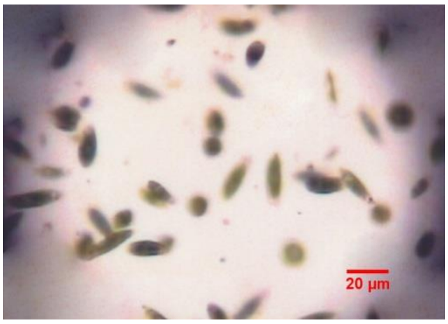
Division: Charophyta

Class: Klebormidiophyceae

Order: Klebsormidales

Family:Klebsormidiaceae

11. *Euglena proxima*



Division: Eulenozoa

Class: Euglenophyceae

Order:Euglenales

Family:Euglenaceae

2.6 Discussion and conclusion

The present survey has enlisted the number of ponds existing in the ‘Pallippuram Panchayath’, their state of use, and indirectly indicated the water quality. As 66% of the ponds are now unused, it is evident that they are not essential for the community from the utilitarian point of view, and therefore grossly neglected. The drinking water ponds were limited to only ward 1 which is an island and had no water supply. The ponds that are still used for drinking purpose are maintained through traditional methods.

The survey result indicated the presence of one pond for every eight house in the Panchayath. 66% of the total ponds are now out of use and is facing the threat of either degradation of water quality or reclamation. Ponds coming under the category of 200-300 m^2 area were negligible in number since large proportion of the ponds in the Panchayath are domestic ponds associated with households. The domestic use includes mainly washing of clothes. Therefore phosphates from detergents could be a strong reason for induction of algal blooms. The region has paddy fields; both cultivated and uncultivated which could also be a source of fertilizer run off. Effluents from the residential areas and the faulty sanitation systems can likely contribute excess organic matter, and consequent nutrient enrichment in the ponds. The land use pattern around the ponds has direct effect on water quality and aquatic vegetation (Akasaka *et al*., 2010).

Ward No. 10 of the Panchayath didn't report any pond with algal bloom. Ward.10 had the highest percentage of managed ponds which are cleaned every year and maintained for domestic uses. This shows that more than the nutrient enrichment from surrounding land plots and from domestic inputs, the management pattern plays important role in controlling the noxious bloom formation. The conventional cleaning procedure is quite sufficient to maintain the water quality of the ponds.

Livestock poisoning reported from water bodies with heavy Cyanobacteria bloom has led to many studies on cyanobacterial toxicity. The common toxic Cyanobacteria in freshwater are *Microcystis* spp., *Cylindrospermopsis raciborskii*, *Planktothrix rubescens*, *Synechococcus* spp., *Lyngbya* spp., *Aphanizomenon* spp., *Nostoc* spp., some *Oscillatoria* spp., *Schizothrix* spp. and *Synechocystis* spp. The most common Cyano toxins are microcystin and neurotoxins [anatoxin-a, anatoxin –a(s) and saxitoxins] (World Health Organization, 2003).

Chorus and Bartram (1999) reported *O. limosa* as a microcystin producing toxic cyanobacterial strain. *Oscillatoria* spp. are reported to produce hepatotoxic microcystins (Ahmed *et al*., 2010). Mez *et al.* (1997) reported that the neurotoxins produced by *O.limosa* as the reason of cattle death in the Alpine grazing lands of Switzerland. The observation of potentially toxic genera of *M.aeruginosa* and *O. limosa* in the present study is of concern. Mycrocystin produced by *Microcystis* is toxic to the fish and animal life in the pond (Sivonen *et al.*, 1990; Imai *et al.*, 2008; Gaikwad *et al.*, 2013). It was observed in the survey that only unused ponds are found colonised by *M.aeruginosa*. According to Almanza *et al.* (2016) stable water bodies without any disturbance accumulate the floating colonies of *M.aeruginosa* forming thick blue-green surface scum.

Dense growth of three species of Charophyta was observed in six ponds in this survey. Charophytes are generally recognized as indicators of clean water ecosystems and they prefer hard alkaline waters rich in calcium. However Charophytes may persist under moderate fertility and turbidity (Klosowski *et al.*, 2006).

Ponds are important in maintaining the water balance and to maintain the ecological integrity of the environment by controlling the water cycle and food web. Ponds are home and shelter to amphibians, reptiles and many other vertebrate and invertebrate species. Comparative studies on the biodiversity on ponds with that of rivers and streams have revealed that they dominate in species richness and diversity irrespective of their small size (Williams *et al.,* 2004; Cottenie and De Meester, 2004). Now ponds are vulnerable freshwater sources because of the contemporary pressure on land. Converting these ponds into useful and profitable resource will ensure their protection. The survey has revealed that the Pallippuram Panchayath has a rich traditional water resource that has to be restored and utilized in sustainable way.

The results of the survey showed that Pallippuram Panchayath has 873 ponds and the highest number of ponds is in ward No. 3 (120 ponds). As it was not possible to study in detail all these ponds within the time frame, and as the Panchayath has a uniform terrain it was decided to restrict detailed investigation to a few ponds. The ponds in ward 3 were close to each other and yet had different types of algal blooms - blue-green, green and yellow green algal blooms, and also clean ponds without algal blooms arousing curiosity on the ecology of the ponds and the phytoplankton blooming. Therefore three ponds with different types of algal blooms and a clean pond situated in ward No. 3 hardly separated by 1 km distance from each other were short listed for further investigation on water quality, phytoplankton ecology and bloom formation.

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Chapter 3

WATER QUALITY AND TROPHIC STATE OF PONDS

Contents

3.1 Introduction

Water quality can be defined as the physical, chemical and biological parameters of a water body which influence the survival and flourishing of the living organisms in it. Water quality testing is an important part of environmental monitoring. Safe and clean water is an essential requirement for the healthy living of organisms. When water quality is poor, it affects not only aquatic life but the surrounding ecosystem as well. The extent of pollution caused by human activities to the aquatic ecosystems generated a need for regular monitoring of the water quality (Poonam *et al.*, 2013).

By recognising the importance of ponds as a freshwater source, numerous studies have been conducted on the water quality and hydrology of permanent and temporary ponds all over the world (Arle, 2002; Kozusko *et al.*, 2006; Rim-Rukeh and Irerhievwie, 2014; Rodrı´guez-Rodrı´guez *et al.*, 2016). Man-made earthen ponds are mainly used for aquaculture, and many studies on pond water quality are related to fish culture and management (Banerjea, 1967; Boyd, 1990; Chowdhury and Mamun, 2006; Singh and Bhatnagar, 2010; Gupta and Dey, 2012; Bhavimani and Puttaiah, 2014; Parvathi and Sivakumar, 2016; Sandhya and Benarjee, 2016).

Even before the world recognised the importance of ponds, ponds have been used as a freshwater source to meet the daily needs and were traditionally maintained and protected in India. Many studies to assess the quality and ecological status of these ponds in the present day were carried vigorously in all parts of India (Baruah *et al.*, 1998; Ghosh, 2006; Shastri *et al.,* 2007; Bhat *et al.,* 2009; Rajalakshmi *et al.*, 2011; Sayeswara *et al.,* 2011; Ankita and Mankodi, 2012; Mahajan and Billore, 2014; Nag and Gupta, 2014; Prajapati, 2014; Jena *et al.*, 2016; Kar and Kar, 2016). Meera *et al.* (2015) studied the pollution status of ponds in Chavara industrial area near KMML, Kollam. Yadav *et al.* (2016) reported severe microbial pollution in urban ponds of Raipur, Chhattisgarh. Many studies were based on the water quality of temple ponds which are considered sacred and are isolated from external disturbances (Sulabha and Prakasam, 2006; Ekhalak *et al.*, 2012; Banita *et al.,* 2013; Elayaraj *et al.,* 2016). A study by Harichandan *et al.* (2016) on the temple ponds of Odisha revealed the poor quality of the pond water. Some of the temple pond studies focussed on the biodiversity along with water quality (Dalal and Gupta, 2014; Anand *et al.,* 2016).

3.1.1 Trophic state

Trophic state is a concept used to classify the biological productivity of an aquatic system (Dodds and Cole, 2007). Trophic state of a water body is defined on the basis of the degree of eutrophication. Any water body at a particular time belongs to a particular nutrient status and it changes with time (Jekatierynczuk-Redczyk *et al.,* 2014). Aquatic ecosystems with low productivity and clear water are considered as oligotrophic in nature. Moderately productive and limited nutrient conditions in a lake are termed as mesotrophic condition. Highly productive water bodies with high nutrient loading and nuisance algal bloom comes under eutrophic (Wetzel, 2001; Offem *et al.,* 2011). Variations in nutrient loading may possibly result in a relative variation in the community structure at each trophic level. Various trophic state indices were developed for measuring these changes in water environment (Jeppesen *et al.,* 2000). Lakes and ponds go through different trophic stages from ultra-oligotrophic to oligotrophic, mesotrophic, eutrophic and finally reaching hyper-eutrophic stage in course of time (Frumin and Krashanovskaya, 2014). Analysis of the trophic state of ponds is important in the assessment and management of ponds (Devi Prasad, 2012). It is especially helpful in developing conservation strategies (Sharma *et al.,* 2010). Both abiotic and biotic indices are used to assess the trophic state of water bodies. Nutrients, oxygen demand and transparency are the abiotic parameters. Biotic parameters consider the aquatic organisms especially algae and macro-invertebrates (Szelag- Wasielewska, 2006; Sharma *et al.,* 2010).

Various studies have been undertaken in different parts of the world for assessing the trophic state of aquatic ecosystems and some multi-parametric indices were developed by incorporating abiotic as well as biotic parameters. It is usually measured in terms of nitrogen, phosphorus, algal abundance and light penetration. Plant nutrients are the major factors which influence the biological production and their concentration has major effect on the transparency of water and algal biomass.

Trophic status of water bodies can be expressed in terms of their productivity (Murthy *et al.*, 2008). The trophic state index developed by Carlson (1977) for temperate lakes was used widely by various lake assessment studies for a long time. In this he used algal biomass as the key descriptor, which was represented by the average values obtained from TSI[SD], TSI[TP], and TSI[Chl *a*]. Kratzer and Brezonik (1981) later developed TSI[TN] based on total nitrogen concentration in lakes. Some studies focussed on the flora and fauna of the water bodies, their seasonal changes and used them as the indicators of different trophic stages (Rosenberg and Resh, 1993; Garcia-criado *et al.*, 2005; Kagalou *et al.*, 2006). Szelag- Wasielewska (2006) studied the trophic status of lakes based on the phytoplankton biomass and community structure.

Padisak *et al.* (2006) developed Q-index for Hungarian lakes based on the phytoplankton functional groups published by Reynolds *et al.* (2002). Jeppesen *et al.* (2011) suggested the use of rotifers as biological indicators for assessing trophic state. Wu *et al.* (2012) developed and tested phytoplankton index of biotic integrity by selecting community metrics which signal the water quality change by assessing their correlation with different environmental variables. A study on the trophic state of the Lake Daihai by Hou *et al.* (2013) reported cultural eutrophication. Many studies for analysing trophic status of lakes incorporated macrophyte biomass as it covers most of the lake area and does not come in the Chl *a* value (Hu *et al.*, 2014). Water quality index is formulated as an aggregation of sub-indices with weight assigned on important water quality parameters (Yan *et al.*, 2015). Tahsin and Chang (2016) developed a new multivariate trophic state index (MTSI) for trophic state assessment of storm water detention ponds with respect to TP, TN and Secchi depth.

Pond water being affected by even day by day activities needs well scheduled surveillance and monitoring of their water quality to ensure the sustenance of ponds as freshwater resources. Relative state of eutrophication can be measured by analysing the effect of physico-chemical characteristics upon dynamics. The result of water quality monitoring of four selected ponds in the Pallippuram Panchayath for the period of 2011 to 2014 is presented in this chapter.

3.2 Specific Objectives

- Analysing the water quality of selected ponds.
- Analysing the trophic states of the ponds.
- Understanding the hydro-dynamics of the pond ecosystems in relation to phytoplankton growth.

3.3 Sampling Procedure

3.3.1 Sampling location

Based on the results of the survey presented in Chapter 2, four ponds were selected from ward No.3 for further study. Selection of ponds was based on the type of algal bloom observed as it is a clear indicator of water quality and trophic state of the pond. Moreover different groups of algae differ in their specific niche requirements and may be their presence or absence can provide information on locational variations in water quality. Accordingly one pond which was observed to develop filamentous green metaphyton was selected as pond 1 for the study. Pond 2 had a bloom of blue-green metaphyton. Pond 3 had unicellular yellow-green floating scum. The fourth pond (pond 4) was maintained by annual cleaning, had clear water and no algal scum or metaphyton. All the ponds are located within a distance of 400-800 metres in ward 3 of the Panchayath (Figure 3.1).

Pond 1

Pond 1 is a shallow seasonal pond with an area of $38m^2$ and a maximum depth of 2 meters during rainy season. The pond is situated near an agricultural field and it was partially shaded by trees on one side. As it fills in the rainy season, it overflows into the paddy field. The pond is used for coconut husk retting.

Pond 2

Pond 2 is a domestic pond with high usage of the pond water for bathing and washing. It is a perennial pond with an area of 113 m^2. The maximum depth of the pond is 5 metres during monsoon. The pond was observed with floating mats of blue-green metaphyton over three fourth of the pond surface and submerged vegetation of *Hydrilla verticillata*. The pond had a good amount of shade over it from the trees inclined to it.

Pond 3

Pond 3 is situated near an abandoned paddy field and it is fully shaded by the surrounding vegetation. This is a seasonal pond that dries up in summer. The pond has an overflow channel to the paddy field. It has an area of 63m^2 and depth of 3-4 metres during monsoon. The pond is used for irrigation.

Figure 3.1: Images of the ponds selected for investigation of water quality (as in November 2011)

Pond 4

Pond 4 is a domestic pond which is selected as a control pond in this study since it is cleaned every year at the beginning of summer. The pond has an area of 78 m^2 and a maximum depth of 4 metres during monsoon. The pond was receiving good light penetration and had only less amount of shade from surrounding vegetation. It has comparatively clear water with floating vegetation of *Pistia stratiotes*. It holds water year round but with a reduced volume in summer. The pond is used mainly for direct bathing and washing clothes.

3.3.2 Water sample collection

Water samples were collected every fortnight from the four ponds during November 2011 to May 2012. Water sampling started towards the end of November when the northeast monsoon receded and the ponds were filled with water. Water samples were collected in clean polythene bottles. Physical parameters such as temperature and secchi depth transparency were measured at the time of sample collection. Dissolved oxygen samples were fixed with Winkler reagents in a BOD bottle immediate to collection and transferred to the laboratory. The samples were brought to the lab for further analysis of water quality. The process of water sampling was repeated during September 2012 to April 2013, and September 2013 to June 2014.

3.4 Methods of Analysis

Physical parameters (temperature and secchi disc transparency) were measured at the site itself. Chemical analysis of the water samples were carried out for pH, electrical conductivity (EC), dissolved oxygen (DO), Biochemical Oxygen Demand (BOD), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), ammonia-nitrogen (NH_4-N), soluble reactive phosphorus (SRP), total phosphorus (TP), and dissolved iron. Chlorophyll *a* (Chl *a*) was estimated as biological indicator of phytoplankton production. The water samples collected during 2012-13 and 2013-14 were analysed for pH, EC and chlorophyll *a*.

3.4.1 Temperature

Temperature is the most commonly measured essential physical parameter of water. Other than it's direct influence on water quality, temperature affects the physiological functioning of organisms. Temperature of the water was measured by a centigrade thermometer.

3.4.2 Secchi disc transparency

Secchi disc transparency is the measure of light penetration into the water body and that of euphotic depth. Transparency of water was measured using a secchi disc. Secchi disc is an intermittently black and white painted circular disc with a diameter of the range 20-30 cm mounted on a rope or chain and lowered down to the water till it disappears from our sight. The point of disappearance and reappearance was marked and the mean distance was computed.

3.4.3 pH

pH is defined as the negative logarithm of hydrogen ion concentration. It has a major influence on the type of organisms living in the freshwater ecosystems. pH was measured with a digital pH meter (Eutech instruments pH tutor). The pH meter was calibrated before use with pH buffers of 4, 7 and 9.2. The pH probe was then placed in the samples and the digital readings were noted. The turbid samples were filtered through whatman No.1 filter paper before taking measurements.

3.4.4 Electrical conductivity

Electrical conductivity is the ability of water to conduct electricity. It gives the measure of dissolved inorganic ions in water. The greater the dissolved ions present in water, the higher will be the conductivity of water. Electrical conductivity was measured with a conductivity meter and expressed in micromhos /centimeter (μmhos/cm).

3.4.5 Dissolved oxygen

Dissolved oxygen refers to the level of free, non-compound oxygen present in water. Dissolved oxygen in water is essential to maintain life in the ecosystem. Good amount of dissolved oxygen in a water ecosystem indicate good health of the aquatic system. A too high or too low dissolved oxygen level in water can harm aquatic life and affect water quality. Dissolved oxygen was estimated by azide modification method.

Dissolved oxygen samples were collected in BOD bottles of 300 mL volume. The samples were fixed with winkler A (1mL $MnSO_4$) and Winkler B (1mL alkali iodide azide) reagents. Mixed it well by inverting the bottle a few times and allowed the precipitate to settle down. Then added 1 mL conc.H_2SO_4. Dissolved the precipitate completely by inverting the bottle several times. 50 mL of the sample was pipetted out and titrated against standardized thiosulphate solution. The end point was found out at the point of colour change from pale yellow to blue by using starch as indicator (Eaton *et al.,* 2005).

3.4.6 Biochemical Oxygen Demand

Biochemical oxygen demand is the amount of oxygen required by bacteria to decompose organic matter under aerobic conditions. The greater the BOD, the more rapidly oxygen is depleted in the water. So less oxygen will be available to aquatic life.

Biochemical oxygen demand is determined by finding out the difference between dissolved oxygen (DO) in the water sample at the time of sampling and after incubating the sample at 20ºC for five days. Samples with less amount of DO were diluted with dilution water prepared according to Eaton *et al.* (2005). Samples were taken in two BOD bottles and the DO in the samples was fixed with 1mL $MnSO_4$ and 1mL alkali iodide azide reagents. The precipitate formed was allowed to settle at the bottom. One was fixed at the time of water sampling and analysed for DO. The other one was placed in the BOD incubator at 20ºC for five days and analysed for DO. Dissolved oxygen was measured following the azide modification method. The precipitate at the bottom of the samplers was dissolved in 1mL H_2SO_4. 50 mL of the samples were taken and titrated against 0.025M $Na_2S_2O_3$ solution using starch as indicator. The end point was noted as the colour change from pale yellow to blue. BOD values were calculated from the following equation.

BOD (mg/L) = D_1-D_2/P

D_1- DO of diluted sample immediately after preparation

D_2- DO of diluted sample after 5days incubation

1/P- dilution factor

3.4.7 Nitrite-nitrogen

Nitrite can be formed by the oxidation of ammonia or the reduction of nitrate. Nitrite in water exists as an intermediate product of the microbial reduction of nitrate or oxidation of ammonia. Nitrite (NO_2^-) produces a reddish purple azo-dye at a lower pH range of 2.0 to 2.5 by coupling diazotized sulfanilamide with *N*-(1-naphthyl)-ethylene diamine dihydrochloride (NNED). 50 mL of sample was filtered through whatman GF/C; added 2 mL of colour reagent and measured the absorbance of purple coloured azo-dye between 10 minutes and 2 hours at 543nm in UV-visible spectrophotometer (Eaton *et al.*, 2005).

3.4.8 Nitrate-nitrogen

Nitrates are the most common form of nitrogen compound found in the environment because of its stable nature. Nitrate-nitrogen in water was measured colorimetrically by hydrazine reduction method (Kamphake *et al.*, 1967). The NO_2^- (originally present) and reduced NO_3^- by hydrazine sulphate is determined by diazotization with sulfanilamide and coupling with *N*-(1-naphthyl)-ethylene diamine dihydrochloride (NNED) to form a highly coloured azo-dye that is measured colorimetrically at 543 nm in UV-visible spectrophotometer.

2 mL of buffer reagent was added to 50 mL of the water sample. Then 1 mL reducing agent was added with rapid mixing and the samples were kept at dark for 20 hrs. Added 2 mL acetone followed by 1 mL sulphanilamide solution. After 2 minutes 1 mL NNED was added and mixed. The colour is allowed to develop for 30 minutes. The absorbance of the pink coloured complex was measured at 543 nm in UV- visible spectrophotometer.

3.4.9 Ammonia-nitrogen

Ammonia is a common form of nitrogen in water bodies and is toxic to fish and other aquatic life. It is excreted by animals and produced during decomposition of plants and animals, thus returning nitrogen to the aquatic system. Ammonia-nitrogen in the water samples were determined by phenol hypochlorite method (Solorzano, 1969). The reaction of ammonia with phenol and hypochlorite at high pH gives blue coloured indophenol. Procedure consisted of addition of 2 mL phenol solution, 2mL sodium nitroprusside solution and 5 mL oxidizing reagent to 50 mL sample. The absorbance of the samples was measured in UV-visible spectrophotometer after one hour at 640 nm.

3.4.10 Soluble reactive phosphorus

Soluble reactive phosphorus (SRP) is a measure of orthophosphate, the soluble, inorganic fraction of phosphorus, which can be directly taken up by the plant cells. Soluble reactive phosphorus(SRP) was estimated by ascorbic acid method (Murphy and Riley, 1962). In this method, ammonium molybdate and antimony potassium tartrate react in an acid medium with orthophosphate in the water sample to form antimony-phospho-molybdate complex. It is then reduced to an intensely blue-colored complex by ascorbic acid and the absorbance of the complex is measured at 880 nm.

For the estimation of SRP, 50 mL of the water sample was taken in an acid washed and dry Erlenmeyer flask. 1 mL phenolphthalein indicator was added to the sample. 5N H_2SO_4 was added drop wise to discharge if red colour is formed. Then 8 mL freshly prepared combined reagent (100 mL combined reagent is prepared by mixing 50 mL 5N H_2SO_4, 5 mL potassium antimonyl solution , 15 mL ammonium molybdate solution and 30 mL ascorbic acid) was added and mixed. The absorbance of the blue coloured complex was measured at 880 nm after 10 minutes.

3.4.11 Total phosphorus

Total Phosphorus is the sum of reactive, condensed and organic phosphorous. Orthophosphates can be determined directly by colorimetric analysis. Other types require a digestion step to convert them into the ortho- form for analysis. This gives the total Phosphorus result. Total Phosphorus was measured by persulfate digestion (Eaton *et al.,* 2005) followed by ascorbic acid method. To 50 mL of the sample 1mL H_2SO_4 solution and 0.5g potassium persulfate was added and autoclaved for 30 min at 15 Pa. After cooling the sample estimation of total phosphorus was performed the same way SRP was measured spectrophotometrically (refer section 3.4.10).

3.4.12 Dissolved Iron

Dissolved iron is an essential micronutrient for phytoplankton primary production (Johnson *et al.,* 1997). For the estimation of dissolved iron the samples collected in acid washed containers were filtered through 0.45µm membrane filter and analysed spectrophotometrically (VARIAN UV-VISIBLE spectrophotometer) by phenanthroline method (Eaton *et al.,* 2005). To a 50 mL thoroughly mixed sample taken in 125 mL erlenmeyer flask, added 2 mL conc. HCl and 1 mL hydroxylamine hydrochloride solution. A few glass beads were added and boiled till its volume was reduced to 15-20 mL. It was then cooled to room temperature. 10mL ammonium acetate buffer and 4 mL phenanthrolein solution were added to the sample and diluted to the 50 mL mark. The orange- red coloured complex formed was measured in UV-visible spectrophotometer after 10 min at 510 nm.

3.4.13 Chlorophyll *a*

Chlorophyll *a* (Chl *a*) estimation gives measure of the phytoplankton biomass. Phytoplankton biomass reflects the trophic status of a water body. Hence Chl *a* is the principle variable to ascertain trophic state of an aquatic ecosystem (Boyer *et al.*, 2009). A high chlorophyll *a* concentration is an indicator of eutrophication.

Chlorophyll *a* was extracted in 90% acetone and absorbance measured in spectrophotometer. 100 mL of the well shaken water samples were taken and added 1 mL of 1% $MgCO_3$ suspension as a precaution to prevent pigment degradation. The samples were filtered through a 0.45 µm membrane filter and the filter paper was folded and kept inside a screw cap bottle. Added 8 mL 90% aqueous acetone for the extraction of pigments and kept in dark at 4ºC overnight. After the extraction period the samples were thawed and centrifuged for 20 min at 5000 rpm. The supernatants were made up to 10 mL by 90% acetone and the absorbance was read at multiple wavelengths of 630, 647, 664 and 750 nm in a spectrophotometer. The amount of Chl *a* was computed by applying the equation of Jeffrey and Humphrey (1975).

Ca = 11.85 E664-1.54 E647- 0.08 E 630

$$\text{Chl } a \text{ (µg/L)} = \frac{\text{Ca } \times \text{v}}{\text{V } \times \text{I}}$$

v- volume of acetone (mL)

V- volume of water sample taken (L)

I – path length in cm

3.5 Data Analysis

3.5.1 Statistical analysis

The temporal variation in water quality was represented through graphical illustrations. The significance of variations of parameters among the ponds was tested by ANOVA and Tukey's test. The variation of mean values of parameters between the years was compared by taking the mean values in pre-monsoon and post- monsoon. Pre-monsoon data included the data from February to May. Post monsoon data included data from October- January. The three year data on pH, EC and chlorophyll *a* were compared graphically. Origin 9.1 was used for ANOVA and Tukey's test of significance.

Association among the parameters were determined by Pearson correlation analysis and multivariate factor analysis. Principal Component Analysis (PCA) was used for the extraction of factors. SPSS ver.16 was used for PCA and correlation analysis. Principal component analysis is a data reduction technique. PCA creates new orthogonal principal components from linear combination of original variables which could explain the data variation in a new coordinate system (Praus, 2007). The components extracted were rotated by varimax rotation to get a simple matrix structure.

3.5.2 Trophic State Index (TSI)

Trophic level classification based on the nutrient status and chlorophyll *a* values were developed by Wetzel (1983) by consolidating the data from a wide range of studies in various lakes in temporal regions. The index proposed by Carlson (1991) for temporal lakes can't be applied to the tropical lakes and ponds. Therefore the TSI derived by Cunha *et al.* (2013) for the reservoirs of tropical and subtropical regions was followed. This relates the trophic state index of the water body to the amount of phosphorus and chlorophyll *a* as given below.

$$\mathrm{TSI(TP)} = 10\left[6 - \left(\frac{-.27637\ln\mathrm{TP} - 1.329766}{\ln 2}\right)\right]$$

$$\mathrm{TSI(Chl)} = 10\left[6 - \left(\frac{-.2512\ln\mathrm{Chl}a + .842257}{\ln 2}\right)\right]$$

$$\mathrm{TSIx_{tsr}} = \frac{\mathrm{TSI(TP)} + \mathrm{TSI(Chl)}}{2}$$

3.6 Results

3.6.1 Water quality of the ponds during 2011-2012

3.6.1.1 Temperature

Temperature of the pond water followed similar trend throughout the sampling period from November 2011 to May 2012 in all the four ponds with values starting between 26.1 ºC to 26.6 ºC in November. The water temperature in pond 1 ranged from 25.3 to 28.9 ºC. Pond 2 had a temperature range of 25.4-29.2 ºC. Pond 3 and Pond 4 was observed with a range of 24.8-27.9 ºC and 25.2-29.4 ºC respectively (Annexure II). The temperature of all the four ponds increased from November to early December but started to decrease in late December – January. The lowest temperature recorded was 24.8ºC. The temperature increased from February to April and reached up to 29.4 ºC. The temperature decreased in late April and May (Figure 3.2).

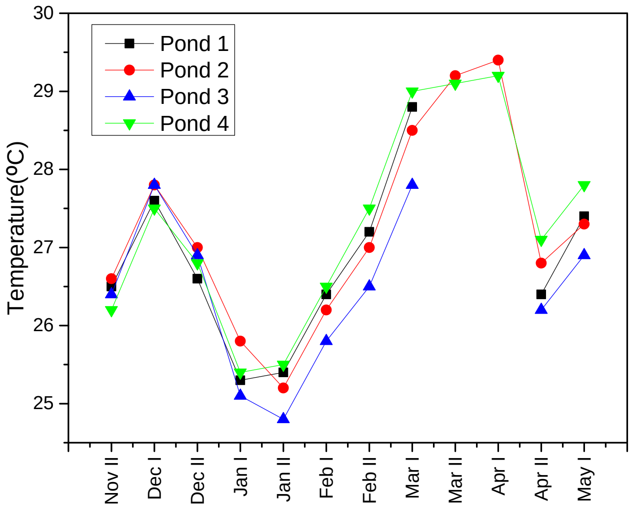

Figure 3.2: Temporal variation of temperature in the four selected ponds of Pallippuram during November 2011 to May 2012

The analysis of variance (ANOVA) of the data on pond water temperature of the four ponds is found to be insignificant at the 0.05 level (Table 3.1).

Table 3.1: Analysis of variance showing the significance of variation in temperature of the four study ponds

ANOVA	DF	Sum of squares	Mean Square	F value	Probability
Model	3	2.27	0.757	0.652	0.589
Error	24	27.87	1.161		
Total	27	30.14			

3.6.1.2 Secchi disc transparency

Changes in biological productivity can cause changes in the colour and turbidity of the pond (Fruh *et al*., 1966). Hence water transparency is a major measure of physical parameter which is used to classify the trophic status of aquatic ecosystems. The ranges of secchi depth visibility were 2 - 61 cm in pond 1, 17 - 69 cm in Pond 2, 29 - 65 cm in Pond 3 and 30-67 cm in Pond 4 (Annexure II). The secchi disc transparency values of all the ponds started with high values during November since the sampling started immediately after the monsoon showers with very clear and transparent water. By the end of December, the SD values of all the ponds started decreasing gradually (Figure 3.3). Pond 1 recorded the lowest secchi disc value during February and early March, further which the pond dried up and rejuvenated with higher transparency towards the end of April. Pond 3 behaved similarly but had higher level of transparency than pond 1. The transparency of water was highest in pond 4 in summer months. Analysis of variance of the data from the four ponds revealed that there is no significant variation at $P \leq 0.05$ (Table 3.2).

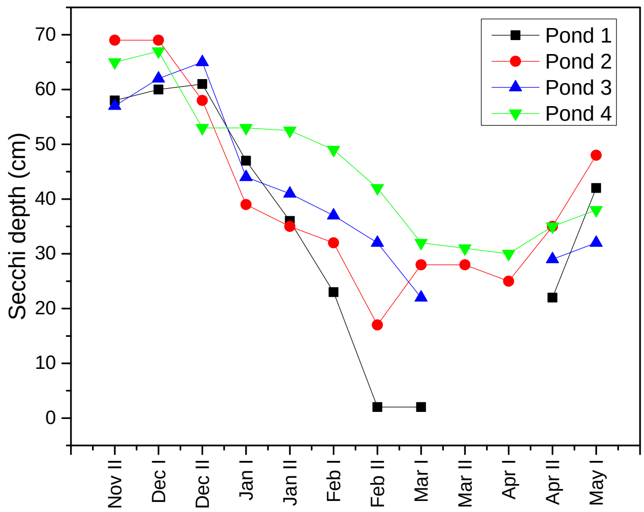

Figure 3.3: Temporal variations in Secchi disc transparency of the four selected ponds of Pallippuram during November 2011 to May 2012

Table 3.2: Analysis of variance showing the significance of variation in secchi disc transparency of the four study ponds

ANOVA	DF	Sum of squares	Mean Square	F value	Probability
Model	3	574.7	191.57	0.624	0.6063
Error	24	7366.6	306.94		
Total	27	7941.3			

3.6.1.3 pH

pH is an important factor in determining the productivity of an ecosystem. The pH of pond 1 was in the range of 5.40-7.77 (Annexure II). Pond 1 recorded a mean pH of 5.49 in November. It maintained pH below 6 till the end of December and recorded 6.36 in January first half. From January onwards pH of the pond water increased recording highest value of 7.74 in February second half. The pH then gradually decreased to 6.81 in May. The pH in pond 2 ranged 6.02-7.09. Pond 2 started with a pH of 6.42 in November. It maintained the pH above 6.0 throughout the sampling. Highest pH in pond 2 was recorded in March. The range of pH in pond 3 was 5.48-6.89. Pond 3 recorded a pH of 5.53 in November. The pH increased gradually to reach its highest value at the end of sampling in May. The pH of the pond 4 water was in the range of 6.22-7.54. The pH in pond 4 started with a value of 6.23 in November. Then an increase in pH was observed till March first half and a decrease thereafter. The pH in all the four ponds followed a specific trend, starting with lower pH values in November and gradually increasing toward February- March (Figure 3.4). The variations in pH of the four ponds were not significant at the 0.05 significance level (Table 3.3).

Table 3.3: Analysis of variance showing the significance of variation in pH of the four study ponds

ANOVA	DF	Sum of squares	Mean Square	F value	Probability
Model	3	1.481	0.493	1.973	0.145
Error	24	6.001	0.250		
Total	27	7.482			

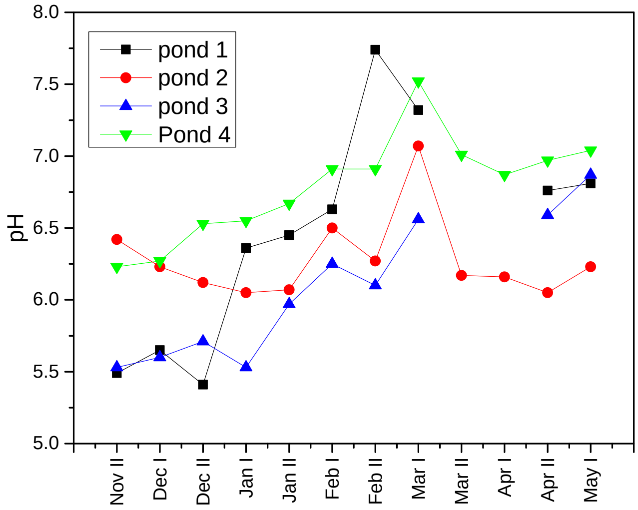

Figure 3.4: Temporal variation of pH in the four selected ponds of Pallippuram during November 2011 to May 2012

3.6.1.4 Electrical conductivity

The electrical conductivity in the ponds remained low in November-December and then started increasing from January with some fluctuations (Figure 3.5). The EC values in pond 1 ranged from 60 to 240 µmho/cm. Pond 1 showed steep increase in EC from December 2nd half towards February. There was reduction in EC during March, but it began to increase towards April- May. The EC range in pond 2 was 68- 221 µmho/cm. EC in pond 2 was low during November- February. Then it gradually increased towards higher values in March- April immediately followed by a reduction in the mid of April and a further increase in May reaching the highest EC value. Electrical conductivity range in pond 3 was between 89.00 µmho/cm and 240.0 µmho/cm. The EC range in pond 4 was between 110 and 281 µmho/cm. Highest EC values were recorded in pond 4 (Annexure II). Variations in electrical conductivity of the ponds are significantly different at the 0.05 level. Tukey's test was performed to further confirm the results of ANOVA. Tukey's analysis showed significant variations between the EC values of pond 2 and pond 4 (Table 3.4).

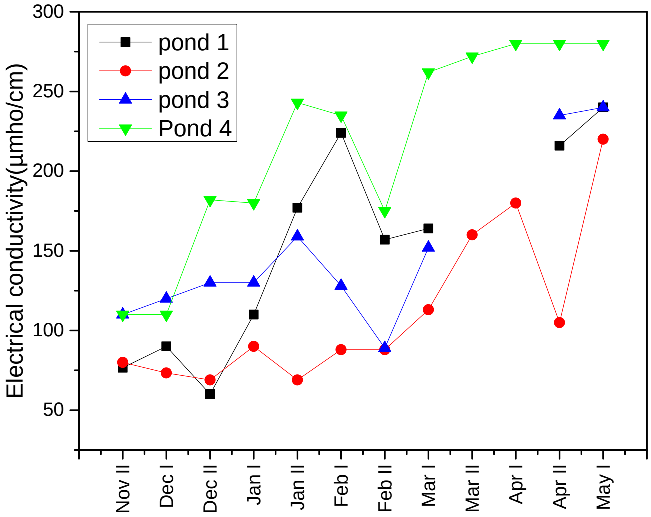

Figure 3.5: Temporal variation of electrical conductivity in four selected ponds of Pallippuram during November 2011 to May 2012

Table 3.4: Analysis of variance showing the significance of variation in Electrical Conductivity of the four study ponds

ANOVA	DF	Sum of squares	Mean Square	F value	Probability
Model	3	33545.3	11181.8	3.049	0.048*
Error	24	88000.7	3666.69		
Total	27	121546.1			

* Population means are significantly different at P ≤ 0.05

Multiple comparison by Tukey's test of significance

Group	Identity	Mean	2 1 3 4
2	Pond 2	116.81	\
1	Pond 1	157.95	. \
3	Pond 3	159.28	. . \
4	Pond 4	214.21	* . . \

*= significant difference (p≤0.05)

3.6.1.5 Dissolved oxygen

Dissolved oxygen in a water body represents the health status of the ecosystem at a glance. Dissolved Oxygen concentration > 5 mg/L favours good growth of flora and fauna (Das, 2000). The level of DO in all the four ponds was comparatively high during November to January. The DO remained low in February- March and then increased towards April- May. The range of DO in pond 1 was 0.40-8.06 mg/L, pond 2 was 0.00- 6.85 mg/L., pond 3 was 1.61-7.26 mg/L, and pond 4 was 3.23-7.26 mg/L (Annexure II). Then it fluctuated over the entire sampling period and recorded lower values during the months of February and March (Figure 3.6). The analysis of variance for the DO data of the four ponds didn't show significant variation between them (Table 3.5).

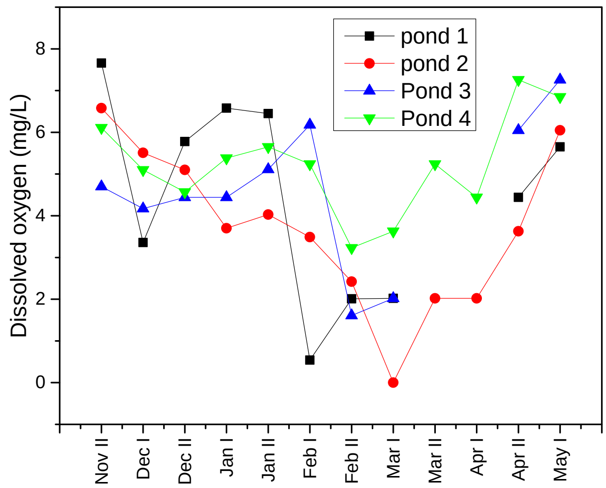

Figure 3.6: Temporal variation of DO concentration in four selected ponds of Pallippuram during November 2011 to May 2012

Table 3.5: Analysis of variance showing the significance of variation in dissolved oxygen of the four study ponds

ANOVA	DF	Sum of squares	Mean Square	F value	Probability
Model	3	5.080	1.693	0.562	0.640
Error	24	72.27	3.011		
Total	27	77.35			

3.6.1.6 Biochemical Oxygen Demand

Biochemical Oxygen Demand is the measure of the amount of oxygen required by microorganisms to breakdown the organic matter present in the water. BOD is the one single test to analyse the organic pollution in water bodies. The BOD in pond 1 ranged 2.42 - 34.25 mg/L. Pond 1 had the lowest BOD in November. Then it showed small increase till January and sudden hike in February (Figure 3.7). The BOD values started decreasing from the second half of February. In pond 2, it ranged from 4.02- 33.80 mg/L. The BOD values in pond 2 were high during

February-April. The BOD values in pond 3 ranged between 5.62 mg/L and 10.52 mg/L. Pond 3 had small fluctuations in BOD values but didn't show much increase. It maintained an average value around 4.00 mg/L throughout the sampling except with a little hike in February. BOD in pond 4 ranged 2.02-16.15 mg/L. The pond went to high concentrations - during February and April. The general trend of BOD values in the ponds were lower BOD during November-January and an increase in February- April (Annexure II). Biochemical Oxygen Demand (BOD) level of the four ponds did not differ significantly (Table 3.6).

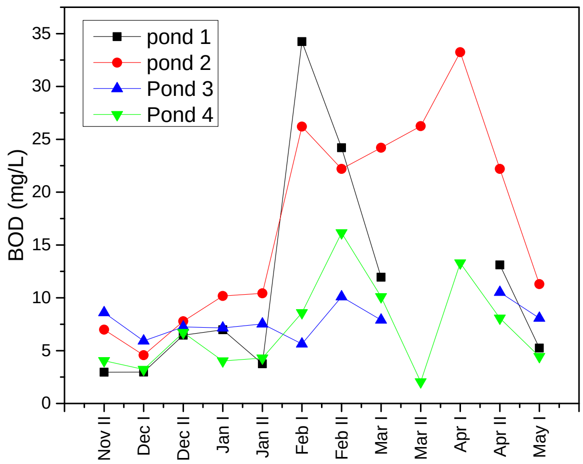

Figure 3.7: Variation of BOD in the ponds of Pallippuram during November 2011 to May 2012

Table 3.6: Analysis of variance showing the significance of variation in BOD of the four study ponds

ANOVA	DF	Sum of squares	Mean Square	F value	Probability
Model	3	351.34	117.11	2.543	0.079
Error	24	1104.9	46.037		
Total	27	1456.2			

3.6.1.7 Nitrite- nitrogen

Generally, nitrites are formed in water due to bacterial action and oxidation of ammonia. Nitrites are readily oxidized to nitrates, though they are seldom present in significant concentration in surface water. The nitrites in water are indicative of organic pollution. Biological decomposition of all nitrogenous organic matter such as sewage and animal wastes contribute nitrite in water. Its presence indicates that the nitrogenous organic matter is undergoing oxidation or nitrification and that the process is not complete. The range of nitrite-nitrogen in pond 1 was 3.00-124.8 µg/L. Nitrite–nitrogen in Pond 1 was low with values below 5.0 µg/L till the first half of January. Then showed a small increase and maintained that level in February. The highest value of NO_2-N in pond 1 was reported during March and then it decreased thereafter. The nitrite-nitrogen in pond 2 ranged between 3.82 and 132.9 µg/L. Pond 2 is characterized with prominent peaks after the first week of March (Figure 3.8). The range of nitrite-nitrogen in pond 3 was 7.09-115.9 µg/L. Pond 3 had intermittently fluctuating nitrite values. The higher nitrite-nitrogen in pond 3 was reported during January-February and a decrease in March. It maintained the value in that range in April followed by a little increase in May. The nitrite-nitrogen level in pond 4 ranged between 3.27- 33.16 µg/L. Pond 4 was the one with the lowest nitrite concentration and it persistently maintained nitrite level at minimum (Annexure II). Analysis of variance didn't show any significant variation among the ponds (Table 3.7).

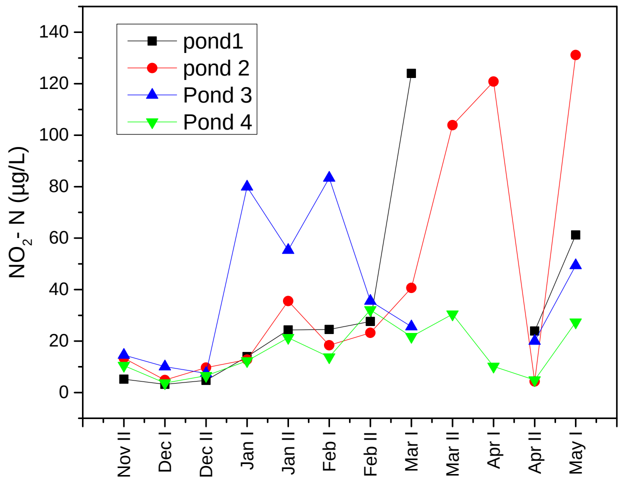

Figure 3.8: Temporal variation of Nitrite-nitrogen in four selected ponds of Pallippuram during November 2011 to May 2012

Table 3.7: Analysis of variance showing the significance of variation in nitrite-nitrogen of the four study ponds

ANOVA	DF	Sum of squares	Mean Square	F value	Probability
Model	3	3415.67	1138.5	1.033	0.395
Error	24	26436.6	1101.5		
Total	27	29852.3			

3.6.1.8 Nitrate- nitrogen

Nitrate-nitrogen is the available form of nitrogen to the plants. An increase in concentration in February-March is the overall trend of nitrate-nitrogen in ponds (Figure 3.9). Nitrate-nitrogen in pond 1 ranged from 17.96 to 255.1 µg/L. Pond 1 recorded the highest nitrate value among the four ponds. The range of NO_3-N in pond 2 was 29.07-236.4 µg/L. Pond 2 recorded the highest value of nitrate-nitrogen during April. In pond 3 the nitrate level ranged from 33.44 to 185.4 µg/L. Highest nitrate-nitrogen in pond 3 was observed during the month of February. The range of nitrate-nitrogen in pond 4 was 29.81-155.0 µg/L (Annexure II). Pond 4 maintained nitrate-nitrogen concentration without much fluctuation except in March. The variations in the nitrate levels among the ponds were insignificant at the 0.05 level of significance (Table 3.8).

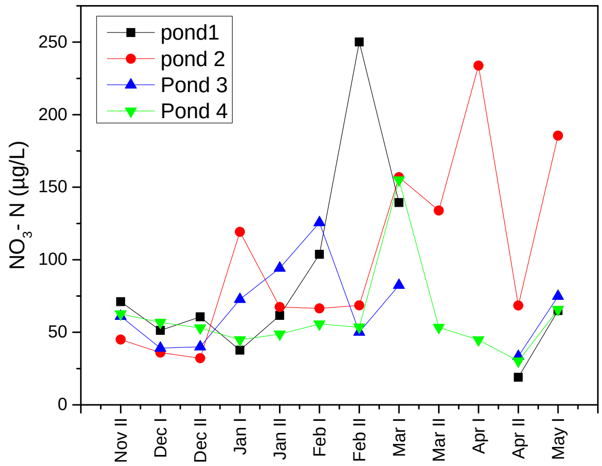

Figure 3.9: Temporal variation of nitrate-nitrogen in four selected ponds of Pallippuram during November 2011 to May 2012

Table 3.8: Analysis of variance showing the significance of variation in nitrate-nitrogen of the four study ponds

ANOVA	DF	Sum of squares	Mean Square	F value	Probability
Model	3	7714.77	2571.5	1.431	0.258
Error	24	43126.7	1796.9		
Total	27	50841.5			

3.6.1.9 Ammonia- nitrogen

Ammonia is generated from aerobic and anaerobic decomposition of nitrogenous organic matter. Increasing ammonium values along with declining DO indicate the presence of decomposition processes in the ponds. Ammonia-N in ponds ranged 5.93 - 42.84 µg/L in pond 1, 4.99-72.62 µg/L in pond 2, 13.19 -178.6 µg/L in pond 3, and 2.51 - 53.49 µg/L in pond 4 (Annexure II). It is evident from Figure 3.10 that the ammonia-nitrogen in pond 3 is very much higher than the other three ponds. In all other ponds, the values were below 80 µg/L. Pond 2 and pond 3 followed a similar pattern of increase in ammonia concentration during the months of January and March, though in the former the peak values was much lower *ie*. 72.62 µg/L. Peak value of ammonia-nitrogen was recorded in pond 3 during January reaching up to 178.6µg/L. The level of NH_4-N was low in pond 4. The ANOVA test revealed significant variations in the ammonia values among the ponds at the 0.05 significance level. Further confirmation of the result by Tukey's test showed that pond 3 had significantly higher level of ammonia-nitrogen (Table 3.9).

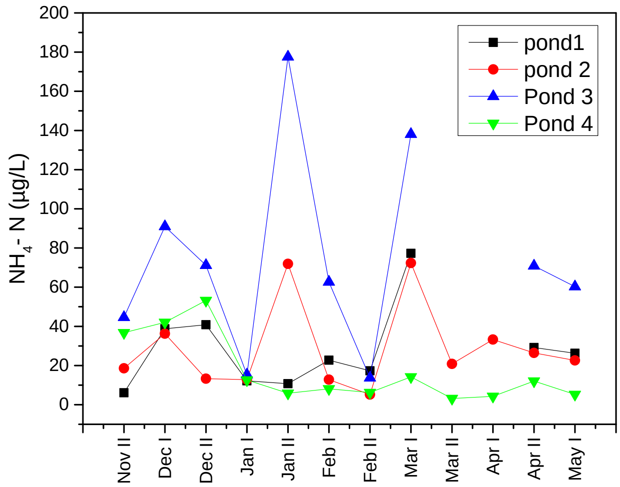

Figure 3.10: Temporal variation of ammonia-nitrogen in four ponds of Pallippuram during November 2011 to May 2012

Table 3.9: Analysis of variance showing the significance of variation in ammonia-N of the four study ponds

ANOVA	DF	Sum of squares	Mean Square	F value	Probability
Model	3	16000.8	5333.6	9.218	3.0806E-4*
Error	24	13886.2	578.59		
Total	27	29887.1			

* Population means significantly differ at P ≤0.01 level

Multiple comparison by Tukey's test of significance

Group	Identity	Mean	2 4 1 3
2	Pond 2	17.55	\
4	Pond 4	17.55	. \
1	Pond 1	30.01	. . \
3	Pond 3	75.64	* * * \

*= significant difference (p≤0.05)

3.6.1.10 Soluble Reactive Phosphorus

Soluble Reactive phosphorus is the inorganic phosphorus which is readily available to the plants. The SRP in pond 1 ranged between 12.16 µg/L and 3833.0 µg/L. Soluble reactive phosphorus in pond 1 started with 48.54 µg/L in November. It gradually decreased to reach the lowest value in January first half. Then the values went above 1000 µg/L in February and marked its highest value in March (Annexure II). The SRP in pond 2 ranged 2.16 - 786.7 µg/L. Pond 2 had lower values till February first half. The SRP in pond 2 increased gradually reaching the highest value in March and observed a gradual decrease thereafter. Pond 3 on the other hand had fluctuating SRP values over the sampling period. The SRP range in pond 3 was 7.23 - 401.0 µg/L. The SRP in pond 4 ranged 289.6 – 215.9 µg/L. Pond 4 had high SRP values in almost all sampling months (Figure 3.11). It started with a mean value of 1477.0 µg/L in November and reached highest value in February. The analysis of variance showed significant variations in SRP values among the ponds.

Tukey's test was performed to find the significant difference among the ponds (Table 3.10). Tukey's test result identified a significant difference in variation of SRP among the ponds. Pond 1 differed significantly from pond 3 and pond 4 from pond 2 and pond 3.

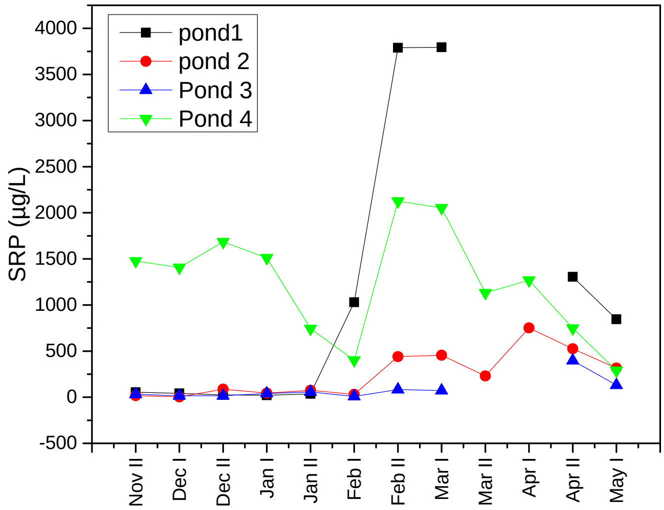

Figure 3.11: Temporal variations of SRP in four selected ponds of Pallippuram during November 2011 to May 2012

Table 3.10: Analysis of variance showing the significance of variation in SRP of the four study ponds

ANOVA	DF	Sum of squares	Mean Square	F value	Probability
Model	3	1.0816E7	3.605E6	5.23	0.0149*
Error	36	2.4773E7	688154.1		
Total	39	3.5590E7			

* Population means significantly differ at P ≤0.05

Multiple comparison by Tukey's test of significance

Group	Identity	Mean	3 2 4 1
3	Pond 3	106.2	\
2	Pond 2	238.3	. \
4	Pond 4	1191.3	* * \
1	Pond 1	1209.6	* . . \

*= significant difference (p=0.05)

3.6.1.11 Total Phosphorus

The source of phosphorus in ponds is mainly agricultural runoff and synthetic detergents. The high concentration of phosphorus is an indication of eutrophication. The total phosphorus concentration in the ponds recorded very high values in pond 1 followed by pond 4 (Annexure II). Total phosphorus concentration in ponds followed the same pattern as that of soluble reactive phosphorus (Figure 3.12). The TP values in pond 1 ranged 105.1-5354 µg/L. Total phosphorus in pond 1 recorded a mean value of 160.8 µg/L in November and decreased gradually till January. The TP values increased by the end of January reaching highest TP in February second half. Total phosphorus in pond 2 was in the range of 88.20 -1477 µg/L. Highest TP in pond 2 was recorded in January first half. The TP in pond 3 ranged from 93.00 - 556.3 µg/L. Pond 3 didn’t show much fluctuation in TP except in April when the pond rejuvenated after the dry period. Highest TP in pond 3 was recorded in April second half. The TP range in pond 4 was 351.4-2889 µg/L. Pond 4 with an initial mean TP of 1574.1 µg/L, maintained a similar trend till January and then slightly decreased to a mean value of 649.5 µg/L. It again increased in February to 2889 µg/L and then gradually decreased to 351.4 µg/L in May. The ANOVA test showed significant variation of TP among the ponds at the 0.05 significance level (Table 3.11). Tukey’s test result identified a significant difference in variation of TP between pond 1 and pond 3.

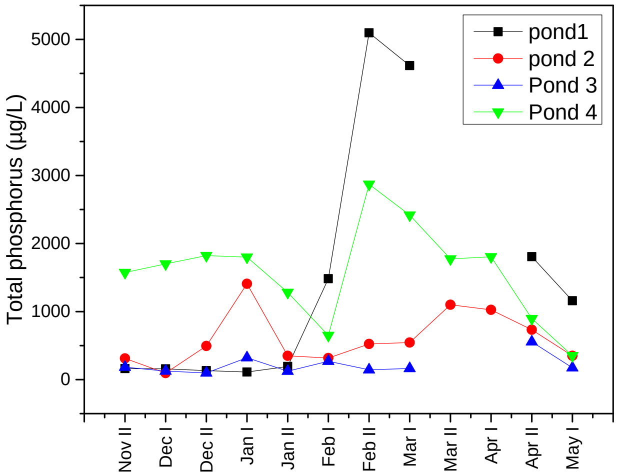

Figure 3.12: Temporal variations in the total phosphorus concentration of four ponds in Pallippuram during November 2011 to May 2012

Table 3.11: Analysis of variance showing the significance of variation in TP of the four study ponds

ANOVA	DF	Sum of squares	Mean Square	F value	Probability
Model	3	9. 8155E6	3.2718E6	3.755	0.024*
Error	24	2.0909E7	871213.1		
Total	27	3.0724E7			

* Population means differ significantly at $P \leq 0.05$

Multiple comparison by Tukey's test of significance

Group	Identity	Mean	3 2 4 1
3	Pond 3	230.85	\
2	Pond 2	565.17	. \
4	Pond 4	1490.7	. . \
1	Pond 1	1618.8	* . . \

*= significant difference ($p \leq 0.05$)

3.6.1.12 Dissolved iron

Dissolved iron has been projected as a determining factor in phytoplankton bloom development by certain studies (Takeda and Tsuda, 2005; Hoppe *et al.*, 2015). Dissolved iron in pond 1 was in the range of 292.3-1670 µg/L. The variation of dissolved iron concentration in pond 1 showed a zig-zag pattern (Figure 3.13). The values of dissolved iron in pond 2 ranged 85.14 - 1383.0 µg/L. Pond 2 had the lowest dissolved iron concentration compared to other ponds. Pond 3 and Pond 4 were marked with high dissolved iron in January and the variations in values were minimal. The values ranged from 198.5 to 594.7 µg/L in pond 3, and from 107.1 to 784.2 µg/L in pond 4 (Annexure II). The result of variance analysis showed significant difference in the dissolved iron concentration among the ponds. Tukey's test revealed that the dissolved iron content of pond 1 is significantly higher than pond 3 and pond 4 (Table 3.12).

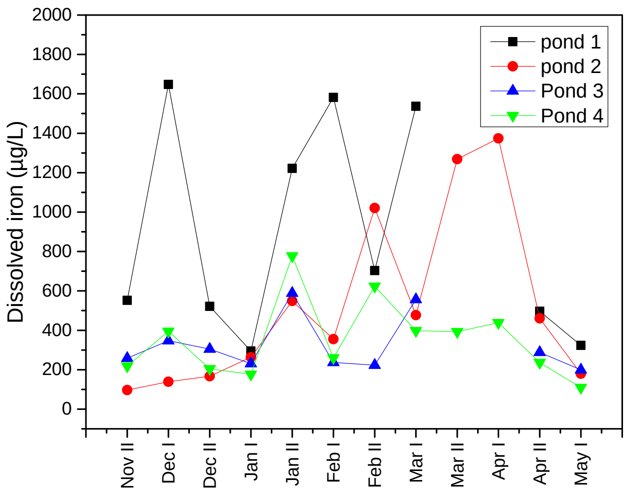

Figure 3.13: Temporal variation of dissolved iron in the water samples of four ponds in Pallippuram during November 2011 to May 2012

Table 3.12: Analysis of variance showing the significance of variation in dissolved iron of the four study ponds

ANOVA	DF	Sum of squares	Mean Square	F value	Probability
Model	3	1.2512E6	417091.3	4.906	0.008*
Error	24	2.0400E6	85001.2		
Total	27	3.2914E6			

* Population means differ significantly at P ≤ 0.01

Multiple comparison by Tukey's test of significance

Group	Identity	Mean	3 4 2 1
3	Pond 3	324.01	\
4	Pond 4	325.74	. \
2	Pond 2	473.35	. . \
1	Pond 1	842.08	* * . \

*= significant difference (p ≤ 0.05)

3.6.1.13 Chlorophyll *a*

The concentration of chlorophyll *a* is an indirect measure of the phytoplankton biomass in water bodies. The values of chlorophyll *a* in pond 1 ranged 6.20 - 667.7 µg/L (Annexure II). Chlorophyll *a* in pond 1 was 20.20µg/L in November and it showed a profound increase in concentration during February and March. Pond 2 had chlorophyll *a* values in the range of 32.18 - 376.0 µg/L. Mean chlorophyll *a* in pond 2 increased from 38.2 µg/L in November to 90.8 µg/L by the end of December. It then decreased to record the lowest value in January second half. Then it gradually increased to reach the highest value in March second half (Figure 3.14). Chlorophyll *a* in pond 3 ranged 15.73 - 273.6 µg/L. The higher values were obtained in March. Pond 4 recorded the lowest amount of chlorophyll *a*. The values were in the range of 4.51 - 223.6 µg/L. According to the analysis of variance results the variance of the chlorophyll *a* values are not significant at the 0.05 level (Table 3.13).

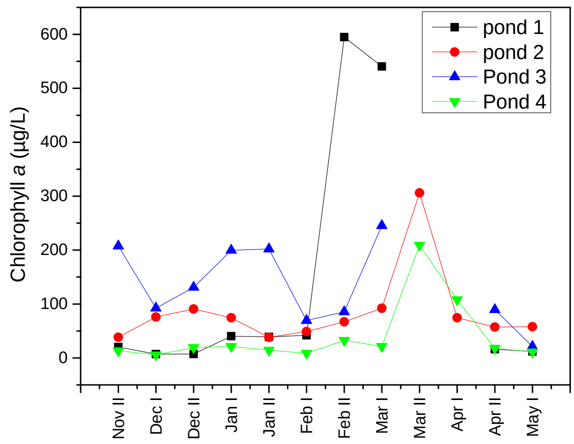

Figure 3.14: Temporal variation of chlorophyll *a* concentration in four selected ponds of Pallippuram during November 2011 to May 2012

Table 3.13: Analysis of variance showing the significance of variation in chlorophyll *a* of the four study ponds

ANOVA	DF	Sum of squares	Mean Square	F value	Probability
Model	3	49539.51	16513.17	1.188	0.335
Error	24	333415.9	13892.33		
Total	27	382955.5			

3.6.2 Water quality of the ponds during 2012-2014

Pre-monsoon (PRM) and post-monsoon (POM) data of three important parameters connected with phytoplankton production were taken for two more consecutive study periods *ie.* 2012 - 2014. The mean values of pH, EC and chlorophyll *a* for the pre- monsoon and post-monsoon period is illustrated graphically.

3.6.2.1 pH

pH in the ponds recorded high values during pre-monsoon season (Figure 3.15). The pH in pond 1 ranged from 5.40-7.77 during 2011-12, 5.87- 7.89 in 2012-13 and 6.23-7.00 in 2013-14. Pond 2 had pH in the range of 6.02-7.09, 6.39-7.47 and 6.40- 7.19 during 2011-12, 2012-13 and 2013-14 respectively. pH of pond 3 were in the range of 5.48- 6.89, 5.57-6.84 and 5.70-6.30 during 2011-12, 2012-13 and 2013-14 respectively. Pond 4 recorded pH ranging 6.22-7.54, 6.22-7.89 and 6.67-7.29 during 2011-12, 2012-13 and 2013-14 respectively (Annexure III). Pond 1, pond 2 and pond 4 recorded highest mean pH during pre-monsoon period of 2012-13. Pond 3 showed a deviation from this trend and recorded highest pH during pre-monsoon period of 2011-12. The lowest mean pH was recorded during post-monsoon period of 2011-12 in all the four ponds.

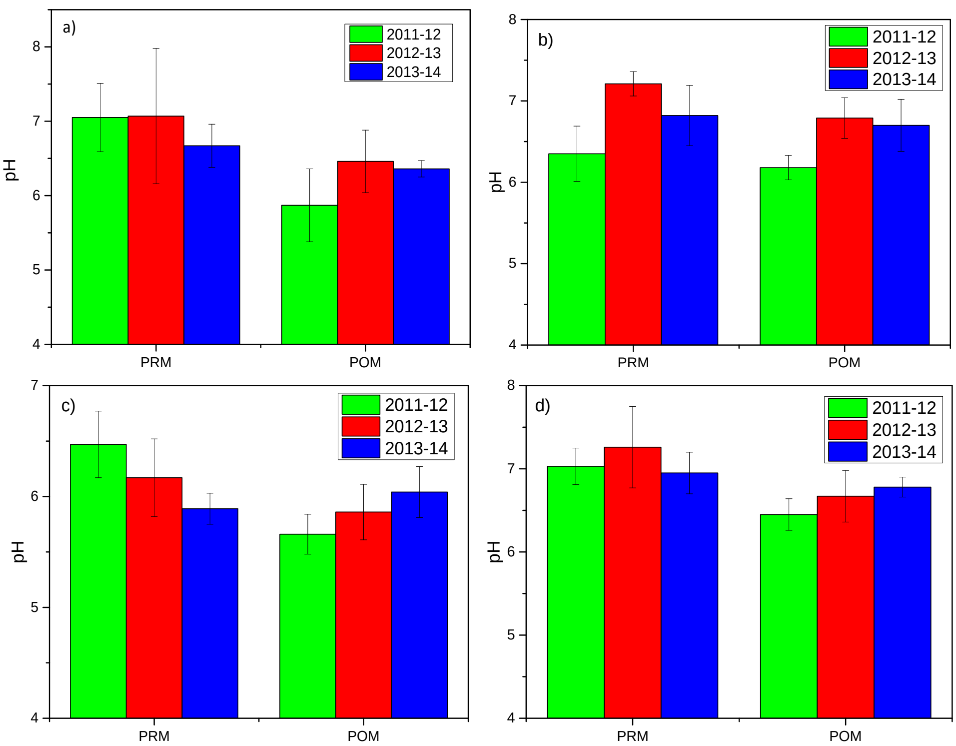

Figure 3.15: The mean pH of pre -monsoon and post- monsoon period of a) pond 1, b) pond 2, c) pond 3, d) pond 4

3.6.2.2 Electrical conductivity

The electrical conductivity of all the ponds was higher during pre-monsoon period (Figure 3.16). Pond 1 recorded EC in the range of 60-240 µmho/cm, 39- 276 µmho/cm and 132-383 µmho/cm during 2011-12, 2012-13 and 2013-14 respectively. Pond 2 ranged 68- 221 µmho/cm

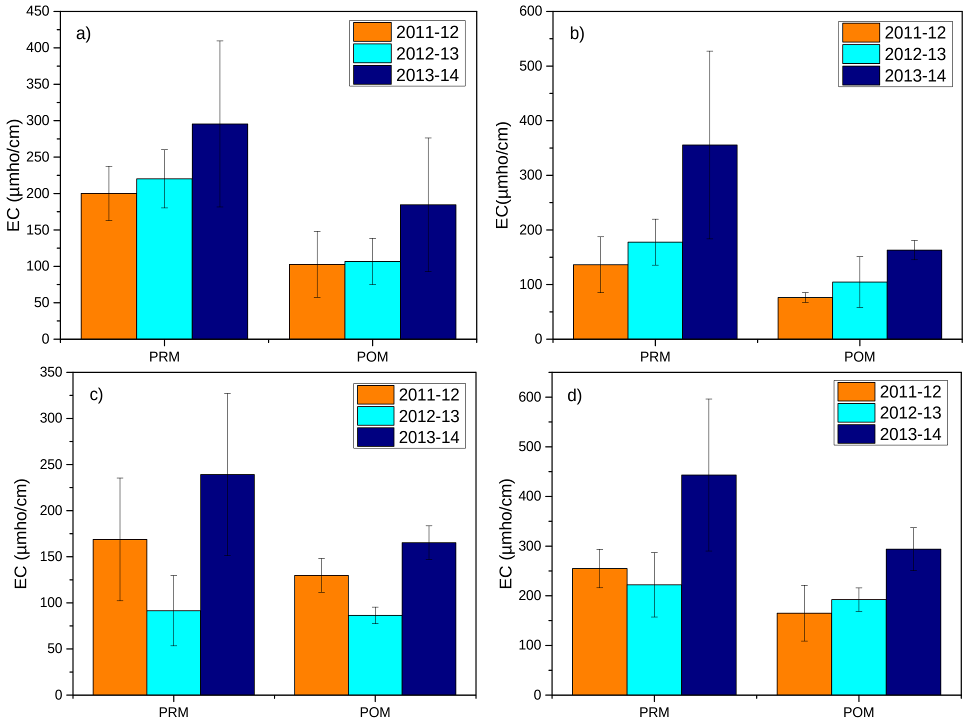

Figure 3.16: The mean EC of pre -monsoon and post- monsoon period of a) pond 1, b) pond 2, c) pond 3, d) pond 4

during 2011-12, 12-212 µmho/cm during 2012-13 and 48- 492 µmho/cm during 2013-14. In pond 3 it ranged 89- 240 µmho/cm, 16- 122 µmho/cm and 98- 306 µmho/cm during 2011-12, 2012-13 and 2013-14 respectively (Annexure IV). The highest EC values in all the four ponds recorded during pre-monsoon period of the study year 2013-14.

3.6.2.3 Chlorophyll *a*

The Chl *a* was higher in pre-monsoon compared to post-monsoon in pond1, pond 2 and pond 4. There were no common trends over the years (Figure 3.17). Chlorophyll *a* in pond 1 ranged from 6.20 µg/L to 667.7 µg/L in 2011-12, 2.19-329.7 µg/L in 2012-13 and 5.07- 160.6 µg/L in 2013-14. Pond 2 had a Chl *a* range of 32.18- 376.0 µg/L, 2.00-155.9 µg/L and 9.85-283.2 µg/L during 2011-12, 2012-13 and 2013-14 respectively. Pond 3 showed Chl *a* range from15.73 µg/L to 273.6 µg/L, 4.69-97.50 µg/L and 7.33-189.0 µg/L in 2011-12, 2012-13 and 2013-14 respectively. In pond 4 it ranged 4.51- 223.6 µg/L, 1.02-173.6 µg/L and 1.04-113.5 µg/L in 2011-12, 2012-13 and 2013-14 respectively (Annexure V).

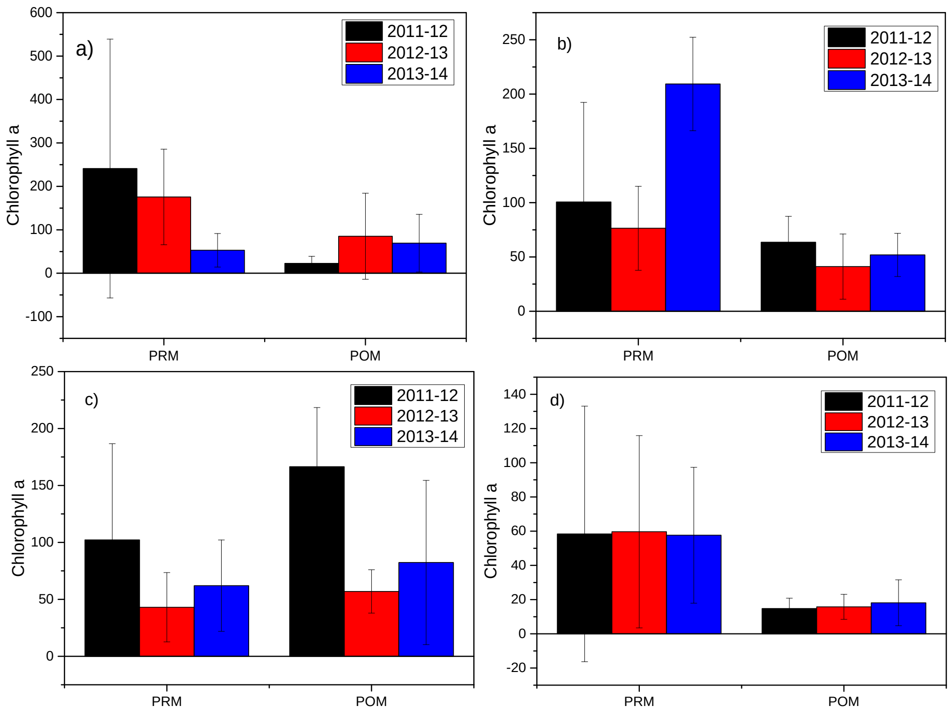

Figure 3.17: The mean chlorophyll *a* of pre -monsoon and post- monsoon period of a) pond 1, b) pond 2, c) pond 3, d) pond 4

3.6.3 Correlation analysis

Pearson correlation analysis was performed to discover the significant relationship between the biotic and abiotic parameters. Chlorophyll *a* was taken as the biotic parameter because it represents quantitatively the entire phytoplankton community of the water column. The abiotic parameters were represented by the water quality characteristics analysed. In pond 1, SD, pH, NO_3-N and TP showed significant correlation with chlorophyll *a* (Table 3.14). The relation of Chl *a* to TP is very strong (r = 0.98). Total phosphorus has the highest positive correlation with chlorophyll *a* followed by nitrate-nitrogen. It implies that high input of TP coupled with nitrate is the major determining factor in primary productivity of pond 1. The significant negative correlation of secchi depth with Chl *a* is indicative of the algal turbidity. In pond 2 only temperature is found to be significantly correlated with chlorophyll *a* value (Table 3.15). It clearly indicates the decisive role of temperature in determining the primary production in the pond. Pond 3 variables didn’t show any significant correlation with chlorophyll *a* (Table 3.16). Even though dissolved iron had correlation coefficients above 0.50 in both pond 2 and pond 3 it was not found significant in the correlation analysis. Pond 4 exhibited a trend similar to that of pond 2. Chlorophyll *a* in pond 4 was found significantly correlated with temperature (Table3.17).

Table 3.14: Pearson correlation matrix for different water quality parameters in pond 1

	SD	Temp	pH	EC	DO	NH4-N	NO2-N	NO3-N	SRP	TP	Iron	BOD	Chl *a*
SD	1												
Temp	-0.363	1											
pH	**-0.939**	0.297	1										
EC	**-0.725**	0.171	**-0.835**	1									
DO	**0.635**	-0.496	-0.477	0.223	1								
NH4-N	-0.322	**0.797**	0.268	0.010	-0.455	1							
NO2-N	**-0.643**	**0.678**	**0.696**	-0.479	-0.337	**0.714**	1						
NO3-N	**-0.680**	0.427	**0.660**	-0.284	-0.533	0.124	0.323	1					
SRP	-0.364	0.537	0.478	-0.345	-0.157	0.225	0.613	0.334	1				
TP	**-0.804**	0.563	**0.769**	-0.342	-0.459	0.413	0.565	**0.859**	0.272	1			
Iron	-0.250	0.383	0.112	0.149	**-0.635**	0.442	0.261	0.192	-0.211	0.257	1		
BOD	**-0.643**	0.051	0.493	-0.443	**-0.815**	-0.013	0.085	0.553	0.058	0.337	0.281	1	
Chl *a*	**-0.800**	0.572	**0.806**	-0.395	-0.481	0.425	0.604	**0.884**	0.337	**0.982**	0.213	0.378	1

* Significant values are given in bold letters

Table 3.15: Pearson correlation matrix for different water quality parameters in pond 2

	SD	**Temp**	**pH**	**EC**	**DO**	**NH4-N**	**NO2-N**	**NO3-N**	**SRP**	**TP**	**Iron**	**BOD**	**Chl *a***
SD	1												
Temp	-0.203	1											
pH	-0.114	0.263	1										
EC	-0.276	**0.588**	-0.020	1									
DO	**0.832**	-0.467	-0.441	-0.141	1								
NH4-N	-0.101	0.068	0.398	-0.045	-0.344	1							
NO2-N	-0.317	**0.598**	-0.047	**0.940**	-0.189	0.075	1						
NO3-N	-0.476	**0.591**	0.147	**0.859**	-0.458	0.194	**0.853**	1					
SRP	**-0.622**	**0.588**	0.098	0.564	**-0.583**	0.134	0.495	**0.671**	1				
TP	-0.475	0.242	-0.295	0.284	-0.460	-0.210	0.266	0.492	0.328	1			
Iron	**-0.753**	**0.591**	-0.114	0.404	**-0.673**	0.012	0.538	0.520	**0.653**	0.502	1		
BOD	**-0.825**	0.542	0.247	0.425	**-0.789**	0.019	0.426	0.564	**0.729**	0.404	**0.787**	1	
Chl *a*	-0.239	**0.593**	-0.054	0.318	-0.377	-0.103	0.396	0.214	0.049	0.459	0.527	0.321	1

*Significant values are given in bold letters

Table 3.16: Pearson correlation matrix for different water quality parameters in pond 3

	SD	Temp	pH	EC	DO	NH4-N	NO2-N	NO3-N	SRP	TP	Iron	BOD	Chl *a*
SD	1												
Temp	0.076	1											
pH	**-0.815**	0.205	1										
EC	-0.453	-0.005	**0.744**	1									
DO	0.071	-0.322	0.267	**0.658**	1								
NH4-N	-0.123	0.037	0.202	0.232	0.010	1							
NO2-N	-0.373	**-0.683**	0.116	0.026	0.299	-0.127	1						
NO3-N	-0.356	-0.416	0.211	-0.048	0.229	0.273	**0.783**	1					
SRP	-0.502	-0.037	0.579	**0.737**	0.286	-0.022	-0.188	-0.386	1				
TP	-0.398	-0.303	0.300	0.506	0.370	-0.255	0.188	-0.114	**0.799**	1			
Iron	-0.158	0.026	0.053	0.013	-0.311	**0.930**	-0.149	0.205	-0.092	-0.282	1		
BOD	-0.439	-0.026	0.347	0.294	-0.197	-0.226	-0.334	-0.494	**0.720**	0.404	-0.127	1	
Chl *a*	0.056	-0.166	-0.409	-0.345	-0.475	0.376	-0.064	0.124	-0.274	-0.149	0.629	-0.035	1

*Significant values are given in bold letters

Table 3.17: Pearson correlation matrix for different water quality parameters in pond 4

	SD	Temp	pH	EC	DO	NH4-N	NO2-N	NO3-N	SRP	TP	Iron	BOD	Chl *a*
SD	1												
Temp	-0.704	1											
pH	**-0.874**	**0.597**	1										
EC	**-0.886**	0.462	**0.803**	1									
DO	0.124	-0.346	-0.221	0.169	1								
NH4-N	**0.690**	-0.244	**-0.641**	**-0.719**	-0.037	1							
NO2-N	-0.466	0.299	0.541	0.352	-0.278	**-0.626**	1						
NO3-N	-0.211	0.400	0.552	0.100	-0.439	0.021	0.269	1					
SRP	0.077	0.190	-0.038	-0.458	**-0.764**	0.355	0.039	0.406	1				
TP	-0.051	0.269	0.052	-0.332	**-0.844**	0.138	0.214	0.337	**0.954**	1			
Iron	-0.070	0.051	0.092	0.048	-0.468	-0.311	0.371	0.029	0.228	0.450	1		
BOD	-0.437	0.327	0.421	0.191	**-0.598**	-0.281	0.155	0.134	0.387	0.457	0.318	1	
Chl *a*	-0.546	**0.613**	0.248	0.393	-0.153	-0.368	0.381	-0.122	0.033	0.208	0.156	-0.068	1

*Significant values are given in bold letters

3.6.4 Principal component analysis

Water quality variables measured in the ponds were segregated and assembled based on their source and nature using principal component analysis.

Pond 1

The total variance in the water quality of pond 1 is given in Table 3.18. Principal component analysis of the variables of pond 1 generated four significant components with eigen values > 1 (Figure 3.18). Component 1 explains 34% of the variance of the data. Four components together accounted for 93% of the variance in the data.

Table 3.18: Explanation of total variance in water quality of pond 1

Total Variance Explained						
Component	**Initial Eigen values**			**Rotation Sums of Squared Loadings**		
	Total	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**
1	6.548	54.566	54.566	4.089	34.078	34.078
2	1.984	16.533	71.099	2.754	22.947	57.026
3	1.353	11.275	82.374	2.284	19.033	76.059
4	1.225	10.210	92.584	1.983	16.525	92.584
5	0.403	3.361	95.945			
6	0.276	2.301	98.246			
7	0.127	1.060	99.305			
8	0.054	0.446	99.751			
9	0.030	0.249	100.000			
10	3.894E-16	3.245E-15	100.000			
11	2.989E-18	2.491E-17	100.000			
12	-1.010E-16	-8.419E-16	100.000			

Extraction Method: Principal Component Analysis.

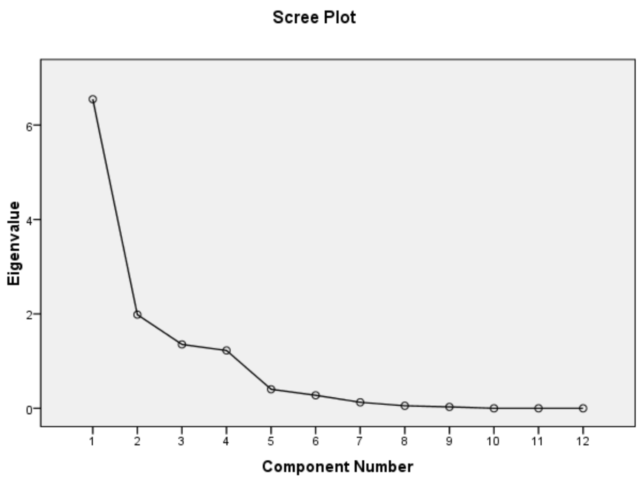

Figure 3.18: Scree plot of components showing eigen values in pond 1

The chemical and biological processes undergoing in the pond ecosystem were elucidated from the significant component loadings (Table 3.19). The components were grouped based on the component loadings. The significant loadings of variables in each component are given below;

C1: Secchi depth, pH, Nitrate- N, total phosphorus and chlorophyll *a*

C2: Temperature, Ammonia-nitrogen and Nitrite-nitrogen

C3: SD, pH and EC

C4: Dissolved oxygen, Dissolved iron and BOD

The four components extracted in the principal component analysis represent the following four processes.

Component 1: Phytoplankton growth component
Component 2: Organic decomposition component
Component 3: Inorganic turbidity component
Component 4: Oxygen consumption component

The significant positive loadings of pH, nitrate- N and total phosphorus in component 1 indicate the increase in phytoplankton production with the nutrients and a consequent rise in pH. The negative loadings of SD indicate the light attenuation due to phytoplankton turbidity. The second component (C2) has positive loadings of ammonia-N, nitrite-N and temperature. Ammonia and nitrite are intermediate products of nitrification and organic decomposition. Component 2 reveals the decomposition processes undergoing in the pond ecosystem with increasing temperature. This might be indicating that summer months have increased rate of organic decomposition. Component 3 attribute to the decreased transparency of water due to high inorganic ions and consequent increase in productivity. Component four has significant negative loadings of oxygen along with positive loadings of BOD and dissolved iron indicating the oxygen consumption by microbes and for the oxidation of iron. Highest loadings of chlorophyll *a* in Component 1 indicate that primary productivity is the major process in the pond and is proportional to pH, Nitrate and TP.

Table 3.19: Principal component loadings of significant components of pond 1

Variables	C1	C2	C3	C4
SD	**-.708**	-.215	**-.589**	-.220
Temperature	.329	**.832**	-.050	.207
pH	**.700**	.159	**.669**	.039
EC	.036	.019	**.966**	.140
DO	-.441	-.232	-.281	**-.794**
Ammonia-N	.081	**.915**	-.007	.243
Nitrite-N	.299	**.791**	.457	-.043
Nitrate-N	**.943**	.045	-.013	.216
TP	**.860**	.365	.300	.144
Dissolved iron	-.035	.357	-.043	**.831**
BOD	.486	-.268	.413	**.645**
Chlorophyll *a*	**.912**	.371	.056	.077

POND 2

Four components were extracted from the principal component analysis of the data on water quality of pond 2. The analysis results are given in Table 3.20. Four components with eigen values above 1 contributed 85% of the variance in the data (Figure 3.19).

Table 3.20: Explanation of total variance in water quality of pond 2

Total Variance Explained						
Component	Initial Eigen values			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	5.530	46.084	46.084	3.595	29.962	29.962
2	1.964	16.368	62.452	3.062	25.516	55.478
3	1.673	13.938	76.391	1.831	15.258	70.737
4	1.019	8.490	84.880	1.697	14.144	84.880
5	.722	6.017	90.897			
6	.574	4.780	95.677			
7	.315	2.628	98.305			
8	.108	.899	99.204			
9	.075	.627	99.831			
10	.018	.147	99.978			
11	.003	.022	100.000			
12	8.963E-18	7.469E-17	100.000			

Extraction Method: Principal Component Analysis.

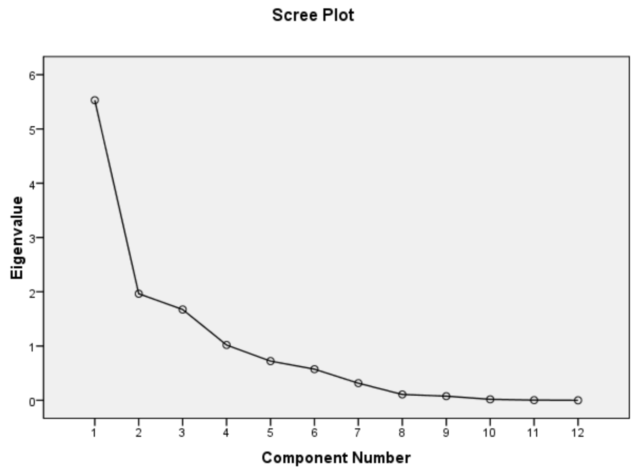

Figure 3.19: Scree plot of components showing eigen values in pond 2

The component loadings are given in Table 3.21. The four components representing the significant variations in variables are;

C1: Secchi depth, DO, TP, dissolved iron, BOD

C2: EC, nitrite-N and nitrate-N

C3: pH and ammonia-N

C4: Temperature and chlorophyll *a*

The components defined and categorised based on the significant variables are;

Component 1: Turbidity component

Component 2: Inorganic nitrogen ion component

Component 3: Organic decomposition component

Component 4: Phytoplankton growth component

Table 3.21: Principal component loadings of significant components of pond 2

Variables	**C1**	**C2**	**C3**	**C4**
SD	**-.948**	-.157	-.049	.049
Temperature	.205	.499	.222	**.733**
pH	.077	-.042	**.878**	.144
EC	.109	**.953**	-.062	.171
DO	**-.871**	-.027	-.383	-.254
Ammonia-N	.092	.090	**.737**	-.161
Nitrite-N	.156	**.936**	-.033	.214
Nitrate-N	.401	**.871**	.117	.049
TP	**.609**	.188	-.481	.196
Dissolved iron	**.759**	.314	-.122	.363
BOD	**.822**	.291	.145	.213
Chlorophyll *a*	.244	.124	-.181	**.867**

Component 1 has negative loadings of SD and DO and positive loadings of TP, dissolved iron and BOD. It explains the effect of increase in TP and dissolved iron in contributing to the turbidity thus by decreasing the water transparency. TP and dissolved iron are two key variables which favour the phytoplankton growth. So the light attenuation may be due to the presence of phytoplankton turbidity. But Chl *a* didn't have positive loading in component 1. This could be due to the failure of subsurface chlorophyll *a* measurements in representing the metaphyton mat over the water surface. Component 2 has positive loadings of nitrite-N, nitrate-N and EC indicating the high nitrogen loading in the pond ecosystem contributing to the major inorganic ion concentration. Positive loadings of ammonia and pH in component 3 attribute to the decomposition processes and a resulting rise in pH. Component 4 has significant positive loadings of chlorophyll *a* and temperature. It shows the role of temperature in the phytoplankton growth.

POND 3

The principal component analysis of water quality variables of pond 3 extracted five components having eigen values >1. The PCA result of percentage variance is given in Table 3.22. The five components together constituted 93% of the variance in the data set (Figure 3.20). First three components were equally important which accounted almost equal percentage of variance (around 20%). The extracted components were categorized according to the significant component loadings (Table 3.23).

Table 3.22: Explanation of total variance in water quality of pond 3

Total Variance Explained						
Component	Initial Eigenvalues			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.368	28.067	28.067	2.536	21.135	21.135
2	2.690	22.416	50.484	2.413	20.111	41.246
3	2.390	19.917	70.401	2.362	19.681	60.927
4	1.523	12.691	83.092	2.060	17.168	78.095
5	1.176	9.796	92.888	1.775	14.793	92.888
6	0.447	3.721	96.609			
7	0.248	2.064	98.673			
8	0.146	1.216	99.889			
9	0.013	0.111	100.000			
10	1.281E-16	1.067E-15	100.000			
11	-8.284E-17	-6.904E-16	100.000			
12	-2.479E-16	-2.066E-15	100.000			

Extraction Method: Principal Component Analysis.

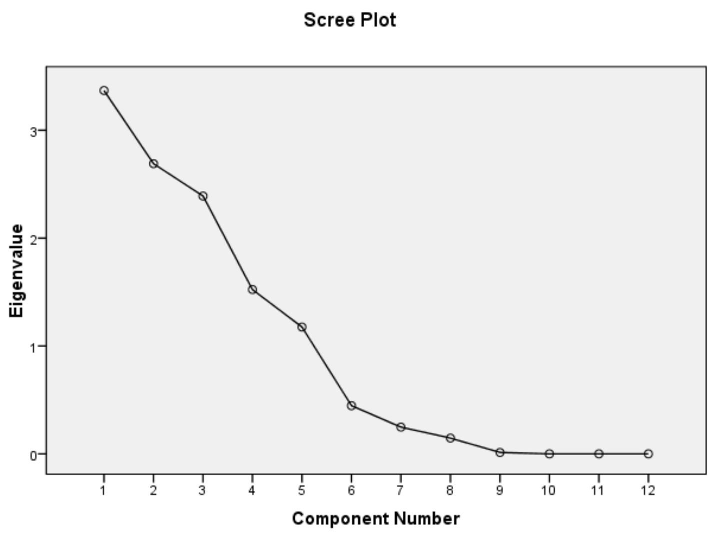

Figure 3.20: Scree plot of components showing eigen values in pond 3

C1: Temperature, nitrite-N and nitrate-N

C2: Ammonia, dissolved iron and chlorophyll *a*

C3: Secchi depth and pH

C4: Electrical conductivity and dissolved oxygen

C5: Total phosphorus and BOD

The components are grouped and defined in accordance with the component loadings as follows;

Component 1: Inorganic nitrogen component

Component 2: Phytoplankton growth component

Component 3: Turbidity component

Component 4: Oxygen saturation component

Component 5: Oxygen demand component

Component 1 has significant positive loadings of nitrite and nitrate and negative loading of temperature. This attribute to the increased nitrogen compounds in the pond in winter time. Component 2 is phytoplankton growth component with positive loadings of dissolved iron, chlorophyll *a* and ammonia. The phytoplankton blooming was influenced by dissolved iron and the ammonia production is indicative of phytoplankton decay. Component 3 indicate a decrease in transparency with an increase in pH. Component 4 with positive loadings of EC and DO reflects increased inorganic ion concentration with high dissolved oxygen content. Component 5 with significant loadings of TP and BOD attribute to the increased biochemical oxygen demand with high phosphorus loading in the pond ecosystem.

Table 3.23: Principal component loadings of significant components of pond 3

Variables	C1	C2	C3	C4	C5
SD	-.279	-.096	**-.896**	.093	-.308
Temperature	**-.786**	-.061	.279	-.129	-.414
pH	-.034	.029	**.935**	.329	.070
EC	-.098	.127	.495	**.765**	.303
DO	.253	-.144	-.040	**.939**	.004
Ammonia-N	-.027	**.930**	.143	.181	-.224
Nitrite-N	**.953**	-.157	.154	.070	-.058
Nitrate-N	**.832**	.192	.259	.024	-.379
TP	.165	-.183	.170	.362	**.744**
Dissolved iron	-.020	**.969**	.094	-.151	-.100
BOD	-.339	-.108	.343	-.161	**.781**
Chlorophyll *a*	.148	**.672**	-.304	-.493	.202

POND 4

The principal component analysis of pond 4 water quality data reduced the variables into four components (Figure 3.21). 83% of the variance of the data is explained by the four components extracted in the principal component analysis (Table 3.24).

Figure 3.21: Scree plot of components showing eigen values in pond 4

Table 3.24: Explanation of total variance in water quality of pond 4

Total Variance Explained						
Component	Initial Eigen values			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4.789	39.909	39.909	3.739	31.157	31.157
2	2.665	22.208	62.118	2.695	22.459	53.616
3	1.370	11.419	73.537	1.841	15.344	68.960
4	1.179	9.825	83.362	1.728	14.401	83.362
5	.938	7.815	91.176			
6	.452	3.769	94.945			
7	.242	2.014	96.959			
8	.155	1.290	98.249			
9	.144	1.200	99.449			
10	.040	.330	99.779			
11	.026	.221	100.000			
12	1.216E-16	1.013E-15	100.000			

Extraction Method: Principal Component Analysis.

The component loadings are presented in Table 3.25. The components with significant loadings of variables are;

C1: Secchi depth, pH, EC, ammonia-N and nitrite-N

C2: DO, TP, dissolved iron, and BOD

C3: Temperature, pH and nitrate-N

C4: Temperature and chlorophyll *a*

The components segregated according to the source and biological processes as given below;

Component 1: Ammonia reduction component

Component 2: Oxygen consumption component

Component 3: Nitrate releasing component

Component 4: Phytoplankton growth component

Component 1 attributes to the reduction of ammonia to nitrite ions contributing to the major ionic concentration in the pond water and a resulting increase in pH and a consequent decrease in transparency. Component 2 consolidates the process of depletion of oxygen with the increase in TP and dissolved iron. Component 3 contribute to the increased nitrate in the summer months with the rise in temperature and pH. Component 4 explains the increase in phytoplankton production with the increase in temperature.

Table 3.25: Principal component loadings of pond 4 variables

Variables	C1	C2	C3	C4
SD	**-.817**	-.023	-.310	-.412
Temperature	.323	.125	**.504**	**.699**
pH	**.805**	.076	**.546**	.123
EC	**.889**	-.240	.142	.208
DO	.085	**-.858**	-.374	-.156
Ammonia-N	**-.918**	-.111	.186	-.057
Nitrite-N	**.601**	.389	-.034	.193
Nitrate-N	.077	.205	**.832**	-.029
TP	-.241	**.845**	.248	.257
Dissolved iron	.246	**.771**	-.319	-.038
BOD	.340	**.595**	.328	-.162
Chlorophyll *a*	.278	.099	-.188	**.923**

3.6.5 Trophic state of ponds

Trophic state index of the ponds were calculated using the TSI equations proposed by Cunha *et al.* (2013). The classification of trophic state by Cunha *et al.* (2013) is given in Table 3.26.

Table 3.26: Trophic state categories proposed for tropical and subtropical reservoirs by Cunha *et al.*(2013)

Trophic state category	Chl *a*(μg/L)	TP(μg/L)	TSI_{TSR}
Ultraoligotrophic	≤ 2.0	≤ 15.9	≤ 51.1
Oligotrophic	2.1-3.90	16.0-23.8	51.2-53.1
Mesotrophic	4.0-10.0	23.9-36.7	53.2-55.7
Eutrophic	10.1-20.2	36.8-63.7	55.8-58.1
Supereutrophic	20.3-27.1	63.8-77.6	58.2-59.0
Hypereutrophic	≥ 27.2	≥ 77.7	≥ 59.1

Pond 1

The TSI_{tsr} calculated for pond 1 had values ranging 57-73. The TSI in pond 1 in November was 59 indicating the supereutrophic state of the pond even in the starting of sampling in November. The pond went to eutrophy in December with TSI_{tsr} value of 57. The pond reverted to hypereutrophic state in January and continued that status till May (Figure 3.22).

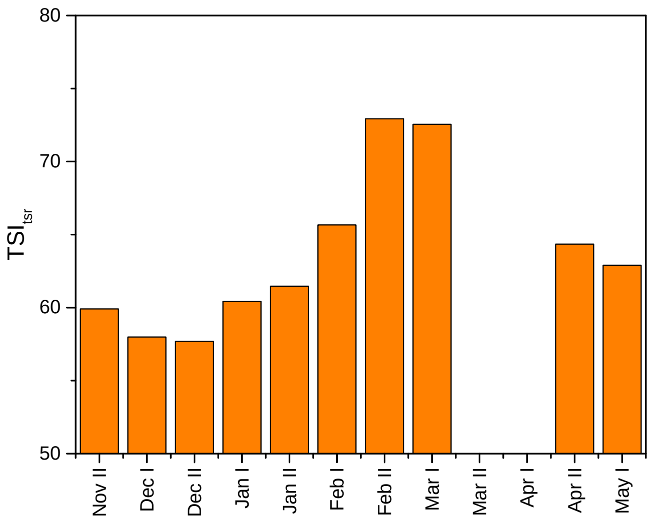

Figure 3.22: Temporal variation in TSI values of pond 1

Pond 2

The TSI_{tsr} values in pond 2 were of the range 61-68. The TSI values classified the pond under hyper eutrophic category throughout the sampling period from November- May (Figure 3.23). The highest TSI was in the second half of March.

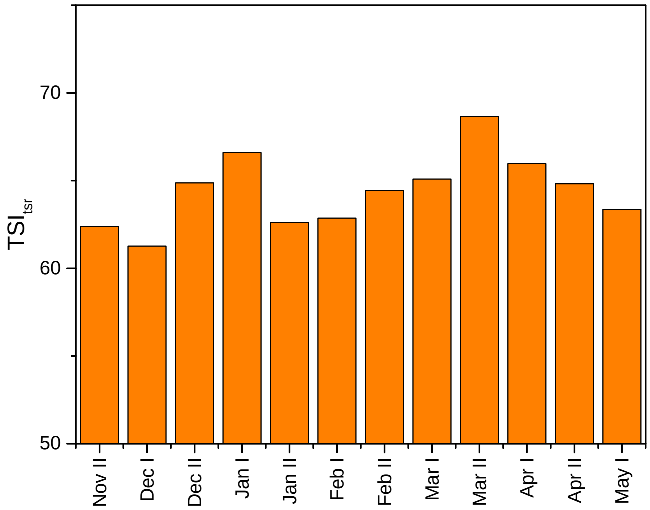

Figure 3.23: Temporal variation in TSI values of pond 2

Pond 3

Pond 3 recorded TSI_{tsr} values in the range of 60-65. The pond was in hypereutrophy from November- May (Figure 3.24). Highest trophic values were recorded in the months of January and April.

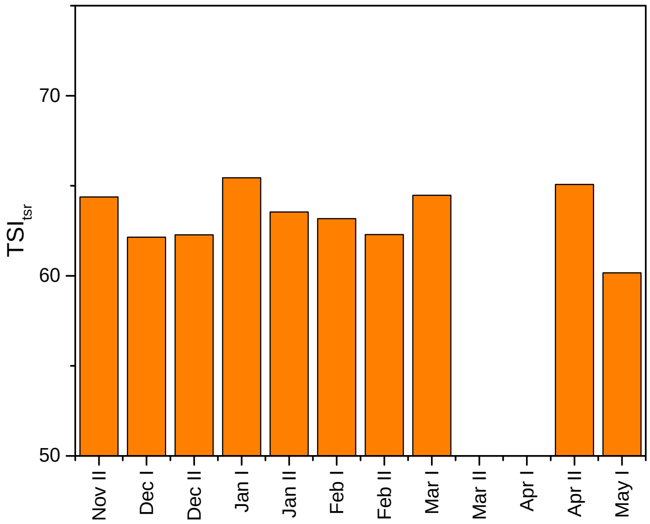

Figure 3.24: Temporal variation in TSI values of pond 3

Pond 4

Pond 4 had hypereutrophic trend from the beginning of sampling in November and continued that trend till the end of sampling in May. The TSI_{tsr} ranged between 60 and 69 (Figure 3.25). The highest TSI value is in March second half followed by April first half.

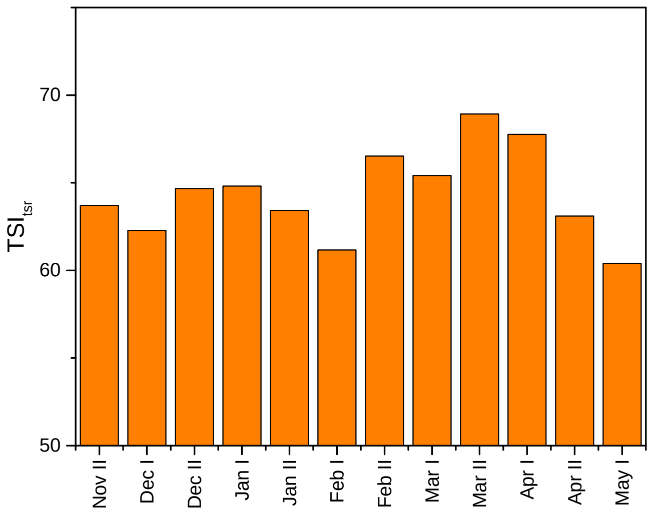

Figure 3.25: Temporal variation in the TSI values of pond 4

3.7 Discussion and Conclusion

Water quality of the four ponds didn't show significant variations with respect to temperature, transparency, pH, DO, BOD, NO_2-N, NO_3-N, and Chl *a*, but there is significant difference among ponds in the parameters EC, ammonia-nitrogen, SRP, TP and dissolved iron. The temperature in the ponds ranges from 24.8°C to 29.4°C. This is in accordance with values from other lentic water bodies of India (Harney *et al.,* 2013; Vanjare and Pai, 2013; Yadav *et al.,* 2013). Transparency of the pond water was in the range of 2 cm to 69 cm. Yadav *et al.* (2013) reported transparency range of 25- 60 cm in a freshwater pond in UP. Thakre *et al.* (2010) reported transparency values as low as 7cm in a natural pond in UP. The pH of pond water ranges from 5.40 to 7.77. Many of the pond studies in India have reported pH values in the range 6-8.5 (Harney *et al.* 2013; Dutta and Patra, 2013; Jipsa *et al.,* 2013; Yadav *et al.,* 2013; Mahobe and Mishra, 2013). Slightly acidic range of pH was also reported in some ponds (Aswathy Ashok *et al.,* 2015; Dalal and Gupta, 2016). Slightly acidic pH reported in the present study might be a result of organic decomposition taking place in the ponds (Chaurasya and Pandey, 2007). Dissolved oxygen ranges from 0.00- 8.06 mg/L. This coincides with other reports from India (Mukhopadhyay and Dewanji, 2005; Parikh Ankita and Mankodi, 2012; Mahajan and Billore, 2014; Dalal and Gupta, 2014; Aswathy Ashok *et al.,* 2015; Meera *et al.,* 2015). Dissolved oxygen in all the ponds got decreased during February-April. Dissolved oxygen decreases in summer with the increase in temperature. Pond 2 went to anoxic condition during March 2012 implying high organic load in the pond during that period. Pond 2 is deeper compared to the other three ponds and had thick growth of submerged vegetation. Probably the decomposition of this large biomass coupled with reduction in volume of water in the summer month of March led to anoxic state. The occurrence of hydrogen sulphide gas was not sensed and as such any abnormal levels of sulphur was not suspected. BOD in the ponds ranged 2.02 mg/L – 34.25 mg/L. This indicates moderate to high organic pollution in the ponds (Adakole, 2000). This result is supported by similar observations in some lakes in India (Ghosh and Salla, 2014; Abhijna, 2016). NO_2-N and NO_3-N in the ponds ranged 3.00-132.9 µg/L and 17.96- 255.1 µg/L respectively. This is in agreement with Khan and Sinha (2002); Muralidharan and Waghode (2014); Mahajan and Billore (2014) and Abhijna (2016).

Ammonia-nitrogen in the ponds ranged from 2.51- 178.6 µg/L. These results were supported by Khan and Sinha (2002) and Jindal *et al.* (2014). SRP range in the ponds varied from 2.16 to 3833 µg/L. This results are in compliance with other studies in lentic ecosystems (Chaurasya and Pandey, 2007; Jeyaraj *et al.*, 2016). Total phosphorus in the ponds had a range of 88.2 to 5354 µg/L. High TP values ranging 2100- 12400 µg/L was reported in still waters by Ramesh and Krishnaiah (2014) and Bhat *et al.* (2015). The high TP values in the lakes and ponds were due to anthropogenic inputs. Khan and Sinha reported TP in ponds ranging 100- 430 µg/L. Dissolved iron in the ponds was in the range of 85.1 – 1670 µg/L. This is a high value compared to ponds in different parts of India (Shrivastava, 2016; Peter and Sridevi, 2013). High iron content was already reported in Vembanadu Lake in Alappuzha district ranging 1490- 2540 µg/L (Sobha *et al.*, 2011).

Electrical conductivity showed significantly high value in pond 4. It indicates high inorganic ion input in the pond from domestic activities. EC values are determined by the concentration and mobility of ions and also on the temperature. High EC values in ponds are due to the leachate filtration from soil (Nag and Gupta, 2014). Significantly high ammonia in pond 3 even in the initial sampling months compared to other ponds is an indication of continuous decomposition process undergoing in the benthic floor of the pond. The pond was fully shaded with surrounding trees. The allochthonous input of leaf litter was high in the pond which settled in the bottom floor and was under intense decay process especially during February- March. At high temperature the process of denitrification and decomposition in polluted waters increases yielding ammonia (Prasad and Singh, 1996). Pond 1 and Pond 4 had high SRP and total phosphorus compared to pond 2 and pond 3. The source of TP in pond 1 might be the agricultural runoff from the nearby cultivation field. In pond 4 it could have been from the addition of detergents through domestic activities. Even though pond 2 was also an actively used domestic pond the detergent addition didn't impart an expected increase in TP values. This might be due to the preferential absorption of TP by *Hydrilla verticillata.* Aquatic macrophytes absorb nutrients from the water body and increase the clarity of water. The inhibitory effect of macrophytes on phytoplankton blooms has significant role in the management of these noxious blooms (Guo-feng *et al.*, 2014). According to De Backer *et al.* (2012) an extensive coverage of submerged macrophytes could maintain a clear water state until the nutrients exceeds a certain level. According to Jeppesen *et al.* (1990) lakes with concentration of TP ranging 25.0 to 100.0 µg/L maintain a clear state with abundant submerged plants. When the nutrients are too high, the SAV could not control phytoplankton growth leading to low light penetration and complete disappearance of SAV and eventually the water body shifts into highly turbid state (Hilt *et al.,* 2006). Submerged vegetation and large zooplankton grazing can weaken the chlorophyll *a* - TP relationship (Teissier *et al.,* 2012).

The dissolved iron in pond 1 was significantly higher than that of other ponds. A nutrient enrichment experiment by North *et al.* (2007) in Lake Erie found that combined addition of iron, phosphorus, and nitrogen yielded higher phytoplankton biomass than phosphorus and nitrogen alone. The growth of specific phytoplankton species influences the iron speciation in water bodies. The increased dissolved iron derives from desorption of the iron adsorbed on suspended solids and dissolution of iron oxyhydroxides due to a decrease in pH (Nagai *et al.,* 2006).

All the four ponds studied maintained hypereutrophic state with respect to total phosphorus and Chl *a* concentrations during 2011-12. Chlorophyll *a* in the ponds during 2011-12 was in the range of 4.51 µg/L to 667.7 µg/L. Pond 1 recorded the highest values during February-March. The Chl *a* in pond 1 was significantly correlated with TP and nitrate. This shows that the combined effect of TP and nitrate had a synergetic effect on the phytoplankton production in the pond (Jensen *et al.,* 1994). A nutrient enrichment experiment by Tang *et al.* (2009) proved that the combination of nitrate and phosphorus gives best result in phytoplankton growth. In pond 2 and pond 4, temperature was the only parameter found significantly correlated with Chl *a*. Seasonal temperature change in water bodies play an important role in phytoplankton growth (Schabhüttl *et al.*, 2013). A study by Lv *et al.* (2011) reported temperature and TP as the major limiting factor in the growth, distribution and community composition of phytoplankton in 15 lakes in China.

The PCA results explained the major processes undergoing in the pond ecosystems. The PCA loadings of pond 1 indicated phytoplankton growth as the major process. The high Chl *a* values in the pond justifies the PCA result. In pond 2 the first component explained turbidity in pond 2. This indicated turbidity in the pond arising from either phytoplankton or suspended solids. The first component of pond 3 accounted for inorganic nitrogen components confirming the organic decomposition process undergoing in the pond. The PCA of pond 4 indicted the process of ammonia reduction taking place in the pond. Even though the pond maintained a clear state in 2011-12 period, the pond was enriched with nutrients from domestic activities and there were decomposition process undergoing in the bottom floor and a consequent production of ammonia.

The comparison of pH, EC and Chl *a* in the ponds between the study years from 2011 to 2014 showed the high values of pH and EC in the pre-monsoon periods of 2012-13 and 2013-14 period respectively. The still water ecosystems receive land runoff be it rain or any sewage inflow and allow it to settle in the bottom, and it gets adsorbed in the bottom sediments and gets locked up there. Over time these nutrients build up in the sediments and will be released back to the water column under different environmental conditions like high temperature and low dissolved oxygen especially in the summer months. The release of nutrients eventually leads to higher algal growth (Hou *et al.*, 2013). The increased EC in 2013-14 correspond to increase in Chl *a* in the ponds except in pond 2. This might be because pond 1 and pond 3 were fully covered by *Pistia stratiotes* and *Salvinia molesta* respectively. Pond 4 on the other hand was well maintained by yearly clean up in 2013-14 reducing the mean Chl *a*. The increased pH in the pre-monsoon period of 2012-13 indicates increased algal growth. But the Chl *a* values didn't show any specific trend unlike pH and EC. This indicates that the average values of Chl *a* could not express the real state of phytoplankton growth. This might be due to the development of periphyton, metaphyton and aquatic macrophytes in the ponds which were not accounted in the subsurface Chl *a* measurements.

Chapter 4

PHYTOPLANKTON AND METAPHYTON BLOOMS IN PONDS

Contents

4.1 Introduction

Ponds and lakes inhabit rich flora of phytoplankton. Phytoplankton include diverse species assemblages having representations from all major algal groups except Phaeophyta and Rhodophyta (Sandgren, 1988). Phytoplankton develop into water blooms at favourable conditions. Algal blooms are formed as a consequence of eutrophication in water bodies. External and internal nutrient load facilitates eutrophication (Shaw *et al.*, 2003). Structure and composition of a phytoplankton community is determined by colonization, speciation, competition, predation and environmental conditions in the aquatic ecosystems. Phytoplankton succession is related to seasonal changes in temperature, nutrient availability, light attenuation and meteorological events (Chalar, 2009). Investigations on the successional development of a water body can be well studied by taking pond as a model system. Ponds undergo successional progression faster than other larger aquatic ecosystems. The species representations in the late-successional stages of a pond will be entirely different from that of mid or early successions (Hassall *et al.*, 2012).

The ecology and succession of phytoplankton in ponds were studied by many Indian limnologists. Seenayya (1971), Rao (1975) and Munawar (1974) made detailed studies on the species turn over's and periodicity of phytoplankton community in freshwater ponds of Hyderabad. These studies unravelled the pattern of species succession and dominance with respect to season and hydrology of ponds. The processes involved in species turn-over of plankton communities in tropical reservoirs were studied by Santos *et al.* (2016).

4.1.1 Phytoplankton dynamics

Phytoplankton are the base of food web in all aquatic systems. The colour, transparency, trophic state, zooplankton and fish production of water bodies depend largely on the phytoplankton. The phytoplankton dynamics and assemblages in water bodies are driven by environmental variations at different time scales (Yang *et al.*, 2016). Physical, chemical and biological factors have major influence on the succession and development of plankton biomass. Each factor listed above play its individual role but the final outcome will be the result of a combination of factors involving interaction of these factors in different proportions (Hulyal and Kaliwal, 2009). According to Haande *et al.* (2011) the phytoplankton assemblages, species composition and dominance pattern in lakes are influenced by the nutrient dynamics. Barinova and Chekryzheva (2014) opined that temperature and nutrients are the major factors affecting the lake ecosystem dynamics. A study of plankton dynamics in a perennial urban pond in Srinagar conducted by Zutshi *et al.* (1984) also reported the positive correlation of phytoplankton biomass with temperature and nitrate. According to Reynolds (1984) the phytoplankton succession in lakes having similar morphometric, trophic state and climatic conditions will be similar. Morphometric differences leads to difference in floral diversity and species composition of lakes (Croome and Tyler, 1986).

There are many studies conducted worldwide to understand the ecology and dynamics of phytoplankton in lakes, ponds, reservoirs etc. Guiral *et al.* (1994) investigated the structure and distribution of planktonic community and their ecological succession during natural recolonization of a tropical pond. The study revealed the fluctuation in phytoplankton structure with water volume, mixing effects, grazing, sedimentation and diurnal productions. Barone and Flores (1994) found hydrological and climatological features as the deciding factors in the phytoplankton dynamics of a hypereutrophic reservoir in Italy. Wieczorek (2006) proposed models to assess the dynamics of plankton with respect to space-size distribution. Venkateshwarlu *et al.* (2011) reported temperature as the controlling factor in the plankton dynamics of two ponds in Shimoga district, Karnataka. Fonseca and Bicudo (2011) analysed the phytoplankton dynamics in a macrophyte dominated pond in Brazil. The phytoplankton dynamics in the pond was found strongly influenced by seasonal stratifications. Haggqvist and Lindholm (2012) investigated the phytoplankton dynamics in a shallow lake in Finland. The result showed the dense vegetation of common water milfoil in the lake affected the phytoplankton dynamics without causing nutrient limitation. Naselli-Flores and Barone (2012) investigated the dynamics and succession of phytoplankton in macrophyte dominated Mediterranean ponds. De Senerpont Domis *et al.* (2013) proposed some predictions on plankton dynamics in different climatic conditions based on modelling. The modelling predictions indicated that the change in phytoplankton dynamics to a great extent is system specific, which profoundly depend on the food-web structure and nutrient loading rather than direct effect of climatic factors. The study of phytoplankton dynamics in Tungabhadra River, Karnataka by Suresh *et al.* (2013) showed highly significant and positive correlation of Cyanobacteria with temperature and phosphate. An investigation on the population dynamics of phytoplankton in Wular Lake by Baba and Pandit (2014) revealed the dominance of Bacillariophyta among the lake plankton. Jalswar and Mehta (2014) reported strong positive correlation of all algal groups with dissolved oxygen. Bhanja *et al.* (2014) found strong influence of physico-chemical parameters on the plankton dynamics of two unmanaged ponds in West Bengal. Ahmed and Wanganeo (2015) opined that despite temperature and nitrate, total dissolved solids also play a decisive role in the dynamics of phytoplankton. Nath *et al.* (2015) studied the phytoplankton succession in relation to the hydrological parameters in a pond in Dhanuvachapuram in Thiruvananthapuram. The study reported high plankton diversity during monsoon. Pastich *et al.* (2016) studied the dynamics and structure of phytoplankton community in a maturation pond in a semi-arid region in Brazil. The study found correlation of surface organic load and N:P ratio with dominance of phytoplankton groups.

4.1.2 Periphyton and metaphyton

Periphyton is a complex community of microbiota including algae, bacteria, fungi etc. that is attached to a substrata. The substrata could be inorganic, organic, living or dead (Wetzel, 2001). The periphytic algae are named based on their type of substrata. Algae that grow on sediments are called epipelic. Those found attached on living organisms are called epiphytic (Annang and Addo-Boadu, 2012). Metaphyton is free-floating or loosely attached filamentous algae that usually arise from epipelic and epiphytic biofilms (Scott *et al.*, 2007; Saunders *et al.*, 2012). Epiphytic and metaphytic algae come under periphytic algal communities. These periphytic algae have faster growth rate and they instantaneously respond to environmental changes (Pfeiffer *et al.*, 2015). Periphyton and metaphyton contribute more to the algal biomass in an aquatic system. Such massive coverings of these algal communities have major role in the aquatic environment by regulating the submerged plants, nutrient dynamics and food web structure (Liboriussen, 2003).

4.1.3 Algal bloom

Phytoplankton benefit the environment as the major primary producers and contributing to the net oxygen production and ammonia assimilation (Akin-Oriola and Jeje, 2001). Excessive nutrient input cause exponential growth of the phytoplankton resulting in blooms. Bloom formation has three stages; pre-bloom, bloom and post-bloom (Meng *et al.*, 2015). Pre-bloom stage is characterized by the even spreading of small phytoplankton in the water column. Picoplankton are the dominant community in a pre-bloom stage. Later on a series of phytoplankton succession occurs prior to bloom formation. Gradually the bloom forming species rises in favourable condition competing with other phytoplankton and concentrate near the surface. In the post bloom stage these aggregated phytoplankton or algal biomass starts sinking to the bottom (Engelsen *et al.,* 2002; Daniels *et al.,* 2015). The blooms often have one or two species of dominant phytoplankton type.

The mechanism involved in the dominance of a particular species is the interaction between the organism and its habitat (Oliver and Ganf, 2000). The factors which trigger bloom formation may differ. Blooms occur naturally in aquatic systems depending on the climatic conditions especially in the summer months. Blooms are often associated with nutrient enrichment and eutrophication of water bodies (Havens *et al.,* 2003). Sometimes blooms may be caused by the natural selection and competition among different species resulting in the uprisal of one particular species out-competing all other species (Bringa, 2015). Some of the algal blooms are noxious and toxic and are detrimental to the aquatic environment. Many countries face the problem of bloom formation in their water ecosystems resulting in unprecedented loss in the aquatic resources.

Many studies were conducted world- wide on the dynamics of algal bloom formation, its devastating effects, factors that trigger bloom formation and the monitoring and control strategies. Kawamiya *et al.* (1996) made a three dimensional model study on the spring blooms of Otsuchi Bay in Japan. A study conducted by Seubert *et al.* (2013) elaborate the seasonal and annual dynamics of toxic bloom events of *Peudo-nitzschia* and seasonal poisoning events from the neurotoxin released in coastal ocean sites of Southern California. Tapolczai *et al.*

(2015) investigated the annual pattern of *Mougeotia* sp. bloom formation in deep peri-Alpine Lakes. Wallace and Gobler (2015) investigated the factors triggering bloom formation in an urban estuary in Jamaica Bay.

Briand *et al.* (2002) studied the toxic Cyanobacteria bloom of *Cylindrospermopsis raciborskii* in a shallow pond in France. The temperature was found to be a crucial factor in the germination and proliferation of *C.raciborskii*. A study by Davis *et al.* (2009) on the dynamics of toxic and non-toxic strains of *Microcystis* during blue-green bloom event reported synergic effect of higher temperature and phosphorus loading in the higher yield of *Microcystis* sp. and consequent toxic bloom formation. Guinder *et al.* (2013) recorded an increase in summer blooms in Bahia Blanca estuary as a result of interacting effects of temperature, turbidity, nutrients and grazing.

The occurrence and bloom formation of Cyanobacteria in two urban fish ponds in Bangladesh were studied by Rahman and Jewel (2008). Somek *et al.* (2008) reported the potential risk of Cyanobacterial toxicity in Egirdir Lake, Turkey. The lake water was used for drinking water supply due to water shortage. The lake was observed with patches of bloom formation and the strains were identified as *Microcystis aeruginosa* and *M. flos-aqua*. A study conducted by Yamamoto and Nakahara (2009) elucidates the factors that trigger *Microcystis* sp. bloom formation in a pond in Kyoto, Japan. Peretyatko *et al.* (2010) assessed the health concerns and risk associated with Cyanobacteria blooms in the urban ponds. Ahmed *et al.* (2010) reported the presence of Microcystin in the *Oscillatoria* bloom developed in the Buriganga River in Bangladesh. An ecological study on the algal bloom formation in two urban ponds in Kano city by Bringa *et al.* (2015) revealed extensive pollution and nutrient enrichment. A study by Vijaya Rani *et al.* (2016) reported bloom formation of *Chroococcus turgidus, Oscillatoria limosa, Microcystis aeruginosa and Anabaena circularis* indicting organic pollution in a temple pond in Kanyakumari.

Raghavan *et al.* (2010) studied the algal blooms in Eastern Arabian Sea. The study used *in situ* hyperspectral radiometer for measurement of surface chlorophyll. It detected the presence of dense algal blooms in Karvar coast (Karnataka) and scattered patches of *Trichodesmium* sp. bloom in Kumbla coast (Kerala). Sarangi and Mohamed (2011) studied the occurrence of seasonal blooms in Kerala coast with the help of remote sensing. Satellite observations combined with *in situ* studies in the Kerala coast revealed dinoflagellate blooms in the Calicut region. Divya *et al.* (2013) reported high dissolved oxygen content in the surface waters of Pazhasi dam (Kerala) and Abbey falls (Karnataka), and a consequent abundance of diatom flora and its bloom formation. Ajin *et al.* (2016) studied heavy algal bloom formation and associated outbreak of diseases in pokkali shrimp ponds adjoining the Cochin backwaters. The disease outbreak was found to be due to the poor water quality management and consequent bloom formation. The algal blooms can harbour more bacteria including the pathogenic one's than the water.

4.1.4 Discrimination of algal phyla using fluorescence spectra

The identification of phytoplankton based on their fluorescence properties has an edge over the laborious and time consuming microscopic examination. *In situ* fluorometers provide quantitative and qualitative data on phytoplankton communities which is helpful in monitoring the toxic algal bloom formation forecasts (Escoffier *et al.,* 2015).

Fluorescence is the absorption of light energy by fluorescent molecules (fluorophores) at one wavelength and instantaneous re-emission of light with lower energy level in the longer wavelength region. Any fluorescent molecule has two characteristic spectra: the excitation spectrum and the emission spectrum. A fluorometer generates the wavelength of light required to excite the analyte of interest. Then it selectively transmits the wavelength of light emitted and measures the intensity of light emitted. Fluorescence spectra of samples are recorded by scanning the emission monochromator for a constant wavelength of excitation light. The fluorescence spectra of each compound will differ from one another giving a characteristic fluorescence fingerprint to each compound. This property is used in the spectral characterization of various fluorophore compounds in nature.

There are two primary photo reactions and two primary pigment systems (Photosystem I and Photosystem II) involved in photosynthesis in green plants and algae. Each photosystem consists of specialized pigment molecules within a large pigment-protein complex called reaction centre. These reaction centres perform the function of photochemical reactions in photosynthesis process. It consists of chlorophyll containing core and a species dependent peripheral antenna (Lutz *et al.,* 2001; Beutler *et al.,* 2002). These include Chl *a*, Chl *b*, Chl *c*, carotenoids and phycobiliproteins. The peripheral antenna imparts colour to the photosynthetic organism and affects the fluorescence excitation spectra also. The chlorophyll *a* found in PSI is long wavelength chlorophylls which are weakly fluorescent. Fluorescent form of chlorophyll *a* belongs to PSII. So the fluorescence properties of photosynthetic plants depend on the chlorophyll *a* of pigment system II (Govindjee and Braun, 1974). Fluorescence results from the re-emission of light reaching the chlorophyll molecule. The light emitted as fluorescence by Chl *a* has long wavelength with peak intensity approximately at 680 nm. Many *in vivo* fluorescence studies adopted the 680 nm wavelength as the emission maxima and a range of 400-650 nm as the excitation wavelength range for analysing chlorophyll *a* fluorescence (Vincent, 1983; Hilton *et al.*, 1989; Poryvkina *et al.*, 2000). The intensity of fluorescence varies with different pigment-protein complexes depending on different algal groups. Figure 4.1 represents the excitation and emission spectra of chlorophyll molecule.

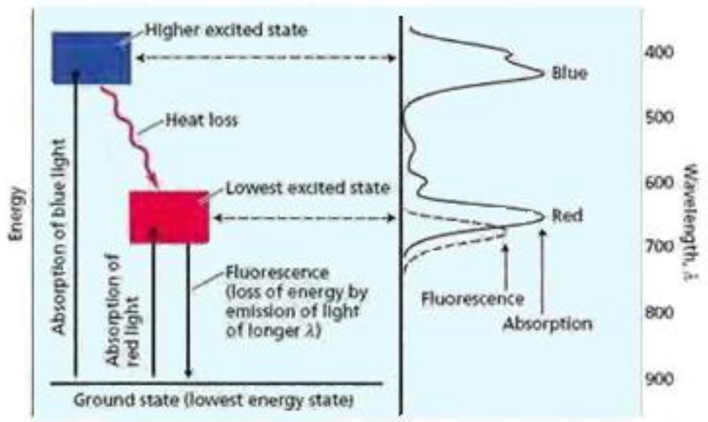

Figure 4.1: Schematic diagram representing the excitation spectra and emission spectra of pigment (chlorophyll) molecules (Reprinted from Misra *et al.*, 2012).

The *in vivo* fluorescence is affected by certain interferences. In natural water samples there will be light scattering particles resulting in the decrease of the effective excitation intensity (Beutler *et al.,* 2002). Rayleigh and Tyndall scatter can be observed in the emission spectrum at the same wavelength as the excitation wavelength, and also at twice this value (second order grating effect). The excitation spectra are hardly affected by the light scattering unlike emission spectra. It does not depend on the light absorption by photo-protective pigments. Hence excitation spectra are widely adopted for the *in vivo* fluorescence discrimination methods (Poryvkina *et al.*, 2000). In very dilute solutions we can observe Raman scatter. The filtrate of the sample is often used as the reference for *in vivo* fluorescence spectra in order to subtract the Raman scattering and the fluorescence of dissolved organic matter from the spectrum (Lazzara *et al.*, 1996). Strehler and Arnold (1951) discovered delayed fluorescence in certain plants and bacteria. In this mechanism, light absorbed by PSI quenches the emission of light caused by the excitation of PSII. When the green plant parts are transferred from darkness to light, Pigment System II (PS II) reaction centres are progressively closed and result in an increase in the yield of chlorophyll fluorescence followed by a fall in fluorescence yield over a time scale of a few minutes. This phenomenon is termed as fluorescence quenching (Maxwell and Johnson, 2000). The natural samples are often kept at dim light for 15-30 minutes for acclimatization of the cells to avoid the fluorescence quenching caused by direct measurement from dark adapted samples.

Different algal groups have characteristic fluorescence excitation and emission spectra. This is attributed to the differences in the accessory pigments or antennae and the organisation of the light capturing apparatus (Vincent, 1983). In addition to the identification of taxonomic position of algae, the *in vivo* spectra give information about the photoadaptation properties that the particular algae of study exhibit (Poryvkina *et al.*, 2000).

Many studies were undertaken in the pure cultures of various marine and freshwater phytoplankton representing different taxonomic groups to optimize the spectral characteristics of different classes of algae. Figure 4.2 shows four chemotaxonomic phytoplankton groups based on the pigment composition.

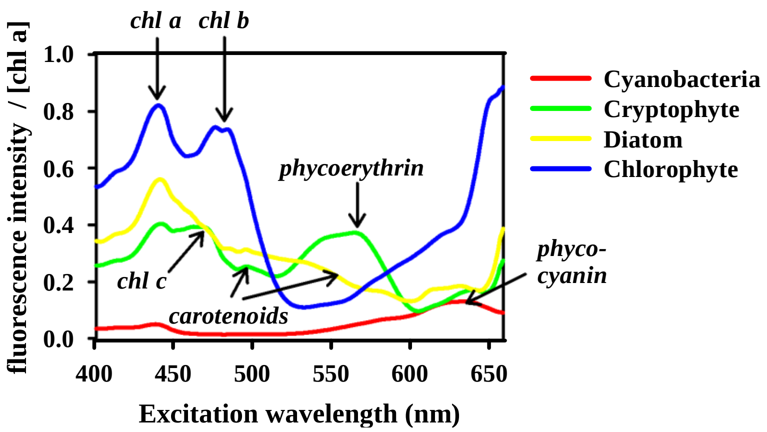

Figure 4.2: Fluorescence-excitation spectra of different chemotaxonomic phytoplankton pigment groups (Reprinted from Seppala, 2003).

Vincent (1983) compared the fluorescence spectral properties of three classes of freshwater algae: Cyanophyceae, Chlorophyceae and Bacillariophyceae. Pure cultures of exponentially growing species from each group were selected for the study and recorded their fluorescence spectra. Hilton (1989) compared the fluorescence excitation spectra of thirty algal cultures for the optimisation of the spectral characters for differentiation of algal groups. Poryvkina *et al.* (2000) studied the marine algal cultures. He divided the fluorescence excitation spectra of algae into three characteristic spectra with respect to their pigment composition. Millie *et al.* (2002) studied the absorbance and fluorescence spectra of phycobilin and non-phycobilin containing algae for the spectral differentiation of algal groups. A study on the excitation-emission fluorescence matrices of selected blue-green algal cultures by Simis *et al.* (2012) revealed highest correlation of community and blue-green algal variable fluorescence in the orange-red excitation wavelength range. Fluoroprobes are used for *in vivo* analysis of phytoplankton community structure. Zamyadi *et al.* (2012) studied the use of fluorimetric probes for the detection of blue-green algae in natural waters. Chen *et al.* (2014) developed a fluorescence analysis method based on parallel factor analysis and CHEMTAX (a matrix factorization program) for the discrimination of three groups of algae (Chlorophyta, Cyanobacteria and Bacillariophyta). A study by Harris and Graham (2015) in the periphyton algae of stream waters in Indian creek basin, Kansas exhibited correlation between field measured fluorescence and the laboratory based Chl *a* biomass, though weak correlation was observed when there was thick algal mat. Peniuk *et al.* (2016) has developed a flow cytometric method using the fluorescence properties of algae and bacteria for instantaneous analysis of the species distribution and growth pattern in mixed cultures used for biofuel and biochemical applications.

4.1.5 Relevance of phytoplankton assemblage study

Phytoplankton are the foundation of food web in ponds. The trophic structure of the ponds is dependent on the phytoplankton species assemblages and their forage value to the immediate consumers. Monitoring the phytoplankton succession over the time is therefore relevant in the context of defining food web, understanding the development of harmful algal blooms, and the ecology of ponds.

4.2 Specific objectives

- Understanding the phytoplankton and metaphyton bloom development in ponds.
- Analysing the phytoplankton dynamics in the ponds.
- Investigate the applicability of fluorescence techniques in the discrimination of algal phyla.

4.3 Methods

4.3.1 Collection of phytoplankton

Subsurface water samples were collected from the four ponds (described in Chapter 3) during 2011 to 2014 to observe the phytoplankton. Samples were collected biweekly in 1 litre polythene bottles in 2011-12 and 2012-13 periods. In 2013-14 period, water samples were collected monthly. The samples of surface scum and floating mats were collected manually. The samples were immediately transported to the laboratory for observation.

4.3.2 Taxonomic identification of phytoplankton

The water samples were centrifuged at 1500 rpm for 10 minutes. The supernatant water was drained out and the pellets at the bottom were dissolved in 2 mL distilled water. Ten subsamples of each were mounted on ten different slides and observed under microscope. Hundred microscope fields of phytoplankton were observed for each sample and the occurrence of the different taxa were tabulated. Microphotographs were taken and the algae were identified with the help of standard monographs and literature (West and West, 1904; Desikachary, 1959; Prescott, 1962; Philipose, 1967; Whitford and Schuacher, 1984; John et al., 2002; Perumal and Anand, 2009) and the guidelines of http://www.algaebase.org. (Guiry and Guiry, 2011).

From the microscope observations, relative frequency of occurrence of each algal phyla were calculated (Rao, 1975). The relative frequency of occurrence of each algal phyla in the pond samples were calculated as given below.

$$\text{Relative frequency}(\%) = \frac{No\ of\ obervations\ of\ an\ algal\ phyla}{Total\ number\ of\ obervations\ of\ all\ the\ algal\ phyla} \times 100$$

4.3.3 Similarity between ponds in terms of phytoplankton composition

The similarity between the ponds with respect to the occurrence of phytoplankton species was calculated using simple matching coefficient of similarity (Krebs, 1999).

$$\text{Simple matching coefficient } = \frac{a+d}{a+b+c+d}$$

Where, a = Number of species present in sample A and sample B

b = Number of species present in sample B but not in sample A

c = Number of species present in sample A but not in sample B

d = Number of species absent in both A and B but present in other samples

The similarity of ponds in terms of phytoplankton species composition were then elucidated through cluster analysis and plotted the dendrogram using **Ky plot**.

4.3.4 Data analysis

Principal component analysis (PCA) and discriminant analysis (DA) was performed to work out the phytoplankton dynamics in the ponds in relation to the physico-chemical variables. The canonical plots (PCA biplots) of the principal components were used for interpreting the phytoplankton variations. PCA was performed using XLSTAT software.

4.3.5 Discrimination of phytoplankton phyla using fluorescence spectra

The comparison of *in vivo* fluorescence measurements for natural waters with the microscopic observations give an account of the efficiency of *in vivo* fluorescence as a quick measuring tool for algal community structure. The *in vivo* fluorescence of the phytoplankton community of the four ponds was measured during the water sampling in January—June 2014.

4.3.5.1 Field sample collection

Water samples were collected from the four ponds in Pallippuram during January 2014—June 2014 for fluorescence measurements for studying phytoplankton. Subsurface water samples were collected monthly in opaque bottles and stored in dark for 2 h to tackle the photoinhibition of fluorescence. Then it was exposed to dim light for 15 minutes to avoid fluorescence quenching. The water samples were filtered through 200 µm filter to obtain the sample of microplankton together with nanoplankton. A portion of the filtrate was further filtered through 20 µm filter to obtain the < 20 µm nanoplankton fraction. The water samples filtered through 0.45 µm GFC was used for blank correction. The fluorescence of the two size fractions of the samples were measured after standardising the slit width.

4.3.5.2 Standardisation of slit width

The fluorescence measurement was done using Shimadzu (RF-5301PC) spectrofluorophotometer. The instrument provides six slit width options (1.5, 3, 5, 10, 15 and 20 nm). The spectral band pass set for the instrument determines the accurate recording of the excitation or emission spectrum shape of the sample. The band pass is determined by the slit width. Slit widths that are too narrow or too wide will lead to reduced resolution and low light intensity respectively. It is important to maintain high wavelength resolution for the spectral analysis of fluorescent compounds that show structural emission. This is achieved by the adjustment of slit width on the monochromator of the instrument. The slit width should be adjusted so that the minute features of the spectra are recorded including the shoulder effects. It is advised to use the widest band pass (slit width) that does not distort the spectral features (Coble *et al.*, 2014). Hence three combinations of slit widths in the middle range (10/5, 10/10 and 15/10) were selected and standardised with the culture suspensions of *Chlorella pyrenoidosa* and *Oscillatoria acuminata*.

a) Algae culture

Chlorella pyrenoidosa and *Oscillatoria acuminata* cultured in the laboratory were used to standardize the excitation /emission slit width for green and blue-green dominated water samples respectively. The culture of *C. pyrenoidosa* was grown in Ward and Parish medium and *O. acuminata* in modified BG-11 medium. The algal cultures were grown in sterile 250 mL Erlenmeyer flasks and illuminated with white fluorescent light with a light and dark period of 12: 12 h at 25 ºC. Exponentially growing cultures were used for measuring fluorescence. The *C. pyrenoidosa* and *O. acuminata* cultures were kept at dim light for 15 minutes before measurements to avoid fast changes in energy flow (state transitions) within the photosynthetic unit (Lutz *et al.*, 2001).

Aliquots of *Chlorella pyrenoidosa* was transferred to culture tubes in volumes of 0.1mL, 0.2mL, 0.3mL, 0.4mL, 0.5mL and 0.6mL. These were made up to 10 mL with the filtrate of the same cultures filtered through Whatsman GF/C of pore size 0.45 µm. *Oscillatoria acuminata* being a filamentous species was sampled out from the culture flask, and mildly ground with mortar and pestle and made up to 10 mL with the culture filtrate. This was diluted to various concentrations as done for

C. pyrenoidosa. Duplicate samples of these dilutions were filtered through 0.45 µm membrane filters and dark-extracted with 90% acetone to determine the concentration of chlorophyll *a* of these samples. The concentration and fluorescence intensity of the sample extract were measured in the fluorimeter at excitation wavelength of 430 nm and emission wavelength of 663 nm (Eaton *et al.*, 2005). The chlorophyll *a* concentrations and their corresponding fluorescence intensity [fluorescence intensity expressed in arbitrary unit (A.u)] were plotted against the dilution values of the culture standards. The fluorescence intensity of *C. pyrenoidosa* showed a linear progression up to 0.12 A.u and a decline in intensity after that (Figure 4.3). The plot of *O. acuminata* had a linear progression up to 0.32 A.u followed by a fall in intensity (Figure 4.4).

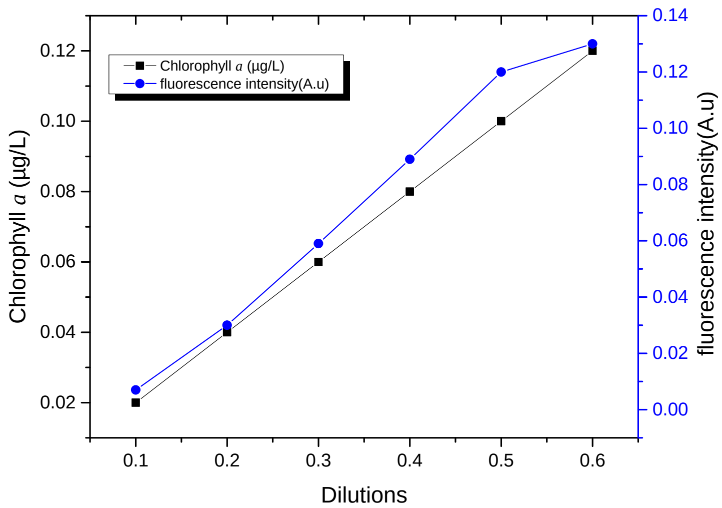

Figure 4.3: A plot of fluorescence intensity against different dilutions of *Chlorella pyrenoidosa*.

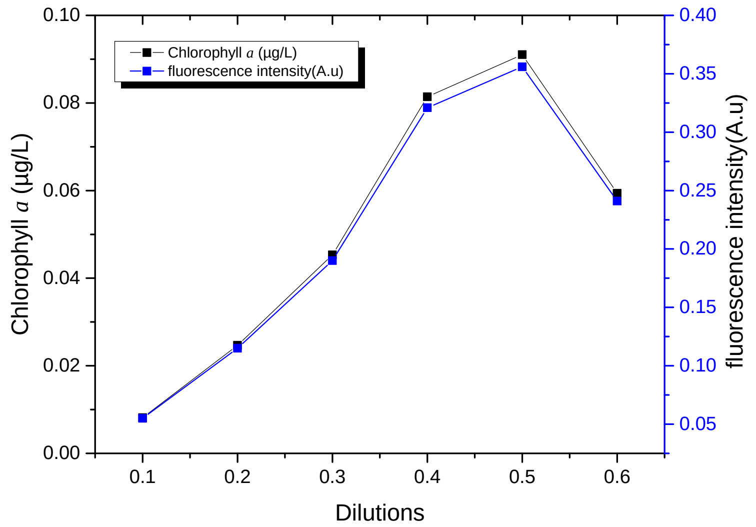

Figure 4.4: A plot of fluorescence intensity against different dilutions of *Oscillatoria acuminata.*

Hence we have selected culture dilutions with 0.1 A.u chlorophyll *a* intensity for the standardization of slit width. Fluorescence spectra of the culture suspensions were taken at excitation/emission slit widths of 10/5, 10/10 and 15/10 (Figure 4.5 and 4.6). The ex/em slit width of 15/10 had higher resolution of the peaks. Hence it was selected for the *in vivo* analysis of water samples.

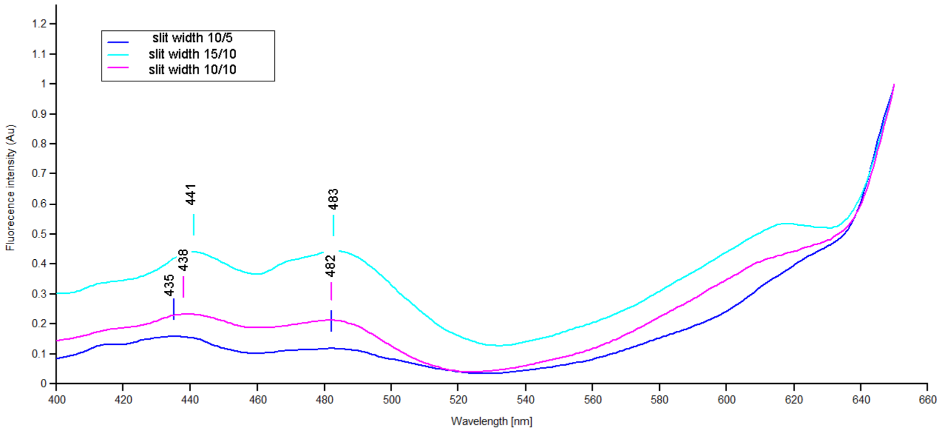

Figure 4.5: ***In vivo*** **excitation spectrum of** ***Chlorella pyrenoidosa*** **with different ex/em slit width (emission at 680nm).**

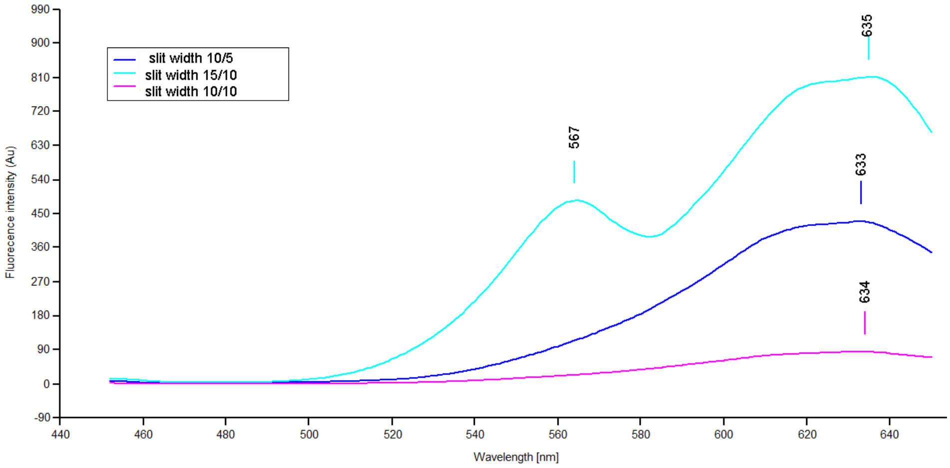

Figure 4.6: ***In vivo*** **excitation spectrum of** ***Oscillatoria acuminata*** **with different ex/em slit width (emission at 680nm).**

4.3.5.3 Fluorescence measurement of pond water

The two size fractions of the pond water samples were measured for their *in vivo* fluorescence. The sample was taken in a 1cm quartz cuvette and placed in the fluorescence measuring chamber of the instrument. The excitation spectra of the samples were taken at an emission wavelength of 680nm and excitation wavelength range of 400-650 nm.

The fluorescence spectral data obtained were taken in ASCII format and transferred to the PC and plotted using the software **spekwin32**. The spectral values were normalized to a peak excitation value of 1 A.u in order to make the comparison of the spectra easier. The 20 µm fraction spectra were subtracted from the 200 µm fraction to remove nanoplankton fraction and to get exclusive microplankton fraction with cell size ranging 20 —200 µm and the fluorescence spectrum of the nanoplankton and microplankton were obtained.

4.4 Results

4.4.1 Gross temporal change in pond 1 during 2011-14

When the investigation started in November 2011, pond 1 had a water depth of 200 cm and the water surface had *Pistia stratiotes*, a floating weed distributed sparsely on the surface. The coverage extended to nearly three fourth of the surface by December 2011 (Figure 4.7). These hydrophytes were manually removed in January 2012. By the second half of February a few plants of *Eichhornia crassipes* were seen in the pond. Periphyton attached to *Eichhornia* plants were seen and epipelic community of green filaments started developing which rose up to the surface forming metaphyton by March first half. By the mid of March the pond completely dried up and it rejuvenated at the end of April. The refilled pond had turbid water in April and May with no visible metaphyton. The south-west monsoon rain in June- September filled the pond water and the overflow flushed out *E.crassipes*.

Figure 4.7: Gross temporal change in pond 1 A) invaded by *Pistia stratiotes* (December 2011) B) *Pistia* removed manually (January 2012) C) Invaded by *E. crassipes* (February 2012) D) *E. crassipes* spreads the entire pond (March 2013).

The observations on algae restarted in September 2012. The volume of water in the pond got reduced by December. Algal scum of green phytoplankton were observed in January- February 2013. The pond was invaded by *E. crassipes* later on which spread fast to cover the entire surface. Metaphyton mats (filamentous algae) did not develop in 2012-13. The pond got dried in April and rejuvenated in May in the pre- monsoon rains.

The observations in 2013 restarted again in September. The pond was filled with clear water in September- October 2013. The hydrophyte

Pistia stratiotes floated over the pond on the peripheral region and spread over to the centre and covered two third of the pond surface by October. The pond became turbid in January- February 2014. The pond was invaded by *E. crassipes* in March. Algal bloom was not observed during this period. The pond got dried in April. The monsoon rain refilled the pond and increased the pond volume and depth in June 2014.

4.4.2 Phytoplankton succession in pond 1

4.4.2.1 Phytoplankton succession during 2011-12

The algal phyla observed in pond 1 during 2011-12 were Cyanobacteria, Chlorophyta, Charophyta, Cryptophyta, Euglenozoa, Ochrophyta and Mezozoa (Figure 4.8). Cryptophyta dominated the pond during the second half of both November (44%) and December (72%). Chlorophyta began to increase over Cryptophyta to dominate the phytoplankton community in the pond during January—February. The highest percentage occurrence of Chlorophyta was observed in January (89%). A shift from Chlorophyta (42%) to Euglenozoa (49%) was observed in the month of March prior to the dry phase. The pond rejuvenated with dominance of Chlorophyta with percentage occurrence of 53% and 71% in April and May respectively. The occurrence frequency of Charophyta varied from 0—5 %, Ochrophyta from 0—22% and Mezozoa from 0—8%.

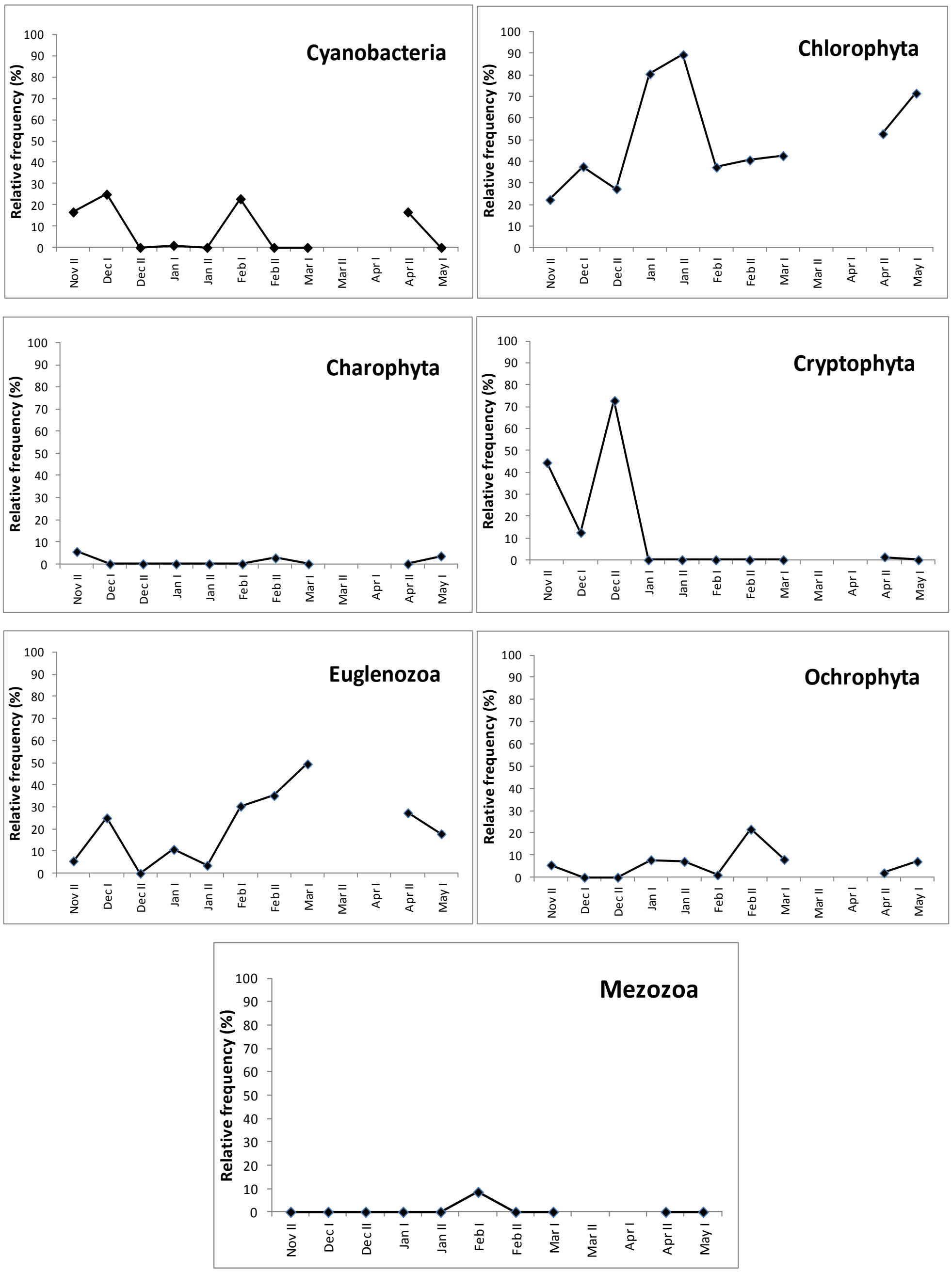

Figure 4.8: Relative frequencies of different algal phyla in pond 1 during 2011-12 (Gap in the graph corresponds to dry period).

4.4.2.2 Phytoplankton succession during 2012-13

The algal phyla observed in pond 1 during 2012-13 period was Cyanobacteria, Chlorophyta, Charophyta, Cryptophyta, Euglenozoa, Ochrophyta and Mezozoa (Figure 4.9). The dominant phyla were Euglenozoa, Cryptophyta and Chlorophyta. The pond was dominated by Euglenozoa in September (56%) and Cryptophyta in the first half of October (90%). As Cryptophyta crashed, phytoplankton dominance shifted to Euglenozoa in November and was succeeded by Chlorophyta dominance till the pond got dried up in April. The highest relative frequency of Chlorophyta was observed in early March with 93% occurrence. The relative frequencies of other algal groups in the pond were < 40%. The frequency of occurrence of Charophyta ranged 0—29 %, Ochrophyta with 0—31 % and Mezozoa with 0—19%.

4.4.2.3 Phytoplankton succession during 2013-14

The dominant phytoplankton phyla observed in pond 1 during 2013-14 were Ochrophyta, Chlorophyta and Euglenozoa. Ochrophyta was the dominant phytoplankton group from September to December. Percentage occurrence of Ochrophyta was highest during October (58%). A shift from Ochrophyta to Chlorophyta occurred in January. The relative frequency of Chlorophyta increased gradually to reach its highest percentage occurrence of 73% in March. Chlorophyta continued its dominance till April. Euglenozoa dominated the pond in May and June with relative frequency of 77% and 87% respectively (Figure 4.10). Other algal phyla in the pond had relative frequencies <15%. Over the years from 2011 to 2014, there is a prepondance of Chlorophyta and Euglenozoa in pond 1.

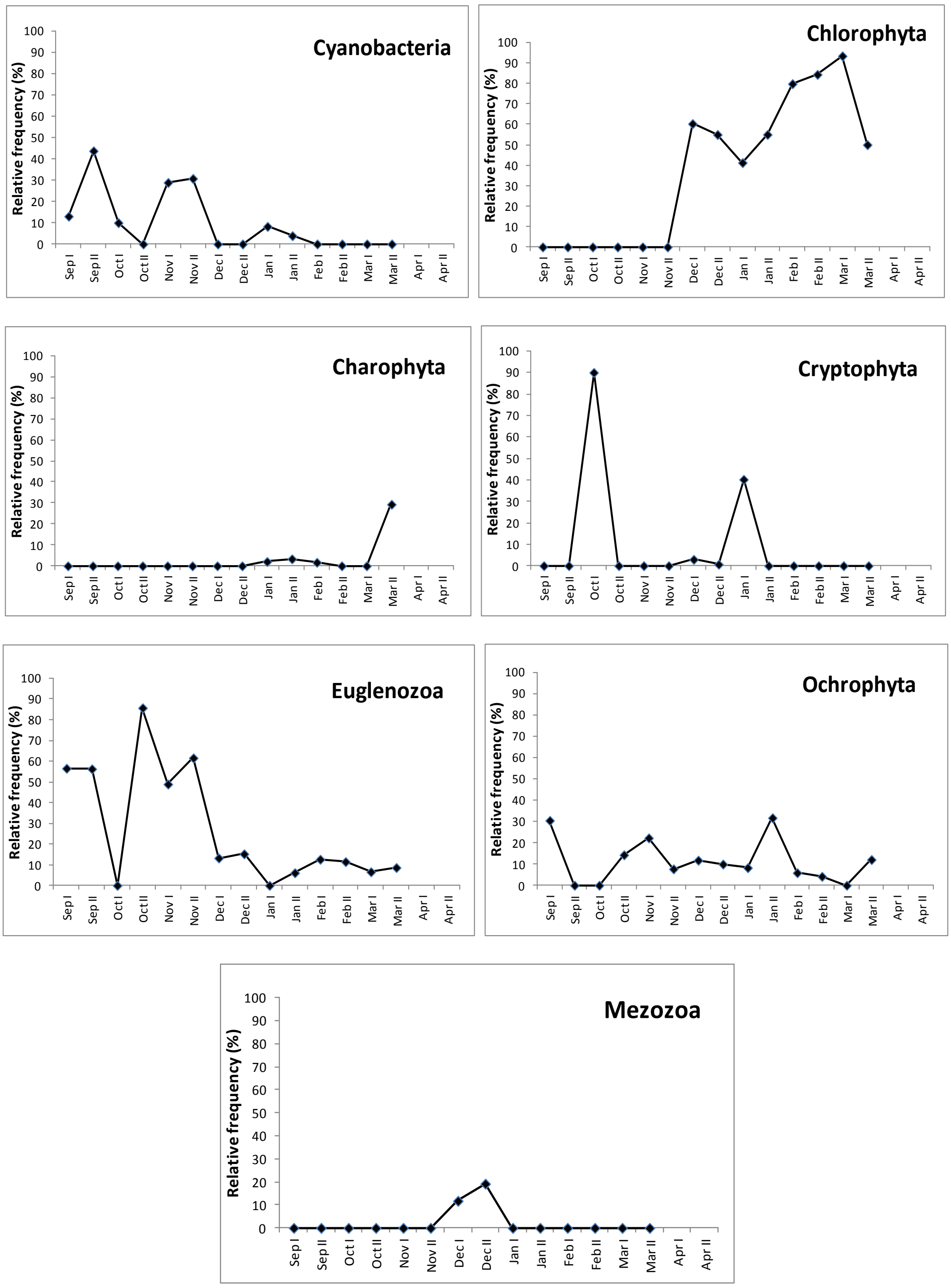

Figure 4.9: Relative frequencies of different algal phyla in pond 1 during 2012-13 (Gap in the graph corresponds to dry phase).

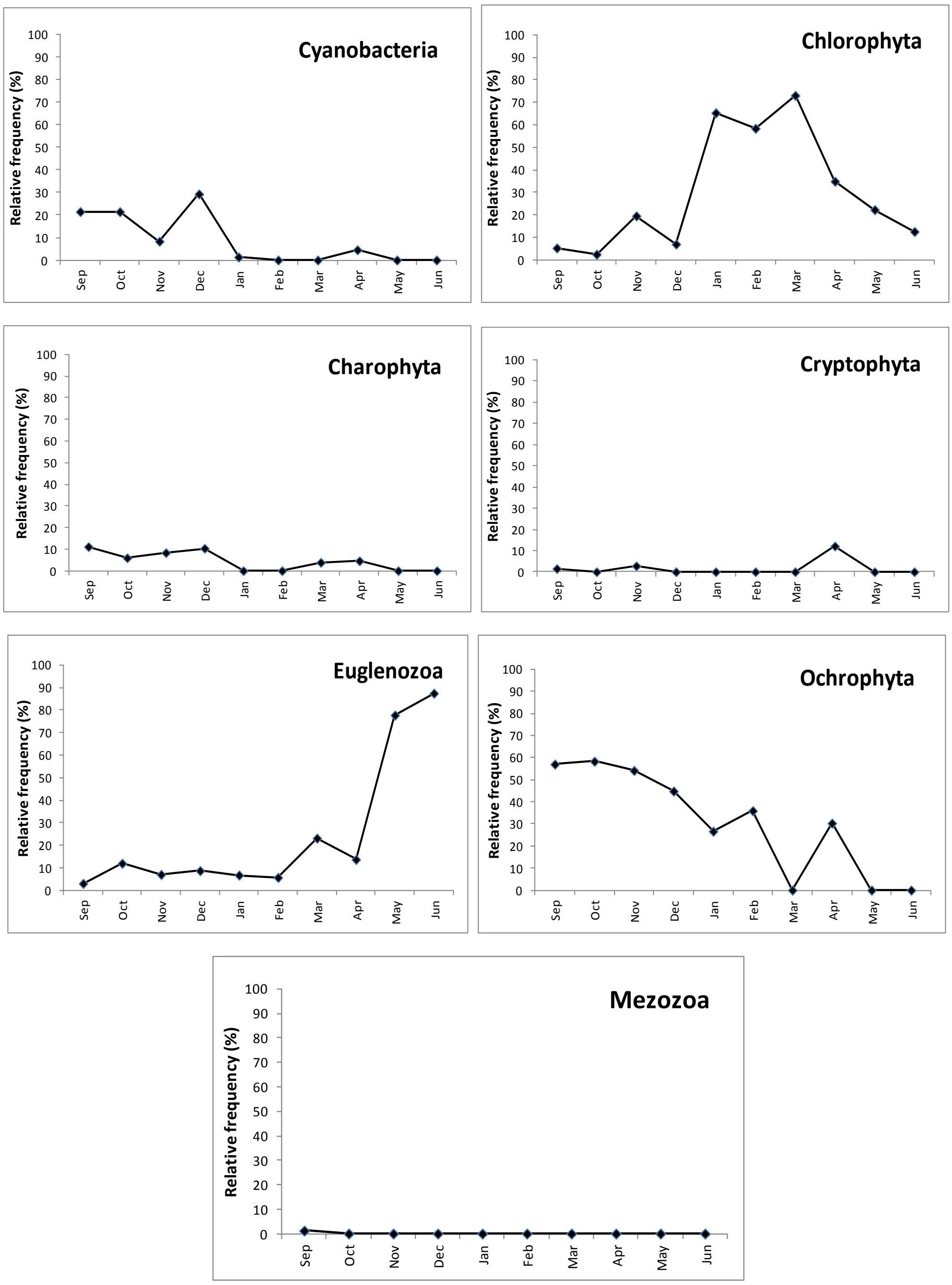

Figure 4.10: Relative frequencies of different algal phyla in pond 1 during 2013-2014.

4.4.2.4 Species composition of phytoplankton

A total of 161 algal species were recorded in pond 1during 2011-14. The highest number of taxa (84) was recorded in 2011-12 period followed by 2012-13 (83) and 2013-14 (76). Chlorophyta constituted most diverse algal group with 83 algal species followed by Ochrophyta with 22 species. The genus *Scenedesmus* was the most diverse among the Chlorophyceans with 19 species (Annexure VI).

The most frequently observed taxa in pond 1 during 2011-12 were *Cryptomonas* sp1, *Chlorella saccharophilum*. and *Lepocinclis globulus*. *Cryptomonas* sp1 dominated the pond with percentage frequency of 14.1% followed by *Chlorella saccharophilum* (10.7%) and *Lepocinclis globulus* (5.8%). *Cryptomonas* sp1 maintained the dominance in 2012-13 also with a relative frequency of 10.6% immediately followed by *Pediastrum duplex* (10.4%) and *Lepocinclis globulus* (8.5%). In 2013-14 period, 20% of the algal taxa were represented by *Trachelomonas volvocina* followed by 7.1% each of *Pediastrum tetras* and *Nitzschia palea*. The Chl *a* in the pond recorded lower values when *Trachelomonas volvocina* and *Nitzschia palea* dominated the pond.

4.4.2.5 Phytoplankton bloom

Chlorophyll *a* in water samples was taken as the quantitative measure of phytoplankton abundance in the ponds. The phytoplankton bloom formation was defined as the period during which the concentration of chlorophyll *a* was >50 µg/L (Zohary *et al.*, 1998). The monthly average of chlorophyll *a* in pond 1 during 2011-12, 2012-13 and 2013-14 is given in Table 4.1.

The concentration of chlorophyll *a* in pond 1 during 2011-12 exceeded 50.00 µg/L in February and March 2012 recording very high values of 318.5 µg/L and 540.5 µg/L respectively. The dominant phytoplankton during these months were identified with relative frequency using the key: present <5%, abundant < 5—40% and dominant ≥ 40% (Zohary *et al.*, 1998). According to this key Chlorophyta and Euglenozoa were the bloom forming phytoplankton phyla in 2011-12. Chlorophyta dominated in second half of February 2012. In March, both Chlorophyta and Euglenozoa dominated equally. During 2012-13, chlorophyll *a* exceeded the phytoplankton bloom limit forming blooms of Euglenozoa and Cyanobacteria in September 2012, Chlorophyta and Cryptophyta in January, and Chlorophyta in February and March 2013. The 2013-14 sampling period had phytoplankton blooms of Ochrophyta during December; Chlorophyta formed bloom in January and March and formed an assemblage with Ochrophyta in April 2014.

Table 4.1: Monthly average of Chl *a* (µg/L) in pond 1 during 2011-14

Months	2011-12	2012-13	2013-14
Sep	--	**72.89**	7.330
Oct	--	2.490	15.70
Nov	20.20	38.79	21.52
Dec	7.220	**110.3**	**81.14**
Jan	39.68	**189.4**	**158.5**
Feb	**318.5**	**184.7**	23.23
Mar	**540.5**	**166.5**	**94.91**
Apr	16.32	--	**76.26**
May	12.03	--	17.21
Jun	--	--	10.11

The phytoplankton bloom species were also identified with the same key for phyla (≥40%). According to this, there was no single species dominance in 2011-12. During 2012-13, the month of September had a bloom of *Trachelomonas volvocina*. *Pediastrum duplex* formed dominant bloom during February 2013 (Table 4.2). In March, *P.duplex* was the dominant bloom forming phytoplankton along with *Scenedesmus dimorphus*. During 2013-14 period a diatom species -*Nitzschia palea* formed blooms in December and April 2013. *Scenedesmus quadricauda* formed bloom during January and this unialgal bloom shifted to the dominance of an assemblage of *Ankistrodesmus falcatus* and *Pediastrum tetras* in March 2014 (Figure 4.11).

Table 4.2: Phytoplankton bloom species in pond 1 during 2011-14

Months	Phytoplankton phyla	Dominant species
	2011-12	
February 2012	Chlorophyta	-
March 2012	Euglenozoa	-
	Chlorophyta	-
	2012-13	
September 2012	Euglenozoa	*Trachelomonas volvocina*
	Cyanobacteria	-
January 2013	Chlorophyta	-
	Cryptophyta	-
February 2013	Chlorophyta	*Pediastrum duplex*
March 2013	Chlorophyta	*Pediastrum duplex, Scenedesmus dimorphus*
	2013-14	
December 2013	Ochrophyta	*Nitzschia palea*
January 2014	Chlorophyta	*Scenedesmus quadricauda*
March 2014	Chlorophyta	*Ankistrodesmus falcatus, Pediastrum tetras*
April 2014	Chlorophyta	-
	Ochrophyta	*Nitzschia palea*

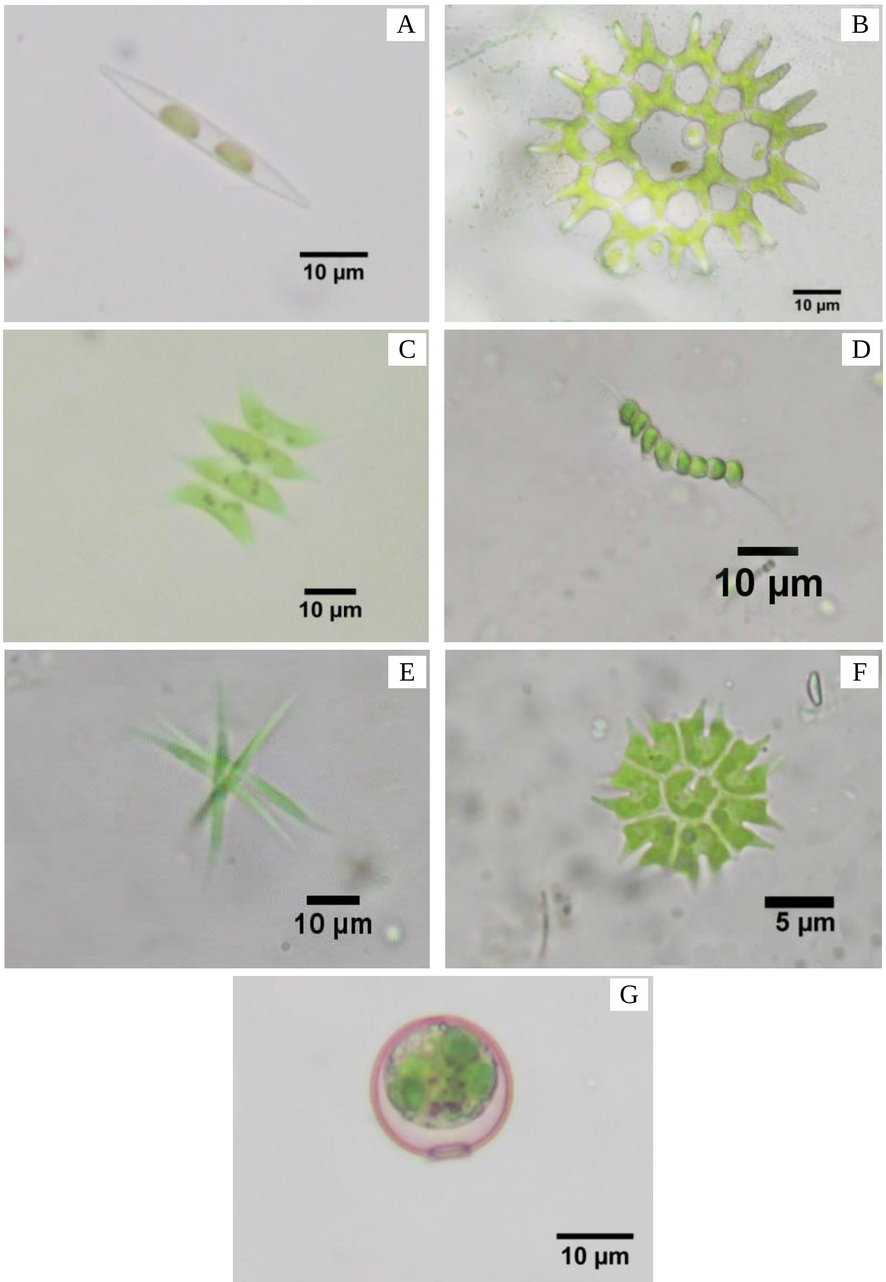

Figure 4.11: Phytoplankton bloom species of pond 1 during 2011-2014. A) *Nitzschia palea* B) *Pediastrum duplex*, C) *Scenedesmus dimorphus* D) *Scenedesmus quadricauda* E) *Ankistrodesmus falcatus* F) *Pediastrum tetras* G) *Trachelomonas volvocina*.

Periphyton were observed in the pond during 2011-12. Epipelic green filaments of *Spirogyra* type 1 developed during February 2012 and it floated on surface as metaphyton. *Spirogyra* type 1 was the single dominant species constituting the metaphyton mat (Figure 4.12). Chlorophyll *a* in the pond water was highest during this period ranging from 540-595µg/L. During 2012-13 period, a thin film of green phytoplankton scum developed on the surface of the pond in January—February 2013 which was a mixed phytoplankton bloom of *Pediastrum duplex* and *Chlorococcum minutum* (Figure 4.13).

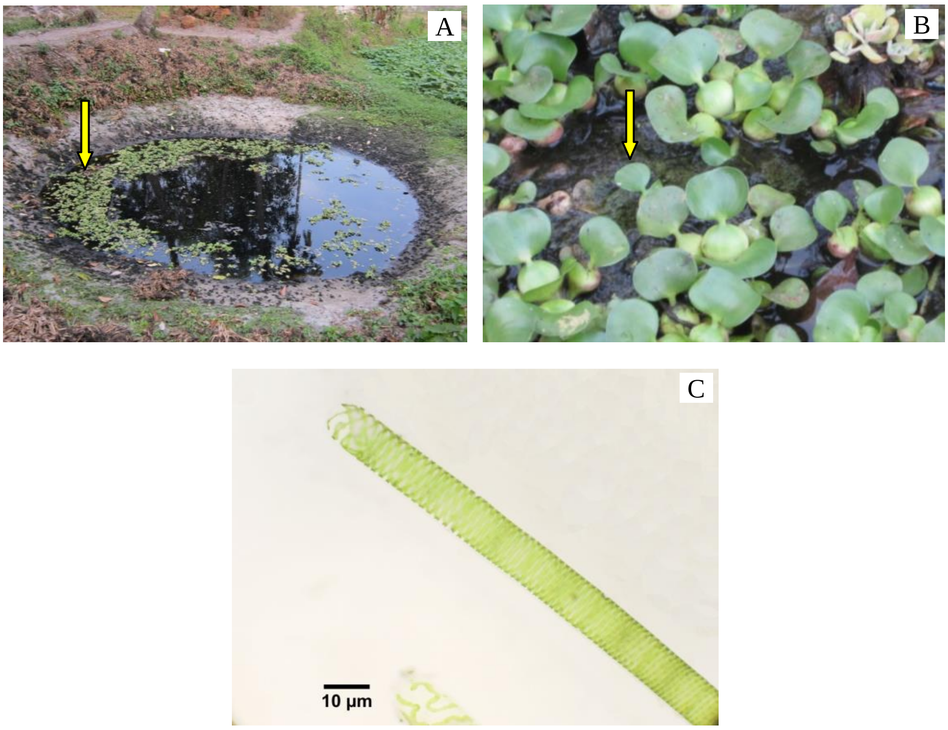

Figure 4.12: A) Pond with *E.crassipes* B) green metaphyton mat of *Spirogyra* type1 on pond surface in between *E.crassipes* C) Microphotograph of *Spirogyra* type 1.

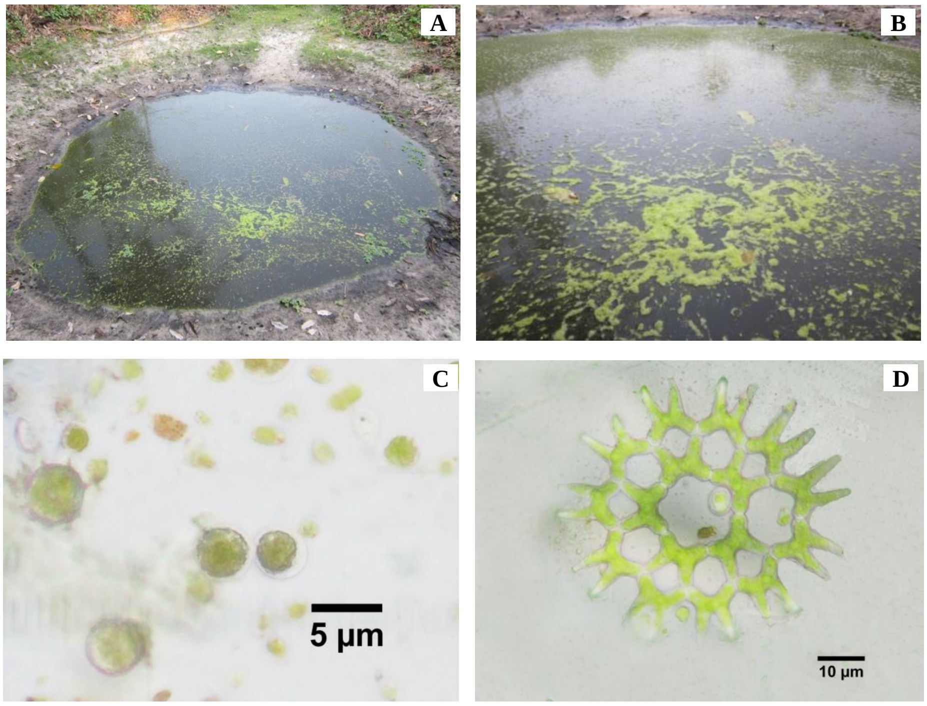

Figure 4.13: A) & B) The phytoplankton scum over the pond surface C) microphotographs of *Chlorococcum minutum* and D) *Pediastrum duplex*.

4.4.3 Phytoplankton dynamics in Pond 1

Temporal variation in the environmental factors and physico-chemical variables contribute to the dynamics of phytoplankton in the ponds. The correlation analysis revealed significant correlation of temperature, pH, TP and NO_3-N with chlorophyll *a* of pond 1(Chapter 3-section 3.6.3). The frequency of algal groups observed during the study year 2011-14 along with the significant variables were analysed by Principal Component Analysis (PCA) and Discriminant Analysis (DA) for elucidating the dynamics of phytoplankton development (Figure 4.14).

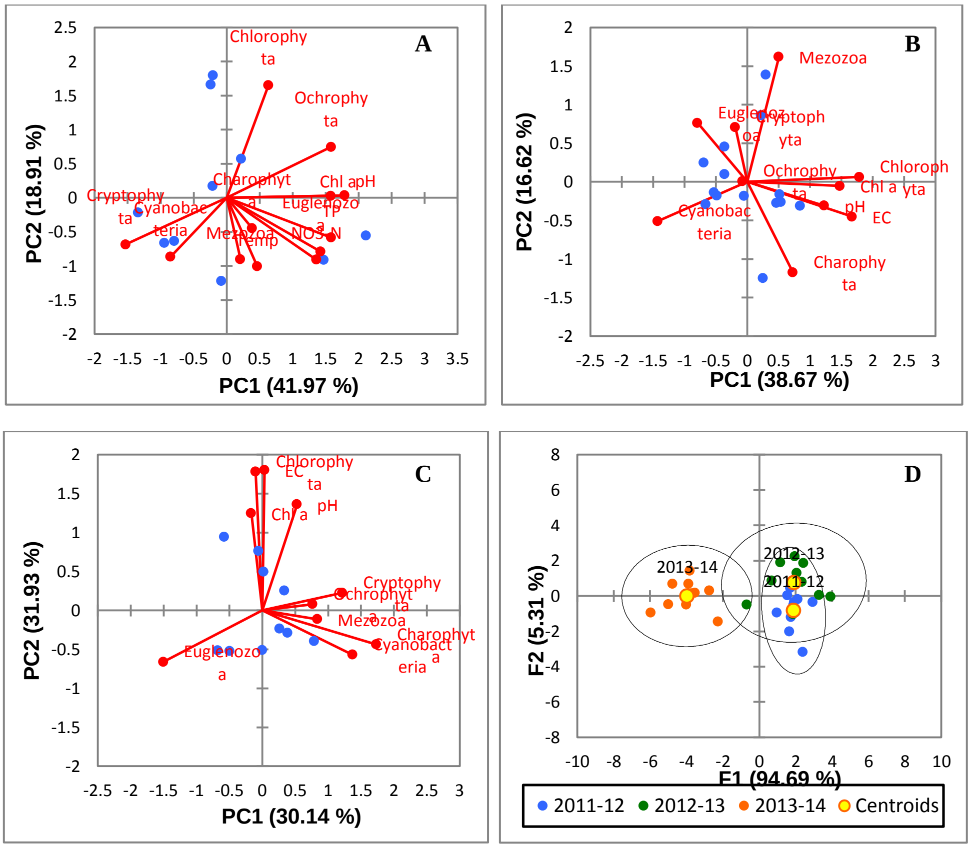

Figure 4.14: PCA biplot of the component loadings of pond 1 variables for the year A) 2011-12, B) 2012-13, C) 2013-14. D) The Discriminant Analysis (DA) of pond 1 variables among the year 2011-12, 2012-13 and 2013-14

PCA using the relative frequency of algal phyla and physico-chemical variables of pond 1 during 2011-12 explained 60% of the variability in the first two ordination axes. The important variables for axis 1 were Ochrophyta, Cryptophyta, Chlorophyta, Cyanobacteria, pH and Chl *a*. Of this, Cyanobacteria and Cryptophyta was found negatively correlated to other variables in axis 1. This indicates that the major portion of the phytoplankton biomass in pond 1 was constituted by Ochrophyta and

Chlorophyta, and when Ochrophyta and Chlorophyta increases in the pond water the blue-green and flagellates decreases. The important parameters represented by ordinate 2 were Euglenozoa, NO_3-N, TP and temperature. The nutrient flux and temperature increase in the beginning of summer months contributed to the growth and proliferation of Euglenozoa in the pond. In 2012-13 period, the PCA component of first two ordinates accounted 55% of the variance in data. The first axis had significant variables of Chlorophyta, pH, EC and Chl *a* (Annexure X). Cyanobacteria was found in the negative axis of the ordinate indicating the negative correlation of blue-green algae with that of Chlorophyta. The second axis had Mezozoa and Charophyta negatively correlated to each other. The PCA of 2013-14 period showed 62% of the variance in data being explained by the first two axis. The first axis had important variables such as Cyanobacteria, Charophyta and Cryptophyta. Euglenozoa was in the negative side of the axis. Axis 2 had Chlorophyta, Chl *a*, pH and EC. This indicates the physico-chemical variables in the pond were directly correlated with the Chlorophycean growth and proliferation. The discriminant analysis (DA) biplot shows that the general trend of the phytoplankton dynamics in pond 1 during 2013-14 varied significantly from that of 2011-12.

4.4.4 Gross temporal change in pond 2 during 2011-2014

The pond was full in November 2011 with submerged plants of *Hydrilla verticillata*. The volume of pond water decreased in December and the water became turbid. From January second half onwards some filaments of blue- green algae started appearing on the surface as floating mat. In February this metaphyton spread over one fourth of the water and it extended to cover three fourth of the pond by the end of March forming thick mat. The pond was very much reduced in volume during March-April. Volume of water increased after receiving the summer rain by the middle of April. The bloom subsided with the monsoon rains in June-September 2012. After a clear period in September- November 2012, the water went to a turbid state in December just like the previous year. The blue -green algal metaphyton started developing on the water surface in January 2013. An epipelic mat of blue-green algae was also found along the periphery of the pond during this period. The epipelic mat disappeared in February 2013. A mat of green algal filaments was observed floating at the centre and the periphery of the pond surface in March first half along with the major blue-green metaphyton. The green algae disappeared by March second half and the blue-green filaments covered almost three fourth of the pond as metaphyton. After the monsoon, the observation restarted in September 2013. The pond was clear during September—November. The pond developed *H.verticillata* during this period. It disappeared in December and the floating weed *Azolla pinnata* invaded and spread over the pond surface. It was short lived and the pond started developing blue-green metaphyton on the surface. It spread over the entire pond by March. The metaphyton mat got dissipated in May- June 2014 by the monsoon rain (Figure 4.15).

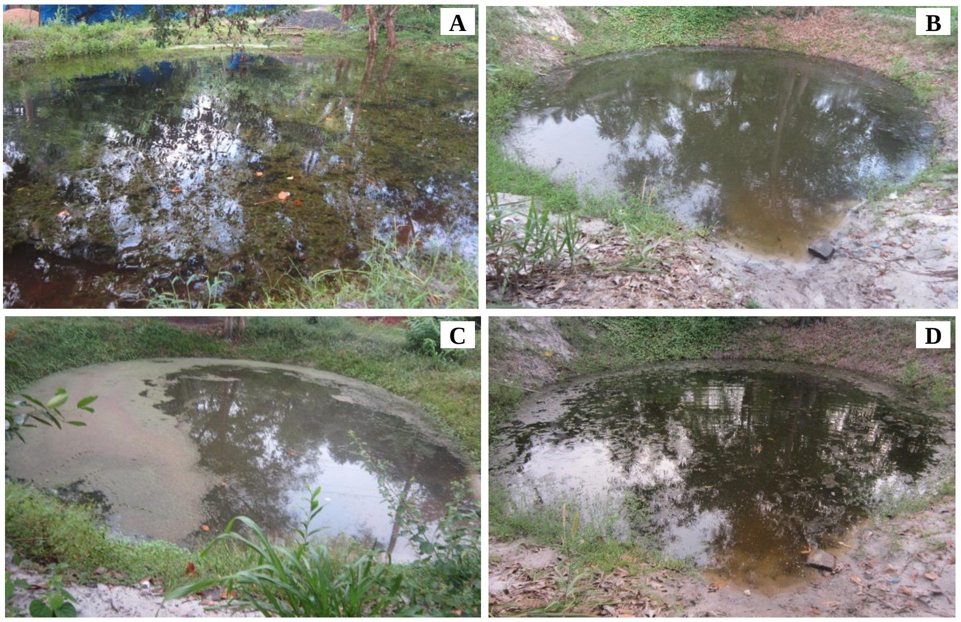

Figure 4.15: Gross temporal change in pond 2 A) submerged *Hydrilla verticellata* (November 2011) B) Algal turbidity (December 2011) C) invaded by *Azolla pinnata* (September 2013) D) Floating Metaphyton (February-2012-14).

4.4.5 Phytoplankton succession in pond 2

4.4.5.1 Phytoplankton succession during 2011-12

Pond 2 during 2011-12 was dominated by Cyanobacteria, Chlorophyta and Euglenozoa (Figure 4.16). Frequent shifts between Cyanobacteria, Chlorophyta and Euglenozoa was observed in the pond. Cyanobacteria dominated the pond in November (59%) and January first half (58.6%). Chlorophyta attained highest frequency of 83% in December and 95% in January. From the second half of January to the end of April the phytoplankton dominance in the pond shifted frequently between Chlorophyta and Euglenozoa. At the end of sampling in May, Cyanobacteria came back to prominent frequency (37%), Chlorophyta exhibited upward trend and Euglenozoa was declining. The relative frequencies of Charophyta, Cryptophyta, Ochrophyta and Mezozoa had frequencies <10%.

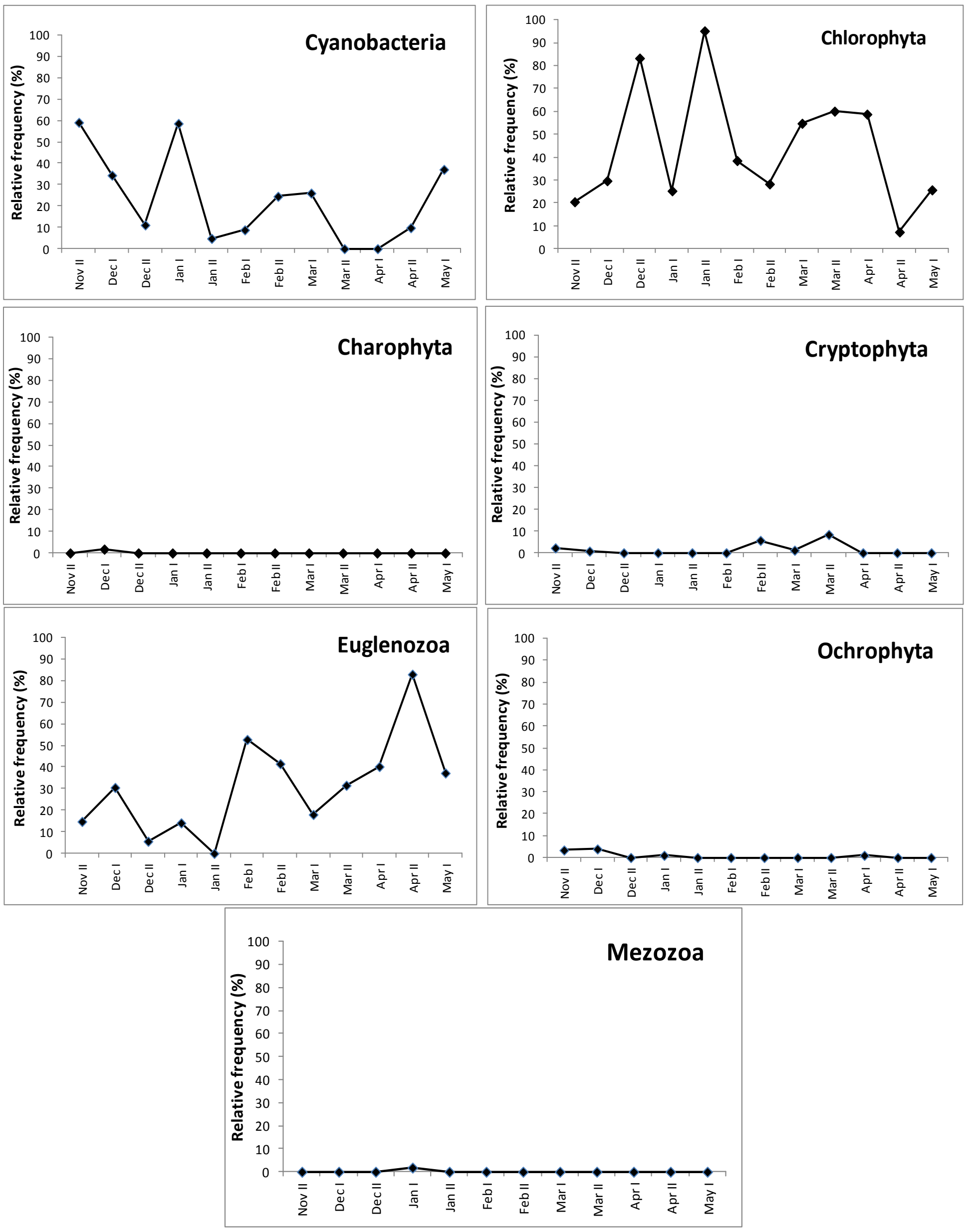

Figure 4.16: Relative frequencies of different algal phyla in pond 2 during 2011-2012

4.4.5.2 Phytoplankton succession during 2012-13

Chlorophyta, Euglenozoa and Cyanobacteria dominated pond 2 during 2012-13. Chlorophyta and Euglenozoa intermittently dominated the pond in two week interval during September —October (Figure 4.17).

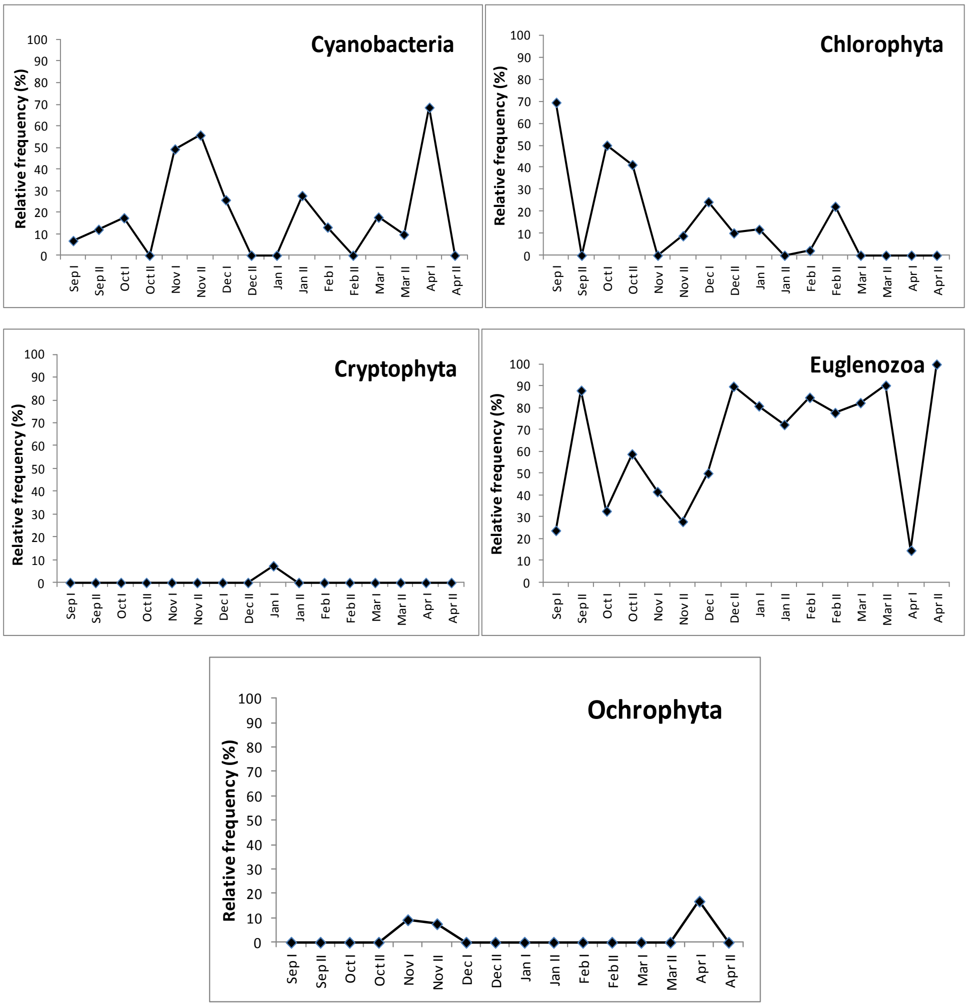

Figure 4.17: Relative frequencies of different algal phyla in pond 2 during 2012-2013

This trend shifted with the dominance of Cyanobacteria in November with a relative frequency of 49% and 56% in the first and second half respectively. Another major shift in phytoplankton from Cyanobacteria to Euglenozoa occurred in December and it continued as the major phytoplankton till the end of March. Cyanobacteria increased to dominate the pond with relative frequency of 68.5% in April first half but couldn't maintain the dominance in the second half. Euglenozoa solely represented the phytoplankton community in this period. The frequency of Cryptophyta was in the range of 0-7% and that of Ochrophyta ranged 0-17%.

4.4.5.3 Phytoplankton succession during 2013-14

The algal phyla observed in the pond during 2013-14 were Cyanobacteria, Chlorophyta, Charophyta, Cryptophyta, Euglenozoa and Ochrophyta (Figure 4.18). The dominant algae among them were Euglenozoa, Chlorophyta and Cyanobacteria. Euglenozoa dominated the pond in the beginning (55%) and end of sampling (63%) in September and June respectively. Chlorophyta dominated in most of the months except in January and April. A marginal dominance of Cyanobacteria was observed in these months. Charophyta, Cryptophyta and Ochrophyta constituted <20%.

4.4.5.4 Species composition of phytoplankton

The pond had recorded a total of 120 taxa during 2011-2014. Chlorophyta was the most diverse group with 64 species. Cyanobacteria and Euglenozoa constituted 17 species each. The genus *Tetraedron* of phylum Chlorophyta was the most diverse with 11 species. The highest diversity in phytoplankton was observed during 2011-12 with 76 species followed by 59 species in 2013-14 and 29 species during 2012-13.

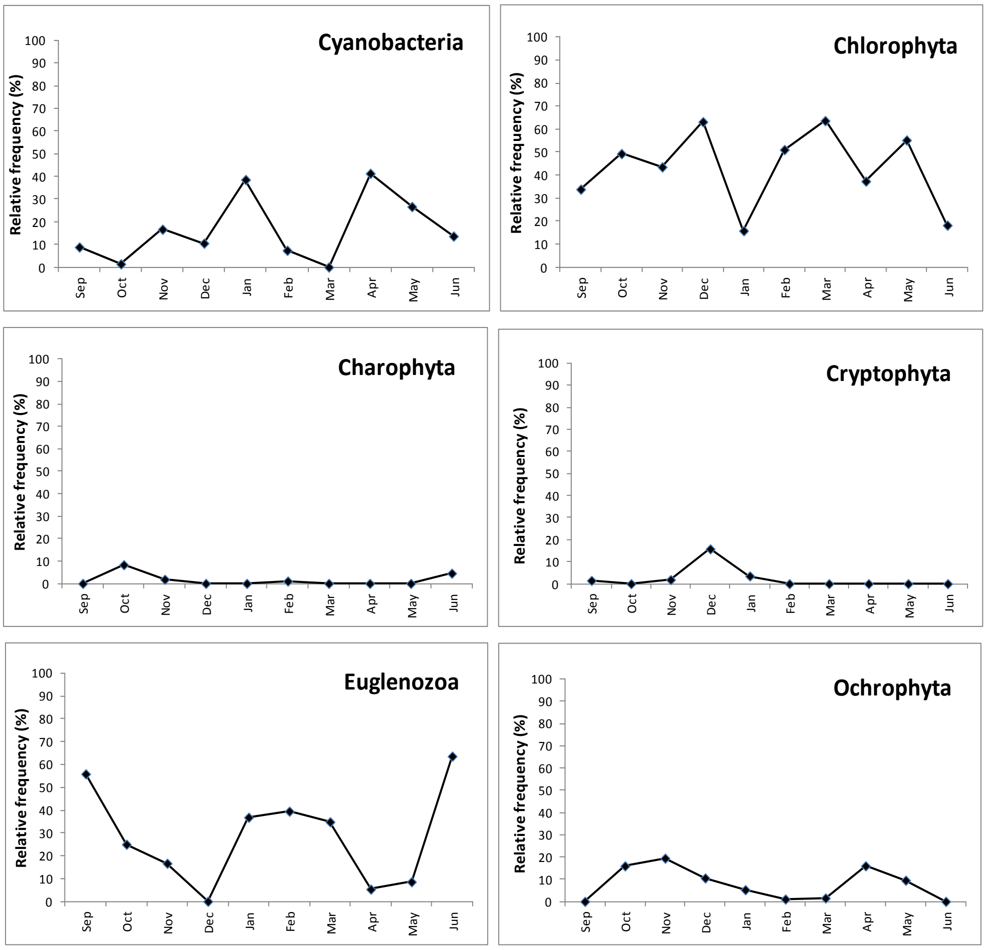

Figure 4.18: Relative frequencies of different algal phyla in pond 2 during 2013-14

The dominant species in pond 2 during 2011-12 were *Trachelomonas volvocina* (17%), *Ankistrodesmus acicularis* (9%) and *Chlorella vulgaris* (8%). During 2012-13, *Trachelomonas volvocina* topped with 51% occurrence frequency. *Lepocinclis globulus* (7%) and *Oscillatoria limosa*

(5%) were the dominant taxa among the Euglenozoa and Cyanobacteria respectively. *Trachelomonas volvocina* (24%) and *Monoraphidium minutum* (20%) dominated in the pond during 2013-14. The Euglenoid, *Trachelomonas volvocina* uninterruptedly continued as the dominant phytoplankton in the pond (Annexure VII).

4.4.5.5 Phytoplankton bloom

Phytoplankton bloom with respect to chlorophyll *a* in pond 2 during 2011-12 was observed from December—May. In 2012-13 and 2013-14 there were phytoplankton blooms in the initial sampling months also (Table 4.3). The pond had phytoplankton blooms in February—April in three consecutive sampling years with respect to the concentration of chlorophyll *a*. Chlorophyta, Euglenozoa and Cyanobacteria were the major bloom forming phyla in pond 2 during 2011-12 and 2012-13. Chlorophyta and Cyanobacteria dominated the pond in 2013-14 (Table 4.4).

Table 4.3: Monthly average of Chl *a* (µg/L) in pond 2 during 2011-14. (Bold font for Chl *a* > 50 µg/L)

Months	2011-12	2012-13	2013-14
Sep	--	**64.33**	10.08
Oct	--	10.02	**73.16**
Nov	38.31	**60.05**	43.49
Dec	**83.37**	**74.27**	**62.68**
Jan	**56.31**	20.24	28.26
Feb	**57.99**	**58.47**	**273.3**
Mar	**199.1**	**97.79**	**195.9**
Apr	**65.91**	**73.00**	**188.0**
May	**57.91**	--	**180.2**
Jun	--	--	36.25

Table 4.4: Phytoplankton bloom species in pond 2 during 2011-14

Months	Phytoplankton phyla	Dominant species
2011-12		
December 2011	Chlorophyta	*Chlorella vulgaris*
January 2012	Cyanobacteria	*Aphanocapsa* sp2
	Chlorophyta	*Ankistrodesmus acicularis*
February 2012	Euglenozoa	*Lepocinclis globulus, Trachelomonas vovocina*
March 2012	Chlorophyta	*Chlorella vulgaris, Ankistrodemus acicularis*
April 2012	Chlorophyta	-
	Euglenozoa	*Trachelomonas volvocina*
May 2012	--	-
2012-13		
September 2012	Chlorophyta	*Ankistrodesmus acicularis*
	Euglenozoa	*Trachelomonas volvocina, Lepocinclis globulus*
November 2012	Cyanobacteria	*Microcystis aeruginosa*
	Euglenozoa	*Trachelomonas volvocina*
December 2012	Euglenozoa	*Trachelomonas volvocina*
February 2013	Euglenozoa	*Trachelomonas volvocina*
March 2013	Euglenozoa	*Trachelomonas volvocina*
April 2013	Cyanobacteria	*Phormidium retzii*
	Euglenozoa	*Trachelomonas volvocina*
2013-14		
October 2013	Chlorophyta	*Scenedesmus obtusus*
December 2013	Chlorophyta	*Monoraphidium minutum*
February 2014	Chlorophyta	*Chlorella vulgaris*
March 2014	Chlorophyta	*Monoraphidium contortum, Monoraphidium minutum*
April 2014	Cyanobacteria	*Oscillatoria limosa*
May 2014	Chlorophyta	*Monoraphidium minutum*

The bloom forming chlorophyceans in pond 2 during 2011-12 period were *Ankistrodesmus acicularis* and *Chlorella vulgaris*. A Cyanobacteria bloom of *Aphanocapsa* sp2 was also observed during January 2012. *Trachelomonas volvocina* and *Lepocinclis globulus* constituted the Euglenozoan members which contributed to the phytoplankton bloom in the pond during 2011-12. During 2012-13, Euglenozoa was the major phytoplankton bloom forming phyla with species comprising *Trachelomonas volvocina* and *Lepocinclis globulus*. Chlorophycean bloom was observed only in September 2012 with a single species composition of *Ankistrodesmus acicularis*. Cyanobacteria species of *Microcystis aeruginosa* and *Phormidium retzii* formed blooms during November 2012 and April 2013 respectively. During 2013-14 period, there were no Euglenozoan bloom. Chlorophyceans were the major phytoplankton bloom forming phyla with species composition of *Scenedesmus obtusus, Monoraphidium minutum, Chlorella vulgaris* and *Monoraphidium contortum*. Cyanobacterial bloom of *Oscillatoria limosa* was observed during April 2014 (Figure 4.19).

The pond developed blue-green metaphyton mat in three consecutive study years from 2011-2014. The metaphyton species was identified as *Oscillatoria limosa*. During 2012-13 study year, the pond was observed with a metaphyton assemblage of *Oscillatoria limosa* and *Spirogyra* sp. (type 2) in March 2013 (Figure 4.20). A visible mat of epipelic algae- *Komvophoron schimidlei* was also observed during January 2013 along with the major metaphyton species of *O.limosa* (Figure 4.21).

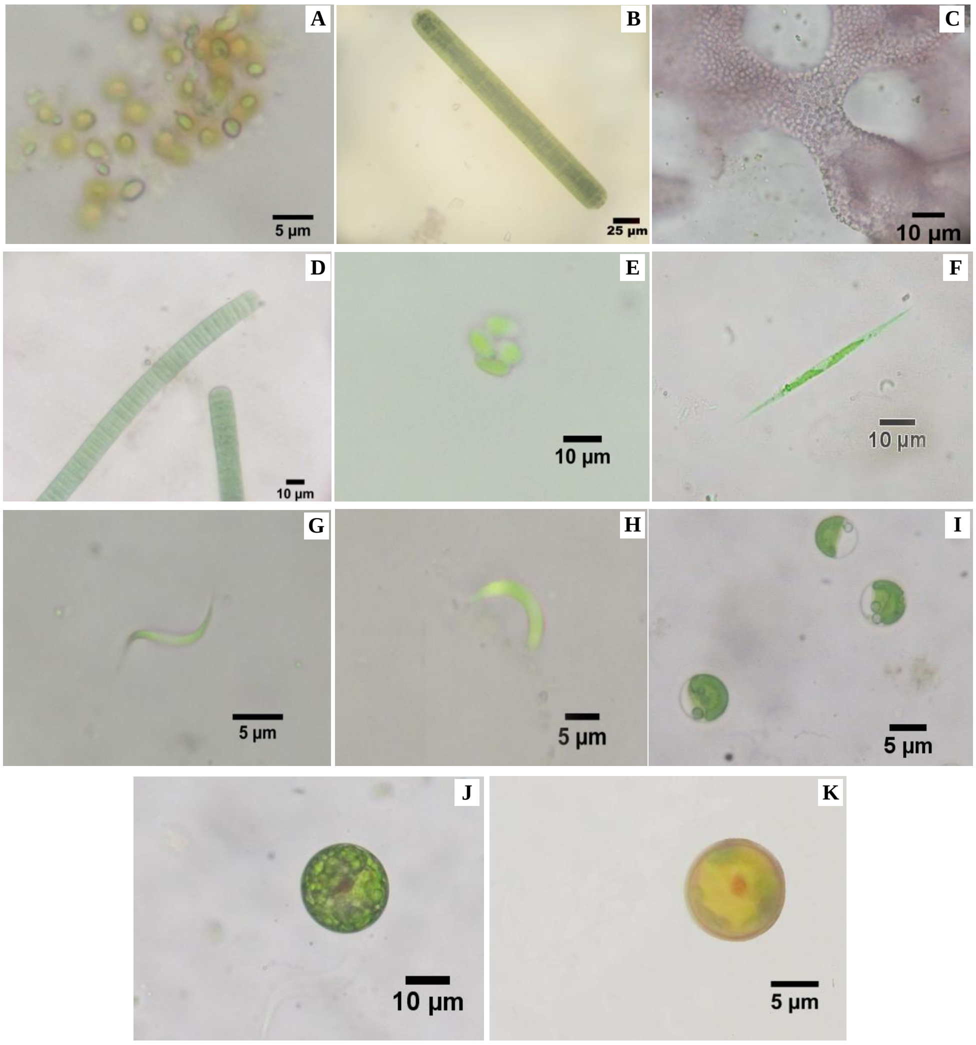

Figure 4.19: Phytoplankton bloom species of pond 2 during 2011-2014. A) *Aphanocapsa* sp2 B) *Oscillatoria limosa* C) *Microcystis aeruginosa* D) *Phormidium retzii* E) *Scenedesmus obtusus* F) *Ankistrodesmus acicularis* G) *Monoraphidium contortum* H) *Monoraphidium minutum* I) *Chlorella vulgaris* J) *Lepocinclis globulus* K) *Trachelomonas volvocina.*

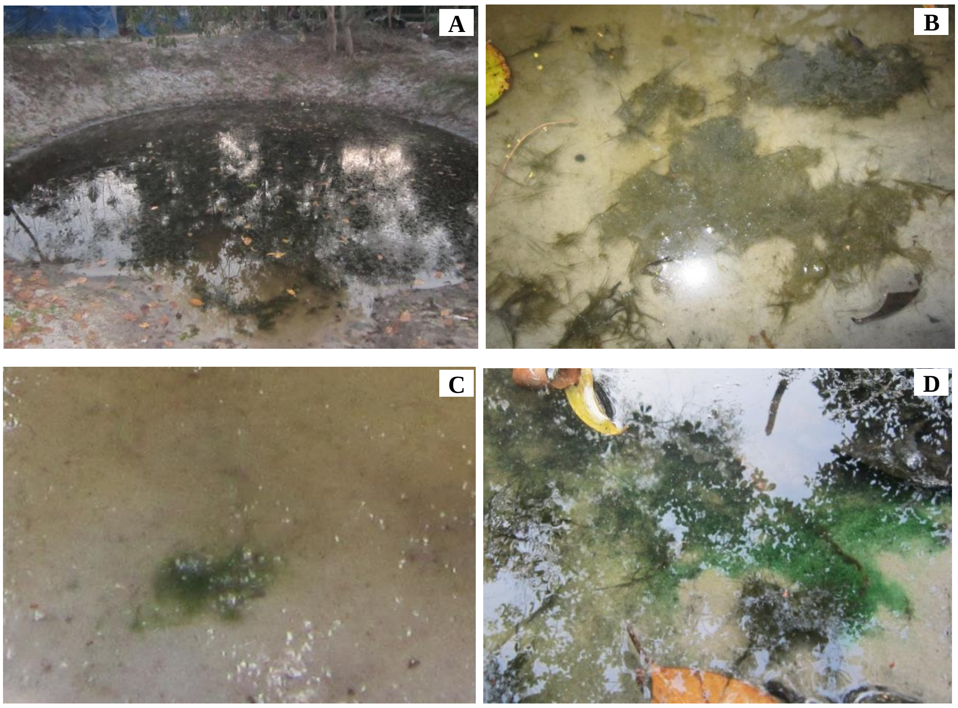

Figure 4.20: A) Metaphyton spread over the pond B) Major blue-green metaphyton- *Oscillatoria limosa* C) Green metaphyton- *Spirogyra* type2 D) Epipelic mat of *Komvophoron schimidlei.*

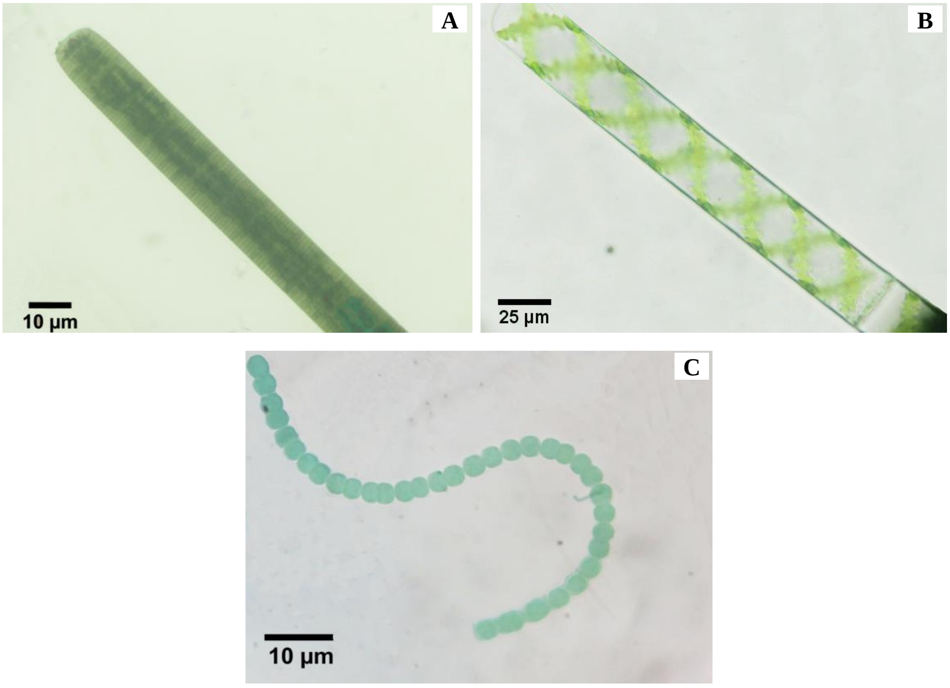

Figure 4.21: Microphotographs of bloom species A) *Oscillatoria limosa* (metaphyton) B) *Spirogyra* type 2 (metaphyton) and C) *Komvophoron schimidlei* (epipelon).

4.4.6 Phytoplankton dynamics in pond 2

The dynamics of phytoplankton in pond 2 during 2011-12 analysed by PCA is represented by canonical plot (Figure 4.22). The PCA result of 2011-12 had the first two ordinates attributing 46% of the variance in data. The important variables in axis 1 were Cyanobacteria, Charophyta, Ochrophyta, TP and NO_3-N. The nutrients were found negatively correlated with Cyanobacteria, Charophyta and Ochrophyta (Annexure XI). This indicates that these algal phyla flourished when there was less nutrient enrichment in the pond. In axis 2 the important variables were Cryptophyta and pH which were negatively correlated with Mezozoa. The PCA of 2012-13 period had explained 45% of variance in data by the first two ordinates. Cyanobacteria and Ochrophyta in the axis 1 was negatively correlated with Euglenozoa. Axis 2 had pH and EC in the positive side of the axis and Chlorophyta in the negative side of the axis. This indicates that Chlorophytes in pond 2 favour low inorganic ions and increased pH. In 2013-14, the first axis of the canonical plot had Charophyta and Euglenozoa. pH and EC had no significant influence in these phyto-groups. Axis 2 had Cyanobacteria and Ochrophyta negatively correlated with pH. The discriminant analysis showed an overlapping diagram indicating no significant deviation over the years.

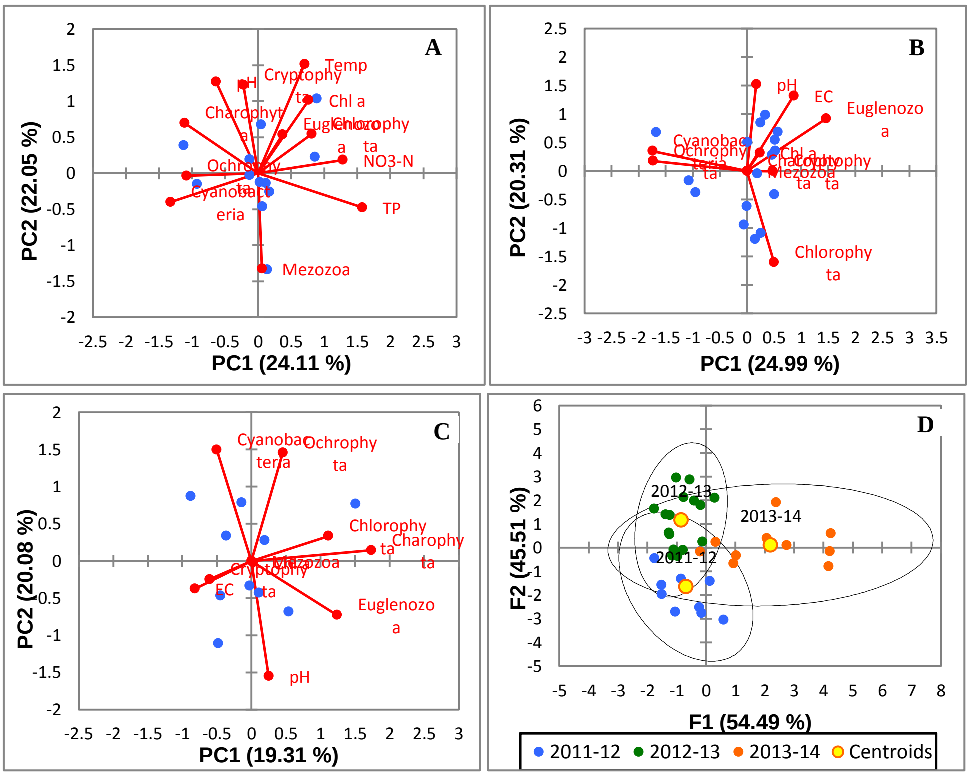

Figure 4.22: PCA biplot of the component loadings of pond 2 variables for the year A) 2011-12, B) 2012-13, C) 2013-14. D) The Discriminant Analysis (DA) of pond 1 variables among the year 2011-12, 2012-13 and 2013-14

4.4.7 Gross temporal change in pond 3 during 2011-2014

Pond 3 had clear water in November- December 2011. In January the floating weed *Lemna minor* invaded the pond and it started to spread over the water surface. A thin film of yellow-green coloured scum was visible in February and it slowly spread over (Figure 4.23). After the monsoon rain in 2012, *Lemna minor* had been washed away and the grass along the periphery began to invade the edge of the water. The same trend was followed in the pond during 2012-13 also. The pond was observed-

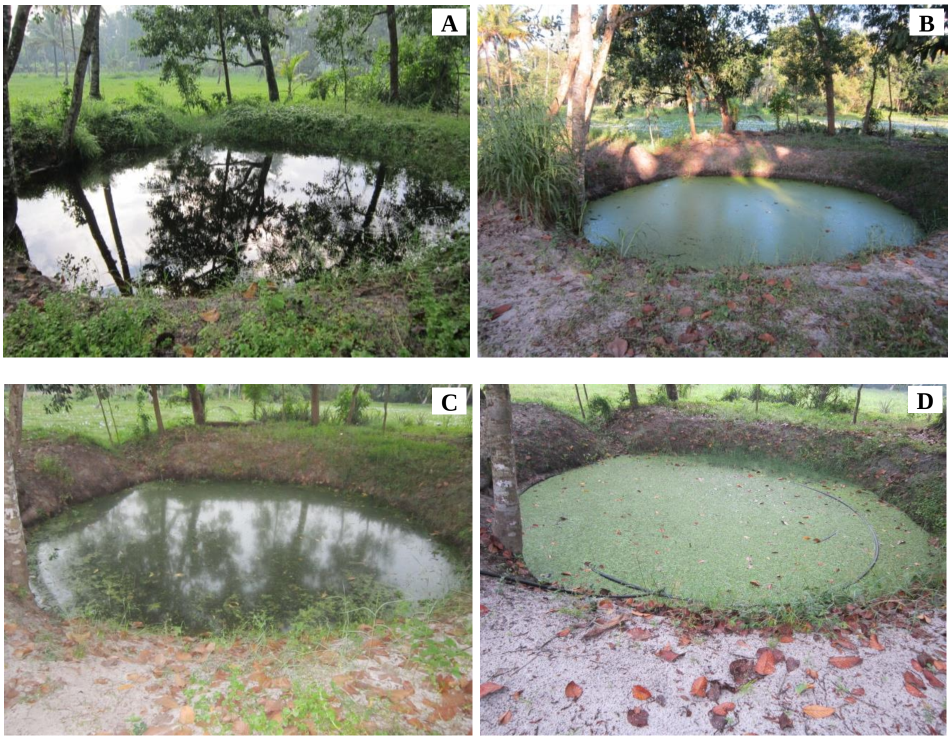

Figure 4.23: Gross temporal change in pond 3 A) Clear pond (November 2011) B) invaded by *Lemna minor* (January 2012) C) Thin green scum over the surface (February 2012) D) *Salvinia molesta* floating over the surface (March 2014).

with the development of *L. minor* immediately after the retreat of monsoon which spread over the entire pond by November 2012. The pond water became turbid in February with the leaf litter from the surrounding and yellow-green scum on the water surface during March 2013. The pond was very much reduced in volume but didn't dry in March and was cleaned in April 2013. During 2013-14, there was shift in hydrophytes from *L. minor* to *Salvinia molesta*. It eventually covered the entire pond. The pond didn't form any visible scum on the surface during 2013-14.

4.4.8 Phytoplankton succession in pond 3

4.4.8.1 Phytoplankton succession during 2011-12

The phytoplankton community in pond 3 was dominated by Euglenozoa, Chlorophyta and Cyanobacteria (Figure 4.24). Euglenozoa dominated the pond in first two sampling. A decrease in Euglenozoans and a corresponding increase in Chlorophyceans were observed in December second half. But the Chlorophyceans were completely replaced by Cyanobacteria in January followed by another turn over from Cyanobacteria to Chlorophyta in the next sampling itself. Euglenozoa came back to dominance by replacing Chlorophyta in February and maintained the dominance till the end of sampling in May. The relative frequency of Euglenozoa was highest during February second half with 98% occurrence. The contribution of Ochrophyta increased towards March.

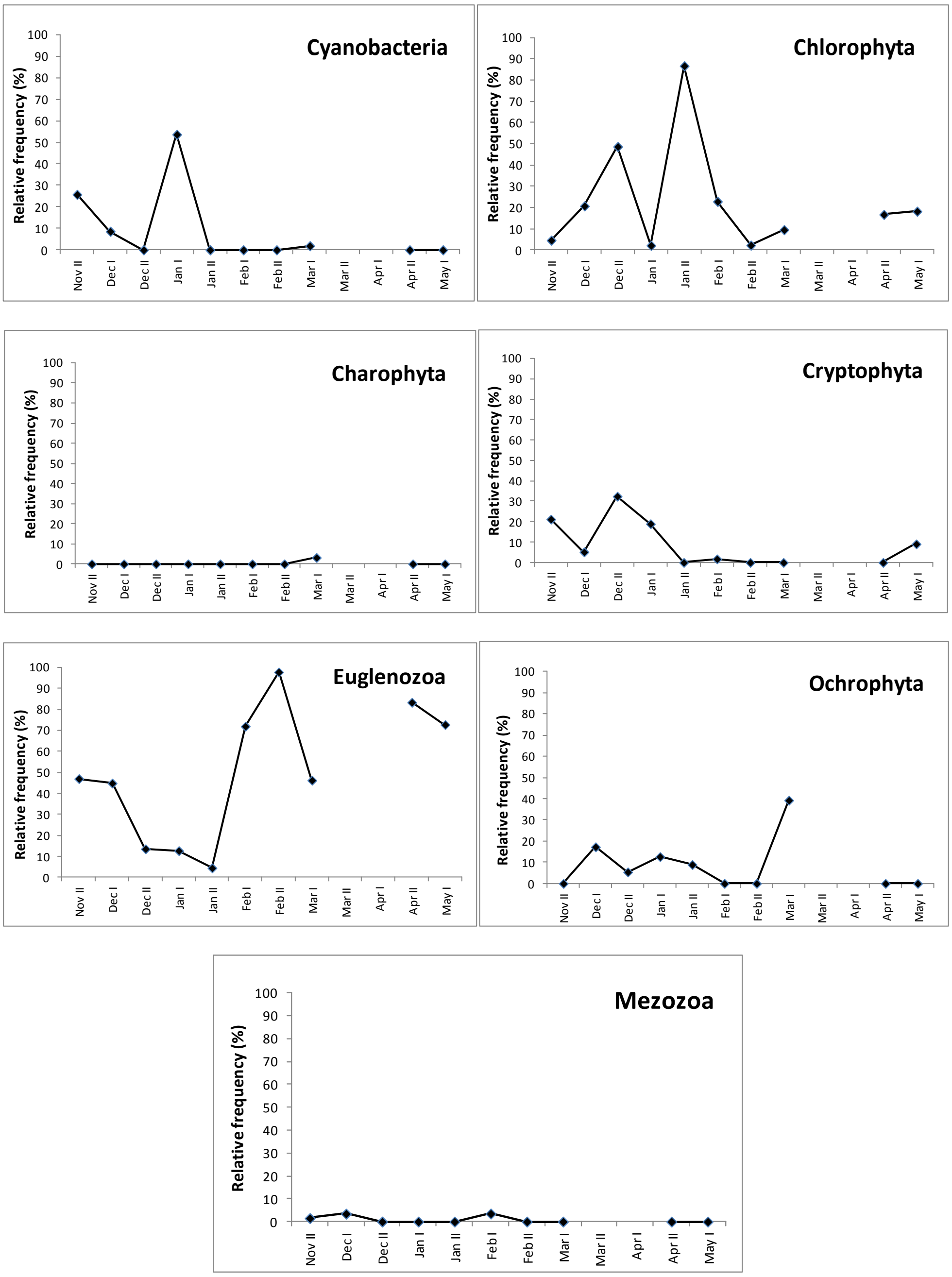

Figure 4.24: Relative frequencies of different algal phyla in pond 3 during 2011-12 (Gap in the graph corresponds to the dry period).

4.4.8.2 Phytoplankton succession during 2012-13

The dominating algal phyla found in pond 3 during 2012-13 were Euglenozoa and Ochrophyta (Figure 4.25). Euglenozoa dominated the

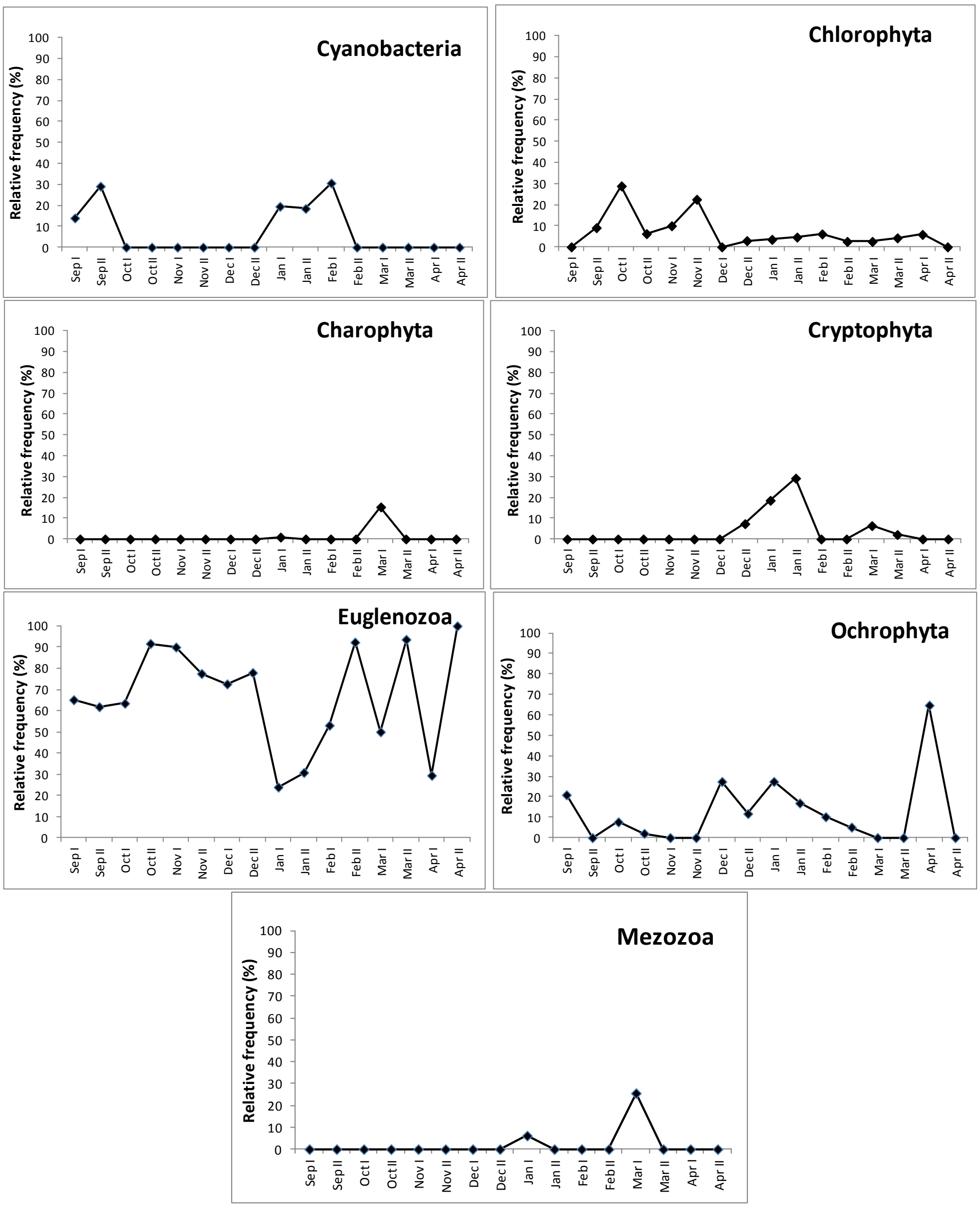

Figure 4.25: Relative frequencies of different algal phyla in pond 3 during 2012-2013

pond in most of the sampling months gradually reaching up to 100% frequency at the end of sampling in April. Ochrophyta dominated over Euglenozoa on a marginal 27.4% in January first half. Ochrophyta again came into dominance with 64.7% relative frequency of occurrence in April first half. Euglenozoa completely occupied the pond with 100% frequency replacing all other algal phyla by the end of April.

4.4.8.3 Phytoplankton succession during 2013-14

Chlorophyta, Ochrophyta and Euglenozoa dominated the pond during 2013 -2014. Chlorophyta dominated the pond from September—December (Figure 4.26). The relative frequency of Chlorophyta gradually increased from September (60.8%) to record its highest frequency of 87% in December. A shift from Chlorophyta to Ochrophyta occurred in January—February. Ochrophyta had a relative frequency of 71% in February. Euglenozoa replaced Ochrophyta in March and dominated the pond till the end of sampling in June. Cyanobacteria and Mezozoa were minor components in the pond.

4.4.8.4 Species composition of phytoplankton

Total number of 86 phytoplankton taxa recorded in pond 3 during 2011-14. Chlorophyta had a marginal dominance over Euglenozoa in terms of species diversity with 29 species and 20 species respectively. Most diverse algal group in the pond during 2011-12 was Euglenozoa with 16 species. During 2012-13 and 2013-14 Chlorophyta was the diverse algal phyla with 15 and 11 species respectively.

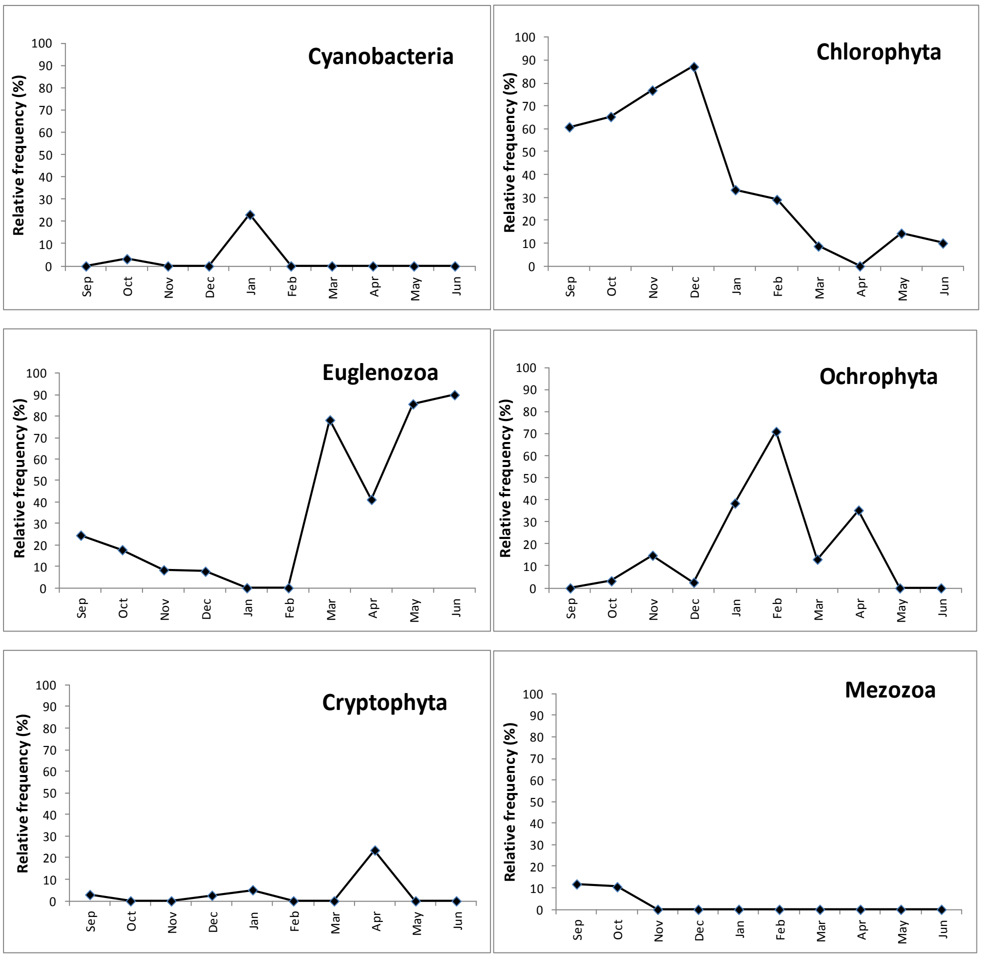

Figure 4.26: Relative frequencies of different algal phyla in pond 3 during 2013-14

The major phytoplankton species observed in pond 3 during 2011-12 were *Trachelomonas volvocina* (10.6%), *Lepocinclis globulus* (7.8%) and *Cryptomonas erosa* (7.6%). During 2012-13 the dominant species were *Lepocinclis globulus* (30.7%), *Trachelomonas volvocina* (11.1%) and *Nitzschia palea* (10.9%). *Lepocinclis globulus* (22.8%), *Chlorococcum infusionum* (21.5%) and *Chlorococcum echinozygotum* (7.1%) dominated the pond during 2013-14 (Annexure VIII).

4.4.8.5 Phytoplankton bloom

The concentration of chlorophyll *a* in pond 3 during 2011-12 showed phytoplankton blooms in almost all months except May 2012 (Table 4.5). During 2011-12, there was no bloom forming dominant species in pond 3 in November 2011. Chlorophycean bloom of *Chlorella vulgaris* occurred in December. It was replaced by *Aphanothece* sp1 and *Leptosira* sp. in the two halves of January 2012 respectively. The Chlorophyta—Cyanobacteria dominance in the pond shifted to Euglenozoa dominance during February—April 2012 with *Lepocinclis globulus* and *Lepocinclis fusiformis* as the bloom forming species. During 2012-13, phytoplankton blooms were caused by Euglenozoa alone. The bloom forming species were identified as *Trachelomonas volvocina,Lepocinclis fusiformis, Phacus curvicauda andLepocinclis globulus.*

Table 4.5: Monthly average of Chl *a* (μg/L) in pond 3 during 2011-14. Bold letters represent Chl *a* > 50 μg/L.

Months	2011-12	2012-13	2013-14
Sep	--	39.02	**51.94**
Oct	--	**56.68**	**61.59**
Nov	**207.6**	**67.02**	**188.5**
Dec	**111.7**	**68.85**	**50.57**
Jan	**200.9**	35.22	28.81
Feb	**77.46**	43.51	**54.35**
Mar	**245.5**	**54.66**	**89.67**
Apr	**89.53**	30.99	**95.73**
May	21.35	--	8.349
Jun	--	--	17.79

Table 4.6: Phytoplankton bloom species in pond 3 during 2011-14

Phytoplankton bloom species in pond 3 during 2011-12		
Months	**Phytoplankton phyla**	**Dominant species**
November 2011	Euglenozoa	-
December 2011	Euglenozoa	-
	Chlorophyta	*Chlorella vulgaris*
January 2012	Chlorophyta	*Leptosira* sp.
	Cyanobacteria	*Aphanothece* sp1
February 2012	Euglenozoa	*Lepocinclis globulus*
March 2012	Euglenozoa	-
April 2012	Euglenozoa	*Lepocinclis fusiformis*
Phytoplankton bloom species in pond 3 during 2012-13		
October 2012	Euglenozoa	*Trachelomonas volvocina, Lepocinclis fusiformis*
November 2012	Euglenozoa	*Lepocinclis globulus*
December 2012	Euglenozoa	*Lepocinclis globulus*
March 2013	Euglenozoa	*Phacus curvicauda, Lepocinclis globulus*
Phytoplankton bloom species in pond 3 during 2013-14		
September 2013	Chlorophyta	*Chlorococcum infusionum*
October 2013	Chlorophyta	*Chlorococcum echinozygotum*
November 2013	Chlorophyta	*Chlorococcum infusionum*
December 2013	Chlorophyta	*Chlorococcum infusionum*
February 2014	Ochrophyta	*Pinnularia interrupta*
March 2014	Euglenozoa	*Lepocinclis globulus*
April 2014	Euglenozoa	*Lepocinclis globulus*

Phytoplankton bloom in pond 3 during 2013-14 was caused by Chlorophyta from September to December 2013 with *Chlorococcum* spp. (*Chlorococcum infusionum* and *Chlorococcum echinozygotum).* An Ochrophycean species *Pinnularia interrupta* replaced the chlorococcales in February 2014. Ochrophyta dominance was short-lived and was replaced by Euglenozoa during March and April with *Lepocinclis globulus* as the bloom forming species (Table 4.6). The microphotographs of the species are given in Figure 4.27.

A visible yellow-green scum of Euglenozoa was observed in the pond during March 2011-12 and 2012-13. The peak concentrations of Chl *a* was recorded during this period ranging 89.5—245.5 µg/L. The bloom was an assemblage of two Euglenozoan species- *Lepocinclis globulus* and *Euglena proxima*. *Lepocinclis globulus* was the major species constituting >90% of the scum (Figure 4.28).

4.4.9 Phytoplankton dynamics in pond 3

Principal component analysis in pond 3 during 2011-12 was represented in the canonical plot in Figure 4.29. The first two ordinates of the biplot explained 48% of the variance. The first axis had Euglenozoa, Cyanobacteria, Ochrophyta, Chl *a* and pH. The second axis had variables Charophyta and Cryptophyta (Annexure XII). The nutrients didn't show any correlation with the phytoplankton growth and development in the pond. In 2012-13, Charophyta, Mezozoa and Cryptophyta was in axis 1. The second axis had Chlorophyta negatively correlated to EC and Ochrophyta. During 2013-14, the PCA explained 52% of the variation by the first two ordinates. Axis 1 had important variables- Chlorophyta, Mezozoa, Euglenozoa and pH. This indicates a resultant rise in pH of the pond water with the flourishing of above mentioned phytoplankton groups.

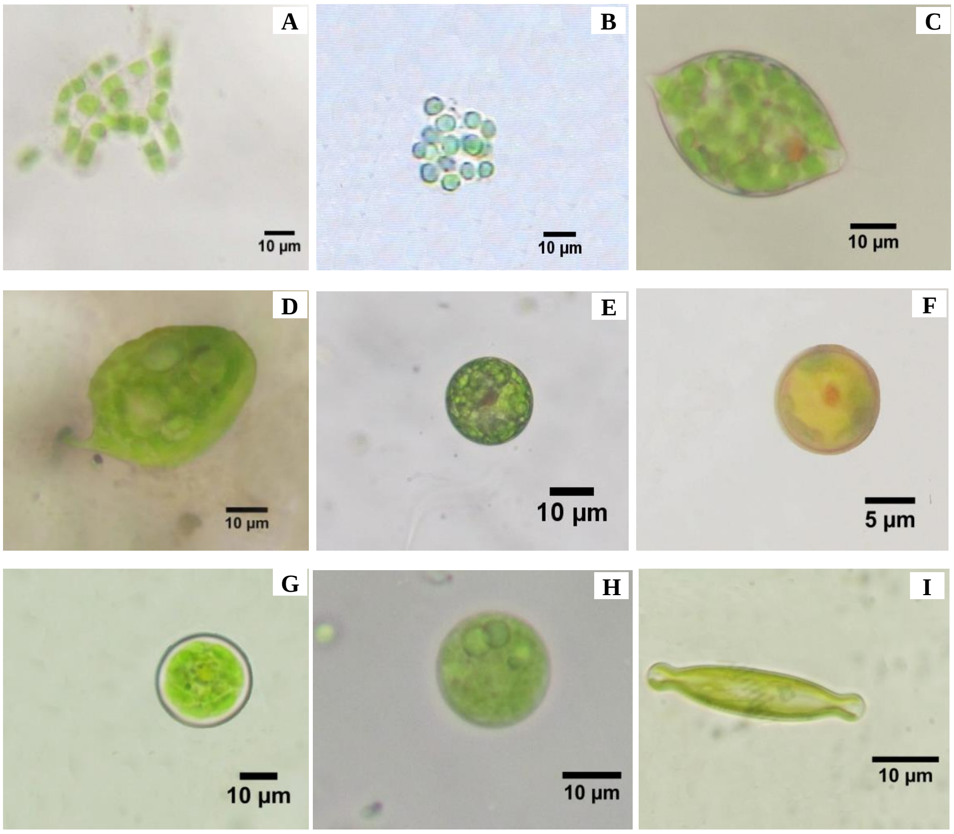

Figure 4.27: Microphotographs of phytoplankton bloom species of pond 3 during 2011-2014. A) *Leptosira* sp. B) *Aphanothece* sp1 C) *Lepocinclis fusiformis* D) *Phacus curvicauda* E) *Lepocinclis globulus* F) *Trachelmonas volvocina* G) *Chlorococcum infusionum* H) *Chlorococcum echinozygotum* I) *Pinnularia interrupta.*

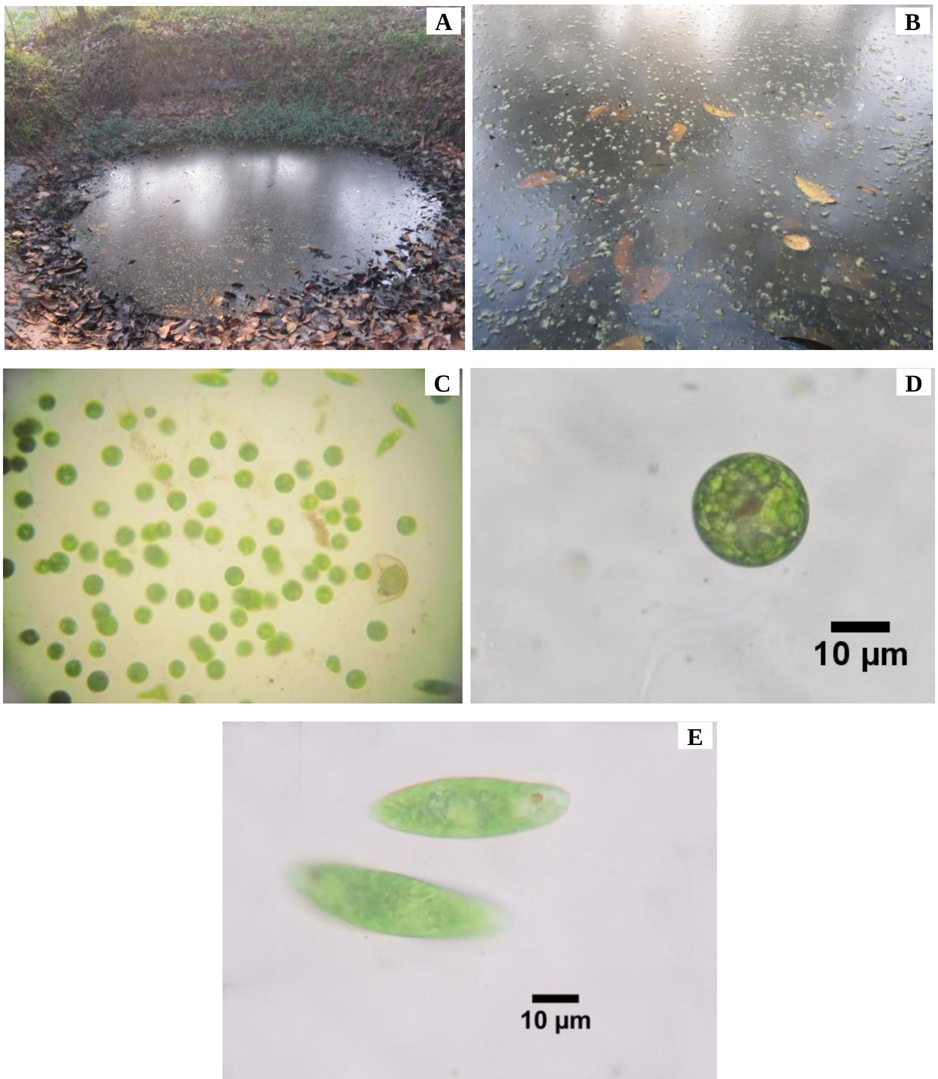

Figure 4.28: **A) and B) Yellow-green scum of *Lepocinclis globulus* and *Euglena proxima* in pond 3 during 2011-12 and 2012-13. Microscopic images of C) Euglenozoa bloom D) *Lepocinclis globulus* E) *Euglena proxima*.**

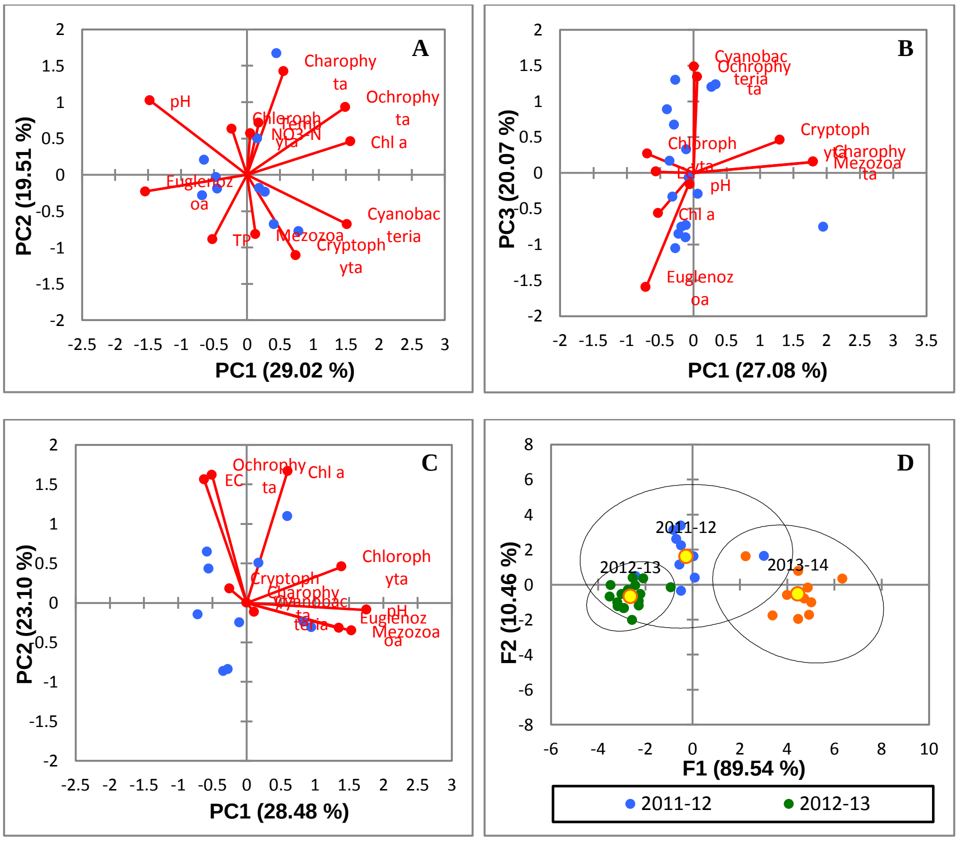

Figure 4.29: PCA biplot of the component loadings of pond 3 variables for the year A) 2011-12, B) 2012-13, C) 2013-14. D) The Discriminant Analysis (DA) of pond 1 variables among the year 2011-12, 2012-13 and 2013-14.

Axis 2 had significant variables consisting Ochrophyta, Chl *a* and EC. This shows a positive correlation of Ochrophyta with the inorganic input in the water. The discriminant analysis (DA) results show that the general trend of the phytoplankton dynamics of the pond during 2013-14 varied significantly from that of 2012-13.

4.4.10 Gross temporal change in pond 4 during 2011-2014

The water in pond 4 was clear in November to December in 2011. It was dredged and the sediments were removed in February 2012. Algal turbidity occurred in March-April. After the monsoon rains the pond was full with water, and as the observation continued the depth of the pond and its water level diminished by November- December 2012 (Figure 4.30). The regular dredging and cleaning of the pond was not done in

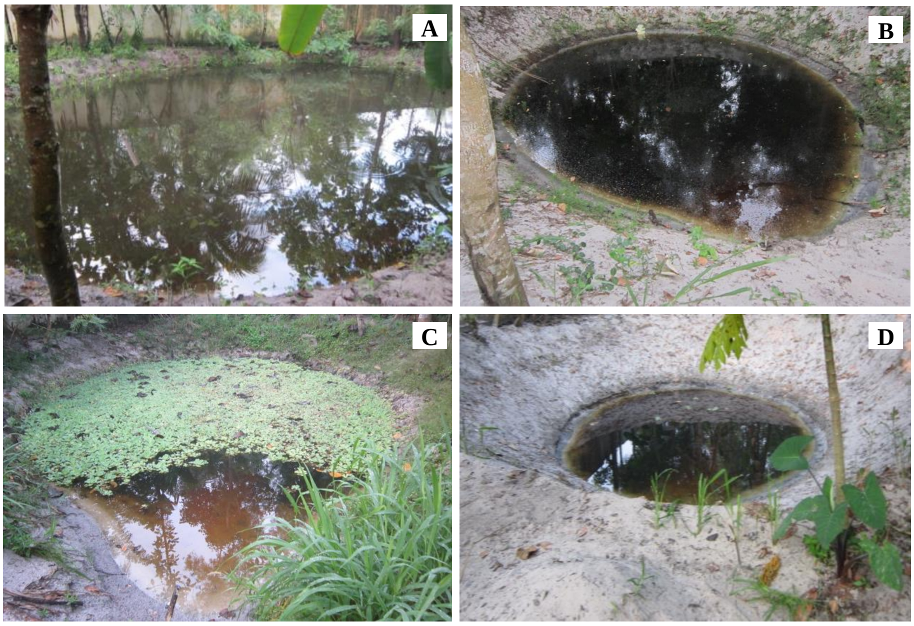

Figure 4.30: Gross temporal change in pond 4 A) Clear water (November 2011) B) water volume decreased and the water got turbid (November 2012) C) *Pistia stratiotes* invaded (March 2013) D) immediately after the pond was dredged and cleaned (February 2014).

February 2013. The littoral zone of the pond developed blue-green epipelic algal mat and periphyton during March which detached from the plants and rose from the bottom to the water surface forming freely floating metaphyton in April 2013. *Pistia stratiotes* also invaded the pond during this period. 2013-14 observations continued after the monsoon rain in September 2013. *Pistia stratiotes* invaded the pond during November and it spread over half of the pond surface by December. The pond was cleaned during February 2014. The pond didn't develop metaphyton as in the previous year, although phytoplankton turbidity was observed.

4.4.11 Phytoplankton succession in pond 4

4.4.11.1 Phytoplankton succession during 2011-12

The algal phyla of common occurrence in pond 4 during 2011-12 were Chlorophyta, Cryptophyta, Cyanobacteria and Euglenozoa (Figure 4.31). Chlorophyta dominated the pond in the starting of sampling in November with a relative frequency of 59%. Then the dominance of algal community shifted from one group to another between Chlorophyta and Cryptophyta. Cyanobacteria became prominent in January first half. In the second half of February Euglenozoans multiplied exponentially to completely occupy and solely represent the phytoplankton community in pond 4. It then gradually got reduced to 37.5% in April first half leading to the dominance of Chlorophyta (50%). But in the next two samplings Euglenozoa came back to dominance with 78% and 45.5% respectively.

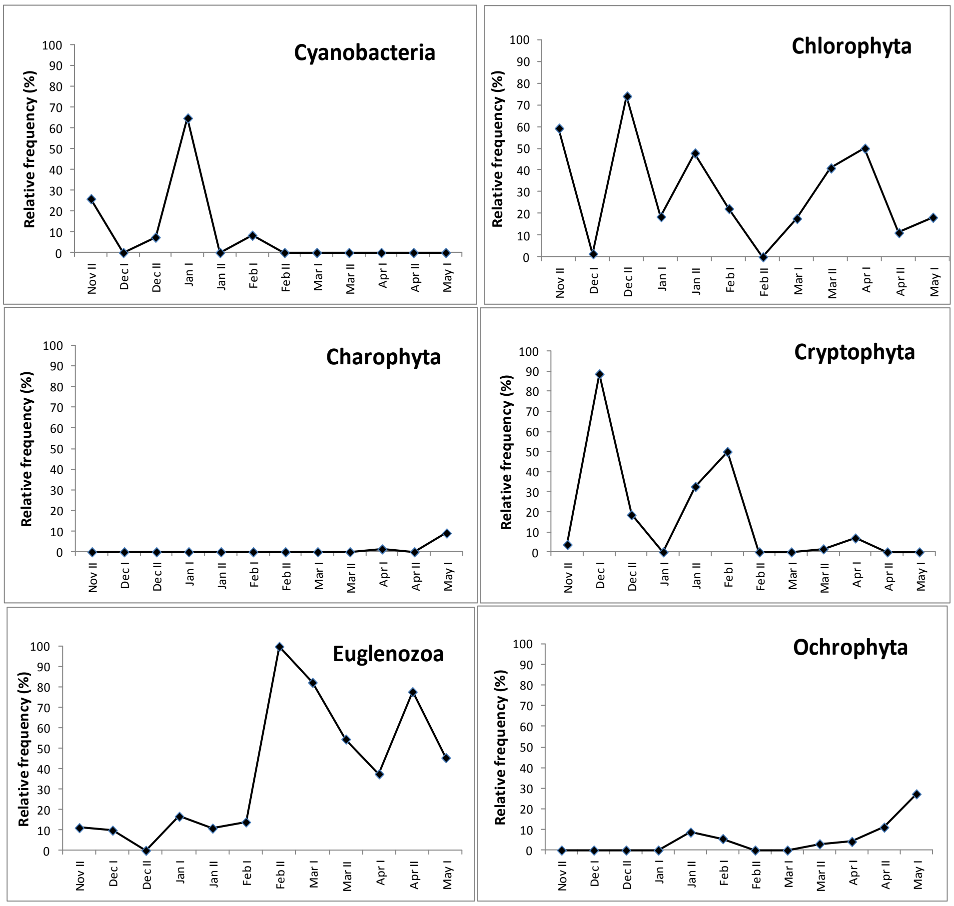

Figure 4.31: Relative frequencies of different algal phyla in pond 4 during 2011-2012

4.4.11.2 Phytoplankton succession during 2012-13

Pond 4 during 2012-13 period was dominated by Euglenozoa, Cyanobacteria, Chlorophyta, Cryptophyta and Ochrophyta (Figure 4.32). Euglenozoa (53%) dominated the pond in September first half. Cyanobacteria (90.5%) replaced Euglenozoa in the latter half of September. The dominance shifted from Cyanobacteria to Chlorophyta in October.

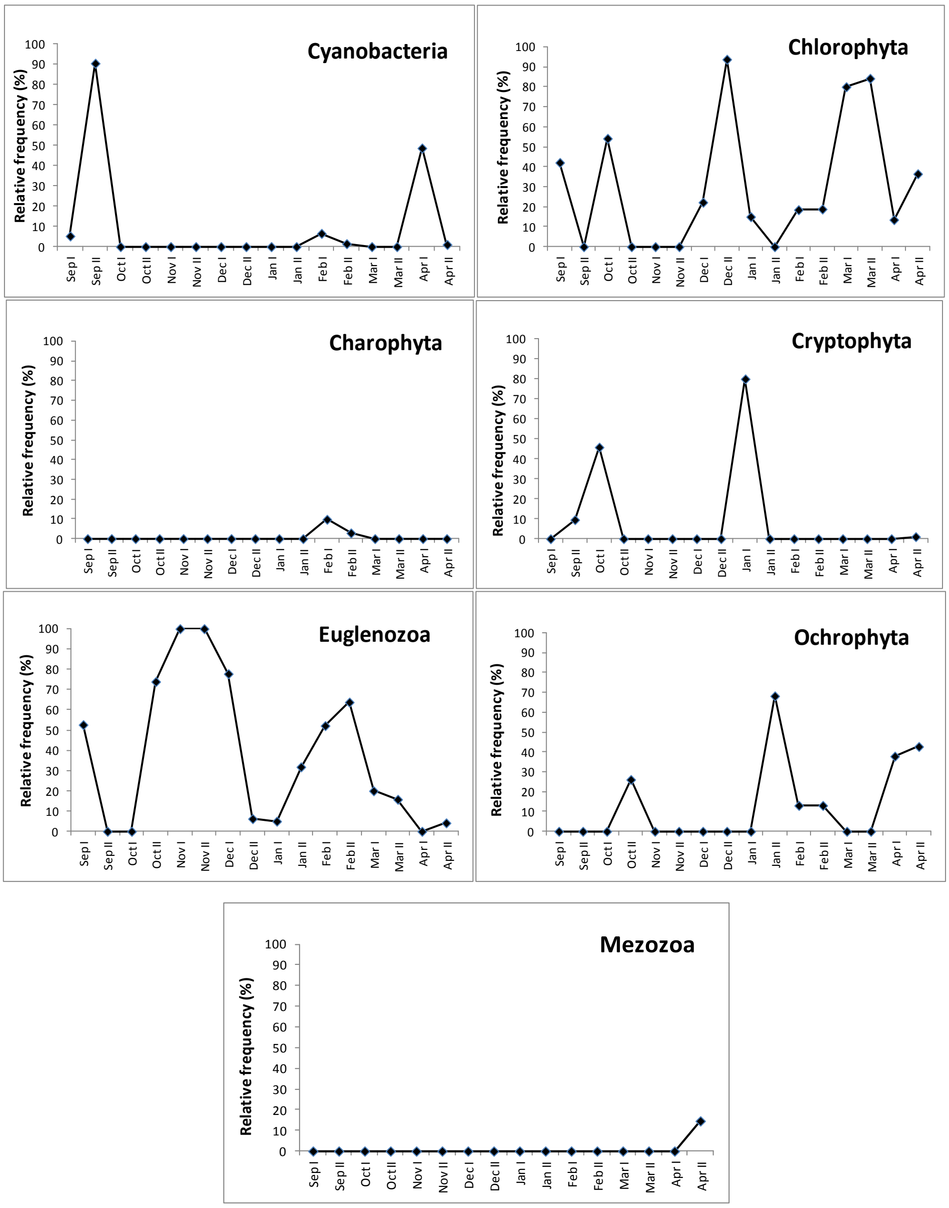

Figure 4.32: Relative frequencies of different algal phyla in pond 4 during 2012-2013

Euglenozoa dominated the pond from October second half to December first half. From December second half onwards the dominance shifted between different algal groups (Chlorophyta, Cryptophyta, Ochrophyta, Euglenozoa and Cyanobacteria). First half of January observed a major shift with the decline of both Chlorophyta and Euglenozoa and Cryptophyta got into the dominance (80%). Euglenozoa and Chlorophyta came back to dominance in February and March respectively. Cyanobacteria dominated the pond water at the end of April with 49% occurrence.

4.4.11.3 Phytoplankton succession during 2013-14

Ochrophyta, Euglenozoa, Cyanobacteria and Chlorophyta were the dominant algal phyla observed in pond 4 in 2013-14 period (Figure 4.33).

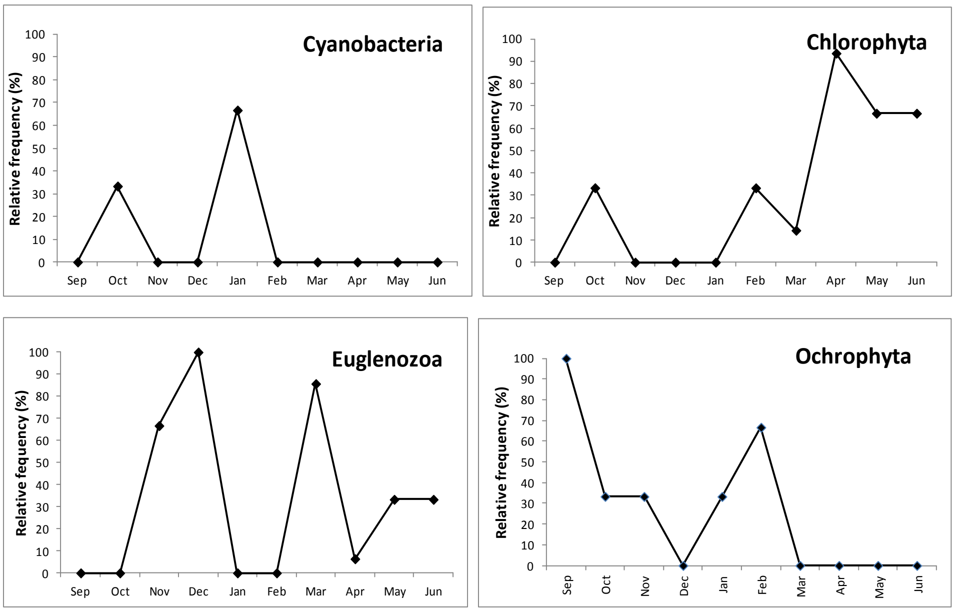

Figure 4.33: Relative frequencies of different algal phyla in pond 4 during 2013-2014

Ochrophyta was the only algal group found in the pond in September (100%). Chlorophyta, Cyanobacteria and Ochrophyta had equal percentage occurrence (33.3%) in October. Euglenozoa slowly increased to dominate over other algal groups in November—December. This was followed by frequent shifts between Cyanobacteria, Ochrophyta, Euglenozoa and Chlorophyta. Chlorophyta maintained its dominance over other phytoplankton groups from April—June.

4.4.11.4 Species composition of phytoplankton

A total of 82 algal species were recorded in pond 4 during 2011-14. Chlorophyta constituted the most diverse algal phyla with 35 species followed by Euglenozoa with 15 species. The lowest species diversity in the pond was recorded during 2013-14. The total phytoplankton species recorded during 2011-12 was 46, during 2012-13 it was 50 and during 2013-14 it was only 13 species all together.

The dominant species in the pond varied annually from 2011 to 2014. During 2011-12 the dominant species observed were *Cryptomonas* sp2 (10.2%), *Gyrodinium* sp. (7.3%) and *Aphanocapsa hyalina* (6.1%). During 2012-13, *Phacus triquieter* (12%), *Trachelomonas volvocina* (11.7%) and *Ankistrodesmus convolutus* (9.8%) were the major taxa observed (Annexure IX). *Trachelomonas volvocina* maintained its dominance in 2013-14 also. But the other two species were replaced by *Chromulina nebulosa* (10%) and *Melosira granulata* (10%).

4.4.11.5 Phytoplankton bloom

The chlorophyll *a* in pond 3 indicates phytoplankton bloom in March and April during 2011-12. During 2012-13, chlorophyll *a* in the pond crossed the bloom forming limit in February 2013 only. The phytoplankton bloom in the pond recurred in February 2014 also with March and May also experiencing bloom during 2013-14 (Table 4.7).

Table 4.7: Monthly average of Chl *a* (µg/L) in pond 4 during 2011-14

Months	2011-12	2012-13	2013-14
Sep	--	15.69	1.110
Oct	--	14.89	13.46
Nov	13.39	24.76	14.45
Dec	12.46	15.13	37.62
Jan	17.79	8.465	7.060
Feb	20.68	**96.30**	**51.32**
Mar	**115.2**	42.69	**107.3**
Apr	**62.93**	40.02	10.50
May	11.24	--	**61.44**
Jun	--	--	34.59

Phytoplankton bloom in the pond during 2011-12 was caused by Euglenozoa and Chlorophyta with *Trachelomonas volvocina* and *Monoraphidium tortile* (Table 4.8). The Euglenozoa bloom in 2012-13 constituted *Phacus triquiter* and *Trachelomonas volvocina.* During 2013-14, the phytoplankton bloom in the pond started with Ochrophyta species-*Chromulina nebulosa* in February 2014. *Trachelomonas volvocina* replaced the Ochrophyte in March. Two chlorophycean species of *Monoraphidium griffithi and Chlorococcum* sp. dominated the pond forming bloom in May 2014 (Figure 4.34).

The pond developed blue-green metaphyton mat in the month of April during 2012-13 (Figure 4.35). The mat comprised of unialgal mat of *Oscillatoria princeps*.

Table 4.8: Phytoplankton bloom species in pond 4 during 2011-14

	2011-12	
Months	**Phytoplankton phyla**	**Dominant species**
March 2012	Euglenozoa Chlorophyta	*Trachelomonas volvocina* -
April 2012	Euglenozoa Chlorophyta	*Monoraphidium tortile* *Trachelomonas vovocina*
	2012-13	
February 2013	Euglenozoa	*Phacus triquiter, Trachelomonas volvocina*
	2013-14	
February 2014	Ochrophyta	*Chromulina nebulosa*
March 2014	Chlorophyta	*Trachelomonas volvocina*
May 2014	Chlorophyta	*Monoraphidium griffithi*, *Chlorococcum* sp.

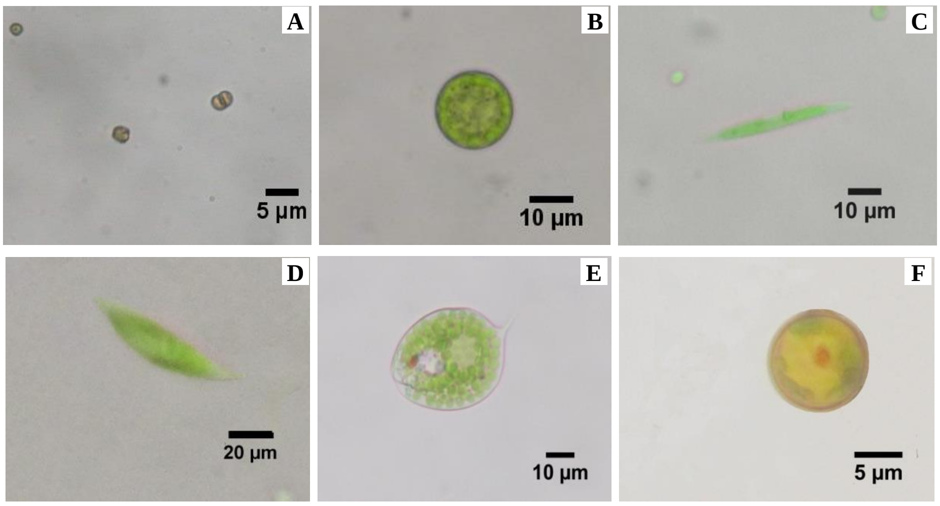

Figure 4.34: Phytoplankton bloom species of pond 4 during 2011-2014.
A) *Chromulina nebulosa* B) *Chlorococcum* sp.
C) *Monoraphidium griffithi* D) *Monoraphidium tortile*
E) *Phacus triquiter* F) *Trachelomonas volvocina*

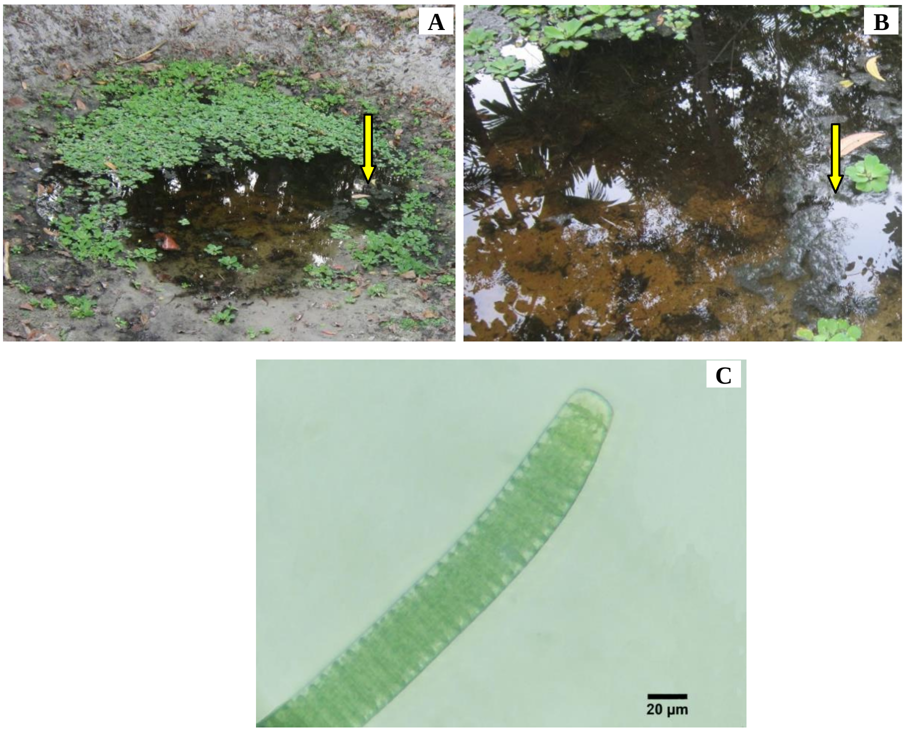

Figure 4.35: A) and B) The metaphyton mat of *Oscillatoria princeps* in pond 4. C) Microphotograph of *Oscillatoria princeps*.

4.4.12 Phytoplankton dynamics in pond 4

The first two ordinates of PCA in pond 4 during 2011-12 attributed 53% of the variance in data. The first axis had Euglenozoa, Cyanobacteria, Chlorophyta, Cryptophyta, Chl *a*, pH and temperature (Figure 4.36). Cyanobacteria, Chlorophyta and Cryptophyta was in the negative side of the axis indicating a decrease in blue- greens and flagellates with an increase in temperature and pH. The second axis had Charophyta and Ochrophyta significantly and inversely related to TP and Chl *a* (Annexure XIII). This indicates that the growth of Charophytes and Ochrophytes are favourable under low TP level. In 2012-13, the first axis of the biplot had

Mezozoa, pH and EC. Cryptophyta and Euglenozoa were negatively correlated to each other in axis 2. During 2013-14 period, the first two-

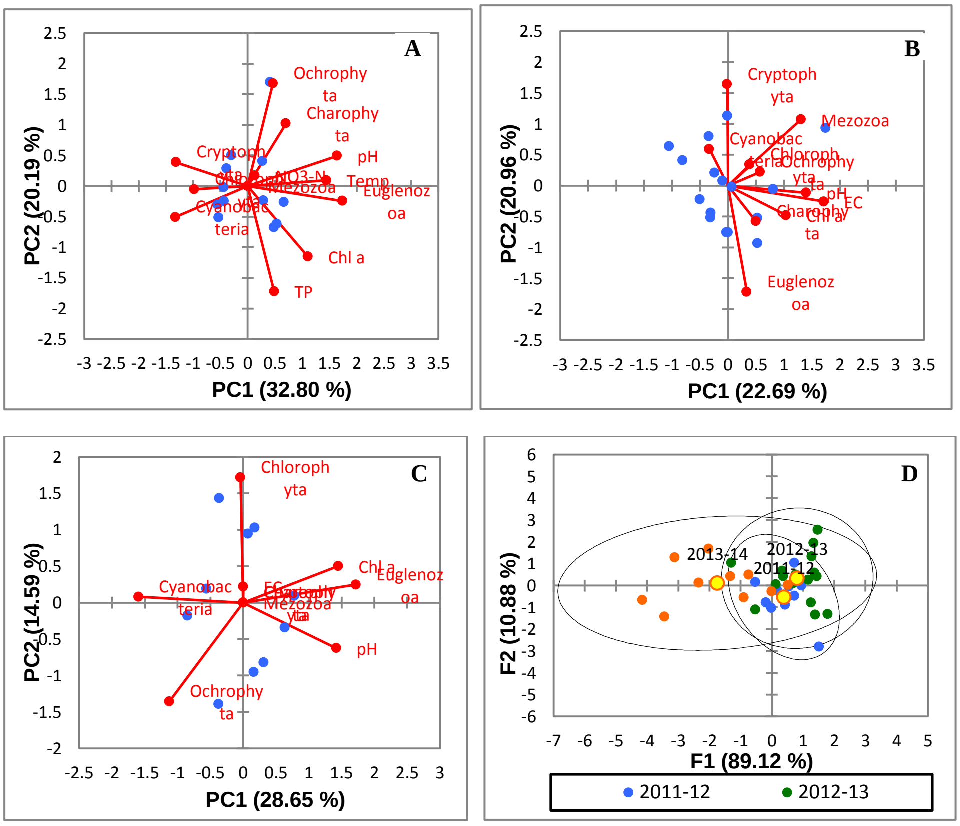

Figure 4.36: PCA biplot of the component loadings of pond 4 variables for the year A) 2011-12, B) 2012-13, C) 2013-14. D) The Discriminant Analysis (DA) of pond 1 variables among the year 2011-12, 2012-13 and 2013-14.

ordinates of PCA explained 43% of the variance. Axis 1 had Euglenozoa, pH and Chl *a* which were negatively correlated to Cyanobacteria and Ochrophyta. In axis 2 Chlorophyta was negatively correlated to Ochrophyta. The discriminant analysis didn't show significant variation between the years.

4.4.13 Similarity between ponds

The cluster analysis result shows that Pond 3 and Pond 4 are the most similar ponds in terms of the phytoplankton composition (Figure 4.37). Pond 1 was found with least similarity with other three ponds. The phytoplankton composition in pond 1 and pond 2 constituted 51-53% Chlorophyta followed by 13-14 % Cyanobacteria. Pond 3 and Pond 4 on the other hand had 33-42% Chlorophyta followed by Euglenozoa with 18-23% occurrence.

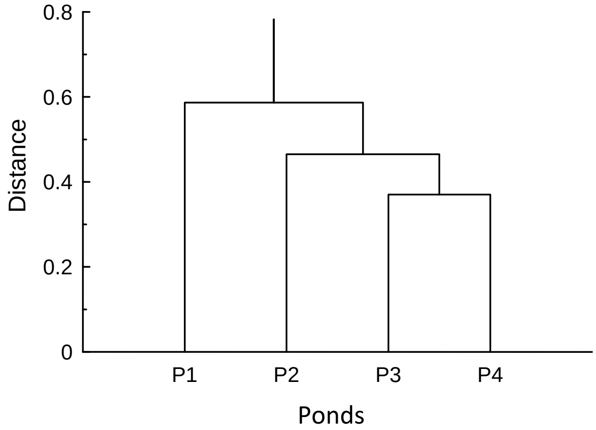

Figure 4.37: Dendrogram showing the similarity between ponds in phytoplankton composition

4. 4.14 Spectral discrimination of phytoplankton

POND 1

The fluorescence spectrum of nano-fractionated sample recorded the dominance of chlorophyll *a* and *b* in January 2014 indicating the presence of Chlorophyta, Charophyta and Euglenozoa (Figure 4.38). In February, the peaks exhibited the presence of Chl *c*, Chl *b*, carotenoids and phycocyanin indicative of an assemblage of Chlorophyta, Cyanobacteria and Ochrophyta. There was clear dominance of Cyanobacteria and

Ochrophyta from March to June. The fluorescence peaks in the microplankton fraction were that of Chl *a* and *b* from January to April and shifted towards Cyanobacteria in May and shifted back to Chlorophyta and Euglenozoa in June.

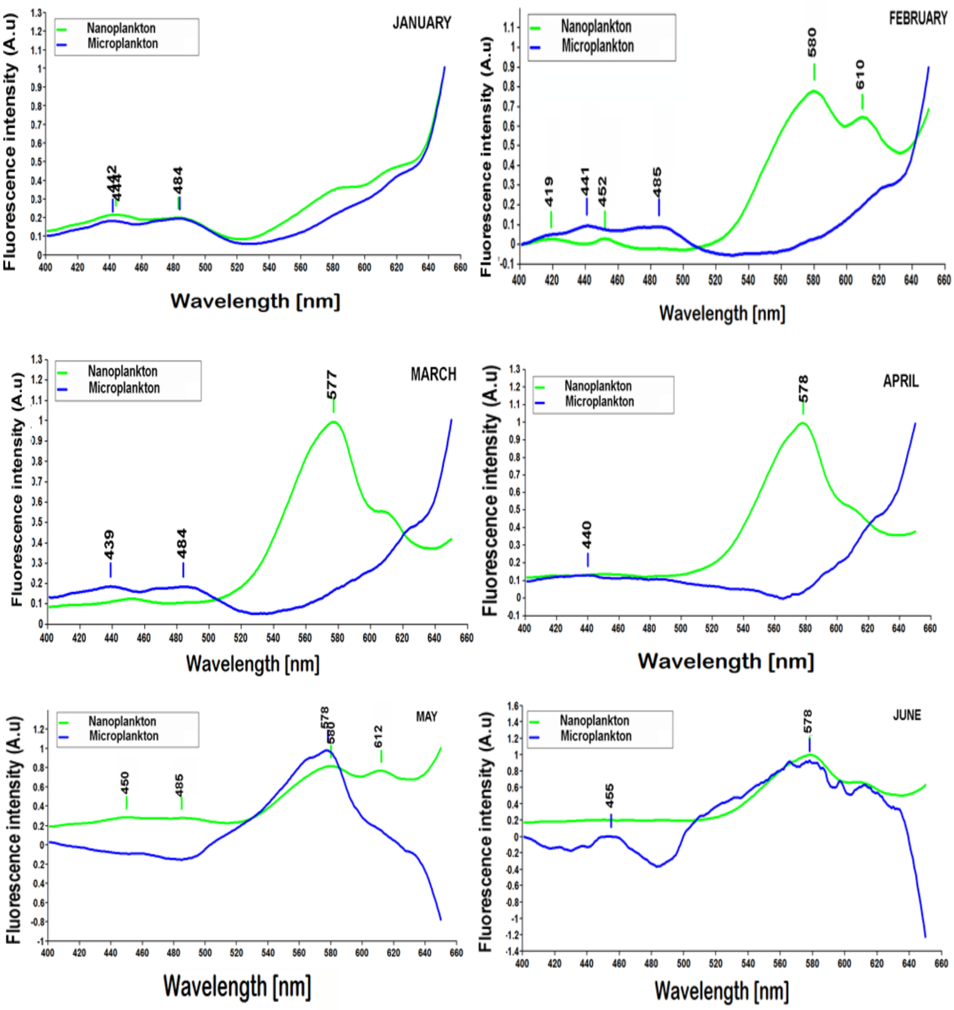

Figure 4.38: Fluorescence excitation spectra of pond 1 samples (nanoplankton and microplankton fractions) during January—June 2014

A comparision of the fluorometric analysis with the microscope observation showed that the Cyanobacteria are better detected by fluorimetry especially in the nanoplankton fraction. However, the fluorescence technique could not differentiate among the Chl *b* containing phyla and similarly among the algal groups with different Chl *c* and carotenoid pigments. Hence the measurement needs further refinement.

POND 2

The nanoplankton in pond 2 samples exhibited peaks in the Phycocyanin and β-carotene region indicating the presence of Cyanobacteria (Figure 4.39). The microplankton fraction had peaks at Chl *a* and Chl *c* region in January indicating the assemblage of Chlorophyta, Euglenozoa and Ochrophyta. The peaks shifted from Chl *c* to Phycoerythrin and Phycocyanin region in February—March indicating a shift from Ochrophyta to Cyanobacteria. The peaks shifted towards the Chl *b* region in April—June representing the Chl *b* bearing groups of algae-Chlorophyta, Charophyta and Euglenozoa dominance.

Certain xanthophyll carotenoids seen in golden-yellow algae included in Ochrophyta exhibit peaks in the 450 nm region (Alberte and Andersen, 1986; Brown, 1987). These carotenoid bearing nanoplankton were not detected in the microscope observations.

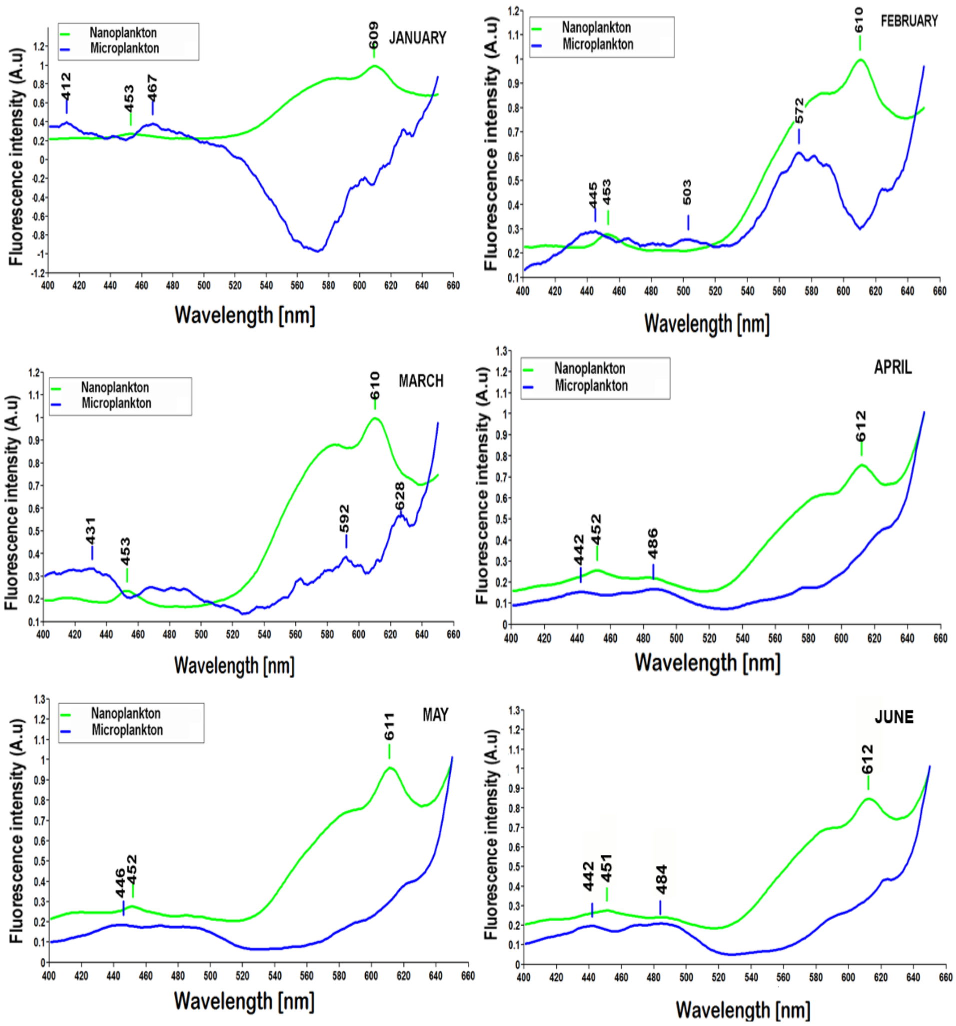

Figure 4.39: Fluorescence excitation spectra of pond 2 samples (nanoplankton and microplankton fractions) during January—June 2014.

POND 3

The nanoplankton fractions of pond 3 samples clearly exhibited peaks in the carotene and Phycocyanin region from January—May (Figure 4.40). This attributes to the dominance of Ochrophyta and Cyanobacteria. In June the peak was observed only at the phycocyanin region indicating the absence of Ochrophyta. The microplankton fraction had peaks at Chl *b*, Chl *c* and carotenoid region during January—April. In May there were peaks at Chl *a*, Chl *b*, fucoxanthin and Phycocyanin region indicative of the assemblage of Chlorophyta, Cyanobacteria, Ochrophyta and Mezozoa. The Chl *a*, Chl *b* and fucoxanthin peaks disappeared in June and it only represented Ochrophyta and Cyanobacteria.

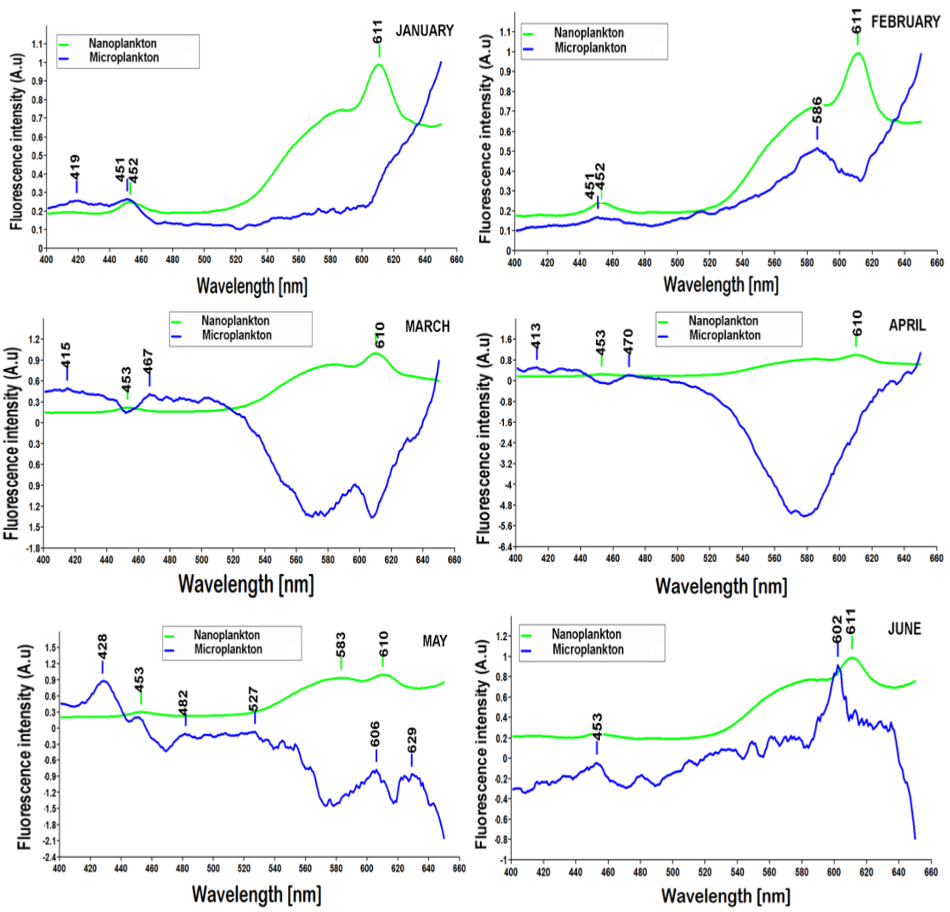

Figure 4.40: Fluorescence excitation spectra of pond 3 samples (nanoplankton and microplankton fractions) during January—June 2014.

The Cyanobacteria was better detected in the fluorescence spectrum when compared to the microscope observations. The fluorescence technique on the other hand could not differentiate Cryptophyta from rest of the algae.

POND 4

The nanoplankton fraction in pond 4 recorded the dominance of carotenoid, phycocyanin and phycoerythrin peaks in January, March and April attributing to the presence of Ochrophyta and Cyanobacteria. The peaks in Chl *a* and Chl *b* region in February, May and June indicated the dominance of Chlorophyta (Figure 4.41).

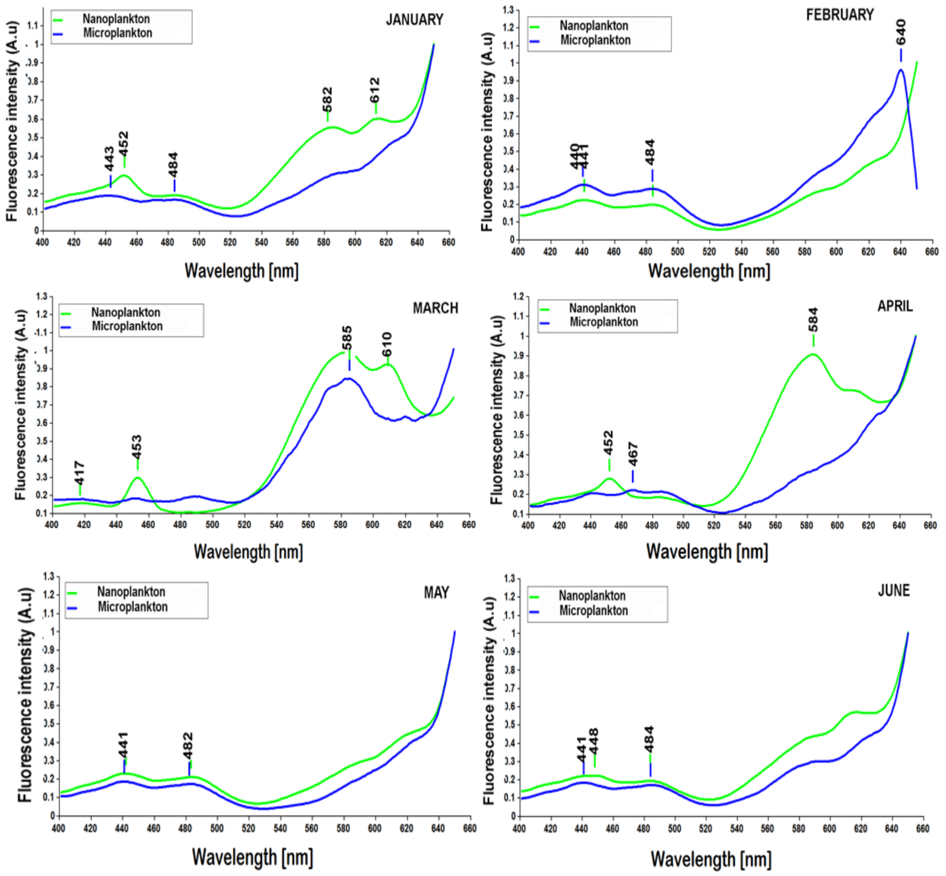

Figure 4.41: Fluorescence excitation spectra of pond 4 samples (nanoplankton and microplankton fractions) during January—June 2014

The Chl *a* and Chl *b* peaks solely represented the microplankton fraction of the spectra in January, May and June indicating the clear dominance of Chlorophyta. In February, phycocyanin peak was also observed in addition to the Chl *a* and Chl *b* peaks in February attributing to the presence of Chlorophyta- Cyanobacteria assemblage. The phycoerythrin peak in March indicates either Cyanobacteria or Cryptophyta. It shifted to Chl *b* region in April.

The comparison of fluorescence results with that of microscope observations indicate that the former is better method for locating Cyanobacteria.

4.5 Discussion and Conclusion

All the four ponds selected for the study exhibited hypereutrophic state. Hence availability of nutrients is not a limiting factor for the selective dominance of phytoplankton species. The ponds were dominated by Chlorophyta, Euglenozoa, Cyanobacteria, Cryptophyta and Ochrophyta. Chlorophyta accounted for the highest percentage occurrence and species diversity in the ponds. Crossetti *et al.* (2008) also reported high Chlorophycean richness in a shallow tropical hypereutrophic reservoir. Arulmurugan *et al.* (2010) reported high diversity of Chlorophyceans in the temple tanks of Palakkad and Thrissur districts of Kerala. A study by Ajayan *et al.* (2013) also reported highest Chlorophycean percentage in Ananthapura temple Lake in Kerala. Paul and Anu (2016) reported the dominance of Chlorophyceae (49%) in Guruvayur temple pond, Kerala. Euglenozoa was the second important algal phyla observed in the ponds. The ecological studies on Euglenoids by Munawar (1972) found inorganic nitrogen as important determinant in their presence and fluctuation. Even though Euglenozoa flourishes in organically polluted environment, he reported active multiplication of Euglenoids in an unpolluted pond at the end of monsoon and in summer. Cyanobacteria was the third important phyla in the ponds. The conditions that favour the dominance of blue-green algae include high temperature, slightly alkaline water, high nutrient concentration and turbidity due to sediment suspension (George *et al.,* 2012).

Phytoplankton dynamics in pond 1 showed the dominance of Chlorophyta accompanied by Ochrophyta. Ochrophyta flourishes in varying environment conditions with turbulence (Havens and DeCosta, 1986). A study by Bhanja *et al*. (2014) reported dominance of diatoms in oligotrophic water bodies. Diatoms are usually associated with low temperature, high DO and low organic matter (Prasad and Singh, 1996). However the dominating Ochrophycean species found in pond 1 was *Nitzschia palea* which is an indicator of eutrophic state and organic pollution in the water body (Jindal *et al.,* 2014). The Euglenozoa dynamics in pond 1 was found influenced by nutrient enrichment. Euglenoids proliferate in water with high ammonia, nitrate and phosphorus concentrations (Kim and Boo, 2001). *Trachelomonas volvocina* was the dominating species among Euglenozoans, it usually flourishes in organically rich stagnant water. It is reported to have positive correlation with nitrate and ammonium concentrations (Santana *et al.,* 2016). Chlorococcales were the major phytoplankton group in pond 1. Chlorococcales usually dominate in nitrogen rich water (Zebek, 2009). *Scenedesmus* was the most diverse genus among them. Paul and

Anu (2016) also reported a similar result in Guruvayur temple pond, Kerala. Pond 1 developed periphyton bloom of *Spirogyra* type 1 during 2011-2012. Eutrophication in water bodies are expected to be accompanied by Cyanobacterial bloom. A switch over from blue-green to green algae occurs at very high nutrient concentrations (Jensen *et al.*, 1994). Filamentous mat of *Spirogyra* sps. frequently occur in small eutrophic ponds. (Bellinger and Sigee, 2010). During 2012-2013 there was no periphyton bloom. A green scum of *Pediastrum duplex* and *Chlorococcum minutum* developed bloom during this period. De Senerpont Domis *et al.* (2013) opined that increased precipitation can reset the seasonal dynamics of plankton communities and support the development of species adapted to highly variable environments. It is reported that at enriched nutrient conditions in shallow lakes and ponds with unstable condition, the fast growing small sized Chlorococcales dominates (Solis *et al.*, 2016).

Even though recurrent blue-green metaphyton bloom occurred in pond 2, the subsurface phytoplankton in the pond was dominated mainly by the Chlorococcales and Euglenozoans. Messyasz (2006) reported that Chlorococcales may accompany dense blue-green blooms. Highly diverse genera of Chlorococcales in water bodies attribute to an unstable environment. The subsurface Cyanobacteria in the pond was found negatively related to the nutrient input. The overall trend of phytoplankton dynamics in pond 2 showed the dominance of Cyanobacteria, Ochrophyta and Charophyta during low nutrient conditions. In hypereutrophic systems, Cyanobacteria dominate at low nitrogen concentrations (Pastich *et al.*, 2016). According to Sinang *et al.* (2015) the dominance of blue-green algae in the water bodies are favoured by lower nutrient concentrations since it is negatively correlated to TP and iron. They found that the relationships between the environmental factors and Cyanobacterial bloom were different for different lakes studied. The pond developed blue-green metaphyton of *Oscillatoria limosa* all through the study period. High accumulation of organic matter in small ponds may result in the depletion of oxygen. In this anoxic condition, the sulphate reducers oxidize organic matter producing H_2S. Hence sulfide resistant Cyanobacteria may dominate the pond. *Oscillatoria limosa* and *Komvophoron schimidlei* observed in the pond are typical examples of this group (Likens, 2010; Stal, 1995). According to Cao *et al.* (2016) high N: P ratio might not be the factors that lead to the blooming of *Oscillatoria* and *Microcystis*. *Oscillatoria* is reported to favour an environment with high organic content and low oxygen (Singh, 2015). The significant correlation of Chl *a* in pond 2 with that of temperature indicate temperature as a controlling factor in the Cyanobacterial bloom. Blue-green algae multiply profusely at a temperature range of 26-29°C (Mohan and Reddy, 1986). They are reported to prefer water with high temperature for bloom formation (Wang *et al.*, 2015)

In pond 3, Euglenozoa dominated when Cyanobacteria and Ochrophyta subsided. Ochrophyta in pond 3 had a positive relation to high inorganic ion content. Large amount of organic matter in the bottom floor of the ponds result in the dominance of flagellates especially Euglenozoa (Evangelista *et al.*, 2007). The pond developed visible blooms of Euglenozoan species *Lepocinclis globulus* and *Euglena proxima* during 2011-12 and 2012-13. Dattagupta *et al.* (2004) reported blooms of Euglena spp. in ponds of Barak valley, Assam, with high concentrations of ammonia, nitrate and iron. Euglenozoans usually grow in organically polluted water. Green euglenoids which prefer nitrogen in the form of ammonia flourishes in such environment (Wehr and Sheath, 2003). According to Rahman *et al.* (2014) euglenoids prefer slightly acidic pH. *Pinnularia interrupta* was the dominant diatom species found in pond 3. The genera *Pinnularia* is abundantly seen in water bodies with low pH, low mineral, and high humate content (Jackson, 1980).

In pond 4, Euglenozoa was positively correlated with pH. Cryptophyta was found increasing when Euglenozoa decreased. Cryptomonads flourishes in a condition which favours diatom flora (Singh, 2015). They are common in oligotrophic lakes. But also reported in mesotrophic as well as eutrophic water bodies (Havens and De Costa, 1986). A study by Jahan *et al.* (2010) found negative correlation of Euglenozoa with that of nutrients in a water body with Cyanobacteria bloom. In that study Euglena spp. were abundant during rainy season and early autumn when all other phyla subsided. So it was assigned as an opportunistic phytoplankton phylum. Pond 4 was free of metaphyton irrespective of its eutrophic state during 2011-12 and 2013-14 due to the annual cleaning. During 2012-13, the yearly cleaning was not performed and the pond developed metaphyton bloom of *Osillatoria princeps* in February- March. This clearly indicates the importance of yearly cleaning and sediment removal to check the algal bloom formation.

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Chapter 5

SUMMARY AND CONCLUSION

Ponds are shallow standing water bodies which are recognised by the Ramsar convention as ‘artificial wetlands’ of national and international importance. Freshwater ponds are vital components of the landscapes. They are integral part of the villages in India with multipurpose uses. Phytoplankton are essential components of pond water occupying the bottom most level of the food web. Under favourable conditions these plankton develop into blooms. The ecology of ponds and the plankton in them are complex seeking constant research.

The present study deals with the trophic state and phytoplankton bloom formation in shallow ponds. The study area is Pallippuram Panchayath in Cherthala Taluk, Alappuzha district of Kerala state. It is a semi island bounded by Vembanadu estuary on three sides. The Panchayath is divided into 17 wards. The study started in 2011 with an elaborate survey of ponds in all the 17 wards of the Panchayath. The survey was carried out in two phases. Phase I (January – February 2011) enumerated the ponds, documented their type, use, transparency, shading condition, presence of algal bloom and macrophyte vegetation and management. The survey recorded 873 ponds in the Panchayath. Highest number of ponds (120) was recorded from ward No.3. Area of the ponds ranged from 12 m^2 to 300 m^2. Ponds with an area ranging 50-100 m^2

dominated among them with 424 ponds. Perennial ponds constituted 59% and the rest of the 41% were seasonal ponds. 57% of the ponds had clear water and the rest were either turbid or moderately turbid. Unshaded ponds constituted only 4.9%. Vast majority of the ponds had good shading indicating good vegetative coverage in the surrounding land plots. 66% of the total ponds recorded in the Panchayath were unused and under the threat of degradation and reclamation. Lack of proper management and nutrient enrichment from domestic and agricultural activities led to the formation of algal blooms in these ponds. Forty eight ponds were observed with algal blooms during phase I of the survey. These ponds were revisited during Phase II of the survey in March 2011. Algal bloom samples were collected from 32 ponds. The rest of the 16 ponds were seasonal ponds which dried in March. Cyanobacteria was the major bloom forming algal phyla which were observed in 24 ponds. Six ponds had blooms of Charophyta and two ponds had Euglenozoan bloom. *Oscillatoria* was the major bloom forming genera which occurred in 19 ponds. Toxic Cyanobacterial strains were also recorded. *Microcystis aeruginosa* and *Oscillatoria limosa* recorded during the survey are toxic and reported to impose serious health concern to human beings, cattle and to the aquatic fauna.

Based on the survey results four ponds were short listed from ward No.3 (with highest number of ponds) for further studies. To understand the ecology of different kinds of algal bloom formation we have selected three ponds observed with different types of algal blooms and a reference pond having no visible bloom. Pond 1 had green metaphyton, pond 2 with blue-green metaphyton, pond 3 with yellow-green scum and pond 4 subject to annual cleaning and sediment removal. Pond 1 was a shallow seasonal pond with an area of 38 m^2 nearby a cultivated field. Pond 2 was a perennial domestic pond with an area of 113 $m^{2.}$. The pond had submerged vegetation of *Hydrilla verticillata*. Pond 3 was a seasonal pond having an area of 63 m^2 situated near by an abandoned field. Pond 4 was a perennial domestic pond with an area of 78 m^2. Water sampling in 2011-12 started in November when the northeast monsoon receded and the ponds were filled with clear water. During 2012-13 and 2013-14 samples were collected from September after southwest monsoon. The samples were collected twice in a month during 2011-12 and 2012-13. During 2013-14, samples were collected once in a month. Water quality parameters: secchi disc transparency, temperature, pH, EC, DO, BOD, NO_2-N, NO_3-N, ammonia-nitrogen, SRP, TP, dissolved iron and chlorophyll *a* were measured during 2011-12. In the next two years *ie.* 2012-13 and 2013-14, the water samples were analysed for only three parameters (pH, EC and chlorophyll *a*). The significant relation of phytoplankton growth with water quality parameters were found out using Analysis of variance and Pearson correlation analysis. The chemical and biological processes undergoing in the pond ecosystems were elucidated through Principal Component Analysis.

Transparency of the ponds varied from 2 cm to 69 cm. The lowest transparency was recorded in pond 1 and highest value in pond 2. The temperature range in the ponds varied from 24.8 to 29.4°C. pH had a range of 5.41 to 7.74. The lowest as well as the highest values of pH were recorded from pond 1. Electrical conductivity in the ponds was observed with a range of 60 to 280 µmho/cm. Highest EC value was recorded in pond 4. Dissolved oxygen in the ponds had a range of 0.00 to 7.66 mg/L. Pond 2 went to anoxic condition during March. Highest DO value was recorded from pond 1. Biochemical Oxygen Demand varied from 2.05 to 34.25 mg/L. The range of ammonia-nitrogen in the ponds varied from 3.15-177.6 µg/L. Nitrite-nitrogen ranged from 3.19 to131.2 µg/L. Nitrate-nitrogen in the ponds ranged from 18.99 to 250.1 µg/L. The lowest and the highest value of nitrate was recorded from pond 1. SRP in the ponds ranged from 2.45 to 2125 µg/L. Total phosphorus range in the ponds varied from 95.54 to 5098 µg/L. Dissolved iron range varied from 96.73 to 1648 µg/L. Chlorophyll *a* in the ponds had a range between 5.60 µg/L and 595.1 µg/L. Highest TP, dissolved iron and Chl *a* values were recorded from pond 1. The analysis of variance showed significantly high EC in pond 4, high TP and dissolved iron in pond 1 and significantly high ammonia and low TP in pond 3. Pond 4 recorded comparatively low TP values than expected even though it was regularly used for domestic purpose including washing clothes and bathing. The reduced TP might be due to the presence of submerged vegetation of *Hydrilla verticillata* which absorbs and stores phosphorus to a certain extent. Phytoplankton biomass in the ponds was represented by Chl *a*. Chlorophyll *a* in pond 1 showed significant positive correlation with TP, nitrate and pH. Phytoplankton biomass in pond 2 and pond 4 was highly correlated with the water temperature. Pond 3 didn't show any significant correlation. The major bio-chemical processes undergoing in the ponds are explained on the basis of the first component of the PCA. The first component of the PCA of the ponds were phytoplankton growth component in pond 1, turbidity component in pond 2, Inorganic nitrogen component in pond 3 and ammonia reduction component in pond 4.

The comparison of pH, EC and Chl *a* over the years from 2011-2014 showed high values of pH and EC in the pre-monsoon period. Electrical conductivity of all the ponds recorded high values in the pre monsoon period of 2013-14. The high EC in pre-monsoon might be due to the release of nutrients trapped in the sediments due to increase in temperature and decrease in DO in the summer months. The trophic state of the ponds were analysed using the TSI equation based on TP and Chl *a* proposed by Cunha *et al.* (2013) for tropical and subtropical reservoirs. According to the trophic state classifications all the four ponds studied fell in the category hypereutrophic.

The phytoplankton in the ponds were analysed by microscope observations (2011-2014) and fluorimetric method (2014). A total of 161 algal species were recorded in pond 1during 2011-14. Chlorophyta was the most diverse algal group with 83 algal species followed by Ochrophyta with 22 species. Pond 2 recorded a total of 120 taxa. Chlorophyta was the most diverse group with 64 species. Total number of phytoplankton taxa recorded in pond 3 was 86. Chlorophyta had a marginal dominance over Euglenozoa in terms of species diversity with 29 species and 20 species respectively. A total of 82 algal species were recorded in pond 4 during 2011-14. Chlorophyta constituted the most diverse algal phyla with 35 species followed by Euglenozoa with 15 species. Pond1 developed green metaphyton bloom of *Spirogyra* type 1 during 2011-12 and a phytoplankton scum of *Pediastrum duplex* and *Chorooccum minutum* during 2012-13. The pond didn't develop any visible metaphyton during 2013-14 since it was completely covered by *Pistia stratiotes*. The synergetic effect of TP and nitrate might be the reason for green algal bloom in pond 1. Pond 2 developed blue-green metaphyton bloom of *Oscillatoria limosa* in all the three years. The pond also developed some patches of green metaphyton *Spirogyra* type 2 and *Komvophoron shimidlei* in 2012-13. Cyanobacteria usually flourish in water bodies with high temperature, high organic matter and low dissolved oxygen. *Oscillatoria limosa* and *Komvophoron shimidlei* reported in pond 2 were reported as sulphur resistant Cyanobacteria which flourish in anoxic conditions. Pond 3 developed *Euglena* spp. scum comprising *Lepocinclis globulus* and *Euglena proxima* during 2011-12 and 2012-13. During 2013-14 the floating hydrophyte *Salvinia molesta* spread over the entire pond surface limiting light for phytoplankton development. Pond 4 is a regularly used domestic pond which was maintained by yearly clean up. The pond developed blue-green metaphyton bloom of *Oscillatoria princeps* in 2012-13 only. The pond was not cleaned during that year. This indicates the role of yearly cleaning in controlling the bloom formation.

The pond water samples were collected for phytoplankton discrimination using fluorescence technique during January- June 2014. The samples were collected in opaque bottles and *in vivo* excitation spectra were taken in Shimadzu spectrofluorophotometer at an emission wavelength of 680 nm and excitation range of 400-650 nm. The comparative analysis of fluorescence excitation spectra with that of microscope observations showed that cyanobacteria was well resolved in the spectrum but could not be detected in microscope observations. The fluorescence technique in turn could not differentiate the Chl *b* bearing groups of Chlorophytes and green Euglenoids and between Chl *c* and carotenoid bearing phytoplankton groups. The study revealed fluorescence technique as an alternate method for laborious microscope observations especially to identify the phytoplankton in the nanoplankton fraction which is often neglected in the microscope observations. However the fluorescence technique needs further refinement to discriminate the algal groups sharing similar pigment combinations.

Recommendations

The survey of ponds in Pallippuram Panchayath recorded a total of 873 ponds in the entire Panchayath. Only 34% of the ponds are actively used and maintained. Rest of the 66% is unutilized and are under threat of degradation and reclamation.

- As observed in the survey the ponds in Ward No.10 of the panchayath are maintained for domestic use by regular cleaning and sediment removal adopting conventional methods and these ponds did not develop algal blooms. Therefore it is recommended that annual cleaning of the ponds may be extended to all the ponds in the panchayath. Annual maintenance of the smaller ponds can be achieved by land owners at low cost, while maintenance of large ponds requires legislative financial support. This can be achieved by undertaking the yearly cleaning of ponds as part of the rural employment guarantee scheme. This process will ensure good water quality and a dependable surface water source for the panchayath.

- There has been no concerted effort to utilise these ponds to create employment opportunity and commercial benefit. It is recommended to start fish culture in these ponds so that there will be a monetary benefit and that in turn will promote the maintenance of these ponds.
- Algal blooms were observed in forty eight ponds in the panchayath. Such dense algal growth results from eutrophication. The source of this nutrient enrichment is domestic effluents as well as paddy field run off. Ways of diverting these effluent sources away from the ponds may be assessed.

Recommendations for future research leads…

- The possibility of occurrence of 'Harmful Algal Blooms' must be assessed as it is directly linked to toxicity to cattle and human beings. This requires detailed study at the level of species, their ecophysiology, culture and toxicological assessment.
- Refinement of fluorescence technique for the discrimination of Chl *b* containing algae and those among Chl *c* and carotenoid bearing algal groups should be pursued.

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Annexures

ANNEXURE I

SURVEY OF PONDS - QUESTIONNAIRE

DATE:

WARD NUMBER**:** ☐ POND NUMBER: ☐

Pond diameter: ☐ Pond area ☐

Pond type: ☐ Perennial ☐ Seasonal

Type of use: ☐ Drinking ☐ Washing ☐ Bathing ☐ Irrigation

Water transparency: ☐ Clear ☐ Moderately turbid ☐ Highly turbid

Shade: ☐ Fully shaded ☐ Partially shaded ☐ Unshaded

Algal bloom ☐ Present ☐ Absent

Macrophyte vegetation

Floating hydrophytes: ☐ Present ☐ Absent

☐

Submerged Aquatic Vegetation: ☐ Present ☐ Absent

☐

Emergent plant: ☐ Present ☐ Absent

☐

Pond Management: ☐ Managed ☐ Unmanaged ☐ Degraded

Remarks: ☐

ANNEXURE II

a) Data on temperature (ºC) of the four ponds during 2011-12

Samples	Nov	Mean ±SD	Dec I	Mean ±SD	Dec II	Mean ±SD	Jan I	Mean ±SD	Jan II	Mean ±SD	Feb I	Mean ±SD	Feb II	Mean ±SD	Mar I	Mean ±SD	Mar II	Mean ±SD	Apr I	Mean ±SD	Apr II	Mean ±SD	May I	Mean ±SD
P1S1	26.5	26.5	27.6	27.6	26.6	26.6	25.3	25.3	25.4	25.4	26.4	26.4	27.2	27.2	28.8	28.8	-		-		26.4	26.4	27.4	27.4
P1S2	26.5	±0.0	27.5	±0.06	26.6	±0.06	25.3	±0.0	25.4	±0.0	26.4	±0.0	27.1	±0.1	28.9	±0.06	-	-	-	-	26.4	±0.0	27.4	±0.06
P1S3	26.5		27.6		26.5		25.3		25.4		26.4		27.3		28.8		-		-		26.4		27.5	
P2S1	26.2	26.2	27.5	27.5	26.8	26.8	25.4	25.4	25.5	25.5	26.5	26.5	27.5	27.5	29.0	29.0	29.1	29.1	29.2	29.2	27.1	27.1	27.8	27.8
P2S2	26.3	±0.1	27.5	±0.0	26.8	±0.0	25.4	±0.0	25.4	±0.06	26.5	±0.0	27.5	±0.0	28.9	±0.06	29.1	±0.06	29.2	±0.0	27.2	±0.1	27.8	±0.0
P2S3	26.1		27.5		26.8		25.4		25.5		26.5		27.5		29.0		29.0		29.2		27.0		27.8	
P3S1	26.4	26.4	27.7	27.8	26.9	26.9	25.1	25.1	24.8	24.8	25.8	25.8	26.5	26.5	27.8	27.8	-		-		26.2	26.2	26.9	26.9
P3S2	26.4	±0.06	27.9	±0.12	26.9	±0.0	25.1	±0.06	24.8	±0.0	25.8	±0.0	26.4	±0.06	27.8	±0.0	-	-	-	-	26.2	±0.0	26.9	±0.0
P3S3	26.5		27.9		26.9		25.2		24.8		25.8		26.5		27.8		-		-		26.2		26.9	
P4S1	26.6	26.6	27.8	27.8	27.0	27.0	25.8	25.8	25.2	25.2	26.2	26.2	27.0	27.0	28.5	28.5	29.2	29.2	29.4	29.4	26.8	26.8	27.3	27.3
P4S2	26.6	±0.0	27.8	±0.0	27.1	±0.06	25.8	±0.0	25.2	±0.06	26.2	±0.0	27.0	±0.06	28.5	±0.0	29.1	±0.06	29.4	±0.0	26.8	±0.0	27.2	±0.06
P4S3	26.6		27.8		27.0		25.8		25.3		26.2		27.1		28.5		29.2		29.4		26.8		27.3	

P1-P4 = ponds, S1-S3 = samples

b) Data on secchi disc depth (cm) of the four ponds during 2011-12

Samples	Nov	Mean ±SD	Dec I	Mean ±SD	Dec II	Mean ±SD	Jan I	Mean ±SD	Jan II	Mean ±SD	Feb I	Mean ±SD	Feb II	Mean ±SD	Mar I	Mean ±SD	Mar II	Mean ±SD	Apr I	Mean ±SD	Apr II	Mean ±SD	May I	Mean ±SD
P1S1	58	58	60	60	61	61	47	47	36	36	23	23	2	2	2	2	-		-		22	22	42	42
P1S2	58	±0.0	60	±0.0	61	±0.0	47	±0.0	36	±0.0	23	±0.0	2	±0.0	2	±0.0	-	-	-	-	22	±0.0	42	±0.0
P1S3	58		60		61		47		36		23		2		2		-		-		22		42	
P2S1	69	69	69	69	58	58	39	39	35	35	32	32	17	17	28	28	28	28	25	25	35	35	48	48
P2S2	69	±0.0	69	±0.0	58	±0.0	39	±0.0	35	±0.0	32	±0.0	17	±0.0	28	±0.0	28	±0.0	25	±0.0	35	±0.0	48	±0.0
P2S3	69		69		58		39		35		32		17		28		28		25		35		48	
P3S1	57	57	62	62	65	65	44	44	41	41	37	37	32	32	22	22	-		-		29	29	32	32
P3S2	57	±0.0	62	±0.0	65	±0.0	44	±0.0	41	±0.0	37	±0.0	32	±0.0	22	±0.0	-	-	-	-	29	±0.0	32	±0.0
P3S3	57		62		65		44		41		37		32		22		-		-		29		32	
P4S1	65	65	67	67	53	53	53	53	52.5	52.5	49	49	42	42	32	32	31	31	30	30 ±0.0	35	35	38	38
P4S2	65	±0.0	67	±0.0	53	±0.0	53	±0.0	52.5	±0.0	49	±0.0	42	±0.0	32	±0.0	31	±0.0	30		35	±0.0	38	±0.0
P4S3	65		67		53		53		52.5		49		42		32		31		30		35		38	

P1-P4 = ponds, S1-S3 = samples

c) Data on pH of the four ponds during 2011-12

samples	Nov	Mean ±SD	Dec I	Mean ±SD	Dec II	Mean ±SD	Jan I	Mean ±SD	Jan II	Mean ±SD	Feb I	Mean ±SD	Feb II	Mean ±SD	Mar I	Mean ±SD	Mar II	Mean ±SD	Apr I	Mean ±SD	Apr II	Mean ±SD	May I	Mean ±SD
P1S1	5.55	5.49	5.64	5.65	5.40	5.41	6.36	6.36	6.45	6.45	6.70	6.63	7.77	7.72	7.33	7.31	-		-		6.77	6.76	6.79	6.80
P1S2	5.47	±0.05	5.63	±0.02	5.44	±0.02	6.36	±0.01	6.46	±0.02	6.57	±0.07	7.65	±0.06	7.29	±0.02	-	-	-	-	6.75	±0.01	6.81	±0.01
P1S3	5.45		5.67		5.40		6.37		6.43		6.63		7.74		7.32		-		-		6.76		6.81	
P2S1	6.45	6.42	6.31	6.23	6.16	6.12	6.10	6.05	6.08	6.07	6.45	6.5	6.26	6.27	7.08	7.07	6.16	6.17	6.16	6.16	6.04	6.05	6.23	6.23
P2S2	6.43	±0.04	6.22	±0.07	6.11	±0.03	6.02	±0.05	6.10	±0.04	6.49	±0.06	6.29	±0.02	7.04	±0.03	6.19	±0.02	6.16	±0.01	6.05	±0.01	6.21	±0.02
P2S3	6.38		6.17		6.10		6.02		6.03		6.56		6.27		7.09		6.15		6.15		6.05		6.24	
P3S1	5.57	5.53	5.71	5.6	5.76	5.71	5.55	5.53	5.98	5.98	6.22	6.25	6.11	6.1	6.57	6.57	-	-	-	-	6.6	6.59	6.89	6.88
P3S2	5.53	±0.05	5.55	±0.1	5.77	±0.09	5.53	±0.02	5.99	±0.02	6.27	±0.03	6.09	±0.01	6.58	±0.01	-		-		6.58	±0.01	6.87	±0.01
P3S3	5.48		5.53		5.61		5.51		5.96		6.25		6.1		6.57		-		-		6.59		6.87	
P4S1	6.24	6.23	6.33	6.27	6.61	6.53	6.59	6.55	6.66	6.67	6.96	6.91	6.86	6.91	7.51	7.52	7.00	7.0	6.86	6.87	6.97	6.97	7.02	7.04
P4S2	6.22	±0.01	6.25	±0.05	6.47	±0.07	6.49	±0.05	6.68	±0.01	6.9	±0.04	6.89	±0.06	7.52	±0.02	6.99	±0.02	6.87	±0.02	6.97	±0.01	7.04	±0.02
P4S3	6.24		6.23		6.51		6.56		6.68		6.88		6.97		7.54		7.02		6.89		6.98		7.05	

P1-P4 = ponds, S1-S3 = samples

d) Data on electrical conductivity (µmho/cm) of the four ponds during 2011-12

sample	Nov	Mean ±SD	Dec I	Mean ±SD	Dec II	Mean ±SD	Jan I	Mean ±SD	Jan II	Mean ±SD	Feb I	Mean ±SD	Feb II	Mean ±SD	Mar I	Mean ±SD	Mar II	Mean ±SD	Apr I	Mean ±SD	Apr II	Mean ±SD	May I	Mean ±SD
P1S1	70	76.67	90	90	60	60	110	110	176	176.7	224	224.3	157	157.7	164	164.3	-		-		216	216	239	239.7
P1S2	80	±5.77	90	±0.0	60	±0.0	110	±0.0	178	±1.15	224	±0.58	158	±0.58	165	±0.58	-	-	-	-	216	±0.0	240	±0.58
P1S3	80		90		60		110		176		225		158		164		-		-		216		240	
P2S1	80	80	70	73.3	68	68.7	90	90	68	68.7	87	87.7	89	88.7	113	113	160	160.3	180	180	105	104.7	221	220.3
P2S2	80	±0.0	80	±5.78	70	±1.15	90	±0.0	69	±0.58	88	±0.58	89	±0.58	113	±0.0	161	±0.58	180	±0.0	105	±0.58	220	±0.58
P2S3	80		70		68		90		69		88		88		113		160		180		104		220	
P3S1	110	110	120	120	129	129.7	130	130	159	158.7	128	127.7	90	89.7	153	152.7	-		-		235	234.7	240	240
P3S2	110	±0.0	120	±0.0	130	±0.58	130	±0.0	158	±0.58	128	±0.58	89	±0.58	152	±0.58	-	-	-	-	235	±0.58	240	±0.0
P3S3	110		120		130		130		159		127		89		152		-		-		234		240	
P4S1	110	110	110	110	183	182	180	180	242	242.7	235	234.7	174	174.7	263	262.7	272	272	280		280	280	281	280.7
P4S2	110	±0.0	110	±0.0	183	±1.73	180	±0.0	243	±0.58	234	±0.58	175	±0.58	262	±0.58	272	±0.0	281		281	±1.0	280	±0.58
P4S3	110		110		180		180		243		235		175		262		272		280		279		280	

P1-P4 = ponds, S1-S3 = samples

e) Data on dissolved oxygen (mg/L) of the four ponds during 2011-12

Sample	Nov	Mean ±SD	Dec I	Mean ±SD	Dec II	Mean ±SD	Jan I	Mean ±SD	Jan II	Mean ±SD	Feb I	Mean ±SD	Feb II	Mean ±SD	Mar I	Mean ±SD	Mar II	Mean ±SD	Apr I	Mean ±SD	Apr II	Mean ±SD	May I	Mean ±SD
P1S1	7.26	7.66	3.23	3.36	5.65	5.78	6.45	6.58	6.45	6.45	0.81	0.54	2.02	2.02	2.02	2.02	-		-		4.44	4.44	5.65	5.65
P1S2	8.06	±0.4	3.23	±0.23	6.05	±0.23	6.45	±0.23	6.45	±0.0	0.40	±0.24	2.02	±0.0	2.02	±0.0	-	-	-	-	4.44	±0.0	5.65	±0.0
P1S3	7.66		3.63		5.65		6.85		6.45		0.40		2.02		2.02		-		-		4.44		5.65	
P2S1	6.05	6.58	5.65	5.51	5.24	5.11	4.03	3.89	4.03	4.03	3.22	3.49	2.42	2.42	0.00	0.00	2.02	2.02	2.02	2.02	3.63	3.63	6.05	6.05
P2S2	6.85	±0.46	5.24	±0.24	4.84	±0.23	4.03	±0.23	4.03	±0.0	4.03	±0.47	2.42	±0.0	0.00	±0.0	2.02	±0.0	2.02	±0.0	3.63	±0.0	6.05	±0.0
P2S3	6.85		5.65		5.24		3.63		4.03		3.22		2.42		0.00		2.02		2.02		3.63		6.05	
P3S1	4.44	4.70	4.03	4.16	4.44	4.44	4.44	4.44	5.24	5.11	6.04	6.18	1.61	1.61	2.02	2.02	-		-		6.05	6.05	7.26	7.26
P3S2	4.83	±0.23	4.44	±0.24	4.44	±0.0	4.44	±0.0	5.24	±0.23	6.45	±0.24	1.61	±0.0	2.02	±0.0	-	-	-	-	6.05	±0.0	7.26	±0.0
P3S3	4.83		4.03		4.44		4.44		4.84		6.04		1.61		2.02		-		-		6.05		7.26	
P4S1	6.05	6.11	5.24	5.10	4.44	4.57	5.65	5.38	5.65	5.65	5.24	5.24	3.23	3.23	3.63	3.63	5.24	5.24	4.44	4.44	7.26	7.26	6.85	6.85
P4S2	6.64	±0.49	5.24	±0.24	4.44	±0.23	5.24	±0.24	5.65	±0.0	5.24	±0.0	3.23	±0.0	3.63	±0.0	5.24	±0.0	4.44	±0.0	7.26	±0.0	6.85	±0.0
P4S3	5.65		4.83		4.84		5.24		5.65		5.24		3.23		3.63		5.24		4.44		7.26		6.85	

P1-P4 = ponds, S1-S3 = samples

f) Data on BOD (mg/L) of the four ponds during 2011-12

Sample	Nov	Mean ±SD	Dec I	Mean ±SD	Dec II	Mean ±SD	Jan I	Mean ±SD	Jan II	Mean ±SD	Feb I	Mean ±SD	Feb II	Mean ±SD	Mar I	Mean ±SD	Mar II	Mean ±SD	Apr I	Mean ±SD	Apr II	Mean ±SD	May I	Mean ±SD
P1S1	2.42	2.96	3.26	2.97	6.46	6.46	6.46	6.98	3.22	3.75	34.25	34.25	24.2	24.2	11.64	11.96	-		-		11.94	13.21	5.24	5.24
P1S2	3.22	±0.47	2.42	±0.47	6.46	±0.0	7.24	±0.45	4.02	±0.46	34.25	±0.0	24.2	±0.0	12.12	±0.28	-	-	-	-	11.94	±2.2	5.24	±0.0
P1S3	3.24		3.22		6.46		7.24		4.02		34.25		24.2		12.12		-		-		15.75		5.24	
P2S1	7.24	6.98	4.86	4.57	7.24	7.79	10.48	10.18	11.94	10.43	26.2	26.2	22.2	22.2	26.44	24.3	26.25	26.25	32.2	33.3	22.2	22.2	12.4	11.3
P2S2	6.44	±0.47	4.84	±0.48	8.06	±0.47	9.58	±0.52	9.66	±1.31	26.2	±0.0	22.2	±0.0	22.00	±2.22	26.25	±0.0	33.8	±0.92	22.2	±0.0	9.20	±1.85
P2S3	7.26		4.02		8.06		10.48		9.68		26.2		22.2		24.44		26.25		33.8		22.2		12.4	
P3S1	8.86	8.6	6.46	5.92	7.26	7.25	7.10	7.15	7.58	7.53	5.64	5.64	10.1	10.1	7.90	7.9	-		-		10.52	10.5	8.08	8.08
P3S2	8.08	±0.45	5.64	±0.47	7.24	±0.01	7.10	±0.08	7.66	±0.17	5.64	±0.0	10.1	±0.0	7.90	±0.0	-	-	-	-	10.52	±0.0	8.08	±0.0
P3S3	8.86		5.66		7.24		7.24		7.34		5.64		10.1		7.90		-		-		10.52		8.08	
P4S1	4.02	4.05	3.22	3.21	7.24	6.71	3.22	4.03	5.64	4.3	8.86	8.6	16.15	16.15	10.10	10.1	2.02	2.04	10.8	13.33	8.08	8.08	4.44	4.44
P4S2	4.06	±0.02	3.22	±0.01	6.44	±0.46	4.04	±0.8	3.24	±1.22	8.86	±0.46	16.15	±0.0	10.10	±0.0	2.08	±0.03	14.6	±2.19	8.08	±0.0	4.44	±0.0
P4S3	4.06		3.2		6.44		4.82		4.02		8.06		16.15		10.10		2.02		14.6		8.08		4.44	

P1-P4 = ponds, S1-S3 = samples

g) Data on nitrite-nitrogen (µg/L) of the four ponds during 2011-12

Samples	Nov	Mean ±SD	Dec I	Mean ±SD	Dec II	Mean ±SD	Jan I	Mean ±SD	Jan II	Mean ±SD	Feb I	Mean ±SD	Feb II	Mean ±SD	Mar I	Mean ±SD	Mar II	Mean ±SD	Apr I	Mean ±SD	Apr II	Mean ±SD	May I	Mean ±SD
P1S1	4.790	5.18	3.700	3.19	6.62	4.7	18.58	13.98	24.22	24.3	24.32	24.52	28.25	27.6	124.2	124.03	-		-		24.06	23.89	61.65	61.23
P1S2	5.180	±0.39	2.570	±0.57	4.48	±1.82	12.1	±4.01	24.67	±0.34	24.56	±0.18	28.36	±1.2	124.8	±0.86	-	-	-	-	24.84	±1.05	61.21	±0.42
P1S3	5.570		3.310		3.00		11.26		24.00		24.67		26.23		123.1		-		-		22.77		60.82	
P2S1	16.89	13.36	3.820	4.84	10.12	9.69	12.44	12.79	35.34	35.53	18.99	18.37	24.39	23.18	41.04	40.65	104.2	103.9	120.1	120.77	3.890	4.31	130.2	131.13
P2S2	11.68	±3.06	5.760	±0.97	9.23	±0.45	13.11	±0.34	36.01	±0.42	18.64	±0.8	22.21	±1.11	39.03	±1.46	102.2	±1.57	119.9	±1.33	4.170	±0.50	130.3	±1.53
P2S3	11.52		4.950		9.73		12.83		35.23		17.47		22.94		41.88		105.3		122.3		4.870		132.9	
P3S1	17.82	14.57	11.56	10.07	7.36	7.46	79.94	79.94	54.78	55.32	66.85	83.37	37.07	35.53	25.62	25.6	-		-		20.09	20.01	48.46	49.38
P3S2	12.18	±2.92	11.37	±2.42	7.09	±0.43	79.22	±0.73	55.51	±0.48	115.9	±28.1	35.51	±1.54	23.72	±1.87	-	-	-	-	19.36	±0.62	48.86	±1.26
P3S3	13.70		7.280		7.94		80.67		55.68		67.35		34.00		27.46		-		-		20.59		50.82	
P4S1	10.67	10.49	4.320	3.72	6.11	6.51	11.09	12.29	21.37	21.29	13.39	13.78	33.16	32.12	22.43	21.73	30.76	30.44	10.24	10.16	4.520	4.84	27.74	27.35
P4S2	9.460	±0.95	3.580	±0.54	7.63	±0.98	14.28	±1.74	21.71	±0.47	14.05	±0.34	31.15	±1.01	21.77	±0.73	30.70	±0.5	10.08	±0.08	4.710	±0.40	27.02	±0.36
P4S3	11.33		3.270		5.80		11.49		20.78		13.89		32.04		20.98		29.87		10.16		5.290		27.30	

P1-P4 = ponds, S1-S3 = samples

h) Data on nitrate-nitrogen (µg/L) of the four ponds during 2011-12

Samples	Nov	Mean ±SD	Dec I	Mean ±SD	Dec II	Mean ±SD	Jan I	Mean ±SD	Jan II	Mean ±SD	Feb I	Mean ±SD	Feb II	Mean ±SD	Mar I	Mean ±SD	Mar II	Mean ±SD	Apr I	Mean ±SD	Apr II	Mean ±SD	May I	Mean ±SD
P1S1	77.22	71.15	44.77	51.29	58.25	60.67	36.84	37.66	60.33	61.64	98.70	103.97	250.6	250.13	140.9	139.33	-		-		17.96	18.99	63.73	64.94
P1S2	63.59	±6.94	57.36	±6.31	62.40	±2.16	38.62	±0.9	62.7	±1.20	106.4	±4.57	255.1	±5.22	137.7	±1.60	-	-	-	-	19.14	±0.97	64.25	±1.67
P1S3	72.64		51.73		61.36		37.51		61.88		106.8		244.7		139.4		-		-		19.89		66.84	
P2S1	44.44	45.04	36.03	36.01	34.33	32.20	119.2	119.2	66.7	67.44	64.03	66.47	65.36	68.52	156.1	156.87	134.6	133.6	229.3	233.8	67.88	68.43	187.1	185.6
P2S2	53.25	±7.92	35.07	±0.93	29.07	±2.77	119.7	±0.45	67.59	±0.68	63.59	±4.62	70.69	±2.8	156.6	±0.93	133.1	±0.87	236.4	±3.91	68.99	±0.56	184.3	±1.41
P2S3	37.44		36.92		33.21		118.8		68.03		71.81		69.51		157.9		133.1		235.7		68.41		185.4	
P3S1	61.49	61.04	37.73	39.14	40.10	40.0	72.18	72.72	95.30	94.28	130.3	125.67	51.66	50.12	82.84	82.42	-		-		33.14	33.44	73.14	74.84
P3S2	60.4	±0.57	40.69	±1.49	41.21	±1.26	72.47	±0.7	93.29	±1.01	160.3	±37.2	48.9	±1.41	82.84	±0.72	-	-	-	-	33.44	±0.3	74.03	±2.22
P3S3	61.22		38.99		38.70		73.51		94.25		86.4		49.81		81.59		-		-		33.73		77.36	
P4S1	62.68	62.62	57.36	56.82	53.21	52.96	43.51	44.82	48.03	48.79	56.03	55.73	61.29	53.76	154.5	154.97	52.47	53.36	45.59	44.75	30.25	30.20	64.77	65.66
P4S2	62.96	±0.37	55.44	±1.20	51.44	±1.42	46.55	±1.56	48.62	±0.86	56.03	±0.51	43.07	±9.51	155.0	±0.45	53.66	±0.79	44.33	±0.73	29.81	±0.37	66.40	±0.83
P4S3	62.22		57.66		54.24		44.40		49.73		55.14		56.92		155.4		53.96		44.33		30.55		65.81	

P1-P4 = ponds, S1-S3 = samples

i) Data on ammonia-nitrogen (µg/L) of the four ponds during 2011-12

Samples	Nov	Mean ±SD	Dec I	Mean ±SD	Dec II	Mean ±SD	Jan I	Mean ±SD	Jan II	Mean ±SD	Feb I	Mean ±SD	Feb II	Mean ±SD	Mar I	Mean ±SD	Mar II	Mean ±SD	Apr I	Mean ±SD	Apr II	Mean ±SD	May I	Mean ±SD
P1S1	6.09	6.09	42.84	38.77	41.00	40.83	11.81	12.17	10.96	10.69	26.79	22.75	15.67	17.12	77.26	77.16	-		-		28.15	29.19	26.09	26.26
P1S2	5.93	±0.16	33.06	±5.09	40.41	±0.37	12.78	±0.53	10.63	±0.25	18.82	±3.99	18.38	±1.37	77.67	±0.57	-	-	-	-	29.97	±0.94	27.56	±1.23
P1S3	6.24		40.41		41.09		11.93		10.47		22.65		17.32		76.55		-		-		29.44		25.12	
P2S1	17.95	18.59	39.41	36.23	12.74	13.26	12.45	12.79	71.8	71.92	11.78	12.83	5.19	5.09	72.44	72.34	28.05	20.81	33.86	33.22	26.41	26.46	22.61	22.61
P2S2	18.29	±0.84	38.73	±4.92	13.68	±0.48	14.12	±1.20	71.86	±0.16	16.29	±3.08	4.99	±0.1	71.95	±0.35	22.16	±8.0	32.98	±0.56	26.65	±0.17	22.65	±0.04
P2S3	19.54		30.56		13.37		11.79		72.10		10.41		5.09		72.62		12.22		32.82		26.32		22.58	
P3S1	48.82	44.63	77.57	90.95	73.62	71.15	15.46	15.3	178.6	177.6	57.57	62.64	14.21	13.70	138.1	138.07	-		-		70.84	70.85	60.46	60.26
P3S2	49.04	±7.45	113.5	±19.6	73.21	±3.93	15.21	±0.14	176.4	±1.11	64.08	±4.52	13.71	±0.51	138.5	±0.45	-	-	-	-	71.12	±0.26	60.32	±0.24
P3S3	36.02		81.78		66.61		15.23		177.8		66.26		13.19		137.6		-		-		70.60		59.99	
P4S1	37.92	36.77	44.46	42.03	52.96	53.19	11.72	12.59	5.610	5.83	8.13	8.07	5.23	6.21	13.96	14.21	3.01	3.15	3.94	4.18	11.86	12.06	5.12	5.25
P4S2	34.83	±1.69	42.78	±2.88	53.12	±0.27	11.99	±1.27	5.420	±0.55	6.61	±1.43	6.87	±0.87	14.11	±0.31	3.93	±0.72	4.37	±0.22	12.31	±0.23	4.97	±0.37
P4S3	37.57		38.85		53.49		14.05		6.450		9.47		6.54		14.55		2.51		4.23		12.02		5.67	

P1-P4 = ponds, S1-S3 = samples

j) Data on SRP (µg/L) of the four ponds during 2011-12

Samples	Nov	Mean ±SD	Dec I	Mean ±SD	Dec II	Mean ±SD	Jan I	Mean ±SD	Jan II	Mean ±SD	Feb I	Mean ±SD	Feb II	Mean ±SD	Mar I	Mean ±SD	Mar II	Mean ±SD	Apr I	Mean ±SD	Apr II	Mean ±SD	May I	Mean ±SD
P1S1	64.04	53.90	43.89	42.11	17.81	24.72	12.16	21.48	35.49	36.70	1023	1029	3784	3789	3741	3789	-		-		1305	1306	845.5	845.6
P1S2	49.12	±8.78	41.87	±1.67	24.62	±6.96	29.69	±8.81	36.36	±1.41	1039	±8.32	3805	±13.8	3833	±46.2	-	-	-	-	1309	±2.65	844.7	±0.95
P1S3	48.54		40.57		31.72		22.59		38.25		1027		3779		3795		-		-		1304		846.6	
P2S1	11.87	17.04	2.160	2.45	86.16	86.64	44.03	44.89	64.42	75.05	39.06	30.57	433.1	439.77	439.9	454.7	228.5	230.47	702.2	751.77	528.3	525.7	316.5	319.8
P2S2	26.07	±7.85	2.740	±0.29	90.51	±3.65	39.68	±5.70	80.36	±9.20	22.30	±8.38	434.3	±10.52	445.5	±20.97	230.7	±1.86	786.7	±44.11	524.9	±2.31	315.6	±6.51
P2S3	13.17		2.450		83.26		50.98		80.36		30.36		451.9		478.7		232.2		766.4		523.9		327.3	
P3S1	27.52	31.87	13.90	15.20	26.65	16.02	38.39	38.89	45.93	56.34	9.550	9.07	86.69	82.71	71.12	72.78	-	-	-	-	397.6	397.77	129.2	131.97
P3S2	30.57	±5.13	15.20	±1.31	7.23	±9.84	39.99	±1.45	47.09	±17.04	8.390	±0.60	81.02	±3.46	70.28	±3.63	-		-		394.7	±3.15	134.3	±2.58
P3S3	37.52		16.51		14.19		41.29		76.00		9.260		80.43		76.94		-		-		401.0		132.4	
P4S1	1478.9	1477.2	1405.1	1406.4	1673.5	1684.3	1511.3	1511.0	739.1	743.4	401.5	401.67	2091	2125	2107	2052.3	1173.6	1131.5	1258.7	1269.1	748.3	747.5	289.6	290.4
P4S2	1276.8	±199.5	1397.4	±9.66	1704.3	±17.34	1506.9	±4.0	747.0	±3.99	403.6	±1.86	2124	±34.51	2019	±47.72	1055.9	±65.64	1235.3	±40.03	747.6	±0.85	290.6	±0.72
P4S3	1675.8		1416.6		1675.1		1514.9		744.1		399.9		2160		2031		1165.1		1313.3		746.6		291.0	

P1-P4 = ponds, S1-S3 = samples

k) Data on TP (µg/L) of the four ponds during 2011-12

Samples	Nov	Mean ±SD	Dec I	Mean ±SD	Dec II	Mean ±SD	Jan I	Mean ±SD	Jan II	Mean ±SD	Feb I	Mean ±SD	Feb II	Mean ±SD	Mar I	Mean ±SD	Mar II	Mean ±SD	Apr I	Mean ±SD	Apr II	Mean ±SD	May I	Mean ±SD
P1S1	172.4	160	154.2	158.9	135.2	132.6	109.5	11.4	368.8	193.7	1485	1483	5102	5098	4435	4616.3	-		-		1802	1807	1159	1158
P1S2	158.04	±10.3	159.6	±4.39	134.7	±4.1	110.7	±2.27	107.2	±151.6	1501	±19.1	4838	±258	4638	±171	-	-	-	-	1817	±8.38	1161	±2.52
P1S3	152.4		162.9		127.9		113.9		105.1		1463		5355		4776		-		-		1803		1156	
P2S1	246.2	310.9	97.70	95.57	494.8	494.3	1357	1408	329.1	350.1	312.8	316.8	526.5	524.4	670.1	544.13	1082	1100	1023	1024	722.2	733.2	349.4	349.3
P2S2	392.1	±74.3	88.20	±6.56	508.7	±14.6	1390	±61.9	348.4	±21.9	302.1	±17.0	524.1	±2.01	588.4	±152.9	1169	±61.9	939.0	±86.5	748.5	±13.6	353.5	±4.20
P2S3	294.5		100.8		479.4		1477		372.8		335.4		522.5		373.9		1049		1112		729.0		345.1	
P3S1	187.3	182.5	125.2	124.3	93.00	96.60	315.4	322.5	117.2	122.9	284.7	269.6	141.3	143.3	163.7	164.2	-		-		555.9	555.4	177.6	174.2
P3S2	172.4	±8.75	119.8	±4.12	99.90	±3.46	320.8	±8.14	123.1	±5.65	211.4	±52.35	144.2	±1.87	167.5	±3.08	-	-	-	-	554.0	±1.23	175.9	±4.44
P3S3	187.8		127.9		96.90		331.4		128.5		312.8		144.8		161.4		-		-		556.3		169.2	
P4S1	1608	1574	1691	1701.3	1818	1822	1802	1799	1232	1281.7	588.2	649.5	2881	2870	2412	2419	1797	1774	1804	1805	899.0	896.9	351.4	366.8
P4S2	1416	±10.5	1701	±10.5	1833	±9.29	1808	±10.3	1318	±44.5	697.5	±55.85	2889	±26.29	2446	±24.3	1789	±33.2	1801	±4.58	883.7	±12.3	389.2	±19.8
P4S3	1698		1712		1816		1788		1295		662.8		2840		2399		1736		1810		908.0		359.9	

P1-P4 = ponds, S1-S3 = samples

l) Data on dissolved iron (µg/L) of the four ponds during 2011-12

Samples	Nov	Mean ±SD	Dec I	Mean ±SD	Dec II	Mean ±SD	Jan I	Mean ±SD	Jan II	Mean ±SD	Feb I	Mean ±SD	Feb II	Mean ±SD	Mar I	Mean ±SD	Mar II	Mean ±SD	Apr I	Mean ±SD	Apr II	Mean ±SD	May I	Mean ±SD
P1S1	547.5	522.3	1753	1648	542.3	521.3	293.2	294.7	1196	1221	1556	1581	702.4	703.3	1536	1538	-		-		495.4	496.6	328.0	325.9
P1S2	548.0	±7.82	1549	±102	500.4	±20.9	298.6	±3.41	1246	±25.0	1489	±107	706.3	±2.71	1543	±4.35	-	-	-	-	502.1	±5.0	326.1	±2.15
P1S3	561.3		1642		521.3		292.3		1223		1699		701.1		1535		-		-		492.3		323.7	
P2S1	108.5	96.74	149.9	138.8	153.2	167.0	260.4	262.9	549.4	548.9	334.7	354.9	1018.9	1021	477.2	477.1	1269	1268	1371	1374	464.1	460.8	181.2	180.0
P2S2	85.14	±11.7	150.4	±19.7	178.9	±12.9	261.3	±3.63	543.2	±5.51	368.9	±17.9	1011.6	±10.3	474.6	±2.40	1258	±10.0	1383	±7.94	458.8	±2.86	179.7	±1.12
P2S3	96.57		116.1		169		267.1		554.2		361.3		1031.9		479.4		1278		1368		459.6		179.0	
P3S1	288.9	258.5	339.9	347.4	293.7	304.3	228.9	230.8	580.4	588.8	243.7	239.9	207.8	222.9	556.6	556.6	-		-		286.1	288.0	204.2	200.9
P3S2	200.4	±50.3	357.5	±9.10	328.9	±21.3	233.2	±2.18	591.3	±7.47	246.6	±9.21	232.3	±13.2	559.7	±3.15	-	-	-	-	293.5	±4.84	198.5	±2.97
P3S3	286.1		344.7		290.4		230.4		594.7		229.4		228.5		553.4		-		-		284.4		199.9	
P4S1	195.6	218.6	402.8	394.8	201.3	204.5	175.1	176.6	775.1	777.4	280.4	260.1	602.2	623.2	404.5	398.0	392.2	393.2	433.4	438.9	233.2	236.2	109.9	109.8
P4S2	235.1	±20.5	419.4	±29.3	212.8	±7.25	184.2	±7.02	784.2	±6.03	308.5	±61.2	622.9	±21.2	389.7	±6.71	387.1	±6.71	439.6	±5.18	238.9	±2.87	112.3	±2.60
P4S3	225.1		362.3		199.4		170.4		772.8		191.3		644.6		399.8		400.4		443.7		236.6		107.1	

P1-P4 = ponds, S1-S3 = samples

m) Data on chlorophyll *a* (µg/L) of the four ponds during 2011-12

Samples	Nov	Mean ±SD	Dec I	Mean ±SD	Dec II	Mean ±SD	Jan I	Mean ±SD	Jan II	Mean ±SD	Feb I	Mean ±SD	Feb II	Mean ±SD	Mar I	Mean ±SD	Mar II	Mean ±SD	Apr I	Mean ±SD	Apr II	Mean ±SD	May I	Mean ±SD
P1S1	20.3	20.2	7.30	7.10	7.730	7.34	38.71	40.32	19.18	39.04	24.5	42.01	539.4	595.1	544.6	540.4	-		-		17.61	16.32	9.320	12.03
P1S2	19.4	±0.75	6.20	±0.82	7.620	±0.58	41.15	±1.39	49.96	±17.23	52.42	±15.3	578.1	±65.8	539.1	±3.69	-	-	-	-	17.90	±2.49	11.20	±3.21
P1S3	20.9		7.80		6.670		41.11		47.98		49.1		667.7		537.6		-		-		13.45		15.57	
P2S1	38.28	38.31	73.07	75.94	89.72	90.80	71.58	74.46	40.36	38.16	58.63	48.89	63.22	67.10	93.14	92.22	376.0	306.1	78.89	74.55	63.12	57.27	61.21	57.91
P2S2	34.89	±3.43	69.30	±8.45	90.75	±1.1	75.36	±2.55	32.18	±5.23	39.54	±9.55	68.34	±3.43	93.47	±1.88	312.2	±73.2	71.41	±3.88	55.66	±5.23	51.97	±5.15
P2S3	41.75		85.45		91.93		76.45		41.93		48.49		69.74		90.05		230.0		73.35		53.03		60.55	
P3S1	174.8	207.6	99.18	92.41	107.3	131.0	208.5	199.7	166.1	202.2	65.06	69.50	77.04	85.40	208.8	245.5	-		-		94.59	89.53	26.23	21.35
P3S2	248.8	±37.7	100.8	±13.2	144.1	±20.6	199.1	±8.56	170.2	±58.9	69.47	±4.46	88.55	±7.31	273.6	±33.2	-	-	-	-	85.81	±4.54	22.09	±5.29
P3S3	199.2		77.25		141.6		191.4		270.2		73.97		90.61		254.0		-		-		88.19		15.73	
P4S1	16.79	13.39	7.310	5.60	19.89	19.32	22.42	21.24	16.07	14.34	9.21	8.71	32.12	32.65	20.37	20.69	223.6	209.9	112.1	108.1	17.37	17.76	14.11	11.24
P4S2	15.14	±4.53	4.510	±1.50	20.17	±1.23	19.95	±1.24	17.01	±3.83	7.08	±1.44	28.76	±4.18	20.04	±0.86	194.9	±14.39	109.5	±4.85	17.04	±0.97	4.890	±5.51
P4S3	8.25		4.970		17.90		21.36		9.95		9.83		37.07		21.67		211.2		102.7		18.87		14.72	

P1-P4 = ponds, S1-S3 = samples

ANNEXURE III

a) Data on pH of the four ponds during 2012-13

Month of sample collection	Sample replicates	pond 1	pond 2	pond 3	pond 4
Sep I	S1	6.63	7.03	5.96	6.84
	S2	6.62	7.03	5.96	6.84
	S3	6.63	7.05	5.96	6.84
Sep II	S1	6.23	6.84	5.82	6.82
	S2	6.23	6.84	5.82	6.82
	S3	6.23	6.84	5.82	6.82
Oct I	S1	6.01	6.80	5.69	6.76
	S2	6.00	6.80	5.69	6.76
	S3	6.01	6.79	5.70	6.76
Oct II	S1	5.86	6.46	5.87	6.77
	S2	5.86	6.46	5.87	6.77
	S3	5.86	6.46	5.87	6.77
Nov I	S1	6.39	6.86	6.35	7.21
	S2	6.39	6.85	6.35	7.21
	S3	6.39	6.86	6.35	7.21
Nov II	S1	6.27	6.44	5.85	6.98
	S2	6.28	6.44	5.85	6.98
	S3	6.27	6.44	5.85	6.98
Dec I	S1	6.49	6.52	5.57	6.23
	S2	6.49	6.52	5.57	6.22
	S3	6.49	6.52	5.57	6.23
Dec II	S1	6.59	6.39	5.63	6.55
	S2	6.60	6.39	5.63	6.55
	S3	6.59	6.39	5.63	6.55

Jan I-2013	S1	7.05	6.96	6.01	7.06
	S2	7.05	6.96	5.99	7.06
	S3	7.05	6.96	6.02	7.06
Jan II	S1	7.02	6.99	5.92	6.76
	S2	7.02	7.00	5.92	6.76
	S3	7.02	6.99	5.92	6.76
Feb I	S1	6.86	7.47	6.23	7.02
	S2	6.86	7.47	6.23	7.02
	S3	6.86	7.47	6.23	7.02
Feb II	S1	7.89	7.10	6.03	7.63
	S2	7.89	7.10	6.02	7.63
	S3	7.89	7.10	6.02	7.63
Mar I	S1	7.67	7.20	5.99	7.89
	S2	7.67	7.20	5.99	7.89
	S3	7.67	7.20	5.99	7.89
Mar II	S1	5.87	7.05	5.84	6.88
	S2	5.87	7.05	5.84	6.88
	S3	5.87	7.05	5.84	6.88
Apr I	S1	--	7.20	6.11	6.64
	S2	--	7.20	6.11	6.64
	S3	--	7.20	6.11	6.64
Apr II	S1	--	7.26	6.84	7.54
	S2	--	7.26	6.84	7.54
	S3	--	7.26	6.84	7.54

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b) Data on pH of the four ponds during 2013-14

Month of sample collection	Sample replicates	Pond 1	Pond 2	Pond 3	Pond 4
Sep	S1	6.29	6.73	6.30	6.71
	S2	6.30	6.73	6.30	6.71
	S3	6.29	6.73	6.30	6.71
Oct	S1	6.25	6.45	6.11	6.67
	S2	6.23	6.44	6.11	6.67
	S3	6.24	6.45	6.10	6.67
Nov	S1	6.44	7.10	6.27	6.88
	S2	6.44	7.10	6.27	6.88
	S3	6.45	7.10	6.27	6.88
Dec	S1	6.47	6.83	6.08	6.89
	S2	6.47	6.82	6.08	6.89
	S3	6.47	6.83	6.08	7.00
Jan	S1	6.29	6.45	5.73	6.68
	S2	6.28	6.45	5.73	6.68
	S3	6.29	6.45	5.72	6.68
Feb	S1	6.99	7.06	5.90	7.29
	S2	7.00	7.06	5.91	7.29
	S3	6.99	7.05	5.90	7.29
Mar	S1	6.79	7.19	6.06	7.01
	S2	6.79	7.19	6.06	7.03
	S3	6.79	7.18	6.06	6.99
Apr	S1	6.59	6.40	5.70	6.80
	S2	6.59	6.40	5.70	6.80
	S3	6.59	6.40	5.71	6.80
May	S1	6.30	6.61	5.92	6.71
	S2	6.29	6.61	5.92	6.71
	S3	6.30	6.61	5.92	6.71
Jun	S1	6.23	6.71	6.01	6.69
	S2	6.23	6.74	6.01	6.70
	S3	6.23	6.74	6.01	6.69

ANNEXURE IV

a) Data on EC (µmho/cm) of the four ponds during 2012-13

Month of sample collection	Sample replicates	pond 1	pond 2	pond 3	pond 4
Sep I	S1	67.00	67.00	65.00	126.0
	S2	68.00	67.00	65.00	126.0
	S3	66.00	67.00	65.00	126.0
Sep II	S1	68.00	73.00	71.00	132.0
	S2	68.00	73.00	71.00	132.0
	S3	68.00	73.00	71.00	132.0
Oct I	S1	39.00	88.00	79.00	160.0
	S2	39.00	88.00	79.00	160.0
	S3	39.00	88.00	79.00	160.0
Oct II	S1	108.0	87.00	77.00	160.0
	S2	109.0	87.00	77.00	163.0
	S3	108.0	87.00	77.00	162.0
Nov I	S1	97.00	13.00	80.00	176.0
	S2	97.00	12.00	80.00	176.0
	S3	97.00	14.00	80.00	176.0
Nov II	S1	107.0	97.00	82.50	204.0
	S2	107.0	97.00	82.50	204.0
	S3	107.0	97.00	84.00	204.0
Dec I	S1	112.0	113.0	91.00	214.0
	S2	112.0	113.0	91.00	214.0
	S3	112.0	113.0	91.00	214.0
Dec II	S1	118.0	129.0	86.00	198.0
	S2	118.0	129.0	86.00	198.0
	S3	118.0	129.0	86.00	198.0

Jan I-2013	S1	122.0	141.0	92.00	204.0
	S2	122.0	141.0	92.00	205.0
	S3	122.0	141.0	92.00	203.0
Jan II	S1	151.0	169.0	104.0	221.0
	S2	151.0	169.0	104.0	221.0
	S3	151.0	169.0	104.0	221.0
Feb I	S1	181.0	193.0	96.00	220.0
	S2	181.0	193.0	96.00	220.0
	S3	181.0	193.0	96.00	220.0
Feb II	S1	210.0	212.0	108.0	242.0
	S2	210.0	212.0	108.0	242.0
	S3	210.0	212.0	108.0	242.0
Mar I	S1	276.0	207.0	16.00	257.0
	S2	276.0	207.0	16.00	257.0
	S3	276.0	207.0	16.00	257.0
Mar II	S1	214.0	206.0	98.00	186.0
	S2	214.0	206.0	98.00	186.0
	S3	214.0	206.0	98.00	186.0
Apr I	S1	--	122.0	109.0	119.0
	S2	--	122.0	109.0	119.0
	S3	--	122.0	109.0	119.0
Apr II	S1	--	126.0	122.0	309.0
	S2	--	126.0	122.0	309.0
	S3	--	126.0	122.0	309.0

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b) Data on electrical conductivity (µmho/cm) of the four ponds during 2013-14

Month of sample collection	Sample replicates	Pond 1	Pond 2	Pond 3	Pond 4
Sep	S1	148.0	141.0	150.0	219.0
	S2	148.0	141.0	150.0	219.0
	S3	148.0	141.0	150.0	219.0
Oct	S1	132.5	144.5	146.5	265.0
	S2	132.0	144.1	146.5	265.0
	S3	133.0	145.0	146.5	265.0
Nov	S1	145.0	158.5	155.0	250.0
	S2	145.0	158.5	155.0	250.0
	S3	145.0	158.5	155.0	250.0
Dec	S1	139.0	162.0	172.0	321.0
	S2	139.0	162.0	172.0	321.0
	S3	139.0	162.0	172.0	321.0
Jan	S1	322.0	187.0	187.5	339.5
	S2	322.0	187.0	189.0	340.0
	S3	322.0	187.0	188.0	339.0
Feb	S1	383.0	312.0	264.0	432.0
	S2	383.0	312.0	264.0	432.0
	S3	383.0	312.0	264.0	432.0
Mar	S1	379.0	492.0	306.0	543.0
	S2	379.0	492.0	306.0	543.0
	S3	379.0	492.0	306.0	543.0
Apr	S1	280.0	48.00	277.0	568.0
	S2	280.0	48.00	277.0	568.0
	S3	280.0	48.00	277.0	568.0
May	S1	140.0	130.0	110.0	230.0
	S2	140.0	130.0	110.0	230.0
	S3	140.0	130.0	110.0	230.0
Jun	S1	132.0	112.0	98.20	184.0
	S2	132.0	112.0	98.00	184.0
	S3	132.0	112.0	98.50	184.0

ANNEXURE V

a) Data on chlorophyll *a* (µg/L) of the four ponds during 2012-13

Month of sample collection	Sample replicates	pond 1	pond 2	pond 3	pond 4
Sep I	S1	36.30	77.64	51.43	21.57
	S2	39.10	72.10	47.08	20.40
	S3	37.90	73.91	48.76	22.11
Sep II	S1	122.1	53.22	26.33	9.09
	S2	107.2	56.01	28.99	9.87
	S3	94.97	53.13	31.53	11.07
Oct I	S1	2.66	17.39	58.17	13.55
	S2	2.84	17.12	57.88	14.96
	S3	2.84	19.46	55.25	16.16
Oct II	S1	2.21	2.00	56.31	14.89
	S2	2.19	2.07	56.25	14.23
	S3	2.20	2.08	56.22	15.52
Nov I	S1	32.50	49.08	67.88	28.01
	S2	36.54	48.97	69.91	27.36
	S3	37.91	49.22	72.39	27.52
Nov II	S1	40.85	71.78	64.11	20.22
	S2	41.50	70.02	65.26	24.17
	S3	43.41	71.20	62.57	21.50
Dec I	S1	112.4	72.80	88.12	16.27
	S2	113.0	73.71	76.55	16.59
	S3	111.9	75.46	86.07	15.95
Dec II	S1	112.3	75.50	56.60	13.39
	S2	107.9	74.49	53.14	14.01
	S3	103.9	73.66	52.62	14.57
Jan I-2013	S1	72.44	6.63	54.77	2.78
	S2	69.92	6.90	56.21	1.020
	S3	71.54	6.93	51.35	2.170

Jan II	S1	312.1	33.10	14.83	14.34
	S2	329.7	32.68	16.72	15.29
	S3	280.6	35.17	17.41	15.19
Feb I	S1	273.2	69.87	33.23	172.1
	S2	261.5	68.40	34.71	173.6
	S3	260.0	71.31	30.25	171.3
Feb II	S1	104.8	46.53	56.04	19.78
	S2	99.87	40.76	53.62	20.17
	S3	108.5	53.92	53.21	20.77
Mar I	S1	275.3	44.91	19.80	52.44
	S2	268.6	46.38	19.77	54.28
	S3	279.9	47.07	19.80	51.23
Mar II	S1	57.44	147.9	87.88	31.06
	S2	58.61	144.5	83.21	32.57
	S3	59.36	155.9	97.50	34.56
Apr I	S1	--	66.71	57.44	39.52
	S2	--	59.66	57.23	36.66
	S3	--	55.28	57.14	38.66
Apr II	S1	--	85.55	4.70	43.32
	S2	--	86.09	4.69	42.17
	S3	--	84.71	4.80	39.76

b) Data on chlorophyll *a* (µg/L) of the four ponds during 2013-14

Month of sample collection	Sample replicates	Pond 1	Pond 2	Pond 3	Pond 4
Sep	S1	7.69	10.02	52.73	1.06
	S2	9.23	9.850	53.16	1.23
	S3	5.07	10.37	49.93	1.04
Oct	S1	15.89	72.56	63.21	13.17
	S2	16.13	73.19	59.70	13.76
	S3	15.05	73.73	61.86	13.45
Nov	S1	21.45	43.44	189.0	12.58
	S2	22.68	43.99	188.2	13.69
	S3	20.40	43.04	188.3	17.08
Dec	S1	88.30	64.12	47.93	38.71
	S2	65.21	65.05	52.16	34.93
	S3	89.91	58.87	51.62	39.22
Jan	S1	155.7	29.14	28.21	6.69
	S2	160.6	27.20	30.07	7.23
	S3	159.2	28.44	28.15	7.26
Feb	S1	21.62	269.7	56.14	47.01
	S2	27.69	283.2	51.26	54.53
	S3	20.38	267.0	55.65	52.42
Mar	S1	93.27	200.1	92.07	95.96
	S2	96.73	193.2	87.51	112.4
	S3	94.73	194.4	89.43	113.5
Apr	S1	79.16	190.8	95.42	10.63
	S2	78.27	186.4	96.73	9.98
	S3	71.35	186.8	95.04	10.89
May	S1	16.79	179.0	8.45	60.14
	S2	18.29	182.4	7.33	64.33
	S3	16.29	179.1	9.26	59.85
Jun	S1	10.22	37.43	17.61	35.20
	S2	9.88	34.60	17.35	29.78
	S3	10.23	36.72	18.41	38.79

ANNEXURE VI

Relative frequency of occurrence of phytoplankton species in **pond 1** during 2011-12, 2012-13 and 2013-14

Species	Relative frequency (annual mean)		
	2011-12	2012-13	2013-14
Cyanobacteria			
Anabaena constricta	--	--	0.68
Anabaena oryzae	--	--	0.06
Anabaena sp1	--	--	0.06
Anabaena volxii	--	0.08	--
Aphanocapsa koordersi	--	--	0.17
Aphanocapsa sp1	1.16	0.93	--
Aphanocapsa sp2	0.06	2.07	--
Chroococcus dispersus	0.05	--	0.71
Chroococcus sp1	0.17	1.43	--
Gloeocapsa sp1	2.26	--	--
Gloethece linearis	--	--	0.06
Lyngbya sp1	--	--	0.06
Merismopedia punctata	--	**2.96**	2.37
Microchaete elongata	--	--	0.35
Microcystis aeruginosa	--	0.07	--
Oscillatoria proteus	--	--	**2.94**
Peudanabaena catenata	**3.08**	--	1.04
Komvophoron schimidlei	--	--	0.06
Spirulina princeps	--	0.73	0.06
Synechococcus sp.	--	0.68	--
Synechocystis aquatis	--	0.05	--
Chlorophyta			
Acanthosphaera sp.	--	--	0.51
Ankistrodesmus convolutus	--	--	0.22
Ankistrodesmus falcatus	0.04	0.22	4.98
Ankistrodesmus nannoselene	0.46	--	--
Asterococcus limneticus	--	--	0.26
Characium sp.	--	--	0.19
Chlamydomonas elongatus	--	--	0.11

Chlamydomonas singulata	0.91	--	--
Chlamydomonas sp.	0.06	0.06	0.13
Chlorella ellipsoida	0.70	0.13	--
Chlorella saccharophilum	**10.67**	3.93	0.13
Chlorella vulgaris	0.08	2.14	--
Chlorococcum minutum	9.16	5.87	0.35
Chlorococcum sp1	0.19	0.18	4.25
Chlorococcum sp2	0.04	0.52	--
Coelastrum microsporum	--	0.95	--
Coelastrum sp.	--	0.05	0.30
Crucigenia fenestrata	--	0.08	0.09
Crucigenia quadrata	0.295	--	--
Cylindrocystis sp.	--	0.10	--
Dictyosphaerium ehrenbergianum	--	0.12	--
Eudorina elegans	0.04	0.40	0.18
Eudorina sp1	1.57	--	--
Golenkinia radiata	0.07	0.14	--
Kerato cocus succiccus	--	0.14	--
Klebsormidium sp.	0.07	--	0.32
Monoraphidium convolutum	0.85	0.28	0.15
Mesotaenium sp.	--	0.05	--
Micractinium pusillum	0.79	--	--
Microspora sp.	1.40	--	0.17
Monoraphidium circinale		1.06	0.44
Monoraphidium contortum	0.64	--	--
Monoraphidium griffithii		0.04	--
Monoraphidium sp1	1.48	0.09	--
Monoraphidium sp2	--	0.04	--
Mougeotia sp.	--	--	1.40
Nephrocytium agardhianum	--	--	0.64
Oedogonium sp.	--	0.10	0.28
Oocystis gigas	--		0.17
Palmellococcus sp.	--	0.07	--
Palmodictyon varium	--	--	0.06
Pandorina morum	0.73	--	0.39
Pediastrum duplex	--	**10.4**	--

Pediastrum gracillimum	0.04	--	--
Pediastrum subgranulatum	--	0.04	--
Pediastrum tetras	--	--	**7.59**
Planktonema lauterbornii	--	0.07	--
Planktosphaeria sp.	0.11	0.22	0.04
Quadrigula chodatii	0.27	--	--
Scenedesmus acuminatus	3.06	1.73	0.30
Scenedesmus arcuatus	0.12	--	--
Scenedesmus armatus	0.42	--	--
Scenedesmus aureus	0.72	0.06	--
Scenedesmus bernardii	0.17	--	--
Scenedesmus Bijuga var. alternans f. parvus	0.08	--	--
Scenedesmus Sp6	0.13	--	--
Scenedesmus denticulatus	0.08	0.04	--
Scenedesmus ecornis	0.08	0.04	--
Scenedesmus ellipticus	--	0.04	--
Scenedesmus irregularis	0.15	--	--
Scenedesmus obtusus	0.07	--	--
Scenedesmus quadricauda	3.92	4.92	6.78
Scenedesmus Sp1	--	--	1.06
Scenedesmus Sp2	--	3.82	--
Scenedesmus Sp3	0.49	1.96	--
Scenedesmus Sp4	0.04	0.09	--
Scenedesmus Sp5	0.08	0.09	--
Scenedesmus quadrispina	--	--	0.91
Schroederia indica	2.02	--	--
Schroederia sp.	1.58	1.13	--
Schroederia spiralis	1.53	--	--
Monoraphidiu minutum	--	0.12	1.43
Tedradesmus smithii	--	--	0.15
Tetmemorus sp.	1.00	--	--
Tetradesmus wisconinense		--	0.18
Tetraedron minimum	0.12	1.11	--
Tetraedron proteiforme	0.30	0.69	--
Tetraedron sp1	--	0.74	--
Tetraedron sp2	--	0.16	--

Tetraedron trosum	--	0.26	--
Tetranabaena socialis	--	0.53	--
Tetrastrum heteracanthum	--	0.40	--
Westella botryoides	--	0.16	--
Charophyta			
Closterium acerosum	--	**2.06**	--
Closterium dianae	--	--	1.25
Closterium kutenzingi	0.42	0.05	0.66
Closterium moniliferum		0.06	--
Closterium strigosum	**0.47**	--	--
Cosmarium corbula	--	--	0.15
Cosmarium cruciatum	0.07	0.02	0.30
Cosmarium subquadrans	--	--	0.06
Euastrum verrucosum	--	0.02	--
Spirogyra sp.	0.07	0.14	0.13
Staurastrum pinnatum	0.06	0.96	0.13
Euglenozoa			
Euglena acus	0.27	1.71	--
Euglena ehrenberg	0.06	--	--
Euglena gracilus	0.64	--	--
Euglena limnophyla	--	0.02	--
Euglena spirogyra	0.05	--	--
Euglena sp1	3.00	0.47	--
Euglena sp2	--	0.07	--
Euglena sp3	--	0.02	0.32
Lepocinclis fusiformis	1.61	3.36	
Lepocinclis globulus	**5.78**	**8.48**	1.51
Phacus chloroplastes	0.08	--	--
Phacus horridus	0.18	0.34	--
Phacus longicauda	--	--	0.97
Phacus pyrum	0.20	0.75	--
Phacus sp1	0.13	--	--
Phacus triqueter	1.88	0.04	--
Trachelomonas bacillifera	0.34	--	--
Trachelmonas volvocina	3.67	4.38	**20.2**
Trachelomonas hispida	1.32	0.71	0.99

Ochrophyta			
Acanthes sp.	--	0.04	--
Asterionella sp.	**2.50**	**--**	--
Chromulina nebulosa	--	1.74	12.8
Cocconeis placentula	--		0.13
Cymbella amphicephala	0.39	0.09	--
Desmogonium gracile	--	--	0.13
Fragilaria construens var. construens	0.12	--	0.69
Fragilaria sp1	--	--	0.30
Gomphonea abbreviatum	--	--	0.52
Gomphonema gracile	0.42	--	--
Melosira sp1	--	--	0.63
Melosira sp2	**2.50**	--	--
Navicula sp1	--	--	3.12
Navicula gregaria	0.37	1.01	2.63
Navicula sp2	--	--	0.13
Navicula sublinearis	0.05	--	--
Nitzschia gracilis	2.37	--	0.17
Nitzschia palea	1.35	**4.52**	**7.09**
Nitzschia sp1	--	**--**	0.51
Nitzschia sp2	--	**--**	0.30
Pinnularia interrupta	0.13	2.57	0.83
Tabellaria flocculoa	--	**--**	0.13
Cryptophyta			
Cryptomonas erosa	0.91	**--**	--
Cryptomonas ovata	--	--	0.26
Cryptomonas sp1	**14.09**	**10.64**	**1.49**
Mezozoa			
Gymnodinium sp.	**--**	**--**	0.06
Gyrodinium sp.	1.60	0.97	--

ANNEXURE VII

Relative frequency of occurrence of phytoplankton species in **pond 2** during 2011-12, 2012-13 and 2013-14

Species	Relative frequency (annual mean)		
	2011-12	2012-13	2013-14
Cyanobacteria			
Anabaena oryzae	--	--	0.055
Anabaena sp2	--	--	0.592
Aphanocapsa endophytica	0.113	--	--
Aphanocapsa sp1	3.483	1.885	--
Aphanocapsa hyalina	7.35	1.038	--
Aphanocapsa sp2	--	1.508	--
Aphanothece sp1	2.176	--	--
Aphanothece microscopica	--	1.21	0.112
Gloeothece rhodochlamys	1.268	0.12	0.71
Komvophoron schmidlei	--	--	0.129
Merismopedia punctata	0.45	--	0.114
Microcystis aeruginosa	**4.155**	6.141	3.637
Oscillatoria limosa	3.299	**5.328**	**5.776**
Oscillatoria sp1	--	--	2.67
Phormidium retzii	--	2.775	0.462
Phormidium sp1	--	--	0.076
Synechococcus sp.	--	0.336	--
Chlorophyta			
Actinastrum hantzschii	0.237	--	--
Ankistrodesmus acicularis	**9.57**	3.47	--
Ankistrodesmus falcatus	1.61	--	--
Chlamydomonas bacillus	0.066	--	--
Chlamydomonas sp.	--	0.12	--
Chlorella ellipsoida	0.167	--	--
Chlorella vulgaris	**8.675**	--	3.69
Chlorella saccharophilum	1.55	--	--
Chlorococcum chlorococcoides	2.62	--	--

Chlorococcum infusionum	2.21	**4.257**	0.166
Chlorococcum hypnosporum	0.17	--	--
Chlorococcum humicola	0.17	--	--
Crucigenia lauterbornii	0.349	--	--
Crucigenia tetrapedia	0.066	--	0.261
Desmococcus sp.	0.093	--	--
Desmodesmus sp1	0.148	--	--
Didymocystis bicellularis	0.085	--	--
Didymocystis inermis	--	0.176	--
Eudorina sp1	0.477	--	--
Golenkinia radiata	0.167	--	--
Gonium quadratum	--	--	0.112
Kirchineriella subsolita	--	--	0.038
Micractinium pusillum	--	--	0.449
Monoraphidium arcuatum	3.728	0.176	0.356
Monoraphidium sp1	0.556	0.90	0.491
Monoraphidium irregulare	1.44	0.931	0.708
Monoraphidium litorale	1.319	--	--
Monoraphidium contortum	--	--	11.17
Monoraphidium sp2	--	--	0.309
Mougaetia sp.	0.242	--	0.055
Oedogonium sp.	0.105	--	--
Oocystis elliptica	--	--	0.055
Pandorina morum	0.278	--	--
Planktosphaeria sp.	0.223	1.301	0.222
Quadrigula closteriodes	0.037	--	--
Scenedesmus acuminatus	0.306	--	--
Scenedesmus arcuatus	--	--	0.467
Scenedesmus armatus	0.272	--	--
Scenedesmus obtusus	--	--	8.096
Scenedesmus quadricauda	0.663	--	--
*Scenedesmus s*p1	1.534	1.39	0.112
Scenedesmus sp2	2.039	--	0.055

Scenedesmus sp3	0.163	--	--
Scenedesmus sp4	0.181	--	--
Scenedesmus sp5	0.038	--	--
Scenedesmus sp6	0.073	--	--
Schroederia sp.	--	--	0.26
Schroederia spiralis	0.738	--	--
Monoraphidium minutum	0.219	2.686	**20.35**
Stichococcus sp.	0.295	--	--
Tedradesmus smithii	--	--	0.331
Tedraedron trigonum var. longispinum	--	--	0.497
Tetmemorus sp.	0.579	--	--
Tetrabaena socialis	--	--	0.11
Tetradesmus wisconsirense	0.386	--	--
Tetraedron incus	0.148	1.35	--
Tetraedron limneticum	0.105	--	--
Tetraedron minimum	--	0.12	0.519
Tetraedron regulare	--	--	0.055
Tetraedron sp1	0.639	--	--
Tetraedron sp2	--	0.393	--
Tetraedron sp3	--	--	0.038
Tetraedron triangulare	0.595	--	--
Tetraedron tumidulum	0.066	--	0.11
Charophyta			
Centritractus belanophorns	0.369	--	--
Closterium gracile	--	--	0.11
Closterium moniliferum	--	1.94	--
Closterium sp1	--	--	0.11
Closterium sp2	--	--	0.531
Cosmarium difficile	--	--	0.055
Cosmarium pygmaeum	0.066	--	0.434
Euglenozoa			
Euglena ehrenbergii	0.185	--	--
Euglena pascheri	0.211	--	--

Euglena acus	0.205	--	0.26
Euglena elastica	0.075	--	--
Euglena polymorpha	0.066	--	--
Euglena sp1	0.103		--
Euglena sp2	0.667	--	--
Euglena sp3	--	--	0.514
Lepocinclis globulus	7.86	**7.10**	--
Phacus longicauda	0.105	--	--
Phacus sp2	--	--	0.076
Phacus tortuosus	0.76	1.06	--
Strombomonas sp.	0.198	--	--
Trachelomonas volvocina	**17.79**	**51.27**	**24.27**
Trachelomonas armata	--	--	0.336
Trachelomonas hispida	0.38	--	--
Trachelomonas bacillifera	--	0.09	--
Ochrophyta			
Cymbella amphicephala	--	--	0.055
Cymbella ventricosa	0.085	--	--
Eunotia curvata	--	--	0.101
Gomphonema olivaceum	--	--	0.129
Gomphonema sp.	--	--	0.885
Melosira granulata	0.105	--	--
Nitzschia acicularis	0.082	--	0.055
Navicula gregaria	--	--	0.055
Nitzschia obtusa	--	--	0.055
Nitzschia palea	0.995	0.982	**6.153**
Cryptophyta			
Chroomonas sp.	0.066	--	0.101
Cryptomonas borealis		--	0.333
Cryptomonas sp1	1.428	0.528	--
Cryptomonas sp2	--	--	1.692
Mezozoa			
Gyrodinium sp.	0.085	--	--

ANNEXURE VIII

Relative frequency of occurrence of phytoplankton species in **pond 3** during 2011-12, 2012-13 and 2013-14

Species	Relative frequency (annual mean)		
	2011-12	2012-13	2013-14
Cyanobacteria			
Anabaena sp3	--	0.381	--
Aphanocapsa delicatissima	--	3.156	--
Aphanocapsa hyalina	2.65	--	--
Aphanothece hegewaldii	0.13	--	--
Aphanothece sp1	5.0	0.98	--
Gloeocapsa sp1	--	0.049	0.221
Gloeothece rhodochlamys	0.13	--	--
Gloeothece sp1	--	2.525	--
Gloeothece sp2	--	1.318	--
Lyngbya sp2	0.13	0.049	--
Microcystis aeruginosa	2.65	3.017	--
Oscillatoria obscura	--	0.297	--
Oscillatoria proteus	--	--	2.00
Oscillatoria sp2	--	--	0.25
Spirulina princeps	--	0.049	--
Chlorophyta			
Chlamydomonas elongatus	--	1.37	--
Chlamydomonas sp.	--	0.993	--
Chlorella sacharophilium	--	--	4.75
Chlorella vulgaris	**5.5**	--	--
Chlorococcum echinozygotum	--	--	**7.13**
Chlorococcum infusionum	--	--	**21.51**
Chlorococcum sp1	2.10	0.635	--
Chlorococcum sp2	2.39	1.523	--
Coenochloris sp.	--	0.049	--
Didymocystis inermis	--	0.391	--
Didymocystis planktonia	--	0.495	--

Eudorina illinoisensis	4.75	0.899	0.412
Geminella sp.	--	0.175	--
Golenkinia radiata	--	0.079	--
Monoraphidium convolutum	0.98	--	0.078
Monoraphidium contortum	0.125	--	2.631
Monoraphidium irregulare	--	--	0.156
Monoraphidium tortile	--	0.176	--
Palmodictyon varium	--	--	0.75
Pandorina morum	1.24	--	2.534
Planktosphaeria sp.	0.35	0.175	--
Schroderia setigera	0.10	--	0.221
Stichococcus sp.	0.35	--	--
Stigeoclonium sp.	--	0.049	--
Tedradesmus smithii	--	--	0.166
Tetraedron sp1	--	0.457	--
Tetraedron sp2	--	0.063	--
Tribonema sp.	0.25	--	--
Leptosira sp.	3.47	--	--
Charophyta			
Closterium leibleinii	--	0.75	--
Closterium moniliferum	0.25	--	--
Closterium gracile	0.38	--	--
Closterium sp3	--	0.049	--
Euglenozoa			
Euglena acus	3.09	0.525	--
Euglena sp3	5.36	0.153	--
Euglena sp4	0.59	2.678	--
Euglena sp5	1.43	0.201	--
Euglena sp6	--	0.407	0.548
Euglena spirogyra	0.27	--	--
Euglena viridis	1.43	--	--
Euglena proxima	3.26	3.895	1.466
Lepocinclis fusiformis	6.30	5.18	--
Lepocinclis globulus	**7.84**	**30.71**	**22.82**

Lepocinclis texta	1.47	--	1.3
Phacus curvicauda	--	5.17	--
Phacus horridus	0.17	--	--
Phacus sp2	0.77	3.19	0.49
Phacus triqueter	2.71	0.528	3.86
Strombomonas sp.	2.52	0.134	--
Trachelomonas volvocina	**10.6**	**11.06**	2.254
Trachelomonas armata	--	--	0.435
Trachelomonas hispida	1.01	--	1.101
Trachelomonas bacillifera	--	0.264	--
Ochrophyta			
Anthophysa sp.	--	--	2.35
Cymbella gracilis	--	--	0.556
Cymbella aspera	--	--	4.53
Fragilaria construens var. construens	--	0.076	1.573
Gomphonema sp.	1.60	--	0.217
Gyrosigma scalproides	0.33	--	
Melosira granulata	0.13	--	2.04
Navicula sp1	--	--	0.997
Navicula gregaria	0.51	--	0.185
Nitzschia palea	3.00	**10.85**	0.435
Pinnularia biceps	0.13	--	--
Pinnularia braunii	--	0.278	--
Pinnularia interrupta	0.55	--	6.671
Cryptophyta			
Cryptomonas erosa	**7.14**	--	--
Cryptomonas sp1	--	1.941	0.234
Cryptomonas sp2	4.65	0.488	0.756
Mezozoa			
Gymnodinium sp.	--	1.188	--
Gyrodinium sp.	0.49	0.881	1.655

ANNEXURE IX

Relative frequency of occurrence of phytoplankton species in **pond 4** during 2011-12, 2012-13 and 2013-14

Species	Relative frequency (annual mean)		
	2011-12	2012-13	2013-14
Cyanobacteria			
Aphanocapsa endophytica	--	0.19	--
Aphanocapsa koordersi	0.27	--	--
Aphanocapsa sp1	1.08	--	--
Aphanothece minutissima	--	--	3.33
Coelosphaerium sp.	1.62	--	--
Aphanocapsa hyalina	**6.07**	**5.66**	--
Oscillatoria princeps	--	1.93	--
Oscillatoria proteus	--	--	**6.67**
Phormidium sp2	--	1.07	--
Chlorophyta			
Actinastrum sp.	--	0.23	--
Ankistrodesmus convolutus	--	**9.76**	6.67
Chlamydomonas elongatus	--	0.16	--
Chlamydomonas sp.	1.89	--	--
Chlorella ellipsoida	--	--	0.65
Chlorella saccharophila	4.00	--	--
Chlorococcum infusionum	**4.27**	2.38	--
Chlorococcum sp2	0.42	--	--
Chlorococcum sp3	2.50	--	--
Chlorococcum echinozygotum	--	--	7.24
Coenochloris sp.	--	0.09	--
Didymocystis inermis	0.24	--	--
Eudorina sp1	--	0.29	0.323
Golenkinia sp1	--	0.42	--
Golenkinia sp2	--	0.83	--
Monoraphidium arcuatum	2.62	0.11	**9.96**

Monoraphidium griffithi	0.60	0.17	6.66
Monoraphidium sp1	1.74	0.09	--
Monoraphidium tortile	3.16	0.95	--
Oedogonium sp.	1.55	--	--
Oocystis sp.	0.28	--	--
Planktosphaeria sp.	0.27	--	--
Quadrigula sp.	--	0.17	--
Scenedesmus sp2	0.48	0.28	--
Scenedesmus quadricauda	--	1.09	--
Scenedesmus sp1	0.82	0.48	--
Schroederia sp.	0.11	0.33	--
Monoraphidium minutum	0.14	1.35	--
Selenastrum gracile	--	1.54	--
Tetraedron incus	1.44	1.16	--
Tetraedron regulare var. incus	0.27	--	--
Tetraedron sp4	2.23	--	--
Tetraedron triangulare	0.27	0.17	--
Tetraedron trigonium	--	2.67	--
Tetrastrum sp.	--	0.17	--
Charophyta			
Closterium strigosum	--	0.23	--
Closterium kutenzingii	--	0.64	--
Closterium acerosum	--	0.23	--
Closterium moniliferum	0.81	--	--
Staurastrum sp.	--	4.51	--
Euglenozoa			
Euglena sp2	1.09	1.34	--
Euglena acus	0. 71	--	--
Euglena sp5	--	0.33	--
Euglena polymorpha	--	4.35	--
Euglena sp1	0.24	--	--
Euglena sp3	0.18	1.74	--

Lepocinclis globulus	**4.64**	4.03	--
Lepocinclis tetra	0.91	0.94	--
Phacus longicauda	3.62	1.25	5.33
Phacus anacoelus	1.99	0.65	--
Phacus triquieter	--	**11.95**	--
Strombomonas sp.	0.14	--	--
Trachelomonas volvocina	23.0	11.79	**27.22**
Trachelomonas hispida	1.18	0.63	--
Trachelomonas bacillifera	0.29	--	--
Ochrophyta			
Chromulina nebulosa	--	--	**10.0**
Cymbella gracilis	--	--	6.66
Cymbella sp1	0.81	0.05	--
Fragilaria sp1	0.14	--	--
Melosira granulata	**1.62**	--	**10.0**
Navicula gregaria	--	--	--
Navicula sp3	0.29	--	--
Neidium sp.	0.24	--	--
Nitzschia dissipata	2.31	--	--
Pinnularia interrupta	--	1.44	--
Cryptophyta			
Chroomonas sp.	--	**7.09**	--
Cryptomonas erosa	--	0.20	--
Cryptomonas sp1	--	0.71	--
Cryptomonas sp2	**10.16**	--	--
Synura sp.	--	1.49	--
Mezozoa			
Gymnodinium sp.	--	**1.41**	--
Gyrodinium instriatum	--	0.17	--
Gyrodinium sp.	**7.31**	--	--

ANNEXURE X

a) PCA biplot loadings of Pond 1 during 2011-12

	C1	C2
Cyanobacteria	**-0.618**	0.133
Chlorophyta	**0.750**	-0.539
Charophyta	0.040	0.305
Cryptophyta	**-0.865**	-0.140
Euglenozoa	0.395	**0.754**
Ochrophyta	**0.905**	0.128
Mezozoa	-0.168	0.454
Chl *a*	**0.698**	0.441
pH	**0.790**	0.498
Temp	-0.086	**0.572**
TP	0.524	**0.710**
NO_3-N	0.332	**0.787**

b) PCA biplot loadings of Pond 1 during 2012-13

	C1	C2
Cyanobacteria	**-0.773**	-0.277
Chlorophyta	**0.969**	0.034
Charophyta	0.395	**-0.637**
Cryptophyta	-0.103	0.384
Euglenozoa	-0.431	0.414
Ochrophyta	-0.041	0.004
Mezozoa	0.273	**0.878**
Chl *a*	**0.800**	-0.029
pH	**0.665**	-0.167
EC	**0.906**	-0.245

c) PCA biplot loadings of Pond 1 during 2013-14

	C1	C2
Cyanobacteria	**0.744**	-0.308
Chlorophyta	-0.056	**0.966**
Charophyta	**0.940**	-0.237
Cryptophyta	**0.662**	0.123
Euglenozoa	**-0.815**	-0.358
Ochrophyta	0.415	0.044
Mezozoa	0.455	-0.061
Chl *a*	-0.091	**0.677**
pH	0.286	**0.738**
EC	0.018	**0.978**

ANNEXURE XI

a) PCA biplot loadings of Pond 2 during 2011-12

	C1	**C2**
Cyanobacteria	**-0.691**	-0.209
Chlorophyta	0.422	0.284
Charophyta	**-0.582**	0.364
Cryptophyta	-0.116	**0.641**
Euglenozoa	0.193	0.280
Ochrophyta	**-0.566**	-0.019
Mezozoa	0.031	**-0.693**
Chl *a*	0.398	**0.531**
pH	-0.332	**0.663**
Temp	0.367	**0.791**
TP	**0.823**	-0.249
NO_3-N	**0.668**	0.096

b) PCA biplot loadings of Pond 2 during 2012-13

	C1	**C2**
Cyanobacteria	**-0.894**	0.180
Chlorophyta	0.257	**-0.822**
Charophyta	0.000	0.000
Cryptophyta	0.242	-0.003
Euglenozoa	**0.749**	0.470
Ochrophyta	**-0.892**	0.091
Mezozoa	0.000	0.000
Chl *a*	0.121	0.163
pH	0.088	**0.780**
EC	0.443	**0.677**

c) PCA biplot loadings of Pond 2 during 2013-14

	C1	**C2**
Cyanobacteria	-0.258	**0.767**
Chlorophyta	0.571	0.172
Charophyta	**0.888**	0.073
Cryptophyta	-0.312	-0.127
Euglenozoa	**0.635**	-0.375
Ochrophyta	0.232	**0.746**
Mezozoa	0.000	0.000
Chl *a*	0.012	-0.012
pH	0.129	**-0.796**
EC	-0.421	-0.192

ANNEXURE XII

a) PCA biplot loadings of Pond 3 during 2011-12

	C1	**C2**
Cyanobacteria	**0.790**	-0.354
Chlorophyta	-0.121	0.330
Charophyta	0.290	**0.744**
Cryptophyta	0.386	**-0.577**
Euglenozoa	**-0.807**	-0.120
Ochrophyta	**0.779**	0.486
Mezozoa	0.067	-0.427
Chl *a*	**0.819**	0.241
pH	**-0.771**	**0.536**
Temp	0.092	0.374
TP	-0.273	-0.463
NO_3-N	0.025	0.297

b) PCA biplot loadings of Pond 3 during 2012-13

	C1	**C2**
Cyanobacteria	0.008	-0.170
Chlorophyta	-0.362	**-0.702**
Charophyta	**0.949**	-0.086
Cryptophyta	**0.686**	0.223
Euglenozoa	-0.375	0.121
Ochrophyta	0.031	**0.527**
Mezozoa	**0.949**	-0.086
Chl *a*	-0.276	-0.013
pH	-0.026	0.233
EC	-0.293	**0.786**

c) PCA biplot loadings of Pond 3 during 2013-14

	C1	**C2**
Cyanobacteria	0.058	-0.061
Chlorophyta	**0.733**	0.240
Charophyta	0.000	0.000
Cryptophyta	-0.131	0.093
Euglenozoa	**0.711**	-0.169
Ochrophyta	-0.266	**0.852**
Mezozoa	**0.809**	-0.186
Chl *a*	0.317	**0.878**
pH	**0.923**	-0.047
EC	-0.327	**0.823**

ANNEXURE XIII

a) PCA biplot loadings of Pond 4 during 2011-12

	C1	C2
Cyanobacteria	**-0.694**	-0.265
Chlorophyta	**-0.513**	-0.028
Charophyta	0.365	**0.533**
Cryptophyta	**-0.687**	0.203
Euglenozoa	**0.909**	-0.127
Ochrophyta	0.241	**0.875**
Mezozoa	0.000	0.000
Chl *a*	**0.576**	**-0.601**
pH	**0.856**	0.258
Temp	**0.754**	0.049
TP	0.253	**-0.898**
NO_3-N	0.069	0.089

b) PCA biplot loadings of Pond 4 during 2012-13

	C1	C2
Cyanobacteria	-0.171	0.305
Chlorophyta	0.198	0.176
Charophyta	0.256	-0.295
Cryptophyta	-0.007	**0.843**
Euglenozoa	0.172	**-0.882**
Ochrophyta	0.296	0.115
Mezozoa	**0.670**	0.550
Chl *a*	**0.533**	-0.245
pH	**0.716**	-0.058
EC	**0.879**	-0.131

c) PCA biplot loadings of Pond 4 during 2013-14

	C1	C2
Cyanobacteria	**-0.818**	0.041
Chlorophyta	-0.021	**0.881**
Charophyta	0.000	0.000
Cryptophyta	0.000	0.000
Euglenozoa	**0.881**	0.127
Ochrophyta	-0.577	**-0.695**
Mezozoa	0.000	0.000
Chl *a*	**0.745**	0.258
pH	**0.729**	-0.320
EC	0.001	0.113

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Publications

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A survey of algal blooms in the ponds of Pallippuram, Kerala, India

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ABSTRACT

A survey of ponds in the Pallippuam Panchayath of Cherthala taluk, Kerala was undertaken from October 2010 to May 2011. Out of the 873 ponds surveyed 66% are unused, while 33% are used for domestic purpose other than drinking and irrigation; 11 ponds are used as drinking water source. Among the unused ponds 48 had algal blooms comprising species of Cyanophyta and Charophyta. They were observed as scums or mat on the surface of the ponds.

Keywords: Pallippuram Panchayath, algal bloom, ponds, Cyanophyta, Charophyta.

1. Introduction

The algae and Cyanobacteria comprising the phytoplankton are the first link in the aquatic food web as primary producers. Their presence in the water is often unnoticed as they are tiny microscopic organisms. Under favorable environmental conditions such as elevated nutrient concentration, warm temperature, shallow and slow moving water, the algal growth is stimulated in the water bodies that will finally result in the formation of algal blooms (Wetzel, 2001). Anthropogenic inputs can alter the algal community such that the health of an ecosystem may be reflected in the algal community and diversity (Lowe and Pan, 1996). Though algal blooms are natural phenomenon, and have occurred throughout the recorded history, recent studies from around the world indicate that they have increased in frequency and geographic distribution over the past few decades (Rejmenkova et al., 2011, Winter et al., 2011).

The lakes have received much attention in ecological studies in relation to nutrient enrichment and algal blooms. However, the domestic land excavated ponds, though small in size, but large in numbers in certain regions are among the most human influenced systems, as well as most vulnerable. These ponds are important as water sources for drinking and irrigation in rural areas. The water quality of the domestic ponds is influenced by the land use practices in the immediate neighbourhood. According to Akasaka et al (2010) macrophyte diversity and water quality of 55 ponds in western Japan were related to land use and morphometric variables. Soni and Bhatt (2008) have described the degradation of an urban pond in Gujarath, India due to sewage disposal. The pond has become unfit for use due to proliferating algae, macrophyte and pathogens. Similar studies on changing water quality of ponds in India have been reported by many authors (Bhuiyan and Gupta, 2007, Upadhyay et al., 2010).

In Kerala state, located at the south west coast of India, village ponds had been the sole source of drinking water along the coastal regions a few decades ago. Continuous maintenance of these ponds through traditional methods ensured the water quality. As

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population increased and urbanization set in many of these ponds were reclaimed for alternate use. The rest of the ponds were neglected as and when public water supply became accessible. Considering that ponds are important freshwater ecosystems and abode of rich biodiversity, the need for their conservation is recognised. Therefore this study is undertaken in 'Pallippuram' a typical coastal village of Kerala which has high density of domestic ponds. The present investigation is part of a study on current state of the ponds, their scope of restoration and utilization. The results presented in this paper are that of the preliminary survey on the status of these ponds.

2. Materials and methods

2.1 Study area

The study area is Pallippuram Panchayath a village situated in the Alappuzha district of Kerala state located in the South West coast of India at $9^0 45' 20''$N and $76^0 21' 39''$ E. It is an administrative entity that is part of an island in the Vembanadu Estuary bounded in the east, west and south by the estuary. The northern side is contiguous with the rest of the island (Fig.1 and Fig.2). The region has tropical monsoon climate with a mean annual temperature of 26.5^0 C (minimum18^0 C in December; maximum 35^0 C in April) and mean annual precipitation of 2500mm. The Panchayath has a population of 27307 in an area of 25.53 km^2as per census of 2001. There are 6202 households. The predominant land use includes paddy fields, coconut gardens and residential. The drinking water source in the village was traditionally ponds. As the population increased and lifestyles changed, there occurred a shift to piped water supplies and wells. As a result, the once prevalent ponds were largely neglected or reclaimed. It is in this context that a survey of those existing ponds was undertaken to provide the primary data on the state of these ponds so as to devise steps to conserve them as clean freshwater sources.

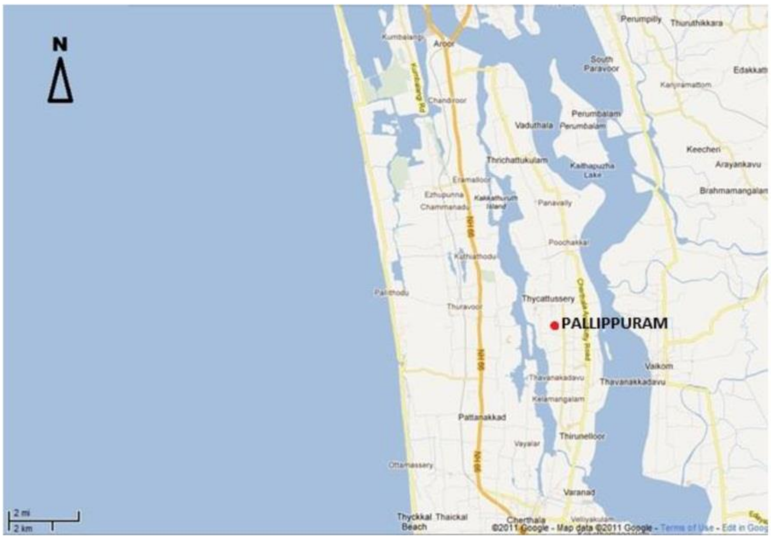


Figure 1: Google map of location of Pallippuram

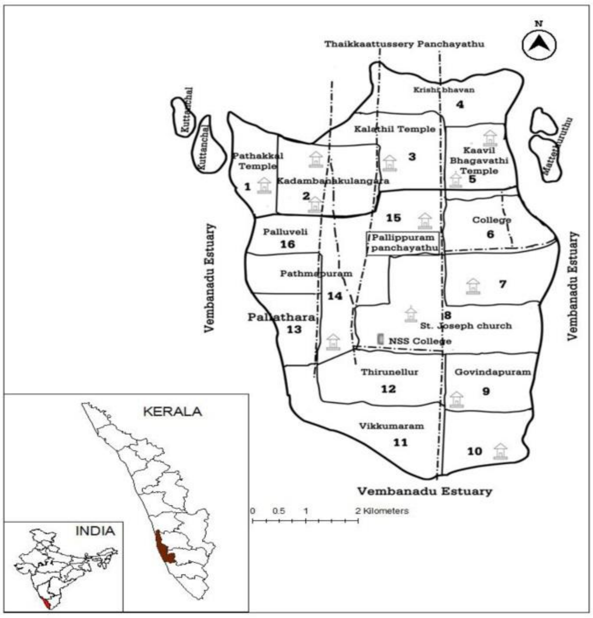


Figure 2: Map of Pallippuram Panchayath showing 17 wards (ward no. 17 is recently formed from ward no. 15 and 16)

2.2 Method of study

The Pallippuram Panchayath which is a local body of the civil administration is divided into17 wards or administrative units. The survey involved collecting data of ponds in each of these 17 wards separately and then compiling it. The period of survey was October 2010 to May 2011. The preliminary survey charted the total number of ponds in each ward, their area, type of use, occurrence of algal blooms, and other aquatic vegetation.

In the second stage of the survey, algal blooms were collected from 32 ponds selected at random; brought to the laboratory, and observed under microscope. The algal blooms were identified based on the existing literature (Desikachary , 1959 , Guiry and Guiry, 2011).

3. Results

A total number of 873 ponds were recorded in the Panchayath during this survey. The area of these ponds ranged from 12 m^2 to 300m^2. The detailed survey results for each ward are given in Table1. It is found that 66% of these ponds are out of use. Eleven ponds out of the fifty one in Ward1 are used for drinking purpose; 33% of all ponds in the Panchayath are used either for irrigation or other purpose such as bathing and washing clothes. Algal blooms were observed in 48 ponds. The blooms appeared as green or blue green turbidity, floating scum, or as thick blue-green floating mats. Submerged Aquatic Vegetation (SAV) occurred in 19 ponds. The SAV comprised of *Vallisneria sp., Hydrilla verticillata and Ceratophyllum* *submersum.* Floating hydrophytes were *Lemna minor, Pistia stratiotes, Eichhornia crassipes, Salvinia molesta and Azolla pinnata* (Fig.3).

Table 1: Survey results of ponds in Pallippuram Panchayath

Ward No.	No. of ponds	Area(m^2)	Type of use			No of ponds with		
			Drinking	Irrigation and other uses*	Unused	Algal bloom	SAV*	Floating hydrophytes
1	51	15-176	11	12	28	1	1	33
2	48	19-153	Nil	19	29	2	2	35
3	120	28-153	Nil	50	70	13	8	84
4	94	19-176	Nil	31	63	7	2	76
5	41	38-300	Nil	12	29	2	-	17
6	57	12-153	Nil	22	35	3	2	35
7	40	19-132	Nil	7	33	3	-	25
8	27	19-132	Nil	6	21	2	-	20
9	46	19-176	Nil	14	32	1	1	37
10	60	12-176	Nil	28	32	-	1	45
11	50	12-176	Nil	17	33	3	-	36
12	20	12-153	Nil	8	12	1	-	14
13	39	19-176	Nil	9	30	1	-	33
14	32	19-176	Nil	8	24	1	-	16
15	82	12-176	Nil	25	57	4	1	53
16	41	12-176	Nil	12	29	2	1	24
17	25	38-153	Nil	6	19	2	-	15

* Irrigation, bathing and washing * Submerged Aquatic Vegetation

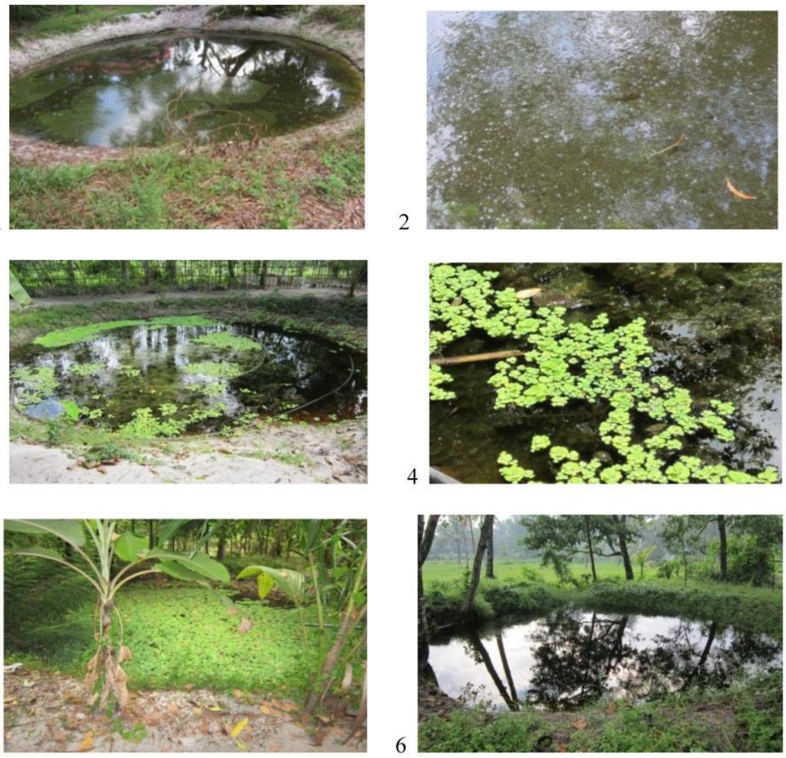


Figure 3: Images of ponds

(Fig.3.1 and3.2 pond with algal bloom, Fig.3.3 and3.4 Pond with floating and submerged vegetation, Fig.3.5 Pond covered completely with *Pistia*, Fig.3.6 A Clean pond under domestic use)

Out of the thirty two ponds sampled for algal bloom, six ponds had blooms of Charophyta, and the rest of the ponds had Cyanophyta. The blooms were mostly of filamentous algae that formed thick floating surface scum or mat (Table 2).

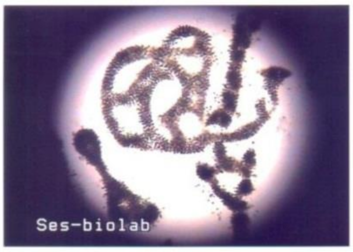
The Charophyta was represented by *Spirogyra* sp., *Klebsormidium* sp., and *Mougeotia scalaris*. *Spirogyra* bloom occurred in four ponds where as *Klebsormidium* and *Mougeotia* were present in one pond each. Blue-green algal bloom occurred in the rest of the twenty six ponds examined.

Oscillatoria occurred in nineteen ponds represented by two species. Blooms of *Microcystis aeruginosa* occurred in three ponds. The species spectrum of the bloom is presented in Fig.4 and 5. Co-existence of hydrophytes and algal bloom was observed in certain ponds.

Table 2: Algal blooms in ponds of Pallippuram Panchayath

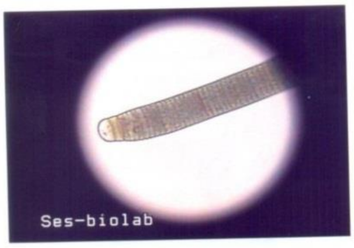
Ward No.	Pond No.	Algal species observed								
		1	2	3	4	5	6	7	8	9
3	1	-	+	-	-	-	-	-	-	-
	29	+	-	-	-	-	-	-	-	-
	38	-	-	-	-	-	-	+	-	-
	52	-	-	-	-	-	-	-	+	-
	56	-	+	-	-	-	-	-	-	-
	58	-	-	-	-	-	-	-	-	-
	65	+	-	-	-	-	-	-	-	-
	83	-	+	-	-	-	-	-	-	-
	106	-	-	-	-	-	-	+	-	-
	108	-	-	+	-	+	-	-	-	-
	112	-	+	-	-	-	-	-	-	-
	118	-	-	-	+	-	-	-	-	-
	120	-	-	+	-	-	-	-	-	-
4	10	-	-	-	-	-	-	-	-	+
	11	-	+	-	-	-	-	-	-	-
	26	-	+	-	-	-	-	-	-	-
	31	-	-	+	-	-	-	-	-	-
	33	-	+	-	-	-	-	-	-	-
	34	-	+	-	-	-	-	+	-	-
	35	-	-	-	-	-	+	-	-	-
	36	-	-	+	-	-	-	-	-	-
	49	-	+	-	-	-	-	-	-	-
	60	-	+	-	-	-	-	-	-	-
5	27	-	-	-	-	-	+	-	-	-
	39	-	+	-	-	-	-	-	-	-
6	25	-	-	-	-	-	-	-	-	-
	26	-	+	-	-	-	-	-	-	-
	27	-	-	+	-	-	-	-	-	-
	28	-	-	-	-	-	+	-	-	-
16	8	+	-	-	-	-	-	-	-	-
17	6	-	-	+	+	-	-	-	-	-
	12	+	+		-	-	-	-	-	-

(1.Spirogyra sp. 2. Oscillatoria princeps 3. Oscillatoria subbrevis 4. Phormidium tenue 5. Anabaena sp 6. Microcystis aeruginosa 7. Coelosphaerium kuetzingianum 8.Mougeotia scalaris 9.Klebsormidium sp.)

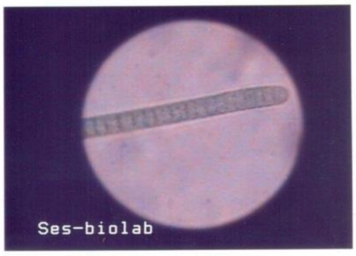


Microcystis aeruginosa-100x

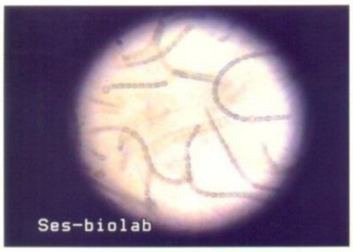
Coelosphaerium kuetzingianum -1000x



Oscillatoria princeps-400x



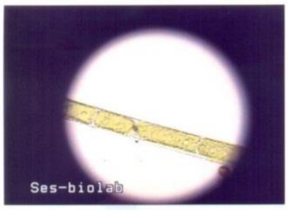
Oscillatoria subbrevis - 400x



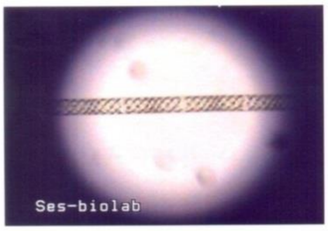
Anabaena sp. - 400x

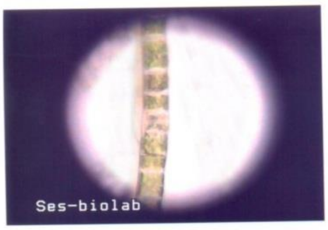
Figure 4: Species spectrum of algae

Phormidium tenue - 1000 x



Mougeotia scalaris – 400 xs





Spirogyra s. - 100x *Klebsormidium sp.- 400x*

Figure 5: Species spectrum of algal bloom

4. Discussion and conclusion

The present survey has listed the number of ponds existing in the 'Pallippuram Panchayath', their status of use, and indirectly indicated the water quality. As 66% of the ponds are now unused, it is evident that they are not essential for the community from the utilitarian point of view, and therefore grossly neglected.

The ponds that are used for drinking purpose are maintained through traditional methods with the funds provided by the civil authority. The domestic use includes mainly washing of clothes. Therefore phosphates from detergents could be a strong reason for induction of algal blooms. The region has paddy fields; both cultivated and uncultivated which could be a source of fertilizer run off. Effluents from the residential areas and the faulty sanitation systems can likely contribute excess organic matter, and consequent nutrient enrichment in the ponds. According to Akasaka et al. (2010) the land use pattern around the pond has direct effect on water quality and aquatic vegetation. The emergence of *Microcystis aeruginosa* bloom in kandy lake, Srilanka has been explained in terms of N-enrichment mainly by ammonium-N, and high turnover rates of dissolved phosphorus(Silva, 2003). Ahmed et al. (2007) relates the periodic cyanobacteria blooms in an urban river to increased dissolved organic nutrients, long sunshine hours and favorable water temperature. Species of *Oscillatoria* are reported to produce hepatotoxic microcystins (Ahmed et al., 2010). Welker et al. (2005) detected microcystins in thirty nine ponds related to occurrence of *Microcystis, Planktothrix* and *Anabaena*. The observation of potentially toxic genera of *Microcystis* and *Oscillatoria* in the present study is of concern.

Dense growth of three species of Charophyta were observed in six ponds in this survey. Charophytes are generally recognized as indicators of clean water ecosystems and they prefer hard alkaline waters rich in calcium. However Charophytes may persists under moderate fertility and turbidity (Klosowski et al., 2006). The survey has revealed the need for conservation, and the scale of restoration to be undertaken.

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