Dedicated
to the sweet memories of
my
beloved parents

Department of Ecology and Environmental Science
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ASSAM UNIVERSITY, SILCHAR
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DECLARATION

I, **Pampi Sarmah**, (Ph.D Registration No.-**Ph.D/1726/11** dated **15/09/2011**), hereby declare that the subject matter of the thesis entitled "**Algal colonization on polythenes and evaluation of biodegradation potentials**" is the record of work done by me and that the contents of this thesis did not formed the basis for the award of any degree to me or to anybody else to the best of my knowledge. The thesis has not been submitted in any other University/ Institute.

Assam University, Silchar **Pampi Sarmah**

Date: 12/09/2018

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Silchar, 12 September, 2018 Pampi Sarmah

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ALGAL COLONIZATION ON POLYTHENES AND EVALUATION OF BIODEGRADATION POTENTIALS

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

By

PAMPI SARMAH

Ph.D Registration No. - Ph. D/1726/11 Dated 15.09.11

To

DEPARTMENT OF ECOLOGY AND ENVIRONMENTAL SCIENCE
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2018

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CERTIFICATE

Certified that the thesis entitled "**Algal colonization on polythenes and evaluation of biodegradation potentials**" submitted by **Pampi Sarmah**, bearing Registration No.-Ph.D/1726/2011 dated 15/09/2011 for the award of the **Degree of Doctor of Philosophy in Ecology and Environmental Science** is the outcome of a bonafide research work. This work has not been submitted previously for any other degree of this or any other university. It is further certified that the candidate has complied with all the formalities as per the requirements of Assam University. I recommend that the thesis may be placed before the examiners for consideration of award of the degree of this University.

Prof. Jayashree Rout
Supervisor
Department of Ecology and Environmental Science

Abstract

Algae are a group of aquatic photosynthetic organisms which are integral part of an ecosystem as they serve as primary producers. Due to their delicate sensitivity to the environment they are also known to act as bioindicator of an ecosystem. Besides aqueous habitats, algae are found in non-aqueous habitats too. The non-aqueous habitat include a variety of terrestrial habitats, including soils, rocks, caves and also living animals and plants. They are known to colonize on submerged polythenes in sewage water and domestic solid waste dumping site. These submerged polythenes gets degraded to some extent into small pieces by bacterial and algal colonization and released into the environment during rain and dry season. The study area Silchar town, the headquarter of Cachar district is located in the southern part of state of Assam in India. It is the second largest city of the state in terms of population and municipal area in the state. Due to lack of inadequate regulations and monitoring of polythene carry bags usage, used polythene bags are generally disposed off indiscriminately. Thrown into landfills or water bodies, such polythenes constitute the principal source of the town municipal solid waste. During monsoon and post-monsoon season, polythene carry bags are found colonized by algae. Study of growth of algae and their colonisation on such polythene substrata are important in the context of sustainable waste management. Set in this backdrop, it is proposed to study the algal colonisation on polythenes from sewage water and solid wastes and their biodegradation potential. A total of 122 algal species were found to colonize on the submerged polythene surface in sewage water and domestic solid waste dumping site. The genus, *Oscillatoria* was found to be the largest genus with 29 species colonizing on the polythene surface. Algal species belonging to Cyanophyceae (57), Chlorophyceae (25), and Bacillariophyceae (38) and Euglenophyceae (2) were found to colonize on the polythene surface. Based on collection, a total 31 species of algae were isolated from the polythene surface. *Chlorella ellipsoidea* was the only green alga isolated. The cyanobacterial species, *Anabaena* (5), *Calothrix* (5), *Cylindrospermum* (2), *Lyngbya* (2), *Nostoc* (5), *Oscillatoria* (4), *Phormidium* (1), *Westiellopsis* (2), *Fischerella* (1), *Anabaenopsis* (1), and *Aphanothece* (1) were also isolated based on collection from the polythene surface. The species *Calothrix fusca*

and *Calothrix parietana* were found to be rich in carbohydrate and *Oscillatoria limosa* was found to be rich in protein, carotenoid and phycobiliproteins. The green alga, *Chlorella ellipsoidea* was found to be rich in lipid. The cyanobacterial species, *Oscillatoria limosa* was found to be rich in enzymatic and non-enzymatic antioxidants. Extracellular polymeric substances (EPS) was found to be maximum in *Anabaenopsis arnoldii* and minimum in *Nostoc linckia*. Besides biochemical analysis, domestic sewage water collected from Silchar town, Assam (India) has been used as a growth media in the present study for the cultivation of three selected species, *Chlorella ellipsoidea*, *Oscillatoria subbrevis* and *Nostoc carneum*. The concentration level of nitrate, phosphate, ammonia and total dissolved solid got significantly reduced at post-stationary phase in the sewage water media. An increased level of dissolved oxygen were observed on 30 days of incubation of these algae. Biochemical constituents of algal species cultivated in sewage water increased by 2-3 folds compared to control. Total lipids increased by 3 folds in domestic sewage water grown algae. The enzymatic and non-enzymatic antioxidants increased by 2-3 folds. Another aspect that drew our attention was the possibility of using some of these species for polythene biodegradation. Five cyanobacterial species, *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum* and *Cylindrospermum muscicola* were employed for biodegradation of LDPE polyethylene. The FT-IR, SEM, NMR features, CHN content, thermal and tensile strength of polyethylene were monitored for structural, morphological and chemical changes of polyethylene. Alteration in bond indices in polyethylene as revealed by FT-IR spectroscopy provided characteristic evidence for biodegradation. Percentage of polymer carbon utilised by cyanobacterial species, substantial weight loss and loss in crystallinity of LDPE polyethylene corroborated efficient biodegradation. Formation of holes and cavities observed on the surface of the polyethylene after six week of incubation attested biodegradation. The CHN analysis evidenced about 3% carbon utilisation by the cyanobacterial species from the polyethylene. The rapid growth of cyanobacterial species on the polyethylene surface *vis-à-vis* that without polyethylene suggested that the microorganisms continued to gain energy during their growth atleast partially from the polyethylene. The reduction in lamellar thickness, weight and crystallinity of the cyanobacterial treated polyethylene pointed to an efficient biodegradation process. That no pro-oxidant additives or pretreatment were employed was a redeeming feature of the biodegradation process. The laccase and

manganese peroxidase enzymatic activity of the cyanobacterium growing on polyethylene substratum got enhanced in the log phase of cyanobacterial species. The characteristic NMR chemical shift patterns associated with the formation of ester, increased carbonyl bond indices and enzymatic activity furnished further evidence for low density polyethylene (LDPE) degradation. The thesis is organized into ten chapters.

Chapter 1 presents an introductory background, significance of the research work and research objectives.

Chapter 2 includes related work carried out by other researchers highlighting informations and status of bacteria, fungi and algae mediated biodegradation of polyethylene. In particular, the gaps in relation to algae based biodegradation has been emphasized.

Chapter 3 furnishes the details of materials and methods, description of study area and various methods employed for laboratory analysis of physico-chemical properties of sewage water, soil of solid domestic waste dumping site, enumeration of algal species, isolation and purification of algal species from the polythene surface, biochemical analysis, enzymatic and non-enzymatic antioxidantsof the algal isolates, use of domestic sewage water as growth medium and phycoremediation, andspectroscopic and analytical tools used for studying biodegradation of LDPE by the cyanobacterial species.

Chapter 4 gives an account of algal colonization on polythene surface and the role of physico-chemical properties of sewage water and soil of domestic solid waste dumping site.

Chapter 5 deals with the isolation and purification of algal species collected from the submerged polythene surface in sewage water and domestic solid waste dumping site. Based on morphological features the identification of the algal isolates have been made.

Chapter 6 gives an account of biochemical composition of algal species isolated from the polythene surface. The identification of heterocystous and non-heterocystous cyanobacterial species based on certain biochemical parameters are also incorporated herein.

Chapter 7 includes the enzymatic and non-enzymatic antioxidants profiling of the algal isolates.

Chapter 8 portrays phycoremediation of domestic sewage water by some of the selected algae including cyanobacteria. The domestic sewage water has also been assessed as growth medium for certain algae.

Chapter 9 describes studies related to polyethylene (LDPE) biodegradation potential of some selected cyanobacteria.

Chapter 10, the concluding chapter provide a general discussion pertaining to the aforementioned chapters. This section also contains a '**Conclusion'** highlighting the salient features of the present PhD research drawing internal comparison.

The relevant literatures pertaining to **Chapter 1-9** are enlisted at the end of the thesis followed by appendix and research publications.

Chapter 1

General Introduction

1.1 Polyethylene and carry bags

The polyethylene molecule consists of long backbone of an even number of covalently linked carbon atoms with a pair of hydrogen atoms attached to each carbon, chain ends are terminated by methyl groups (**Fig.1.1**).

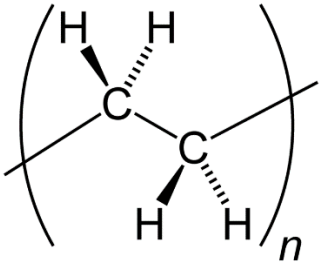

Fig.1.1 Structure of polyethylene

Polyethylene consists of alkane with the formula $C_{2n}H_{4n+2}$, where n is the degree of polymerization, i.e., the number of ethylene monomers polymerized to form the chain. Usually, the degree of polymerization is well in excess of 100 and can be as high as 250,000 or more.

There are three types of polyethylene based on the mode of polymerization, linear high-density polyethylene (HDPE), branched low density polyethylene (LDPE), and linear low-density polyethylene (LLDPE), very low density polyethylene (VLDPE) and Ethylene-Vinyl Ester copolymer (Peacock, 2000).

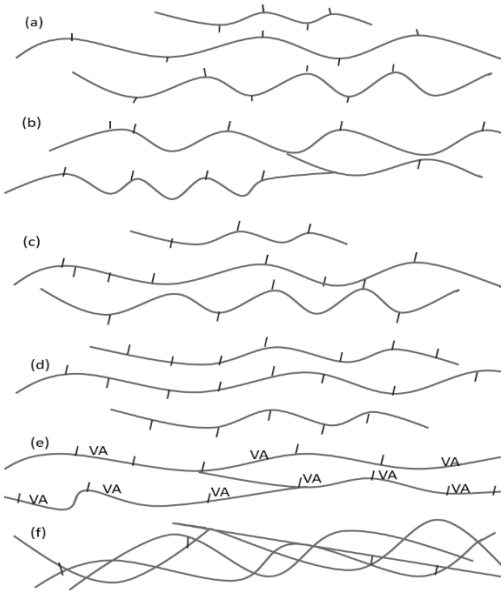

Fig 1.2 Schematic representation of different types of polyethylene (a) HDPE; (b) LDPE; (c) LLDPE; (d) VLDPE; (e) ethylene vinyl acetate copolymer; (f) cross-liked polyethylene (modified after Peacock 2000)

There are some polymer properties based on which the polyethylene are considered for packaging (Barnetson, 1996). The properties of polyethylene are as follows

(a) Density

Polyethylene known to have very low density when compared to other packaging materials. HDPE and LDPE density found to be 0.917-0.930g/cm^3 and 930-970kg/m^3, respectively.

(b) Crystallinity

Crystallinity has a very definite effect on the properties of polymer. Polyethylene consists of some degree of crystallinity, it also consists of some amorphous content.

(c) Clarity

The clarity of polyethylene plays an important role in food packaging. When the density of polyethylene are found to be increase, the clarity is found to decrease.

(d) Mechanical strength

The tensile strength, thermal properties and glass transition temperature of the polyethylene plays a vital role in transportation and packaging of materials.

1.2 Commercial carry bags and disposal

Commercial carry bags usually made from polyethylene consists of long chain of ethylene monomers, has widespread use owing to their role in consumer products and packaging. The recalcitrant nature of polythene due to high molecular weight, complex three dimensional structures and hydrophobic nature cause these polythene bags resistant to natural environment. Consisting of carbon and hydrogen polymers, polythenes are remarkably resistant to biological decay. They can be degraded to some extent by sunlight and oxygen resulting brittleness and loss of tensile strength without proportionate loss of mass, while degradation by mechanical forces may merely lead to smaller pieces (Potts, 1984). Due its nature, polyethylene carry bags are not easily broken down to smaller pieces thus endangering wildlife. Currently both marine ecosystem and urban areas are beset with problems of polyethylene disposal posing severe environmental threats (Caruso, 2015). Durability and undesirable accumulation of synthetic polymers in the natural ecosystem and habitats continue to be major concerns. A recent study suggested that each year millions of polyethylene carry bags are discarded improperly to the environment (Danso *et al.,* 2018). Of late, polyethylene bags containing prooxidant additives have been introduced in the market as a new material promising biodegradability, in conjunction with the continual use of existing production and cost-effective processing technologies (Wiles and Scott, 2006). Primary applications lie in agricultural greenhouse construction, mulching films, packaging films as well as in other products with a limited lifetime. As yet, only a little data exists to support the idea of such materials' biodegradability (Chiellini *et al.,* 2003; 2006). The understanding of the microbiology of the process is still elusive.

1.3 Colonisation of microorganisms including algae on polyethylene surface

A group of microorganisms are known to colonise on polyethylene surface. The microorganisms attached to the surface of the material could extract and utilize oxidation products of polyethylene, a broad spectrum of compounds; mainly various fatty acids like substances, but other functional groups such as esters, ketones, alcohols and double bonds can also be found (Albertsson *et al.,* 1994; Khabbaz *et al.,* 1999).Thus the adherent microorganisms can be regarded as a potential biological agent in the proposed scheme for degradation of polyethylene with prooxidant additives (Koutny *et al.,* 2006b).

Some of the bacterial species, *Pseudomonas* sp., *Acinetobacter* sp., *Rhodococcus* sp., *Flavobacterium* sp., *Stenotrophomonas* sp., *Delftia acidovorans*, *Ralstonia* sp. were found to colonise on polyethylene surface (Koutny *et al.,* 2009). Some of the fungal species, *Aspergillus*, *Fusarium*, *Penicillium*, *Phanerochaete* were known to proliferate on polyethylene surface (Pathak and Navneet, 2017).

Algae are known to colonise on such polythenes submerged in waste water by mucilagenous secretion of extracelluar polymeric substances (EPS) (Suseela and Toppo 2007; Sharma *et al.,* 2014; Kumar *et al.,* 2017).The gelatinous substance, mucilage helps in the colonisation of algae on the polyethylene surface as mucilage is the precipitation formed by the surface and organisms intend to establish on the surface (Boney, 1981).

In a recent field based study conducted in oligotrophic water bodies of Lucknow, Uttar Pradesh, fifteen algal genus viz., *Chaetophora*, *Coleochaete scutata*, *Coleochaete soluta*, *Aphanochaete*, *Gloeotaenium*, *Oedogonium*, *Oocystis*, *Oscillatoria*, *Phormidium*, *Chroococcus*, *Aphanothece*, *Fragillaria*, *Cocconis*, *Navicula* and *Cymbella* were found to be colonised on the surface of polythene (Suseela and Toppo, 2007).

Several species of algae, *Phormidium tenue*, *Oscillatoria tenuis*, *Navicula cuspidata*, *Monoraphidum contortum*, *Microcystis aeruginosa*, *Closterium constatum*, *Chlorella vulgaris* and *Amphora ovalis* were found to be colonise on waste polythene materials in various ponds, lakes and water bodies of Kota city in the state of Rajasthan, India (Sharma *et al.,* 2014).

1.4 Biodegradation of polyethylene

Biodegradation is the process by which organic substances are broken down by living organisms. The term is often used in relation to ecology, waste management,

environmental remediation (bioremediation) and to plastic materials, due to their long life span. Organic material can be degraded aerobically, with oxygen, or anaerobically, without oxygen. A term related to biodegradation is biomineralisation, in which organic matter is converted into minerals-CO_2, H_2O and CH_4 (Fig 1.3).

Microorganisms such as bacteria and fungi are involved in the degradation of both natural and synthetic plastics (Gu *et al.*, 2000a; Gu 2003; Shah *et al.*, 2008; Gu, 2013). The biodegradation of plastics proceeds actively under different soil conditions according to their properties, because the microorganisms responsible for the degradation differ from each other and they have their own optimal growth conditions in the soil. Polymers especially plastics are potential substrates for heterotrophic microorganisms (Glass and Swift, 1989).

Biodegradation is governed by different factors that include polymer characteristics, type of organism, and nature of pretreatment. The polymer characteristics such as its mobility, tacticity, crystallinity, molecular weight, the type of functional groups and substituents present in its structure, and plasticizers or additives added to the polymer all play an important role in its degradation (Artham and Doble, 2008; Gu *et al.*, 2000a; Gu *et al.*, 2000b).

The polyethylene properties such functional group on the surfaces, crystallinity, molecular weight distribution, hydrophobicity, surface topography, mechanical properties, are usually monitored for polyethylene degradation. Polyethylene comprises of both crystalline and amorphous regions. The amorphous regions of the polyethylene are usually consumed first by the microorganisms as it is presumed to be more accessible to microorganisms. The alteration of crystallinity are linked to the consumption of amorphous region, the smaller crystals of the polyethylene are also consumed by the microorganisms (Manzur *et al.*, 1997; Santo *et al.*, 2012; Restrepo-Flórez *et al.*, 2014). Size exclusion chromatography and high temperature gel permeation chromatography are useful techniques in determination of molecular weight distribution. Due to wide distribution in chain length, a typical polymer chain is rarely symmetric and single molecular weight of a polymeric material cannot be characterized. The molecular weight, branching and dispersity are known to exert significant effect on the mechanical strength and other related physical properties of the polymer. Alteration in the molecular weight

distribution of polyethylene are observed upon colonisation of microorganisms on the polyethylene surface (Hadad *et al.*, 2005; Santo *et al.*, 2012).

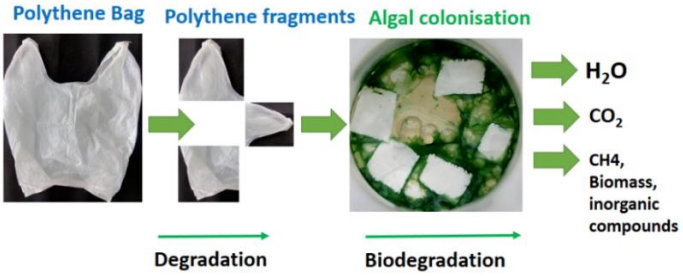

Fig 1.3 Algal colonisation and biodegradation process

1.5 Chemistry of Biodegradation of polyethylene

The study of degradation pathway and products formed can provide a clear understanding of polyethylene degradation. The end product of biodegradation of polyethylene are more likely to be sorbed by the microorganisms. The amount of carbonyl residues usually increases in the polyethylene after incubation with the microorganism. Carboxylic acid, ethanol, ketone thus formed are believed to enter into β-oxidation process of tricarboxylic acid (TCA) cycle (Albertsson *et al.*, 1987; Oprea *et al.*, 2018). Polyethylene are hydrophobic in nature and play an important role favouring algal colonisation (Gilan *et al.*, 2004). Surfactants produced by some microorganisms help in attachment of microbes including cyanobacteria to polyethylene surface (Karlsson *et al.*,1988; Tribedi and Sil, 2013).

During polymer degradation, large unit of polymer break down to monomer, monomer are mineralized and pass through cell membrane of microbial cells. The simpler monomer unit are then absorbed and biodegraded within the cells. The breakdown of polymer to monomer unit utilise various forms of physical and biological forces (Swift, 1997). Various forces are likely to reduce the mechanical strength of the polymer. When microorganism are found to colonise on the polymer surface, the microbes are found to penetrate the polymer (Kamal and Huang, 1992).

Low molecular weight polymer are expected to get biodegraded readily relative to that of high molecular weight. Following conversion of polymer to monomer, it passes through the cell wall and are degraded by cellular enzymes. Extracellular and intracellular depolymerases are the enzymes that actively participate in polymer degradation (Gu *et al.*, 2000b). Extracellular enzymes are responsible for

the break down of complex polymers into smaller molecules of short chains e.g., oligomers, dimers, and monomers, small enough to pass through the semi-permeable outer bacterial membranes eventually to be utilized as carbon and energy sources. The process of breakdown of polymer is known is as depolymerization. End products such as CO_2, H_2O, or CH_4, are produced as a process of mineralization (Frazer, 1994; Hamilton *et al.,* 1995). A small percent of the polymer is usually degraded by the microorganisms, because the degraded portion will be assimilated into microbial biomass, humus and other natural products (Atlas and Bartha, 1997; Narayan, 1993). Environmental factors also play important role in the polymer degradation by microorganisms. Various types of polyethylene have been subjected to biodegradation studies in recent times (Restrepo Flórez *et al.,* 2014; Sen and Raut, 2015).Various types of enzymes present in living cell are associated with the polyethylene degradation. Laccase and manganese peroxidases are found to be main polyethylene degradation enzymes (Bhardwaj *et al*., 2012b). Microbial enzymes capable of degrading lignin with oxidizable C-C bonds have been reported to be involved in the biodegradation of polyethylene (Restrepo-Flórez *et al.,* 2014). Some lignin degrading enzymes, laccases, manganese peroxidase (MnP) and lignin peroxidases (LiP) are found to occur in microbes. The redox potential required for lignin degradation is lower than that required for the cleavage of C-C backbone of polyethylene, so in polyethylene degradation process, the lignolytic enzymes found to be play vital role (Krueger *et al.*, 2015).

Polyethylene consists of both crystalline and amorphous regions. Microorganisms are found to prefer the amorphous regions. The degree of crystallinity of the polyethylene can affect the degradability rate, the amorphous region of the polyethylene are believed to degrade more rapidly than the crystalline region (Restrepo-Flórez *et al.,* 2014).Different techniques used in monitoring the biodegradation are mentioned in Fig 1.4 (Arutchelvi *et al.*, 2008; Nguyen *et al.*, 2016; Pathak and Navneet, 2017). The mechanical changes, surface, physical, chemical and reactive intermediates of polyethylene in the biodegradation process can be monitored. The mechanical properties include tensile strength, percentage of elongation at break and modulus of elasticity of polyethylene. The surface properties helps in monitoring the colonisation pattern and cracks, holes on the surfaces of the polyethylene. The physical properties such as stability, crystallinity

and more importantly molar mass distribution of the polyethylene are key parameters. Addition of keto, ester and carbonyl group in polyethylene chain alters the chemical properties. Reactive intermediates that are formed during the biodegradation process also play an important role (Fig 1.5) (Arutchelvi *et al.*, 2008; Restrepo-Flórez *et al.*, 2014).

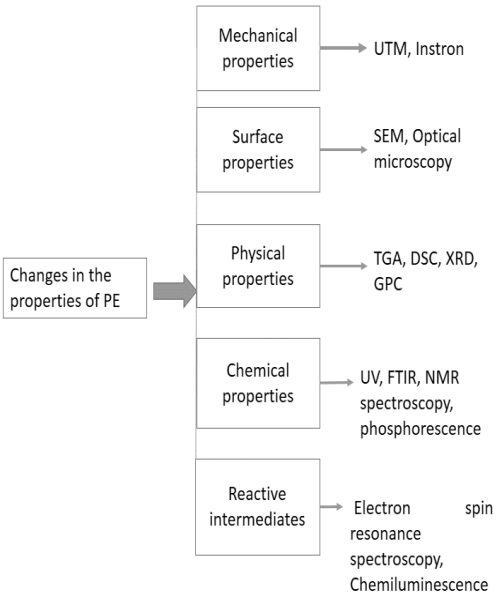

Fig 1.4 Different techniques used in the monitoring of polyethylene degradation (modified after Arutchelvi *et al.*, 2008)

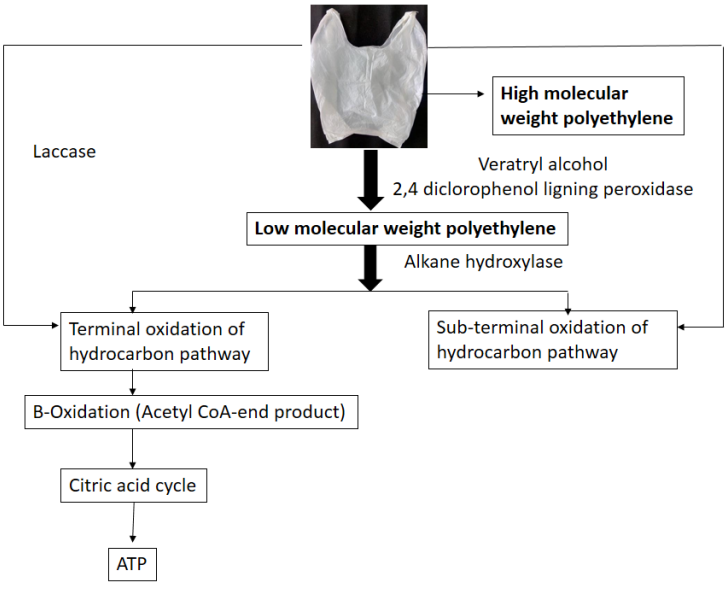

Fig 1.5 Hypothetical mechanisms of polyethylene degradation (modified after Restrepo-Flórez *et al.*, 2014).

1.5.2 Microbial communities associated with polyethylene degradation

Microorganisms communities usually break down a polymer into a monomer form through biochemical transformation. Table 1.1 illustrates the group of microorganisms associated with polyethylene degradation. A number of microorganisms such as actinomycete, bacteria and fungi have been reported to degrade polythene by utilizing its carbon content (Bhardwaj *et al.*, 2012a; Skariyachan *et al.*, 2016; Pathak and Navneet, 2017; Ahmed *et al.*, 2018), but studies pertaining to the role of algae has received only sporadic attention (Suseela and Toppo, 2007; Kumar *et al.*, 2017). Cyanobacteria, as a case in point, have been used in bioremediation and biodegradation agents as they grow rapidly and produces oxidative and lignolytic enzymes (Nayak and Tiwari, 2011; Liu *et al.*, 2016; Gupta *et al.*, 2017).The polymer biodegradation is defined as any alteration of the polymer properties in molecular weight, mechanical strength and surface features triggered and mediated by the microbial enzymes. Enzymatic activities of the microorganisms and bond cleavage of the polymer are the key steps. Biodegradation of polymer can take place in a series of steps, bio-deterioration (altering the chemical and physical properties of the polymer), bio-fragmentation (polymer breakdown in a simpler form via enzymatic cleavage) and assimilation (uptake of molecules by microorganisms) and mineralization (production of oxidized metabolites (CO_2, CH_4, and H_2O) after degradation (Fig 1.6) (Singh and Sharma, 2008).

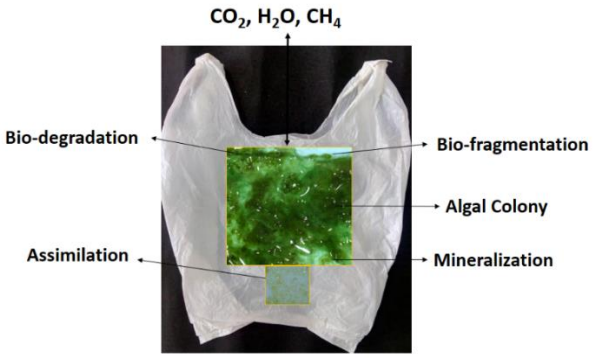

Fig 1.6 Different steps of polyethylene degradation by algae

Table 1.1 Group of microorganisms associated with the polyethylene degradation (Arutchelvi *et al.,* 2008; Restrepo-Flórez *et al.,* 2014; Pathak and Navneet, 2017; Kumar *et al.,* 2017; Ahmed *et al.,* 2018)

Group of microorganisms		
Bacteria	Fungi	Algae
Brevibacillus borstelensis, Comamonas acidovorans, Pseudomonas chlororaphis, P. aeruginosa, Rhodococcus rubber, Staphylococcus cohnii, S. epidermidis, Streptomyces badius, S. setonii, Bacillus brevis, B. cereus,B. circulans, B. halodenitrificans, B. pumilus, B. sphaericus, Arthrobacterparaffineus, A. viscosus, Acinetobacterbaumannii, Microbacterium paraoxydans, Nocardia asteroids ,Micrococcus luteus, Lysinibacillus xylanilyticus	*Aspergillus niger, A. flavus, Cladosporium cladosporioides, Fusarium redolens, Penicillium simplicissimum, P. pinophilum, P. frequentans, Phanerochaete chrysosporium, Mucor circinelloides, Phanerochaete chrysosporium*	*Scenedesmus dimorphus, Anabaena spiroides, Navicula pupula*

1.8 Potentials of algae in biotechnology and biodegradation of polyethylene

Algae are useful to mankind in various ways. They constitute a vast potential resource in varied applications such as food and feed, fine chemicals, pharmaceuticals, biofertilizers, biofuel, degradation of organic pollutants accumulation of polycyclic aromatic hydrocarbons (PAHs), phenanthrene (PHE) and fluoranthene (FLA), biodegradation of xenobiotic compounds and biodegradation of polythene (Gatenby *et al.,* 2003; Hong *et al.,* 2008; Kumar *et al.,* 2017; Bhayani *et al.,* 2018).

The biochemical composition of algae varies with species and growth condition in a batch culture. The use of algae as a supplement in food and feed due to their balanced nutritional composition and highly valuable compounds such as pigments, polyunsaturated fatty acids, and other biologically active compounds gain attention from the researcher around the globe. Algae are also used as an effective bioremediation agent. They are known to accumulate various organic pollutants in aquatic environment. The algal species, *Skeletonema costatum* and

Nitzschia sp. are known to accumulate the phenanthrene and fluoranthene from an aquatic environment. The tolerance of *Skeletonema costatum* was found to be higher than *Nitzschia* sp. The algal species, *S. costatum* and *Nitzschia* sp. are capable of accumulating and degrading the two typical PAHs simultaneously. The accumulation and degradation abilities of *Nitzschia* sp. are higher than those of *S. costatum* (Hong *et al.,* 2008).

Several algal species, *Scenedesmus dimorphus*, *Anabaena spiroides* and *Navicula pupula* were found to be effective in polyethylene degradation. The algal species were found to grow profusely on polythene sheets. The rate of degradation was maximum (8.18%) in *Anabaena spiroides* treatment. The scanning electron microscopy of the treated polyethylene samples revealed adherence of the cyanobacterium on the polyethylene surface. Minute hole on the LDPE sheets were observed after the treatment. The diatom, *Navicula pupula* treated LDPE sheets was found to be partially eroded.

As a part of a long-drawn program on enumeration and development of monocultures of algal species from submerged polythene surface, domestic sewage water of Silchar town (Assam), studies related to morphology, physiology, biochemistry, and colonisation pattern on different substrates including polythenes are currently underway in our laboratory. Of the several species found to be growing on polythene surface in sewage water, the cyanobacterial species like *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Cylindrospermum muscicola* and *Nostoc carneum* were found to be most dominant and fast growing relative to the rest. This prompted us to undertake a detailed systematic investigation on biodegradation of polyethylene using these species. Preliminary results indicate quite efficient biodegradation of polyethylene for these species.

Hence, the present Ph.D. research work portrays an account of investigation on distribution, identification and evaluation of biodegradation potentials of polyethylene by using of algal communities isolated from degraded polythene bags of Silchar town, Cachar district of Southern Assam.

The following **OBJECTIVES** were set for the present PhD research-

1. Physico-chemical characterization of domestic sewage water and solid waste dumping site soil samples.
2. Exploration of the algal species colonized on polythene surfaces, isolation and study of their biochemical and antioxidant profiles.
3. Phycoremediation of sewage water by some selected algal species.
4. Assessment of biodegradation potential of Low Density Polyethylene (LDPE) by some selected cyanobacterial species.

Chapter 2

Review of Literature

2.1 Distribution of algae

Algae are the group of aquatic photosynthetic organisms, serve as primary producers and also bioindicator of an aquatic ecosystem (Eggs and Aksnes, 1992; Chellappa *et al.*, 2008; Anitha Devi *et al.,* 2013). The distribution of algae in an aquatic ecosystem are mainly dependent on the physicochemical parameters of water, nutrients present in water, carbon exchange and biological interaction (Vareethiah and Haniffa, 1998; Bianchi *et al.*, 2003; Tiwari and Chauhan, 2006; Rajagopal *et al.,* 2010).

Algae are found to occur in every terrestrial and aquatic habitat on our planet. They are also present in some of the most extreme terrestrial environments, such as rocks, hot and

cold deserts in addition to natural substrata (Friedmann and Ocampo-Friedmann, 1984; Caneva et al., 1992a, b; Ortega Calvo et al., 1993a, b; Rindi 2007).

2.2 Diversity of algae in polluted habitats

Blue green algae, green algae, diatoms, desmids, euglenoids, silicoflagellates, coccolithophytes are found to distribute in all aquatic ecosystems including polluted habitats also (Reynolds, 2006). Diwedi (2010) studied the algal communities in river Pandu which receives waste water from municipality and industry of Kanpur. The algal species, *Cyclotella fumida*, *Asterionella formosa*, *Cladophora glomerata*, *Pediastrum simplex*, *Scenedesmus bijuga*, *Cladophora glomerata*, *Mycrocystis aeruginosa*, *Merismopedia minima* and *Oscillatoria salina* were found to be occur in river water of Pandu. The concentration of dissolved oxygen in the river water was found to be zero. Due to release of domestic sewage water, the higher concentration of phosphate and nitrate were observed.

Algae have a short life cycles and their distribution are most likely to be affected by physico-chemical parameters of water. A variety of ecological factors influence and control the seasonal distribution and composition of algal communities. Algal population is influenced by various factors such as pH, temperature, heavy metal content, organic matter content and other pollutants that are added by anthropogenic activities in the basin (Lata Dora *et al*., 2010). Investigation of algal distribution in some water bodies of Delhi were carried out (Gupta and Pamposh, 2014). A total of 18 algal genera were found to be distributed in the water bodies of Delhi. Some species, *Lyngyba* sp., *Anacystics* sp., *Tetraderon* sp., *Anabaena* sp., *Agmenellum* sp., *Navicula* sp., and *Nitzschia* sp. were found to be occur in the water bodies indicating the high load of pollution. Chlorophyceae were recorded as the largest group. The dissolved oxygen concentration of water was likely to influence the green algae distribution.

Surface water pollution is a serious threat to environment. Large quantities of organic pollutants are released to the environment as solid and liquid waste causing serious environmental problems. Usually, organic pollutants are released from residential houses, industries and farm houses. Algae are known to serveas good indicator of water pollution. Most of the members of desmids are found to be distributed in oligotrophic waters, while some of these species found to bepresent in eutrophic water as well. Cyanobacteria are

found to occur frequently in nutrient rich water but they are also found in nutrient-poor waters. Some members of algae *Chlamydomonas*, *Euglena*, *Navicula*, *Synedra*, *Oscillatoria* and *Phormidium* are known to tolerate organic pollution (Sen*et al.*, 2013). *Euglena*, *Scenedesmus*, *Closterium lunula*, *Microcystis aeruginosa*, are well known as indicators of organic pollution (Hosmani, 2014). Studies on algal community andecological status of a temple pond in Kanyakumari district has revealed a total of 52 species distributed in the temple pond. Some species of algae were common, *Anabaena circularis*, *Oscillatoria limosa*, and *Chroococcus turgidus* (Vijaya Rani *et al.*, 2016).

2.3. Diversity of algae in urban habitats

Algae are known to occur almost in all urban habitats**.** They are found to colonise on walls, masonry and other man-made substrata. The urban habitats are exposed to harsh environmental conditions such as very high/low temperatures, prolonged dry periods and extreme light intensity and UV radiation. Rindi *et al.*, 1999 and Rindi and Guiry, 2002 studied the composition of algal assemblages at urban sites and observed the Prasiolales and Trentepohliales to be dominant in those habitats. Cyanobacteria are among the most widespread photosynthetic organisms which occur in all urban areas. They are common on surfaces of both modern and ancient buildings (Tripathi *et al.*, 1990; Crispim and Gaylarde, 2004); due to their capacity to resist very harsh conditions, they are among the most important pioneer microorganisms (Grant, 1982; Hoffmann, 1989; Whitton, 1992). The colonisation of cyanobacteria on building surfaces produce grey or black discolorations on exposed surfaces. *Myxosarcina*, *Gloeocapsa*, *Synechococcus*, *Microcoleus*, *Phormidium*, *Leptolyngbya*, *Lyngbya*, *Microcoleus*, *Oscillatoria*, *Calothrix*, *Nostoc*, *Scytonema* and *Tolypothrix* were found to profusely colonise on the urban habitats (Rindi 2007). The species *Pleurococcetum vulgaris*, *Pleurococcetum*, *Protococcus*, *Pleurococcus*, *Desmococcus*, *Apatococcus*, *Desmococcus olivaceus*, *Chlorella*, *Chlorococcum*, *Coccomyxa*, *Stichococcus* and *Trebouxia* were the green algae found to assemblage on the urban habitats (Barkman, 1958; John, 1988; Ettl and Gärtner, 1995; Gärtner and Stoyneva, 2003). The species *Printzinala genifera*, *Trentepohlia aurea* and *Trentepohlia umbrina* were found to colonise on the artificial substrata of urban area (Crispim *et al.*, 2004).

2.4 Diversity of algae on polythene bags

Polythene bags has a wide range of applications in daily life due to its durability, corrosion resistant and inexpensive nature. Polythene bags causes severe pollution to the environment. Algal biofilms are commonly found on submerged objects such as on waste polythene bags.Used polythene bags generally thrown to water bodies or used in landfills. Algae profusely growing on polythene bags in water bodies due to nutrients available. In landfill, algae cannot grow vigorously due to low water amount and light. Algal colonization on submerged polythenes of different water bodies in and around Lucknow city were observed (Suseela and Toppo, 2007). Fifteen algal taxa, including *Chaetophora*, *Coleochaete scutata*, *Coleochaete soluta*, *Aphanochaete*, *Gloeotaenium*, *Oedogonium*, *Oocystis*, *Oscillatoria*, *Phormidium*, *Chroococcus*, *Aphanothece*, *Fragillaria*, *Cocconis*, *Navicula* and *Cymbella* were found to profusely colonise on the surface of polythene. Sharma *et al*., (2014) worked on the algal colonisation on degrading polythene waste of Kota city, Rajasthan. *Amphora ovalis*, *Chlorella vulgaris*, *Microcystis aeruginosa*, *Monoraphidium contortum*, *Navicula cuspidate*, *Oscillatoria tenuis*, *Phormidium tenue*, *Scenedesmus acuminatus*, *Selanestrum minutum* grown on degrading polythene waste.

2.5 Algae and its applications

Microalgae are the powerhouse of highly valuable compounds such as pigments, polyunsaturated fatty acids, and other biologically active compounds. These microalgae have a significant commercial market for their biotechnological applications which is further expected to increase to new areas of nutra-cosmeceuticals (Pulz and Gross, 2004; Paliwal *et al.,* 2015). The carbohydrates and their immediate derivatives play an important role to acclimate algae in stressed environment. There are two types of photosynthetic cycles present in algae (Calvin-Benson cycle (C_3) and Hatch-Slack (C_4) photosynthesis). Usually, C_3 cycle only operates in algae but in stress condition C_4 cycle also found to be occur. Shifts in photosynthetic pathways under abiotic stress have been considered to contribute to the adaptation of plants to environmental stress (Ehleringer *et al.,* 1997). The pharmaceutical and cosmetic markets have consistently benefited from the emerging variety of microalgal products (Pulz and Gross, 2004; Richmond, 2012). Some

blue-green algae, *Arthrospira* has anti-carcinogenic effect as well as hypocholesterolemic properties (Reinehr and Costa, 2006).One of the most distinct characteristic of algae is its colour. In general, each phylum has its particular combination of pigments (Paliwal *et al.*, 2016).These pigments have excellent antioxidant and free radical scavenging activity, and are relevant as potential photo-protector substances (Rastogi and Madamwar, 2016).Reactive oxygen species (ROS) are the oxygen-based free radicals that are capable of producing an unpaired electron. Overproduction or inadequate removal of ROS can result in oxidative stress, leading to altered metabolism, abnormal signal transduction events and other molecular damage, which leads to pathological changes in cell and tissue function. Increased ROS levels results in lipid peroxidation, DNA mutation, and enzyme inhibition. ROS exposure promotes genetic alterations leading to abnormal cell function and immune suppression.

2.6 Algae in bioremediation of sewage water

Algae have immense capability to sorb metals and there is considerable potential wastewater remediation (Mehta and Gaur, 2005). The metal ions are adsorbed over the cell surface very quickly just in a few seconds or minutes; this process is called physical adsorption. Then, these ions are transported slowly into the cytoplasm via chemisorptions (Dwivedi, 2012). In recent year, researchers have used various algae, *Oscillatoria tenuis* (Ajavan *et al.,* 2011), *Oscillatoria quadripunctulata* (Rana *et al.,* 2013; Azizi *et al.,*2012), *Spirogyra hatillensis* (Dwivedi, 2012), *Spirogyra hyaline* (Kumar and Oommen, 2012), *Cladophora glomerata, Oedogonium rivulare* (Dwivedi, 2012), *Chlorella vulgaris*, *Spirulina maxima* (Chan *et al.,* 2014) for removal of heavy metal from contaminated sites. Craggs *et al*., (1997) studied wastewater nutrient removal by *Phaeodactylum tricornutum*, and *Oscillatoria* sp., grown on a corrugated raceway. Silva-Benavides and Torzillo (2012) studied the nitrogen and phosphorus removal through laboratory batch cultures of microalga *Chlorella vulgaris* and cyanobacterium *Planktothrix isothrix* grown as monoalgal and as co-cultures of *Chlorella vulgaris* and *Planktothrix isothrix*. The co-cultures of *Chlorella* and *Planktothrix* showed highest nutrient removal rate. Park *et al*., (2012) studied the biomass productivity of *Chlamydomonas debaryana*, *Chlorella sorokiniana* and *Micractinium* sp., when cultivated mixotrophically in secondary municipal wastewater supplemented with glycerol. This indicated that the exogenous

supplement of an organic carbon source helped to strengthen the biomass production of microalgae.

Significant changes in pigment and biochemical constituents on phycoremediated microalgal biomass by *Scenedesmus obliquus* and *Chlorella vulgaris* were noted (Elumalai *et al.,* 2014).Biochemical analysis of the microalgae biomass of *Scenedesmus obliquus, Chlorella vulgaris* and its consortium revealed higher amount of protein and lipid in ascending order. Sangeetha *et al.,* (2015) studied the role of three microalgae consortia *Oscillatoria* sp., *Chlorella vulgaris* and *Chroococcus* sp. in feasible, cost effective and ecofriendly method in fish waste water treatment. Sivasubramanian *et al.,* (2009) studied on the *Chroococcus turgidus* phycoremediation of acidic effluent from an alginate industry. Sivasubramanian *et al.,* (2012) worked on phycoremediation of effluent from a soft drink manufacturing industry with a special emphasis on nutrient removal. The microalgae *Chlorococcum* sp, *Chlorella conglomerata* and *Desmococcus* sp. were able to remove nitrate and phosphate rapidly. These micro algae can be employed in large scale effluent treatment systems for effective remediation. Sethupathy *et al.,* (2015) studied phycoremediation of sewage waste water of Indira Institute of Engineering and Technology, Chennai by using micro-algal strains.The waste water was treated with three micro-algal species like *Chlorella, Scenedesmus,* and *Nostoc* in waste stabilization pond. The *Chlorella* species showed lowering the BOD, COD, TDS, PO_4^{2-}, and NH_{4+}-N. The *Nostoc sp.* exhibited the increasing phosphate and ammonium concentrations. Sharma and Khan (2013) studied bioremediation of sewage wastewater using *Chlorella minutissima, Scenedesmus* sp., and *Nostoc* sp. The maximum biomass was observed in *Scenedesmus* sp., and while minimum percentage of removal of nitrogen and phosphorus was maximum in *Chlorella minutissima*.

Ramsundar *et al*., (2017) studied cultivation of *Chlorella sorokiniana* in municipal wastewaters. Municipal wastewater are usually loaded with higher nitrogen, phosphorus and organic carbon. The growth of *Chlorella sorokiniana* in sewage water was found to be higher as compare to media.

Chokshi *et al.,* (2017) studied *Acutodesmus dimorphus* cultivation of microalgae in wastewaters. The species was effective in reducing the level of pollutants and producing maximum biomass. Biomass production was increased by 5-6 folds in dairy wastewater

as compared to control. The species *A. dimorphus* produced 25% lipid and 30% carbohydrate when grown in dairy wastewater.

2.7 Bacteria in biodegradation of polyethylene

The biodegradation studies of starch-polyethylene degradable plastic films were made using *Streptomyces badius*, *S. setonii* and *S. viridosporus* (Pometto *et al.*, 1993). The films. Reductions in the mechanical properties of polyethylene films and changes in FT-IR spectrum were observed. The enzymatic activities have evidenced the biodegradation of starch-polyethylene plastic films.

The biodegradation of blends of polycaprolactone and polyethylene was screened by a consortia of *Aspergillus niger*, *Penicillium funiculosum, Chaetomium globosum, Gliocladium virens* and *Aureobasidium pullulans* (Tilstra and Johnsonbaugh, 1993). The significant reduction of weight loss and molecular weight were observed. The mechanical strength of the film was found to reduce after exposure to the consortia. Commercial photodegradable polyethylene have been examined with respect to rate and extent of oxidation measured by carbonyl (carboxylic acid and ester) formation, molar mass reduction (Arnaud *et al.*, 1994). Formation of carboxylic acid and other byproduct of polythene degradation reduces the molar mass of the polythene and facilitate carbon assimilation. Biodegradation of starch-filled polyethylene were screened by *Cladosporium cladosporioides*, *Nocardia asteroids* and *Rhodococcus rhodochrous*.

Biodegradability of starch films was monitored by microbial colonization on the film, film surface analysis (performed after sterilization) by SEM (), molar mass distribution by GPC (Gel permeation chromatography) and FT-IR analysis. Ohtake *et al.*, (1998) worked on LDPE films collected from garden soil, incubated in soil for a period of 30 years. The FT-IR analysis showed C=C double bonds ($1640cm^{-1}$) around the surface of the whitened part of the LDPE. Significant level of hydroperoxide and hydroxide absorption bands were also observed in the LDPE films. It can be concluded that the biodegradation of thin LDPE film in soil was unexpectedly fast because of the synergistic action of oxidative and/or photo-oxidative degradation, which is probably linked to the increased hydrophilicity of the film surface. Some bacterial and fungal strain such as *Rhodococcus rhodochrous, Nocardia asteroids, Cladosporium cladosporoides* were used to degrade the polyethylene. The samples after incubation with these microorganisms

were monitored by SEM, FT-IR, fluorescence microscopy and GPC. The colonisation of microorganisms were observed in all the heat pre-treated and normal samples. Formation of carbonyl, alkane, new double bonds and polysaccharides peaks were observed (Bonhomme *et al.*, 2003). A bacterial strain of *Rhodococcus ruber* was found to utilize the polyethylene films as sole carbon source after a period of 30days (Gilan *et al.*, 2004). In liquid bacterial culture, the bacterium formed a biofilm on the polyethylene surface and degraded up to 8% weight loss of the polyolefin. The bacterial attachment to polyethylene surface showed that the cell-surface hydrophobicity of the bacterium was higher than that of three other isolates which were obtained from the same consortium but were less efficient than C208 in the degradation of polyethylene. Mineral oil present in polyethylene films enhanced the colonization of polyethylene and increased biodegradation by about 50% Some bacterial, fungal actinomycetes, *Bacillus*, *Clostridium*, *Micrococcus*, *Aspergillus*, *Penicillium* and *Mucor*, were found to be colonized in the soil for polymer composites biodegradation. Electret-thermal analysis (ETA) was used to assess the end products of degradation. The tensile strength of the polymer showed a negligible changes by 60-150% increasing of elongation value. The FT-IR spectra showed a broad O-H stretching band of starch hydroxyl groups and adsorbed water at 3100-3500cm^{-1}, the C-O stretching band at 1000-1200cm^{-1} and the H-O-H bending band of adsorbed water at 1645-1655cm^{-1} indicating the role of microorganisms towards the polyethylene degradation (Pinchuk *et al.*, 2004).

A thermophilic bacterium, *Brevibaccillus borstelensis* was found to utilize the branched low-density polyethylene as the sole carbon source and degraded it. The bacterium, *B. borstelensis* (30 days, 50ºC) reduced the polyethylene gravimetric and molecular weights by 11 and 30%, respectively. The bacterium also degraded polyethylene in the presence of mannitol (Hadad *et al.*, 2005). Prooxidant additives present in the commercial polyethylene provide a promising solution to the problem of the environment pollution as microorganism can readily utilized the carbon from the polymer (Koutny *et al.*, 2006a).

Koutny *et al.*, 2006b studied the biodegradation of polyethylene containing additives by *Rhodococcus rhodochrous* and *Nocardia asteroids*. Following an abiotic pre-treatment consisting of photooxidation and thermo-oxidation for about 3 years of outdoor weathering, incubated up to 200 days and their metabolic activities were followed by

measuring ATP content. Simultaneously the cultures were also monitored by optical microscopy and FT-IR spectroscopy. Approximately 15 million tons of styrene are utilized annually in the chemical industry, both as a starting material for synthetic polymers and as a solvent in the polymer processing industry. As one of the xenobiotic compound and polyethylene serves as a potent toxic pollutant to the environment including human health. Its origin is traced to industrial practices that involve polymer and petrochemical processing. The bacterial isolates polycarbonate *Pseudomonas fluorescens, Xanthobacter, Pseudomonas* sp., *Corynebacterium, Escherichia coli* were found to colonize on polycarbonate and were effective in styrene degradation (Mooney *et al.,* 2006).

The bacterial isolates were found to colonize on polycarbonate were *Acinetobacter, Proteobacteria, Arthrobacter* and *Enterobacter*. The degraded products of polycarbonate were analysed by FT-IR and TLC. The FT-IR spectra of control polycarbonate was evidenced by a stretching vibration corresponding to C-H at 3411cm^{-1}, which got shifted to 3451cm-1 for treated polycarbonate (Goela *et al.,* 2008).

Bacterial species growing on polymeric materials with low value of specific growth rate of *Nocardia corynebacterioides* were observed by Pan *et al.,* (2009). Some bacteria such as *Alcaligenes xylosoxidans, Pseudomonas aeruginosa,* and *Nocardia corynebacterioides* were able to utilize rubber products as a sole source of carbon and energy. Formation of dense biofilm of *N. corynebacterioides* was observed on the polymeric surfaces. The protein content in *A. xylosoxidans*, *P. aeruginosa* and *N. corynebacterioides* culture were 38, 64 and 86$mgml^{-1}$, respectively. Biodegradation of polyethylene by *Pseudomonas aeruginosa* were based on thermo-oxidation for a period of 4 years before biodegradation (Reddy *et al.,* 2009). The molecular weight was found to reduce after the biodegradation. The concentration of oxidation products in treated samples were monitored by FT-IR. Microorganisms grow on polyethylene surface and utilise the oxidation products, ketones, ester, alcohols and double bonds of polyethylene (Albertsson et al. 1995; Khabbaz *et al.,* 1999). The bacterium, *Rhodococcus* sp. was found to colonise on polyethylene surface with high cell density. Among the isolates, *Pseudomonas* and *Rhodococcus* are found to be the most studied degraders of various plastics (Koutny *et al.,* 2009).

Fungal biodegradation of linear low-density poly (ethylene) PE-LLD films containing pro-oxidant were studied by Corti *et al.*,(2010). The PE-LLD films were first exposed to the sunlight for a period of 93 days during the summer months followed by their incubation with four fungal strains isolated from the soil. An increase in carbonyl index, crystallinity and melting temperature (T_m), and a concomitant increase in the weight of the residues. The level of oxidation of the PE-LLD was directly proportional to the aging temperature. The PE-LLD films with pro-oxidant exposed to sunlight followed by thermal aging showed even higher rate and extent of oxidation when subsequently subjected to fungal biodegradation. The higher oxidation rate also correlated well with the CO_2 production in biodegradation by fungal species. The biodegradation of low density polyethylene by *Aspergilus* spp. with or without yeast is on record (Pramila and Ramesh, 2011). The formation of cracks on the surfaces have been revealed upon incubation with this fungus. Muthukumar *et al.,* (2011) worked on polyurethane, silicone rubber, polyester, glass fiber reinforced polymer, carbon fibre reinforced plastic and syntactic foams degradation in marine environment. Carbonyl indices were found to decrease for PET (polyethylene terephthalate) indicating biotic degradation. Attachment of macrofoulants were found to be higher during monsoon season. The TGA analysis of degraded products revealed significant weight loss. Another studies on bacterial and fungal degradation of polyethylene films in different soils under laboratory conditions were studied by Nowak *et al.,* (2011). The bacterial genus, *Bacillus*, and the fungal genus, *Gliocladium viride*, *Aspergillus awamori* and *Mortierella subtilissima* were easily able to colonise both polyethylene and polyester. The number of microbial strains were higher in polyester films as compared to polyethylene. The rate of polyethylene biodegradation was dependent on environmental conditions and the growth of microorganisms colonising on the polyethylene and polyester surface. Even polymers designed to degrade under specific conditions (e.g., composting) remain unchanged while exposed to unfavourable conditions for many years.

Potential of microorganisms isolated from compost soil showed the polyethylene degradation (Mahalakshmi *et al.,* 2012). FT-IR spectra of the treated samples showed absorbance for dehydrated dimer of carbonyl group (1720 cm^{-1}), CH_3deformation (1463 cm^{-1}), and C=C conjugation band (862cm^{-1}) indicating biodegradation.

Kyaw *et al.,* (2012) worked on the biodegradation of LDPE by *Pseudomonas aeruginosa* PAO1 (ATCC 15729), *Pseudomonas aeruginosa* (ATCC 15692), *Pseudomonas putida* (KT2440 ATCC 47054) and *Pseudomonas syringae* (DC3000 ATCC 10862). The surface morphology of the polyethylene have showed significant changes. The fungal cells in the media and on the surfaces have showed a similar growth pattern. The weight loss of the polyethylene was found to be 20% when incubated with this fungus for about 40 days. The tensile strength of the polyethylene was found to decrease, so also the carbonyl indices. Santo *et al.,* (2012) reported the role of the copper-binding enzyme, laccase in the biodegradation of polyethylene by the actinomycete *Rhodococcus ruber*. The FT-IR analysis of degraded film indicated an increased carbonyl bond index. Addition of copper (II) ions to the culture *Rhodococcus ruber* containing polyethylene was found to enhance the rate of degradation by 75%. In addition of the extracellular enzyme laccase to the media with the polyethylene, found to be accelerate the rate of degradation lowering the molecular weight by about 20%. This seminal work unfolded a key role of laccase in degradation process. Infact, laccase is considered as the best known polyethylene degrading enzyme.

Kale *et al.,* (2015) worked on polyethylene degradation by *Pseudomonas* spp., *Streptomyces* spp. and *Aspergillus* spp. *Brevibaccillus borstelensis* and *Rhodococcus ruber* were reported to have capacity to degrade the CH_2 backbone and use polyethylene as its sole carbon source (Hadad *et al.,* 2005). Fungal strains like, *Mucor rouxii* and *Aspergillus flavus* (El-Shafei *et al.,*1998), *Penicillium simplicissimum* YK (Yamada-Onodera *et al.,* 2001) were already reported to be used in degradation of polyethylene. Maximum 61% (*Microbacterium paraoxydans*) and minimum 50% (*Pseudomonas aeruginosa*) polythene degradation recorded using FT-IR within two months (Rajandas *et al.,* 2012), while in another report 47% weight loss was recorded after 3 months of incubation with the *A. oryzae* (Konduri *et al.,* 2011). Konduri *et al.,* 2011 worked on biodegradation of low density polyethylene by *Aspergillus oryzae* using a pro-oxidant. Pro-oxidants are known to increase the rate of degradation. The elongation (%) and tensile strength (%) of the low density polyethylene found to decrease by 62% and 51%, respectively. The FT-IR spectra of the treated polyethylene have showed generation of more carbonyl and carboxylic groups indicating biodegradation. The pro-oxidant

activities such as manganese stearate treatment caused maximum degradation of polyethylene. The bacterial species, *Pseudomonas* sp. was comparatively fast and can degrade 5% of low-density polyethylene after a period of 45 days of incubation without any pro-oxidant. This weight loss was found to be enhanced up to 14% with the addition of mineral oil to the growth medium. Sterilized LDPE films were used as sole carbon source to the medium. The surface morphology of the treated LDPE became rough, cracks and grooves were also appeared on the surface as compared to control LDPE. (Tribedi and Sil, 2013).

Polyhydroxyalkanoates degradation by microbial strains in tropical soils were studied by Boyandin *et al.,* (2013). The bacterial genus, *Bacillus*, *Cupriavidus*, *Mycobacterium* and *Nocardiopsis* and such micromycetes as *Acremonium*, *Gongronella, Paecilomyces*, and *Penicillium*, *Trichoderma* have been identified as major PHA degraders. Polyhydroxyalkanoates degradation was attested by decrease in the polymer molecular mass, crystallinity changes, suggesting preferential degradation of the amorphous phase of the polymer. Isolation of mesophilic bacterium, *Stenotrophomonas panacihumi* for degradation of polypropylene were studied by Jeon and kim (2013). Low molecular weight polypropylene was used as carbon source in biodegradation studies.

Esmaeili *et al.,* (2013) worked on biodegradation of LDPE by consortia of *Lysinibacillus xylanilyticus* and *Aspergilus niger* in soil environment for about 126 days without prooxidant. The process of biodegradation was monitored by measuring the growth curve of the population, pH and respiration in the soil, and the mechanical properties of the films. The population of the consortia was found to be increased when incubated with the polyethylene. It was assumed that pH play an important role in the process. The value of pH was found to increase than the control. The tensile strength of the polyethylene was found to be decrease to 7.4% and 48% in the inoculated treatment after 63 and 126 days incubation. The FT-IR analysis of polyethylene have showed the decrease in carbonyl index and increase in double bond indices. The crystallinity and the crystal sizes of the polyethylene were also found to be decrease. A study carried out by Biffinger *et al.,* (2014) revealed that *Pseudomonas protegens* efficiently degraded the polyurethane films. The *Pseudomonas protegens* rapidly degraded the impranil coatings and formed the clear

zone on the surfaces. The pH value of the culture medium used in the biodegradation assay was 8.5. It is pertinent here to mention that the alkaline pH is linked to increased esterase and lipase activity (Howard *et al.,* 2012). The bacterial species, *Enterobacter* spp. and *Pantoea* spp. were isolated and biodegradation potential of these two bacteria were screened. The degradation results of this consortia was compared with some well-known polythene degrading bacteria *Pseudomonas putida* MTCC 2445 (designated as MTCC1), *Pseudomonas stutzeri* MTCC 2643 (designated as MTCC2), and *Bacillus subtilis* MTCC 9447(designated as MTCC3). The polythene degradation results were analyzed by SEM, FTIR, tensile strength, and GC-FID. The *Enterobacter* spp. and *Pantoea* spp. formulated bacterial consortia have revealed the weight loss 81 and 38 % for LDPE strips and LDPE pellets, respectively. The consortia designed by MTCC strains have demonstrated the weight loss 49 and 20 % for LDPE strips and pellets, respectively (Skariyachan *et al.,* 2016). A consortia of *Bacillus vallismortis*, *Psuedomonas protegens* bt-dsce02, *Stenotrophomonas* sp., and *Paenibacillus* sp. were isolated from plastic-contaminated cow dung waste have showed the ability to degrade the low and high-density polyethylene (Skariyachan *et al.,* 2017). Moreover, this consortia have revealed a weight loss of 75, 60, 55, and 43% for LDPE strips, HDPE strips, and pellets of LDPE and HDPE, respectively. The bacterial biofilm formation was revealed by the scanning electron microscopy. The high-density polyethylene (HDPE) degradation potential of this was screened and was found to effective. Enhanced weight loss of HDPE was revealed to be 18.4%. The tensile strength was found to reduce to 60%. The SEM images of HDPE after degradation showed cracks and grooves on the surfaces (Awasthi *et al.,* 2017b).

To date, *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Streptomyces badius*, *Streptomyces setonii*, *Rhodococcus ruber*, *Comamonas acidovorans*, *Clostridium thermocellum* and *Butyrivibrio fibrisolvens*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium lini*, *Pycnoporus cinnabarinus* and *Mucor rouxii* have been known for polythene degradation. The microorganisms produces lignolytic enzymes which play an important role in degradation process (Pathak and Navneet, 2017). Several research studies on degradation of polyethylene and polypropylene with novel thermophilic consortia of *Brevibacillus* sps. Scanning electron microscopy (SEM), nuclear magnetic resonance (NMR), atomic force microscopy (AFM), energy dispersive spectroscopy

(EDS), and gas chromatography-mass spectroscopy (GC-MS) were used to study the end products of degradation. The weight reduction for LDPE, HDPE and PP strips treated was found to be 58, 46 and 56%, respectively and LDPE, HDPE and PP pellets were revealed to be 45, 37 and 44%, respectively. NMR analysis of the degraded products of LDPE, HDPE and PP have revealed methyl and aldehyde moieties. The GC-MS analysis of the degraded products have revealed fatty acid end-products of LDPE, HDPE and PP (Skariyachan *et al.,* 2018). Current scenario and future prospects for environmental safety on plastic degradation has been reviewed by Ahmed *et al.,* (2018). In their review, various biodegradation agents were mentioned. Several factors that affect the biodegradation process such as the polymer properties, the enzyme characteristic and, the exposure conditions. Molecular weight, size, additives and shape of polyethylene may play an important role in degradation process.

The selection of microorganism that can efficiently grow on polyethylene play an important role. The biodegradation of polyethylene by eubacteria and archeabacteria are quite extensively studied (Skariyachan *et al.*, 2018b). Some bacterial genus such as *Pseudomonas*, *Brevibacillus borstelensis*, *Flavobacterium* spp., *Micrococcus* spp., *Bacillus* spp., *Staphylococcus* spp., *Chelatococcus* spp. were found to degrade polyethylene efficiently. Several environmental parameters such as pH, temperature, salinity, sunlight, humidity, stress, water, culture conditions, and presence or absence of oxygen are involved in the mechanism of degradation of polymers. In nature polyethylene are hydrophobic, availability of functional group in tested polyethylene plays an important role which increases the hydrophobicity of polyethylene as hydrophobic nature of the polyethylene resists its adherence to microorganisms. Some bacterial species, *Acinetobacter baumannii*, *Arthrobacter viscosus*, *Bacillus amyloliquefaciens*, *Delftia acidovorans*, *Flavobacterium* spp., *Micrococcus luteus*, *Mycoplasma mycoides*, *Paenibacillus macerans*, *Pseudomonas aeruginosa*, *Rhodococcus erythropolis*, *Staphylococcus cohnii*, *Xylosus* spp., were found to be as an effective biodegradation agent. *Aspergillus* genus and actinomycetes *Amycolatopsis* spp. were also found to be effective.

Chinaglia *et al.,* (2018) studied the rate of biodegradation of plastic at molecular level. Microplastic less than particle size < 5 mm were causing threats to aquatic ecosystems. Microplastics are formed due to break down of larger plastics. The degradation of microplastics (polyethylene pellets, 2-4 mm in size) in artificial sea water were observed. After incubation with the artificial sea-water, the FT-IR spectra of the treated polyethylene have showed absorption for carbonyl. But this peak was absent in control polyethylene. The thermal stability of the polyethylene was found to decrease. Formation of holes and irregular surface morphology was observed after the polyethylene incubated with articial sea-water (Da Costa *et al.*, 2018).

The biodegradation of Polylactide or polylactic acid (PLA) by the bacteria, *Stenotrophomonas pavanii* and *Pseudomonas geniculate* from wastewater sludge have been studied (Bubpachat *et al.,* 2018). The two bacteria were potent source various enzymes PLA-degrading enzyme. The treated PLA showed a significant weight loss, *P. geniculate* was found to be more efficient than the *S. pavanii*. The molecular weight of PLA got reduced when incubated with these two bacteria. Lactic acid content in both liquid culture found to increase when incubated with PLA, the weight loss of the plastic bags were directly proportional to lactic acid production.

Suzuki *et al.,* (2018) worked on *Pseudomonas pachastrellae* biodegradation of poly (ε-caprolactone) (PCL) in a coastal environment. The bacterial strain was found to be colonise on degraded PCL film at a rate of $1.3 mg cm^{-2} day^{-1}$. The growth of *Pseudomonas pachastrellae* on PCL film was showed better growth on presence of carbon source and also showed PCL hydrolytic activity. The molecular weight of the PCL film was found to be decrease when incubated with *Pseudomonas pachastrellae*. The SEM images of pre and post treated PCL film showed significant differences. The smooth surface of control PCL after incubation with *Pseudomonas pachastrellae* showed much rougher appearance.

2.8 Potential of fungi in biodegradation of polyethylene

Fungal isolates, *Phanerochaete chrysosporium* and *Trametes versicolor* were able to degrade the high molecular weight polyethylene (Iiyoshi *et al.,* 1998). The results of degradation were compared with the MnP enriched media which showed enhanced rate

of degradation of polyethylene. Pretreated polyethylene with MnP showed maximum degradation in the presence of Tween 80, Mn(II), and Mn(III) chelator. Raaman *et al.,* (2012) studied the biodegradation of plastic by *Aspergillus niger, Aspergillus japonicus* and *Aspergillus terreus* isolated from polythene polluted sites around Chennai. The degradation potential of commercial carry bags of low density was established and it was found to be 8 to 12% weight loss after a periods of 4 weeks. The scanning electron microscopy results showed that the porosity and fragility on the polythene surface. The *Aspergillus japonicus* have showed 12% weight loss as compared to *Aspergillus niger* of weight loss (8%) in one month period. Melting point was found to be to 1.11ºC in comparison to control polythene. The colonisation of *Aspergillus niger* (ITCC No. 6052) and degradation of polyethylene isolated from plastic waste dumpsite were studied by Mathur *et al.,* 2011. The increase in growth of *Aspergillus niger* was observed on the surface of high density polyethylene. The weight loss (%) of polyethylene was 3.44% when incubated with *Aspergillus niger* for about 30days. The tensile strength of the polyethylene was reduced to 61%. The fungal strains used for biodegradation of plastics revealed that alkanes, alkenes, ketones, aldehydes, alcohols, carboxylic acid, keto acids, dicarboxylic acids, lactones, and esters are released as end products of biodegradation (Ghosh *et al.,* 2013). The fungi genera such as *Acremonium*, *Cladosporium*, *Debaryomyces*, *Emericellopsis*, *Eupenicillium, Fusarium, Mucor*, *Paecilomyces*, *Penicillium*, *Pullularia*, *Rhodosporidium* and *Verticillium* are well established for plastic degradation. The *Trichoderma harzianum* was used as in biodegradation of polyethylene (Sowmya *et al.,* 2014). Activities of laccase and manganese peroxide were quite prominent in the biodegradation of polyethylene by *Trichoderma harzianum.* Another studies with fungal strain, *Aspergillus terreus* was found to be efficient in degradation high-density polyethylene. The degradation end products were analysed by weight loss and FT-IR spectroscopy. Keto carbonyl, ester carbonyl, terminal bond, internal bond indices were found to increase in treatment with *Aspergillus terreus*. The *A. terreus* was found to degrade the polyethylene to the extent of 16% in basal minimum medium (BMM) media. (Balasubramanian *et al.,* 2014). Iggui *et al.,* (2015) studied biodegradation of poly (3-hydroxybutyrate-co-3-hydroxyvalerate)/ organoclay nanocomposites in various environmental conditions. Hydrolytic and enzymatic

processes were responsible for biodegradation process. The two endophytic fungi, *Psychotria flavida* and *Humboldtia brunonis* which produces laccase enzymes isolated from two endemic plants capable in degrading low density polyethylene. It was found to be believed that amorphous phase was first attack by the fungi and causing the crystallinity increase and the separation of crystalline blocks of the crystalline mosaic. There was negligible difference for T_m and T_0 in gamma irradiated LDPE films treated with/without fungi, but crystallinity and heat of fusion found to significant changes. (Sheik *et al.*, 2015).

Some fungi play a crucial role in degradation of polythene. Two *Penicillium* species, *Penicillium oxalicum* and *Penicillium chrysogenum* were found to degrade polyethylene. The LDPE and HDPE plastic sheets were tested for biodegradation by the two *Penicillium* species. Culture media with and without carbon source were used for the experiment. The weight loss of the HDPE and LDPE sheets on 90 days of incubation were found to be 58% and 34%, respectively, *Penicillium oxalicum* NS4 (KU559906) and *Penicillium chrysogenum* NS10 (KU559907) (Ojha*et al.*, 2017). Pramila and Ramesh (2017) studied the biodegradation of LDPE by *Aspergilus flavus* and *Mucor cicinelloides* isolated from municipal landfill area. An increase biomass of fungi was reported when incubated with the polyethylene, leads to the biodegradation of polyethylene. The surface morphology of the polyethylene got changed when incubated with the fungi. *Rhizopus oryzae* was found to be capable in degrading low density polyethylene. The fungi was found to grow on polyethylene surface when incubated in potato dextrose broth at 30ºC for a period of 30days. The weight loss of polyethylene was around 9% and a reduction of 60% tensile strength was observed. The fungus, *Rhizopus oryzae* formed a dense mat on the polyethylene surface (Awasthi *et al.*, 2017a). Anchal Rani and Singh (2017) screened polyethylene degrading fungi from polyethylene dump site. They isolated *Aspergillus flavus, Fusarium, A. fumigatous, A. terrus* from a polyethylene dumping site. The species *Aspergillus* and *Fusarium* showed maximum growth on the supplemented minimal salt medium indicating use of polyethylene-carbon as a sole source. Antibiotic susceptibility of selected fungus has been evaluated for four antifungal discs for the safety purpose. *Fusarium* was able to degrade the HDPE and LDPE, respectively, to the extent 77% and 43%.

2.9 Potential of algae in biodegradation of polyethylene

Algae are one of the potential organisms, which are useful to mankind in various ways. Algae constitute potential resource in varied applications such as food and feed, fine chemicals, pharmaceuticals, biofertilizers, biofuel, degradation of organic pollutants accumulation of polycyclic aromatic hydrocarbons (PAHs), phenanthrene (PHE) and fluoranthene (FLA), biodegradation of xenobiotic compounds and biodegradation of polythene (Gatenby *et al.*, 2003; Hong *et al.*, 2008; Kumar *et al.*, 2017; Bhayani *et al.*, 2018). The biochemical composition of algae varies with species and growth condition in a batch culture. The use of algae as a supplement in food and feed due to their balanced nutritional composition and highly valuable compounds such as pigments, polyunsaturated fatty acids, and other biologically active compounds received attention from the researcher around the globe. Algae are also used as an effective bioremediation agent. Algae are known to accumulate various organic pollutants in aquatic environment. The algal species, *Skeletonema costatum* and *Nitzschia* sp. are known to accumulate the phenanthrene and fluoranthene from environment. The tolerance of *Skeletonema costatum* was found to be more than *Nitzschia* sp. The algal species, *S. costatum* and *Nitzschia* sp. were capable of accumulating and degrading the two typical PAHs simultaneously. The accumulation and degradation abilities of *Nitzschia* sp. were higher than those of *S. costatum* (Hong *et al.*, 2008).

Several algal species, *Scenedesmus dimorphus*, *Anabaena spiroides* and *Navicula pupula* were found to be effective in polyethylene degradation. The algal species were found to be grow profusely on polythene sheets. The rate of polyethylene degradation was maximum (8.18%) in *Anabaena spiroides* treatment. The scanning electron microscopy of the treated polyethylene samples revealed that adherence of the cyanobacterium on the polyethylene surface. Minute hole on the LDPE sheets were observed after the treatment. The LDPE sheets treated by *Navicula pupula* was found to be partially eroded (Kumar *et al.*, 2017).

Chapter 3

Materials and methods

3.1 Study Area

3.1.1 Location of the site

The study area Silchar town is located in the Cachar district of Southernmost part of Assam. It is one of the second largest city in the state of Assam. Silchar town is located at 92.24° E and 93.15′E longitude and 24.22° N and 25º8′N latitude **(Fig. 3.1)**. The area of Silchar town is 257.5km^2. It has an average elevation of 25meters (82feet). Cachar district is bounded on the North by Barail and Jayantia hill ranges, on the South by the State Mizoram, on the East by the State Of Manipur and on the West by sister districts Hailakandi and Karimganj. Located in plain and an apparently mountainous region, the district bears

a cultural link-up with South East Asia on one hand and mainland India on the other. Total geographical area of the district is 3,786 Sq. KM.

3.1.2 Climate

The district falls under sub Himalayan regiion zone II and subsequently sub-regionalized under Barak valley zone.The district receives four season in a year i.e.Winter (Dec-Feb) occasional rain and morning fog, Hot season winter (March-May) temperature during the season is not to high due to cloudiness and pre-monsoon rain,Monsoon season (June-Sept) relatively cool and humid and post monsoon season (Oct-Nov) though rainfall occurs during the early part of the season fog also occurs,night temperature gradually falls with reduced rate of humidity.The average rainfall is 3000 mm and average humidity is 85 %. In winter climate is cold and dry. Winter generally begins towards the end of November and lasts till February. Maximum temperature ranges from 30-40°C and minimum temperature is in between 6-12°C.Climate of the Silchar is tropical by nature. Summer is hot and humid in Silchar.During the month of June and July, heavy rainfall is witnessed. Silchar witnesses maximum temperature 32ºC in summer, minimum temperature 12ºC during winter.

3.1.2 Domestic sewage water

Domestic sewage water and algal colonised submerged polythene were collected from twenty sites of Silchar town. Major portions of the domestic sewage water are the outlets of houses and apartments. The sewage water from residence and institutions carries used water such as washing water, food preparation wastes, laundry wastes, sanitary waste and other waste products of normal living from houses and apartments. The present work was carried out to understand the diversity of algae on polythene surface in twenty domestic sewage water drains of Silchar town and the physico-chemical analysis of domestic sewage water of the area is undertaken a specific view to strengthen the domestic waste water quality database which is wish to help the people to know about the serious water pollution problems. The details of the study sites are presented in Table 3.1.

Table 3.1: Geographical location of the study site

Serial No	Name of study sites	Latitude	Longitude	Elevation
1	Link Road 1st	24°48′15.6″N	92°47′43.1″E	120m
2	Link Road 2nd	24°47′59.3″N	92°47′36.4″E	62.9m
3	Sonai Road	24°98′21.7″N	92°47′36.6″E	65m
4	National Highway (NH) Road	24°58′31.7″N	92°36′46.1″E	48m
5	Rangirkhari	24°49′44.4″N	92°47′26.7″E	68m
6	Kanakpur Road	24°40′35.4″N	92°07′22.3″E	145m
7	Tarani Road	24°48′14.9″N	92°44′14.9″E	105m
8	Bilpar Road	24°10′32.7″N	92°34′37.0″E	94m
9	ONGC Colony	24°48′21.6″N	92°47′52.4″E	123m
10	Chenkoorie Road	24°49′35.1″N	92°47′55.3′E	29m
11	Ambikapatty	24°49′45.6″N	92°48′00.9″E	20m
12	Premtola	24°59′59.3″N	92°48′38.4″E	52.9m
13	Vivekananda Road	24°38′31.7″N	92°47′46.6″E	75m
14	Park Road	24°49′31.7″N	92°47′46.1″E	28m
15	Club Road	24°49′47.4″N	92°48′07.7″E	58m
16	Station Road	24°49′14.9″N	92°47′52.1″E	104m
17	Trunk Road	24°49′10.9″N	92°47′45.3″E	140m
18	Koombhirgram Road	24°49′32.7″N	92°47′37.0″E	94m
19	Karimganj Road	24°49′23.6″N	92°47′51.4″E	48m
20	Ramnagar GC college	24°48′21.6″N	92°47′36.6′E	39m

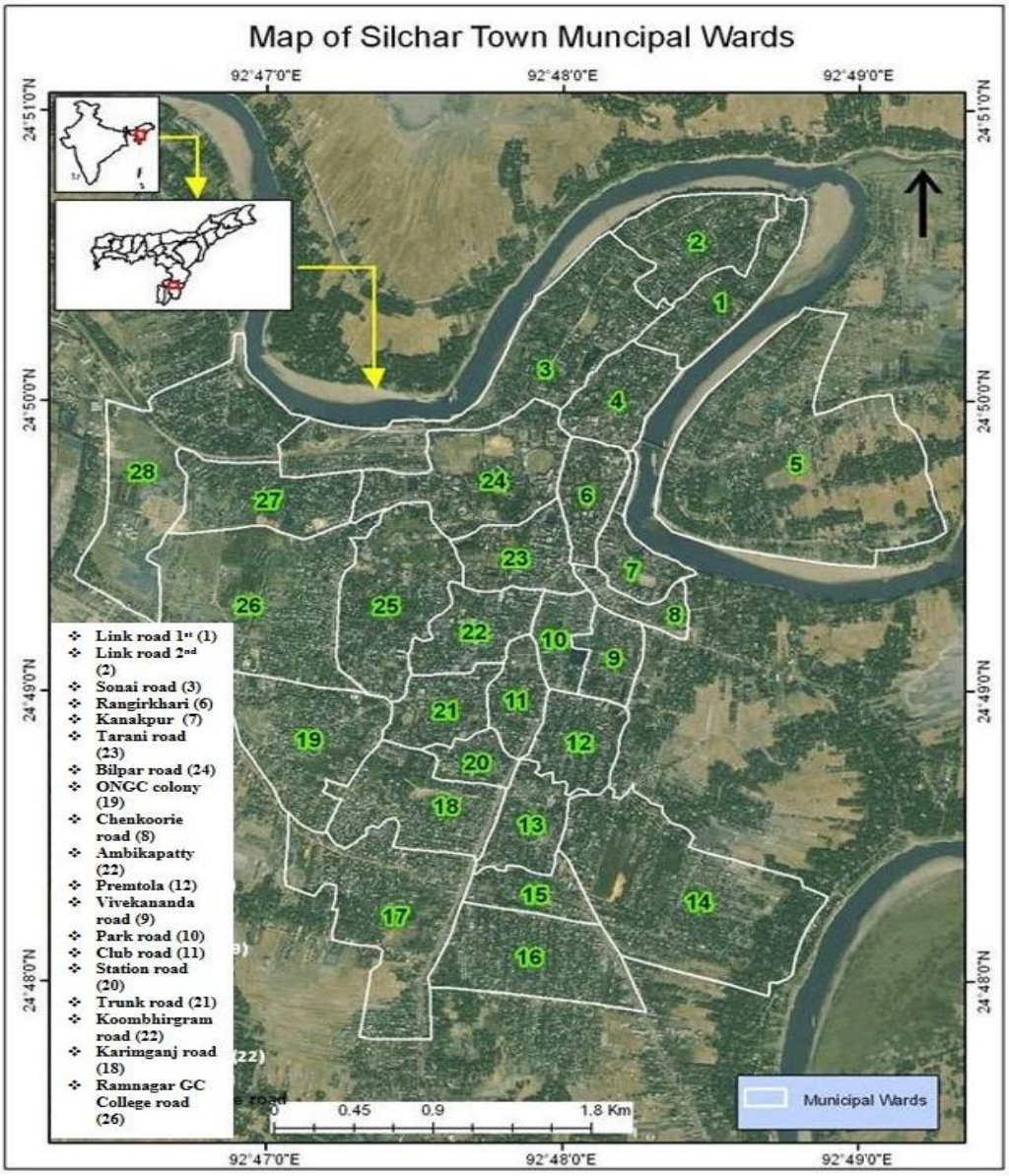

Fig. 3.1: Map of the study area showing the location of study sites

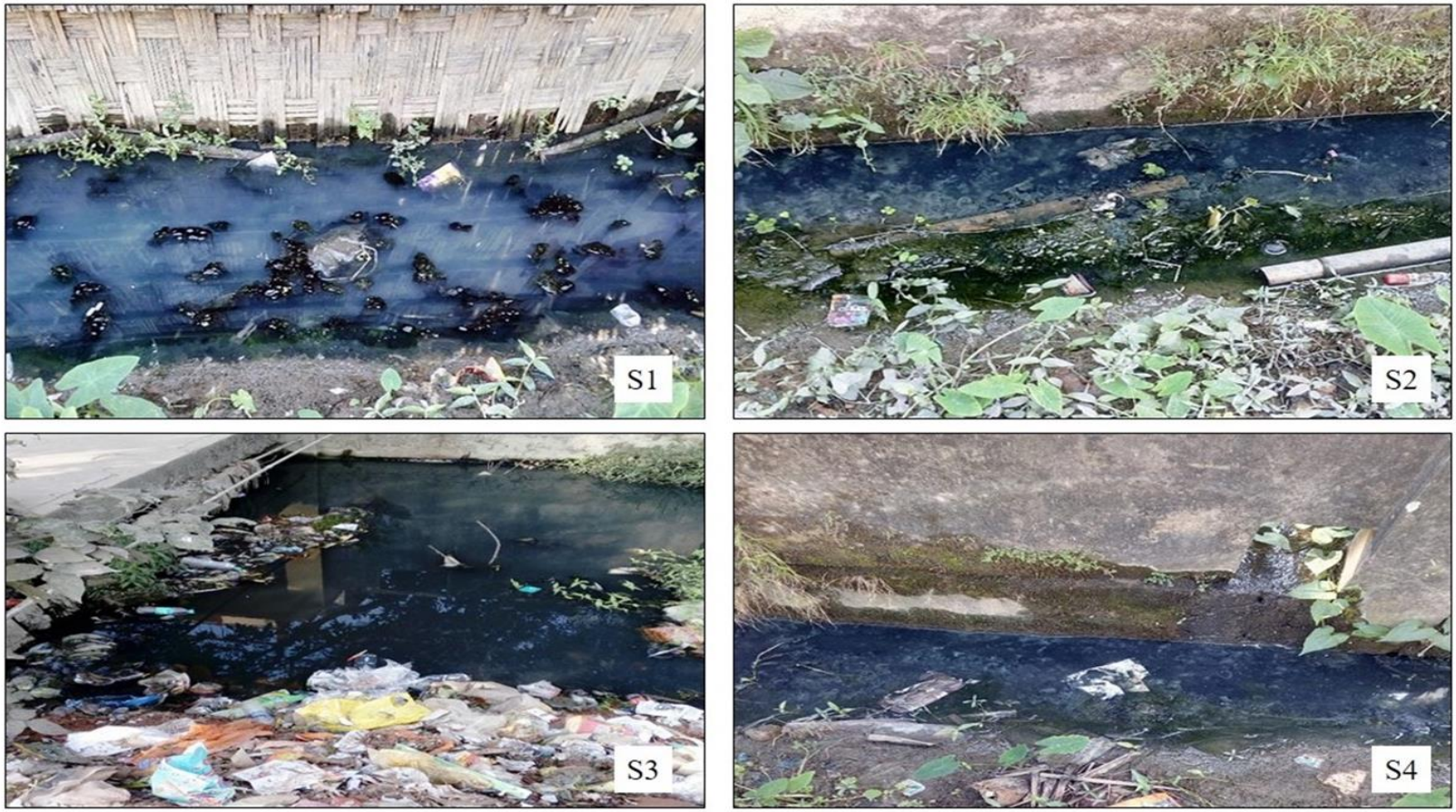

Plate 3.1: The overview of algal colonisation on polythene in domestic sewage water of the study sites Site 1 (S1), Site 2 (S2), Site 3 (S3), and Site 4 (S4)

Plate 3.2: The overview of algal colonisation on polythene in domestic sewage water of the study sites Site 5 (S5), Site 6 (S6), Site 7 (S7), and Site 8 (S8)

Plate 3.3: The overview of algal colonisation on polythene in domestic sewage water of the study sites Site 9 (S9), Site 10 (S10), Site 11 (S11), Site 12 (S12), Site 13 (S13), and Site 14 (S14)

Plate 3.4: The overview of algal colonisation on polythene in domestic sewage water of the study sites Site 15 (S15), Site 16 (S16), Site 17 (S17), Site 18 (S18), Site 19 (S19), and Site 20 (S20)

Plate 3.5: The overview of the algal colonisation on polythene in domestic solid waste dumping study sites Site 1 (S1), Site 2 (S2), Site 3 (S3), Site 4 (S4), Site 5 (S5) and Site 6 (S6)

Plate 3.6: The overview of the algal colonisation on polythene in domestic solid waste dumping study sites Site 7 (S7), Site 8 (S8), Site 9 (S9), and Site 10 (S10)

3.2 Methodology

Geographical location of the twenty study sites of Silchar town, Assam were recorded using GPS (Garmin etrex). The habitat characterization where algae were colonised on polythene bags of the selected sites was done in terms of physico-chemical parameters of water and soil using the standard procedures given below.

3.2.1 Physico-chemical analysis of water

The pH was monitored using a digital pH meter. Biological oxygen demand (BOD) and dissolved oxygen (DO) measured by titrimetric method (APHA, 2005). Chemical oxygen demand (COD) was measured by open reflux method (APHA, 2005). Alkalinity, free CO_2 and magnesium and calcium were measured by titrimetric method (Saxena, 1987). Total dissolved solid (TDS) and suspended solid (SS) were measured by gravimetric method (APHA, 2005). Chloride was measured by argentometric method (Trivedy and Goel, 1986). Sulphate was estimated by turbidimetric method (APHA, 2005). Nitrate was measured by brucine method (APHA, 2005). Soluble reactive phosphate was estimated by molybdate blue method (Wetzel and Likens, 1979). Ammonia was determined by phenol-hypochlorite method (Weatherburn, 1967).

3.2.2 Soil analyses

The algae and soil samples were collected from each sites during the June 2012-May 2013. The soil pH was monitored using Chemline digital pH meter. The conductivity of soil samples were measured using a Systronics direct reading conductivity meter, Type CM-82. Soil bulk density was estimated by soil corer method (Brady and Weil, 2004). The moisture content of the soil was determined by oven drying method (Soil Survey Standard Test Method) (Gupta, 1999). Soil organic matter was analyzed by Walkey and Black's rapid titration method (Jackson, 1958). Nitrogen was analyzed by alkaline permanganate method (Subbiah and Asija, 1956).Available Phosphorous was analyzed by Olsen's method (Olsen *et al.,* 1954). Available Potassium was analyzed by flame photometric method (Black, 1965).

Table 3. 2: Details of the Physico-chemical properties of domestic sewage water

Sl. No	Water Parameters	Unit	Method/Instruments	Reference
1	Water temperature	ºC	Mercury thermometer	-
2	Colour and odour	-	-	-
3	Turbidity	NTU	Turbidity meter	APHA, 2005
4	pH	-	pH meter	-
5	BOD	mg/l	Titrimetry	APHA, 2005
6	COD	mg/l	Open reflux method	-
7	DO	mg/l	Titrimetry	APHA, 2005
8	Alkalinity	mg /l	Titrimetry	Saxena, 1987
9	Free CO_2	mg/l	Titrimetry	Saxena, 1987
10	TDS	mg/l	gravimetric technique	APHA, 2005
11	Suspended solids	mg/l	gravimetric technique	APHA, 2005
12	Chlorides	mg/l	Argentometry	Trivedi and Goel, 1984
13	Ca	mg/l	Titrimetry	Saxena, 1987
14	SO_4^{-2}	mg/l	Turbidimetric method	APHA, 2005
15	Nitrate	mg/l	Brucine method	APHA, 2005
16	Mg	mg/l	Titrimetry	Saxena, 1987
17	Soluble reactive phosphorous (SRP)	mg/l	Molybdate blue method	Wetzel and Likens (1979)
18	Ammonia	mg/l	Phenol-hypochlorite method	Weatherburn,1967

Table 3.3: Details of the Physico-chemical properties of soil of domestic solid waste disposal sites

Sl. No	Soil Parameters	Unit	Method/Instruments	Reference
1	pH	-	pH meter	-
2	conductivity	µS/cm	Conductivity meter	-
3	Soil bulk density	g/cm^3	Soil core method	Brady and Weil, 2004
4	moisture content	%	Oven drying method	Gupta, 1999
5	organic carbon	%	Walkey and Black's rapid titration method	Jackson, 1958
6	Available Nitrogen	%	Alkaline permanganate method	Subbiah and Asija, 1956).
7	Available Phosphorous	%	Olsen's method	Olsen *et al.*,1954
8	Available Potassium	%	Flame photometric method	Black,1965

3.2.3 Algal Analysis

3.2.3.1 Collection and identification of algae from polythene surfaces

Algal samples colonised on polythene bags in domestic sewage water and domestic solid waste dumping sites were collected from different sites randomly. Three replicates of each algal colonised polythene bags sample were collected in every time during the collection. The algae samples from polythene surfaces were scrubbed with a sterilized brush and observed under microscope. A part of collected algae were preserved in 4.5% formalin for identification. Photomicrographs were taken on a Leica application suit (LM1000 LED). The algal samples were identified using standard keys (Presscott, 1952; Desikachary, 1959; Sarode and Kamat, 1984).

3.2 Algal quantitative analysis

Algal colonisation and distribution on polythene bags were monitored by observed under microscope, diversity analysis and estimation of pigments. The polythene bags were cut into 1cm^2 size pieces with a sterilized blade. The algal samples were homogenized in sterile water with glass beads, centrifuged at 3000 rpm for 10 minutes with repeated washing. The algal samples were preserved in 5ml volume using 4.5% formalin. The algal samples were counted using Lackey's drop method (Trivedi and Goel, 1986). A colony as well as a filament more than ¾ was considered as one individual. Chlorophyll *a* of collected samples were measured using trichromatic equation mentioned by Strickland & Parson (1968).

3.2.4 Isolation, identification and growth study of algae

The polythene bags were cut into 1cm^2 size pieces with a sterilized blade. The algae samples from polythene surfaces were scrubbed with a sterilized brush and observed under microscope. The algal samples were homogenized in sterile water with glass beads, centrifuged at 3000 rpm for 10 minutes with repeated washing. The pellets were suspended in sterilized BG-11 medium and placed onto the agar petri plates by pour plate method. The plates were incubated for 15 days under continuous illumination (2000lux) at 24±1^{0}C. The pure colonies developed in the agar plates were picked up and sub-cultured in 500ml Erlenmeyer flask. The method used for isolation and purification of cyanobacteria was according to Rippka *et al.,* (1979). The non-heterocystous isolates were maintained in nitrogen enriched (N^+) and heterocystous isolates were maintained in nitrogen deficient (N^-) medium. The cultures were observed under microscope and the isolated cyanobacterial species were identified using standard keys.

3.2.5 Growth kinetics

Growth rate of isolated algae from polythene surfaces was measured in terms of chl *a* as biomass component (Kobayasi, 1961). Growth kinetics in terms of specific growth rate (K) and generation time (G) were evaluated (Myers and Kratz, 1955).

3.2.6 Biochemical analyses

In the early stationary phase of the growth period, algal samples were harvested for analysis of biochemical and phytochemical analysis. The total carbohydrate was determined

according to anthrone method (Spiro, 1966). Total protein was estimated by modified method of Herbert *et al.,* (1971).The chl *a* and carotenoids were estimated by the standard methods of Strickland and Parsons (1968) and Parson *et al.,* (1984), respectively. Phycobiliproteins estimation has been carried out as per Bennet and Bogorad (1973). Lipid content was estimated by the standard method of Bligh and Dyer (1959). Vitamin C content was evaluated using the method of Roe and Keuther (1943).

The total phenolic content of the methanol extract was estimated by the Folin-Ciocalteu method (Singleton and Rossi, 1965).Total flavonoid content of the culture was determined by aluminium chloride method (Jia *et al.*, 1999). Polyphenol content of the methanol extract was estimated by the Malick and Singh, 1980. The extracellular polymeric substances (EPS) of the cyanobacterial species were estimated by the method of Underwood et al., (1995). Tannin content of the methanol and acetone extract was estimated by the Vanillin-HCl method outline by Makkar and Becker, 1993.

Table 3.4: Details of the biochemical analyses of isolated algae

Sl.No	Biochemical parameters	Unit	Method	Reference
1	Chlorophyll *a*	µg/ml	spectrophotometrically	Strickland and Parsons (1968).
2	carotenoides	µg/ml	”	Parsons *et al.,* (1984)
3	total soluble proteins	µg/ml	”	Herbert *et al.*, 1971
4	total carbohydrates	µg/ml	”	Spiro, 1966
5	phycobiliproteins	µg/ml	”	Bennett and Bogorad (1973)
6	Lipid	µg/ml	”	Bligh and Dyer 1959
7	Ascorbic acid	µg/ml	”	Roe and Keuther, 1953
8	Total phenolic content	µg/ml	”	Singleton and Rossi,1965)
9	Polyphenols	µg/ml	”	Malick and Singh, 1980
10	Tannins	µg/ml	”	–
11	Flavonoids	µg/ml	”	–

3.2.7 Enzymatic antioxidants and non-enzymatic antioxidants

Standard protocol were followed for catalase activity (Aebi, 1984), peroxidase activity (Kar and Mishra, 1976) and glutathione peroxidase activity (Rotruk *et al.*, 1973). Ascorbic acid content was estimated as per Roe and Keuther (1943). Glutathione reductase was assayed following the method of Scaedle and Bassham (1977).

3.2.8 DPPH free radical scavenging activity, hydroxyl radical scavenging activity, total antioxidant activity

DPPH free radical scavenging activity was measured according to Sanchez-Moreno *et al.*, 1995. Hydroxyl radical scavenging activity was measured by the method outlined by Ruch *et al.*, 1989 and that of total antioxidant activity was assessed by the method of Prieto *et al.*, 1999.

Table 3.5: Details of the enzymatic antioxidants and non-enzymatic antioxidants of isolated algae

Sl.No	Enzymatic antioxidants and Non-enzymatic antioxidants	Unit	Method	Reference
1	Catalase	µg/ml	spectrophotometrically	Aebi (1984),
2	Peroxidase	µg/ml	”	Kar and Mishra (1976)
3	Glutathione peroxidase	µg/ml	”	Rotruk *et al.*, 1973
4	Ascorbic acid	µg/ml	”	Roe and Keuther (1943)
5	DPPH free radical scavenging activity	%	”	Moreno-Sanchez *et al.*, (1995)
6	Hydroxyl radical scavenging activity	%	”	Glucin *et al.*, (2004)
7	total antioxidant activity	%	”	Prieto *et al.*, 1999

3.3 Phycoremediation of domestic sewage water

3.3.1 Algal isolates used for remediation of sewage water

Three algal isolates viz. *Oscillatoria subbrevis*, *Nostoc carneum* and *Chlorella ellipsoidea* were isolated from submerged polythene surface in domestic sewage water were used for phycoremediation of sewage water.

3.3.2 Experimental Design

Domestic sewage water was collected from house outlets of Vivekananda road of Silchar town, Assam. A treatment series was made to screen the remediation potentials of algae in which distilled water and sewage water were mixed in different proportions (Table 3.5).

Table 3.6 Details of treatment series used for phycoremediation

Serial no	Treatment series	*Oscillatoria subbrevis*	*Nostoc carneum*	*Chlorella ellipsoidea*
1	BG11	1a	2a	3a
2	DSW	1b	2b	3b
3	BG11 (10%) + (90%) DSW	1c	2c	3c
4	BG11 (20%) + (80%) DSW	1d	2d	3d
5	BG11 (40%) + (60%) DSW	1e	2e	3e
6	BG11 (50%) + (50%) DSW	1f	2f	3f
7	BG11 (60%) + (40%) DSW	1g	2g	3g
8	BG11 (80%) + (20%) DSW	1h	2h	3h
9	BG11 (90%) + (10%) DSW	1i	2i	3i

3.3.3 Growth study during phycoremediation

The growth of algae in BG-11 and sewage water only and in mixture of sewage water and media were measured separately by estimating the chlorophyll *a* on each alternate day for a period of 6 weeks (Kobayasi, 1961). The growth curves, specific growth rates (μ, h^{-1}) were calculated in log period as per Myers and Kratz (1955). The total carbohydrate (Spiro, 1966) and protein (Herbert *et al.*, 1971) content were analysed. Enzymatic antioxidants and non-enzymatic antioxidants were also measured according to standards mentioned in Table 3.4.

3.3.4 Analytical Procedures for phycoremediation

Physico-chemical characteristics of sewage water viz. pH, total dissolved solids (TDS), electrical conductivity (EC), dissolved solids (DO), chlorides, nitrate and phosphate were measured according to standards given in Table 3.1. The rate of removal of nitrate and phosphate was calculated by following equation:

Removal Rate (mgd^{-1}) $=C_i-C_f$ / t; where C_i and C_f are initial and final concentration of nutrient of interest and t is the time duration.

After 20 d, 10mL of homogenised culture was filtered through pre-weighed Whatman No. 41 (ashless) filters and dried at 60°C, until constant weight was achieved. Dry cell weight (DCW) was calculated by subtracting the initial filter paper weight from final weight of filter paper containing dried algae and was expressed as mgL^{-1}.

Chla *a* was estimated according to equation given by Strickland and Parsons (1968). Physiological Stress Index (chlorophyll to phaeophytin ratio) was measured according to the method given by Megateli *et al.,* (2009). Conversion of phaeophytin from chlorophyll was carried out by addition of 10 µL HCl (35% GR, Merck) to 3ml of extract. Ratios of D430/D410 (Phaeopigment Index), D430/D665 (Margalef Index I), D480 /D665 (Margalef Index II) were also recorded by the method of Martinez-Abaigar and Nùñez-Oliveira (1998).

3.3 Biodegradation of polythene

The ASTM (2000) standards were followed for the present study. The ASTM D 5338-98 has been chosen for the tests in determining the aerobic biodegradation of polyethylene materials under controlled composting conditions. The aqueous test method, Test Method D 5247 (specific microbe test) which uses pure microbial cultures to assess the biodegradability of materials based on weight loss has been used in the present study. For ascertaining degradation, the standards used were: ASTM D 3593 Test Method (FTIR and NMR), ASTM D 638 Test Method (SEM), ASTM D 638 Test Method (tensile properties), and ASTM D 5247 Test Method (weight loss).

According to IUPAC Gold book (2006) “Breakdown of a substance catalysed by enzymes *in vitro* or *in vivo*. This may be characterized for purposes of hazard assessment as: primary biodegradation, alteration of the chemical structure of a substance resulting in loss of a specific property of that substance”.

3.3.1 Materials

Low Density Polyethylene (LDPE) strips preparation

Polythenes bags (LDPE) (Kirana bags, made by North East plastic products) were collected from a stationary shop in Silchar medical college & Hospital point, Silchar, Assam, India. Polyethylene (LDPE) sheets of 20µ thickness were dried under ambient conditions and cut into strips (1cm×1cm), washed with 70% ethanol followed by distilled water.

3.3.2 Visual observations

3.3.3 Optical microscopy

The changes in the surface morphology of polyethylene (PE) and formation of algal colonisation on the surface were monitored using an optical microscope, StereoZoom Leica S8 APO.

3.3.4 Scanning electron microscopy (SEM)

The surface morphology of the polyethylene (PE) such as cracks, de-fragmentation, changes in color has also been observed through Scanning Electron Microscopy (A JEOL equipment Japan, Model: JSM 6390 LV) to check any structural changes on the strip. The Pt-coated polythene strips were placed on the sample holder and scanned at magnification of 1000x, 3000x, 5000x and 10000x.

3.3.5 Fourier transform infra-red (FT-IR) analysis

The structural changes in polyethylene (PE) surface was analyzed by a Nicolet, USA equipment, (Model: Impact 410) at a resolution of $1cm^{-1}$, in the frequency range of 4000-$400cm^{-1}$. Relative absorbance intensities (I) of the ester carbonyl bond at $1740cm^{-1}$, keto carbonyl bond at $715cm^{-1}$, terminal double bond (vinyl) bond at $1650cm^{-1}$ and internal double bond at $908cm^{-1}$ to that of the methylene bond at $1465cm^{-1}$ were evaluated from the intensity ratio of characteristic vibrational transitions (Albertsson *et al.*, 1987): keto carbonyl bond index (KCBI)= I_{1715}/I_{1465}, ester carbonyl bond index (ECBI)=I_{1740}/I_{1465}, vinyl bond index (VBI)= I_{1650}/I_{1465}, internal double bond index (IDBI)= I_{908}/I_{1465}.The crystallinity (%) of the polyethylene were measured based on the method of Zerbi *et al.*, (1989) and calculated by using the formula: crystallinity (%) = $100-[(I-(I_a/1.233I_b)/I+(I_a/I_b))\times100]$, where crystallinity is the percentage of the amorphous content, I_a and I_b are

the intensities of absorption bands at 1474 and 1464cm^{-1}, respectively. The constant 1.233 corresponds to the relations of intensity bands of fully crystalline polyethylene.

3.3.6 Carbon, hydrogen, nitrogen (CHN) analysis

The percentage of carbon, hydrogen and nitrogen was measured using 0.1g of dried, ground and homogenized polyethylene into a clean, carbon free combustion boat using a CHN analyser (Perkin Elmer, USA model: 2400 series 2).

3.3.7 Determination of rate of biodegradation by weight loss study

To study the rate of biodegradation by weight loss, the initial weight of polyethylene were measured using an electronic a balance (Mettler Toledo RS 231C). To assess the reduced weight loss in every week, polyethylene were removed, air dried and compared with those of the respective samples before biodegradation. Every time polyethylene were washed with 2% SDS solution and air dried to ensure the complete removal of cyanobacterial biomass.

Weight loss (%) = (Initial weight-final weight)/Initial weight × 100

3.3.8 Nuclear magnetic resonance (NMR) spectroscopy

The nature of chemical composition of polythene and the treated ones were analyzed using ^{13}C NMR spectroscopy (JEOL equipment, Japan, Model: ECS-400). For analysis, polyethylene of both control and treated were dissolved in 1, 2, 4-trichlorobenzene in 10mm tubes at 120ºC and a few drops of dimethyl sulfoxide (DMSO) were added as an internal lock. Hexamethyldisiloxane (HMDS) was used as chemical shift reference. Spectra were recorded in complete decoupling mode under following conditions: pulse interval, 1s; pulse delay, 5s; spectral width 500Hz; number of accumulations, 1500-2000; number of data points per spectrum 8000.

3.3.9 Mechanical properties

The tensile strenght, percentage of elongation at break and modulus of elasticity measures the stress at fracture of the specimen and the extension of the material under load measured by UTM Ratankar Enterprises Model : KUT-100 (E) UTNSRL.For tensile strenght, polyethylene were cut into 5cmx20cm. For, tensile strenght a separate series of conical flasks were maintained.

3.3.10 Thermo-gravimetric (TGA-DSC) analysis

Melting point (T_m) of the 0.1g LDPE polyethylene and enthalpy changes were analysed by a Shimadzu Thermal Analyser (TGADSC) (Model: TGA-50 & DSC-60). The lamellar thickness, L_c ($L_c=2\sigma e/\Delta h$ ($T_{m0}/T_{m0}-T_m$) was estimated from the melting point following a modified procedure (Hoffman *et al.*, 1976).

3.3.11 Enzyme Activity

A 1ml culture from cyanobacterial treatment was added separately to 1ml of 2mM guaiacol and 3ml of sodium acetate buffer (pH 4.6). The reaction mixture containing guiacol and sodium acetate was incubated at 30ºC for 15min and the absorptions were recorded in a UV-VIS spectrophotometer at 450nm. One unit of laccase activity was defined as amount of enzyme required to hydrolyze guiacol during incubation period. For manganese peroxidase, a rather similar procedure was followed (Papinutti and Marttinez, 2006).

3.4 Statistical analysis

All the data were given as mean with standard deviation to have an essence of the whole dataset. Quantitative characterization of algal community (species density, abundance and frequency) was done following Dash and Dash (2009). The algal community structure was analyzed using different diversity indices. Shannon-Wiener diversity index-H, Simpson's dominance index-D, Pielou's evenness index-J were calculated with the help of statistical software, PAST V-3. The same statistical software was also used for performing multivariate analysis of ecological data by Canonical Correspondence Analysis (CCA). All other statistical analyses like Analysis of variance (ANOVA), Tukey multiple comparisons, bivariate correlation, Principal Component Analyses (PCA), and hierarchical cluster analysis were carried out by SPSS V-21.

Chapter 4

ALGAL COLONISATION, DIVERSITY AND DISTRIBUTION PATTERN ON DEGRADING POLYTHENE BAGS

As already discussed, algae are among the most diverse and ubiquitous organisms on earth occupying an enormous range of ecological conditions from lakes and rivers to acidic peat swamps, inland saline lakes, snow and ice, damp soils, wetlands, desert soils, wastewater treatment plants, and are symbionts in and on many plants, fungi, and animals (Fogg *et al.*, 1973). Exploration of algae from a particular habitat in relation to biodiversity of that habitat, productivity, interactions with other organisms, water quality, and influence of physio-chemical properties on algae is of utmost significance.

Each year milion tonnes of polythene waste are buried in landfill sites. Polythene waste includes used polythene bags, carry bags, and the polythenes used for packaging purposes.

Due to its resistance, low cost, and easy to carry polythene bags gained widespread popularity especially in packaging purposes. The increasing use of polythene bags creates major environmental pollution and problems in waste material issues and hazardous to soil and water flora and fauna.Polythene bags when disposed to landfill or domestic sewage water drains, they creates problems in soil water holding capacity or clogged the drains. When the polythene bags are incinerated, they can emit poisonous gases such as dioxins abetting air pollution.

Algae are the primary colonizers of building materials, wall, rocks, etc. and play a significant role in their biodeterioration. Extracellular polymeric substances (EPS) excreates by algae helps in adherence on surfaces. According to Ford and Mitchell (1990), phytoplankton species along with other aquatic microbes are the primary colonizers that form biofilm and serve as cue for other larger organisms to colonize on the surface. In summer months, when the water bodies are dry, the partially decomposed / degraded polythenes exposed to the sun, split into small pieces with bacterial and algal attachment and are released into the environment (Seneviranlne *et al.*, 2006). In the present investigation, physio-chemical properties of both water and soil, seasonal variation of algal communities with special reference to diversity of cyanobacteria had been investigated and correlated.

4.2 Methodology

A calendar year was divided into four seasons viz. monsoon (Jun to August), post monsoon (September to November), winter (December to February) and pre monsoon (March to May) based on the rainfall of the study area. For habitat characterization, water samples were collected bimonthly during 2012-2013. The soil samples were collected and analyzed only one time during September to November. Details of the methods of physico-chemical analysis of water and soil, collection and identification and quantitative analyses of algae were already described at Chapter 3. Algal diversity on the polythene bags were studied from February, 2012-March, 2013 from the 20 different study sites. The algal community structure was analyzed using the diversity indices. Shannon-Wiener diversity index-H, Simpson's dominance index-D and Pielou's evenness index-J were calculated by using the statistical software, PAST V-3. For soil data, one way ANOVA was applied. To establish

the interrelationship between different variables bivariate correlation, Principal Component Analyses (PCA) and Hierarchial cluster analysis were made. The statistical analyses were carried out using the statistical software package, SPSS V-21. The statistical software PAST V-3 was used for performing multivariate analysis of ecological data by Canonical Correspondence Analysis (CCA). The method operates for data on occurrences or abundances (e.g. counts of individuals) of species and data on environmental variables at sites.The number per taxon was logarithmically transformed so as to down weight large numbers. The problem of taking the logarithm of zero was circumvented by adding one to each number before transformation.

4.3 Results and discussion

4.3.1.1 Physico-chemical analyses of water

The variation of physico-chemical parameters of the water of the study sites during the sampling period are shown in **Fig.4.1–Fig.4.36**. The mean water temperature of the sewage water was in the range 17.52-34.94°C. The pH values was in the range 5.4-8.4. The value of pH was found to be maximum in Park road (8.4 ± 0.51) during premonsoon and minimum in Kanakpur during postmonsoon season (5.4 ± 0.31). The value of BOD was in the range 329-608mg/L. The value of BOD was found to be maxiumum in Koombhirgram (608 ± 1.2mg/L) road during postmoonsoon, minimum in Park road (329 ± 2.5mg/L) in premonsoon. The DO was in the range 1.0-2.4 mg/L, maxiumum being in Bilpar road (2.4 ± 0.2mg/L) during postmonsoon season, minimum in NH road (1.0 ± 0.02mg/L) during premonsoon season. The COD was in the range 1193-2192mg/L. COD was found to be maximum in Hospital road (2192 ± 3.2mg/L) during premonsoon season, minimum in Club road (1193 ± 2.2mg/L) in postmonsoon season. The total alkalinity of sewage water was in the range 6.8-11.0mg/L. Total alkalinity was found to be maximum in Club road, Station road, Trunk road, Koombhirgram and Karimganj road (11.0 ± 1.2mg/L) during premonsoon season, minimum in Premtola and Chenkoorie road (6.8 ± 1.1mg/L) during monsoon season. Free CO_2 was in the range 34-62 mg/L. The value of free CO_2 was found to be minimum in Ambikapatty and Link road 1st (34 ± 0.2mg/L) during postmoon season, maximum in Sonai road (62 ± 1.2mg/L) during monsoon season. The TDS was in the range 265-3200mg/L. The value TDS was found to be maxiumum in Link road 2nd (3200 ±

4.2mg/L) in premonsoon season, minimum in Karimganj road (265 ± 0.32mg/L) in premonsoon season. The SS of sewage water ranged from 23-694mg/L. The SS was found to be maxiumum in Rangirkhari (694 ± 2.3mg/L) and minimum in Trunk road (23± 0.12mg/L). The chloride concentration was in the range 14-895 mg/L. The maximum chloride was found to be in NH road (895±1.3mg/L) during premonsoon and postmonsoon season. Sulphate concentration of sewage water ranged from 345-907 mg/L. Maximum sulphate was found to be in Station road (907 ±1.2mg/L) in monsoon season, minimum was found to be in Kanakpur road (345 ± 1.4mg/L) in winter season. Nitrate concentration was in the range 13-789mg/L. Maximum nitrate concentration was found to be in NH road (789 ± 1.2mg/L) in premonsoon season, minimum was found to be in Club road (13 ± 0.02mg/L).Calcium concentration of sewage water was in the range 21-497 mg/L. Maximum calcium concentration was found to be in Sonai road (497 ± 0.34mg/L) in premonsoon season, minimum was found to be in Ramnagar GC College road (21 ± 0.06mg/L) in premonsoon season. Protein and carbohydrate present in the domestic sewage water of Silchar town were presented in Fig.4.27 and Fig.4.28. Carbohydrate content of domestic sewage water was found to be maximum in Link road 2nd (54.4 ± 0.32µg/ml) and minimum in Tarani road (10 ± 0.02µg/ml). Protein content of domestic sewage water was found to be maximum in Link road 1st (53.9 ± 0.56µg/ml) and minimum in Trunk road (16.4 ± 0.21 µg/ml).Turbidity was found to be maximum in Link road 1st during premonsoon season (543 ± 0.23 NTU) and lowest in postmonsoon season in Sonai road (18 ± 0.06 NTU). The ammonia concentration of sewage water was found to be maximum in NH road (158 ± 0.06 mg/L) in premonsoon season and minimum in Bilpar road during winter (30 ± 0.12 mg/L). The phosphate concentration was found to be maximum in NH road during premonsoon season (198 ± 0.04mg/L), minimum in Bilpar road during winter (29 ± 0.01mg/L). Magnesium concentration of sewage water was found to be maximum in Link road 1st during premonsoon season (98 ± 0.06mg/L), minimum in Park road (31 ± 0.02mg/L) during postmonsoon season. All the physico-chemical properties of water concentration were varied significantly ($p < 0.05$) between the sites and the seasons.

4.3.1.2 Physico-chemical analyses of domestic solid waste dumping site soil

The different soil parameters of domestic solid waste dumping site of Silchar town have been shown in **Fig. 4.31 to 4.38**. Soil pH was found to be maximum at Sonai road (7.47 ± 0.02) and minimum at Tarani road (5.6 ± 0.03). Electrical conductivity of soil was found to be maximum in Link road 2nd (160 ± 0.34µS/cm) and minimum in Link road 1st (97.6 ± 0.23µS/cm). Moisture content of domestic solid waste dumping site soil was found to be maximum in Kanakpur (58.4 ± 0.21%) and minimum in Bilpar road (34 ± 0.32%). Bulk density of soil was found to be maximum in NH road (3.44 ± 0.03g/cm^3) and minimum in Rangirkhari (0.86 ± 0.02g/cm^3). Percent of organic matter was found to be maximum in Link road 2nd (2.67 ± 0.03%) and minimum in Chenkoorie road (0.96 ± 0.01%).Total nitrogen content of soil was found to be maximum in Link road 1st (0.78 ± 0.04%) and minimum in NH road (0.07 ± 0.03%). Available phosphorus of soil was found to be maximum in Sonai road (5.57 ± 0.04mg/kg) and minimum in Bilpar load (2.8 ± 0.03mg/kg). Potassium of domestic solid waste dumping site soil was maximum in Tarani road (192 ± 0.34ppm) and minimum in Bilpar road (141 ± 0.04ppm). Most of the mean difference between the sites were observed to be significant ($p<0.05$).

4.3.2 Algal distribution

Algal abundance is usually determined by three principal methods: measurement of pigments and direct observation. However, not one is completely satisfactory (Roger and Kulasooriya, 1980). In this study two techniques were applied in order to have a more acceptable picture of algal distribution.

4.3.2.1 Distribution and diversity analysis of algal population from polythene bags of domestic sewage water drains

A total of 122 algal species belonging to 38 genera under 4 classes had been enumerated from the study sites. Cyanophyceae was the best represented group encountered in the present study. The class Cyanophyceae was represented by 13 genus and 58 species, Chlorophyceae (genus-15, species-25) and Bacillariophyceae (genus-11, species-31). Euglenophyceae was represented by only two species and formed a small component of the algal community. The microscopic photographs of algae encountered in this study are shown in plate **4.5 to 4.7** (Cyanophyceae), **plate 4.8-4.9** (Chlorophyceae), **plate 4.10-4.13** (Bacillariophyceae), **plate 4.14** (Euglenophyceae). Highest number of blue green algal species belonged to the genus

Oscillatoria (29) followed by *Anabaena* (4), *Phormidium* and *Lyngbya* (3) **(Table 4.4).** The densities, abundance, frequency of the algal species are presented in **Table 4.6-4.25**. The ubiquitous forms of blue green algae found in all the sites were mostly of the genus *Anabaena, Cylindrospermum*, *Lyngbya*, *Nostoc, Oscillatoria* and *Phormidium*. The blue green algae were more abundantly found to colonise during the post monsoon season on the degraded polythene bag surfaces in domestic sewage water. The genus *Oscillatoria* was found to be profusely colonised on surfaces of degraded polythene bags. Relative abundances of twenty study sites of four algal groups are given **Fig.4.45-Fig.4.46**. Relative abundances of Cyanophyceace are shown in **Fig. 4.47**. Relative abundances of Chlorophyceae are shown in **Fig.4.48**. Relative abundances of Bacillariophyceae are shown in **Fig.4.49**. Relative abundances of Euglenophyceae are shown in **Fig.4.50.**

Biological diversity can be quantified in many different ways. The three main factors taken into account while assessing diversity are richness, evenness and dominance. Shannon diversity index takes into account the number of individuals as well as number of taxa (varies from 0 for communities with only a single taxon to high values for communities with many taxa), each with few individuals. Dominance index measures the probability that two individuals randomly selected from a sample will belong to the same species. Dominance index ranges from 0 (all taxa are equally present) to 1 (one taxon dominates the community completely). Equitability, a measure of the evenness with which individuals are divided among the taxa present. Highest algal diversity was observed in Link road 1st (Site 1) during premonsoon season with maximum Shannon-Wiener diversity index (H=2.81 ± 0.15), minimum Simpson's dominance index (D = 0.035 ± 0.14) and maximum Pielou's evenness index (J = 0.98 ± 0.01). The algal distribution in Club road during post monsoon was observed to be least diverse with minimum Shannon Wiener diversity index (H = 0.98 ± 0.14), maximum Simpson's dominance index (D = 0.70 ± 0.03) and minimum Pielou's evenness index (J = 0.26 ± 0.33) (**Table 4.28**).

4.3.2.3 Algal biomass analysis in terms of chlorophyll a

Chlorophyll content and abundance of algae are directly related. Chlorophyll *a* is the electron acceptor for photosystem I. In photosynthesis, chlorophyll *a* is the key pigment molecule. Chlorophyll *a* is directly related with the primary productivity. In winter season, chlorophyll *a* concentration was found to be maximum in Link road 1st and Trunk road. In postmonsoon season, maximum chlorophyll *a* content found to be in club road, while Link road 2nd and Trunk road showed second maximum chlorophyll *a* content (**Fig.4.51; Fig.4.52**).

4.3.3 Interrelations among the physico-chemical and distribution of algal groups on polythene surface in domestic sewage water

Pearson's correlation coefficients calculated between various physico-chemical properties of water and total Cyanobacterial species, Chlorophyceae species and total Bacillariophyceae species present on submerged polythene bags in domestic sewage water drains have been presented in **Table 4.29**. Domestic sewage water temperature has positive correlation with total Cyanobacterial species ($r=0.583^{**}$, $p < 0.01$), total Chlorophyceae species ($r=0.311^{**}$, $p <0.01$) and negative correlation with total Bacillariophyceae species (r=0.583**, $p < 0.01$). pH has positive correlation with total Cyanobacterial species ($r =.942^{**}$,$p < 2.21$), negative correlation with total Chlorophyceae and Bacillariophyceae species (r =**-0.992****,**-0.959******,** $p < 0.01$), respectively, in the domestic sewage water. BOD has positive correlation with total Cyanobacterial species (r =**.311****, $p <0.01$) and negative correlation with total Chlorophyceae species ($r =-0.226^{*}$, $p <0.05$) in the domestic sewage water. The DO has positive correlation with total cyanobacterial species ($r=0.656^{**}$, $p< 0.01$) and negative correlation with total Chlorophyceae species ($r=-0.626^{**}$, $p< 0.01$). Total alkalinity (TLK) has positive correlation with total cyanobacterial species (r=0**.923****, $p< 0.01$), negative correlation with total Chlorophyceae species and Bacillariophyceae species ($r=-0.995^{**}$,**-0.938****, $p< 0.01$), respectively. Suspended solid (SS) has positive correlation with total Cyanobacterial species (r=0.**960****, $p < 0.01$) and negative correlation with total Chlorophyceae species and Bacillariophyceae species (r=**-0.974****,**-0.941****, $p < 0.01$), respectively. Sulphate, nitrate, calcium and free CO_2 has positive correlation with

total Cyanobacterial species (r=0.585**, 0.446**, 0.442**, 0.609**, p <0.01), negative correlation with total Chlorophyceae species and Bacillariophyceae species.

In the present study, PCA has been used as useful technique to evaluate the relation between physico-chemical parameters and distribution of algal groups. The loading plots for the PCA of the relative abundance of the algal groups are shown in the Fig. **4.53** and component loading scores with the two dimensions are given at Table **4.30.** In the present study, the 15 variables were analysed i.e., pH (F1), BOD (F2), COD (F3), DO (F4), Alkalinity (F5), free CO_2 (F6), TDS (F7), SS (F8), Chloride (F9), Sulphate (F10), Nitrate (F11), Ca (F12), total cyanobacterial species (F13), total Chlorophyceae species (F14), and total Bacillariophyceae species (F15). The parameters pH, BOD, COD, DO, and alkalinity were found to inter-related. The contribution of five component on the total variance of the data (28.56, 23.34, 17.61, 12.42, 10.18% for F1 and F5) shows that water parameters defined the environmental pollution status of domestic sewage water. Free CO_2 (F6), TDS (F7), and SS (F8) are found to be interrelated. Final component such as, chloride (F9), sulphate (F10), nitrate (F11), Ca (F12), total cyanobacterial species (F13), total Chlorophyceae Species (F14), and total Bacillariophyceae Species (F15) are found to correlate. It is clear from the percentage of total variance that each component and their relationships could specify the colonisation of algae on submerged polythene surfaces. For example, the strong positive loadings of pH, alkalinity, chloride, Ca, total Chlorophyceae species and total Bacillariophyceae species, with negative loadings of BOD, COD, DO, free CO_2, TDS, Nitrate, total Cyanophyceae species for the first component indicate that high levels of dissolved organic matter consume large amounts of oxygen. Decomposition of organic matter in domestic sewage water that leads to formation of acids that leads to formation of organic acids, CO_2 and ammonia. The high SS, sulphate, Ca loadings of domestic sewage water was found to influence the colonisation of algae on submerged polythene surfaces.

Nitrate and phosphate concentration present in domestic sewage water affected the colonisation pattern and distribution of algal species. Water temperature of sewage water also affected the distribution of cyanobacterial species. This findings are consistent with the observation made by Robarts and Zohary (1987), who explained that cyanobacteria

blooms are likely to occur during the summer in temperate water. The ranged of pH and concentration of free CO_2 of domestic sewage water influenced the distribution pattern of total cyanobacterial species. The growth of cyanobacterial population were influenced by high pH and low free CO_2 (Shapiro, 1992).

Due to heavy polluted discharge by household and small scale industries to domestic sewage water drains, nitrate, sulphate and calcium were found to be present in high amount. Nitrate, sulphate and calcium showed positive correlation with total cyanobacterial species. Higher concentration of ammonium and phosphate were ascertained to be favourable for bloom of cyanobacterial populations (Chellappa *et al.*, 2000; Gabriela and Alessandra, 2004; Vidya *et al.*, 2014). Nitrogen and phosphorus are both responsible for abundance of phytoplankton polulation (Gabriela and Alessandra, 2004). Nitrogen and phosphate concentration were found to be directly proportional to phytoplankton abundance in water (Coelho *et al.*, 2003).

In the present investigation, dissolved oxygen ranged between 1.3-0.4mg/l revealing domestic sewage water is highly polluted. Low dissolved oxygen primarily results from excessive algae growth caused by phosphorus. The DO has positive correlation with total Oscillatoria species. Calcium concentration of domestic sewage water ranged between 54-69 mg/l. The abundance of cyanobacterial population is known to be influenced by calcium concentration (Sarojini, 1996). Lower DO concentration and other physiochemical parameters showed that domestic sewage water is highly polluted. The genus *Oscillatoria* has been earlier noted to be tolerant to pollutants in water (Rai and Kumar, 1976). Free CO_2 value in the range 36-40mg/l in the present study and showed positive correlation with total cyanobacterial species. Lower free CO_2 value influenced the growth of cyanobacterial population. It has been observed earlier that green algae cannot thrive in low free CO_2 concentration (King, 1972). Effect of anthropogenic stressors, effluent influenced the growth of phytoplankton diversity. In our present investigation, high nutrients conditions influence the abundance of *Oscillatoria* population. The domestic sewage water drains of Silchar town carries domestic waste, municipal waste and small scale industries waste. Water qualities of the domestic sewage water were analysed and showed high amount of organic and inorganic substances present. Singh *et al.*, (2013) reported that physiochemical

parameters greatly affected the cyanobacterial population in river Gomati. The domestic sewage, industrial waste, municipal waste water, human excreta, agricultural runoff and burning of corpse polluted the Gomati river in Uttar Pradesh. A total number of 35 genera of algae, Cyanophyceae (11 genera), Chlorophyceae (10 genera), Euglenophyceae (1 genera) and bacillariophyceae (11 genera) were recorded in their observation. Jaiswal *et al.,* (2017) reported the occurrence of organic pollutants in different water habitats and correlated the physico-chemical parameters with palmer algal genus index. They found that *Oscillatoria* alongwith frequently with other algal genera constituted around 49% of the total algal cover. This matches with the present findings of *Oscillatoria* occuring as largest genus on submerged polythene bags in domestic sewage water. A study on algal colonization on polythenes and their degradation revealed fifteen species including *Oscillatoria* in and around water bodies of Lucknow city in Uttar Pradesh (Suseela and Toppo, 2007). Sharma *et al.,* (2014) detected ten algal species *Phormidium tenue, Oscillatoria tenuis, Navicula cuspidata, Monoraphidium contortum, Microcystis aeruginosa, Closterium costatum, Chlorella vulgaris* on polythenes on waste water of Kota city, Rajasthan. The species *Oscillatoria* thus may be useful in some monitoring programme (Cairns and Dickson, 1971; James and Evison, 1979) and also in the bidegaradation of polythene (Kumar *et al.,* 2017, Sharma *et al.,* 2014).

In the present investigtion, total cyanobacterial species showed positive correlation with total nitrogen, available phosphrus and available potassium present in soil of domestic solid waste dumping site. Soil algae can also bind with Na^+ and K^+ ions and thus reduces the soil salinity (Subhashini and Kaushik, l98l). Algae are also reported to bring down the level of oxidizable matter in soil especially sulphate and iron content (Aiyer *et al.,* 1971). Many algae have been found to be -capable of solubilizing insoluble phosphate in the soil (Bose *et al*., 1971). The concentration and quality of nutrients are probably more important in the blue-green algal diversity. Availability of phosphates and nitrates are important factors that favor the abundance of cyanophyceace in wetlands (Zancan *et al.,* 2006). The N:P ratio has great influence on cyanobacterial abundance (Hoyos *et al*., 2004). Total number of blue green algal isolates was found positively correlated to the amount of total N and P in soils as observed in the present study. The total number of blue green algal

isolates showed negative correlation with organic carbon. This is in conformity with the report that low carbon favors richness of cyanobacteria in soils (Ohtani *et al*., 2000).

The present study indicated that pH is positively correlated with distribution of algae on polythene surface consistent with the observation of Brady and Weil (2005) who reported that a pH range from 6.0 to 8.3 enhanced the nutrient availability for the growth of plants. A change in pH beyond this limit inhibited the availability of nutrients for the plants as soil tied up large quantities of nutrients and thus would not be available for plants, even though they remain in the soil (Charman and Murphy, 2000). Soil pH less than 6.0 increases the solubility of aluminium, manganese and iron, which can be toxic (Gardner *et al.,* 2003).

In the present study, soil organic carbon showed positive correlation with total cyanobacterial species present on the polythene surfaces. Presence of humic substances enhance algal population in soil without acting as direct source of nutrients (Lee and Bartlett, 1976). Another closely related observation was that organic matter content increases the algal incidence due to higher moisture content of the soil (Friedman and Galum, 1974). The ameliorative effect of cyanophycean algae on soils are well documented by Kaushik and Subhashini, (1985). Due to their nitrogen fixing capability, cyanobacteria have a role in soil improvement (Roger and Kulasooriya, 1980). A common factor of microalgal community structure among the sectors of the study area was the dominance of cyanobacteria. The colonization ability of *Oscillatoria acuminatus*, *Arthospira platensis*, *Nostoc muscorum* was confirmed by its presence in a good number in the six study sites. It has been observed (Fritsch, 1907; John, 1988) that Cyanophycean algae represent the major component of the terrestrial microalgal vegetation in tropical regions, whereas Chlorophyceae are the dominant forms in temperate regions.

Plate 4.1 Photomicrographs of some of the Cyanophyceae species
a- *Oscillatoria curviceps*, b- *O.princeps* v. *pseudolimosa*, c- *O.princeps*, d- *O.subbrevis*, e- *O.amoena*, f- *O.chalybea*, g- *O.peronata*, h- *O.tenuis*, i- *O.willlei*, j- *O.rubescens*, k- *O.vizagapatensis*

Plate 4.2 Photomicrographs of some of the Cyanophyceae species
a- *O. geitleriana*, b- *O. formosa*, c- *O. splendida*, d- *O. limnetica*, e- *O. okeni*, f- *O. earlei*, g- *O.salina*, h- *O.laetevirens* v. *minimus*, i- *O.acuminata*

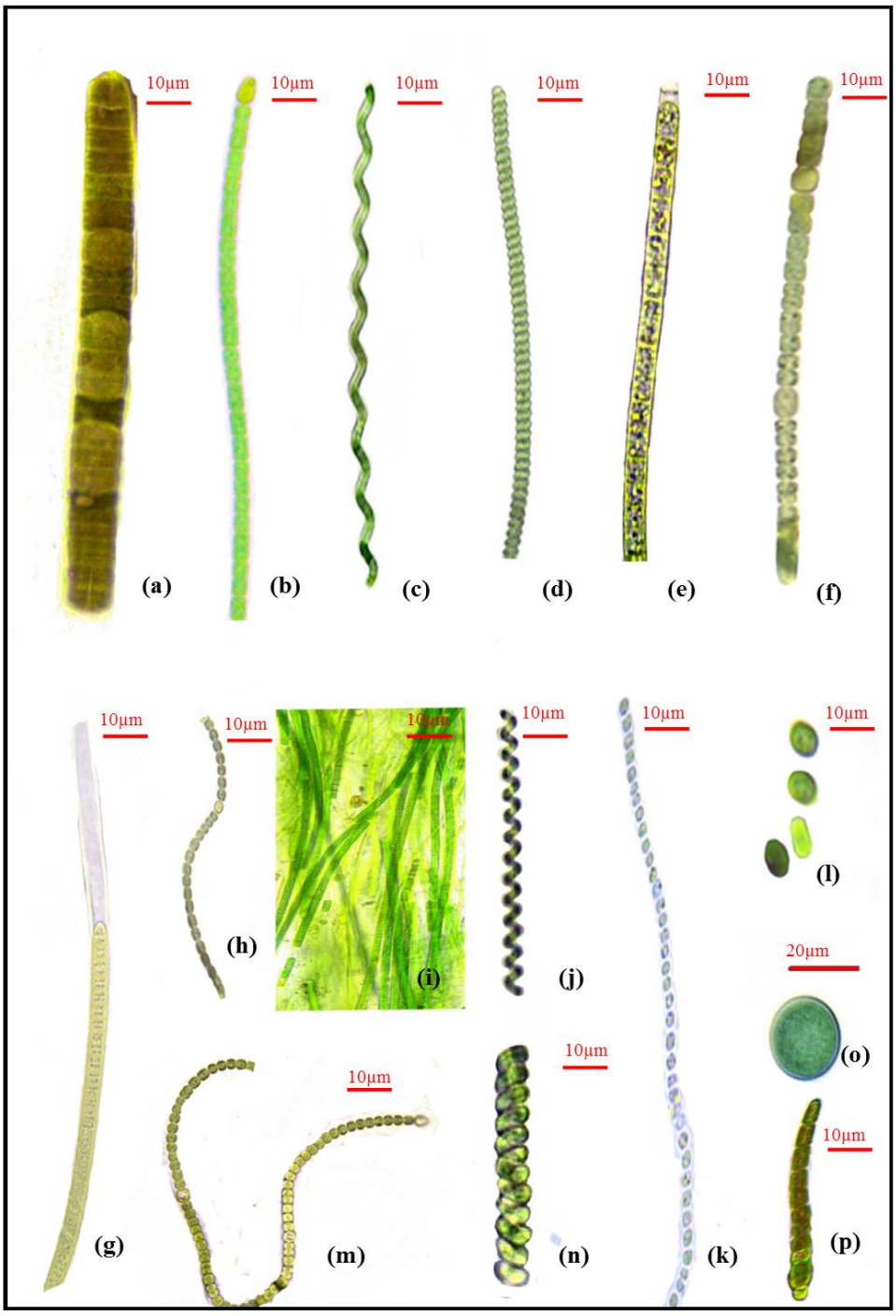

Plate 4.3 Photomicrographs of some of the Cyanophyceae species
a- *O. limosa*, b- *Cylindrospermum muscicola*, c- *Arthospira platensis*, d- *Spirulina major*, e-*Phormidium calcicola*, f- *Anabaena orientalis*,, g- *Lyngbya diguetii*, h- *Anabaena doliolum*, i- *Hydrocoleum sp.*, j- *Spirulina platensis*, k- *Anabeana spiroides*, l- *Aphanothece microscopia*, m- *Nostoc carneum*, n- *Spiruluna tenuissima*, o- *Chrococcus sp.*, p- *Calothrix fusca*

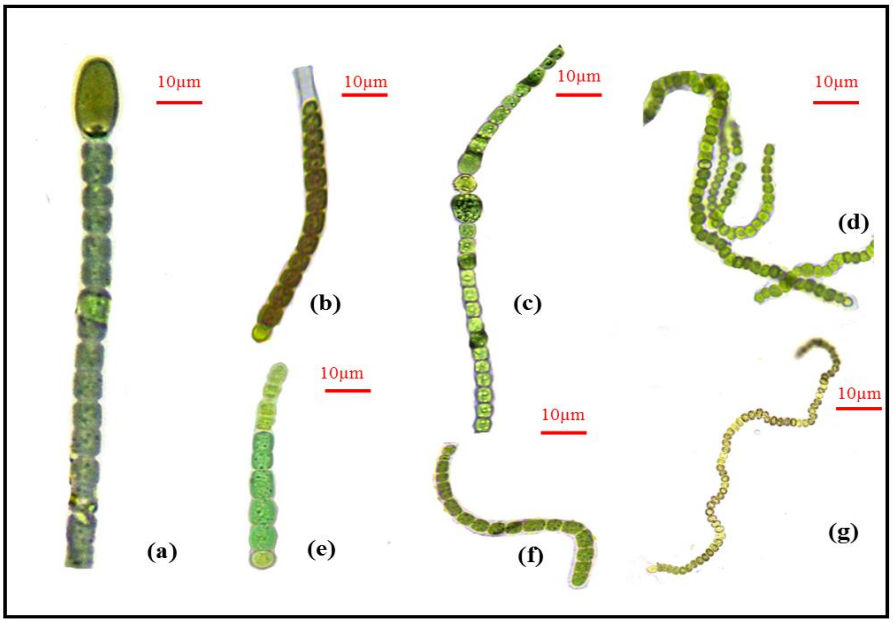

Plate 4.4 Photomicrographs of some of the Cyanophyceae species a- *Cylindrospermum licheniformae*, b- *Calothrix elenkini*, c- *Anabaena oscillatoriales*, d- *Westiellopsis prolifica*, e- *Calothrix parietana*, f- *Anabaena flos-aquae*, g- *Nostoc linckia*

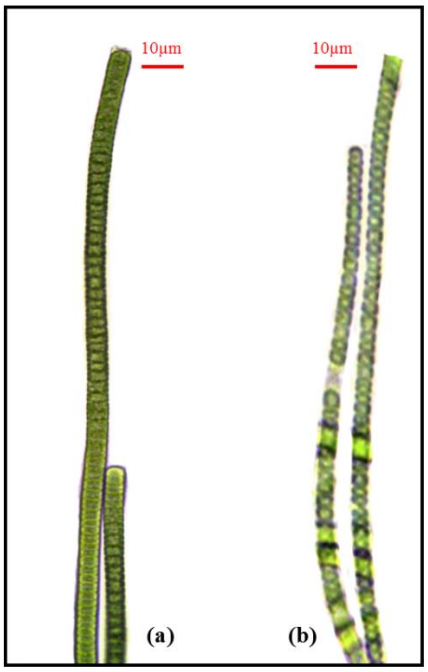

Plate 4.5 Photomicrographs of some of the Cyanophyceae species a- *Phormidium lucidum*, b- *Lyngbya nordgardhii*

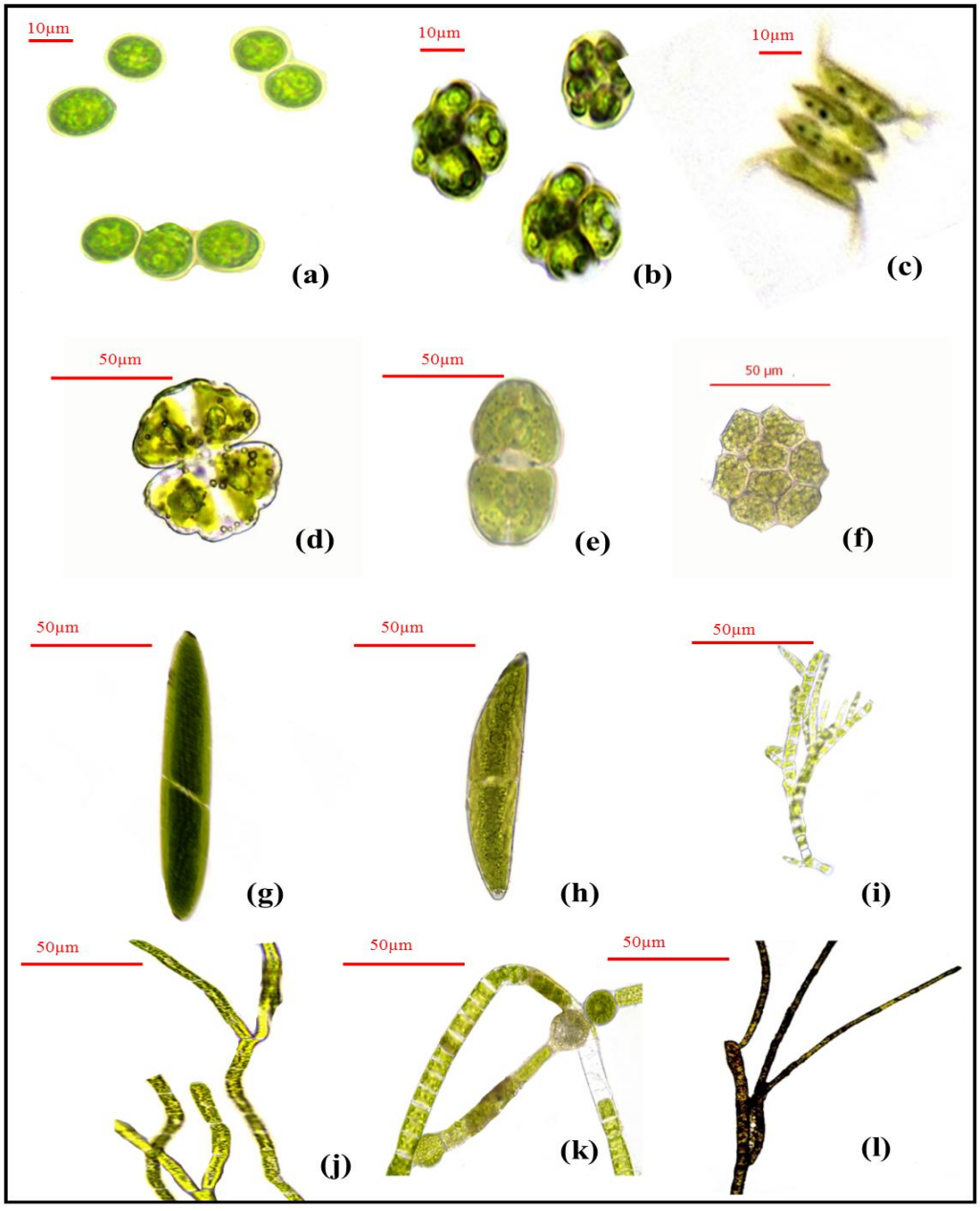

Plate 4.6 Photomicrographs of some of the Chlorophyceae species
a- *Chlorella ellipsoidea*, b- *Chloroccum sp.*, c- *Scenedesmus quadricauda*, d- *Pediastrum integrum* , e- *Cosmarium constrictum* f- *Pediastrum tetras* , g- *Closterium acerosum*, h- *C. costatum*, i- *Stigeoclonium tenue*, j- *Stigeoclonium sp.*, k- *Oedogonium sp.*, l-*Cladophora pellucida*

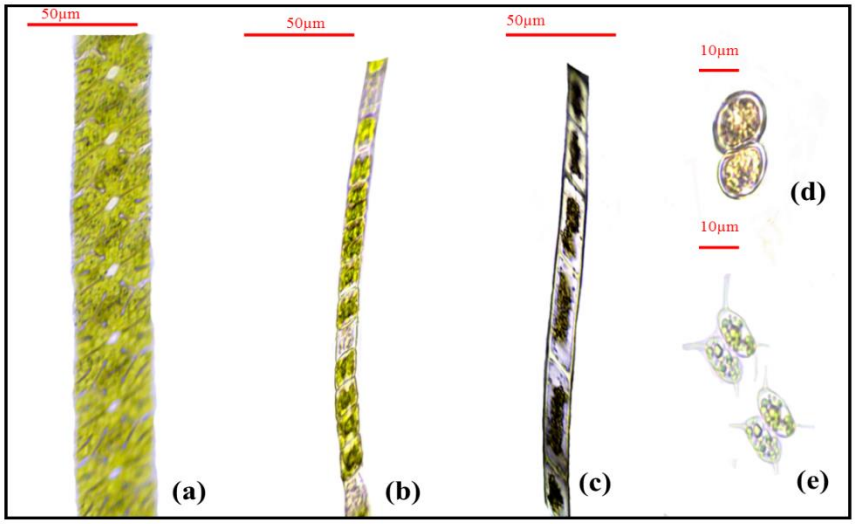

Plate 4.7 Photomicrographs of some of the Chlorophyceae species a- *Micrasterias denticulata,* b-*Hyalotheca dissiliens,* c-*Zygnema sp.,* d-*Cosmarium sp.,* e- *Scenedesmus denticulatus*

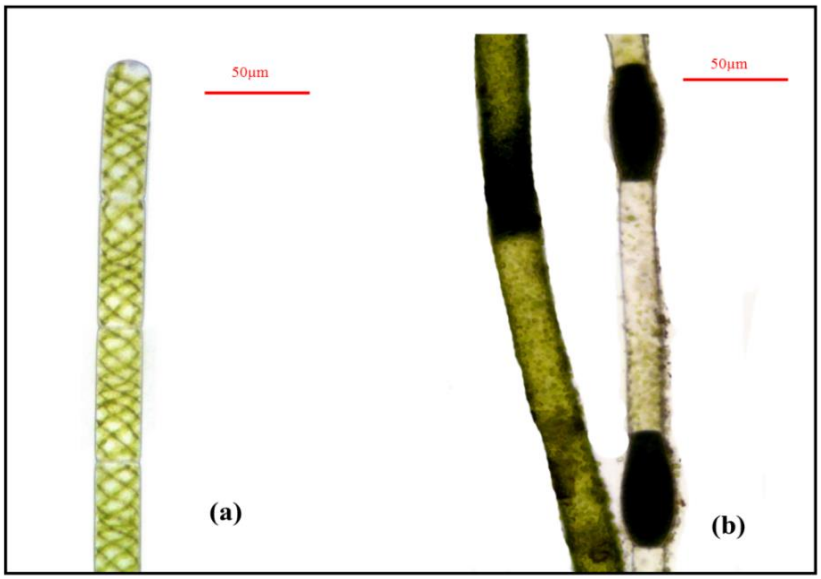

Plate 4.8 Photomicrographs of some of the Chlorophyceae species a- *Spirogyra punctiformis,* b- *Pitophora kewensis*

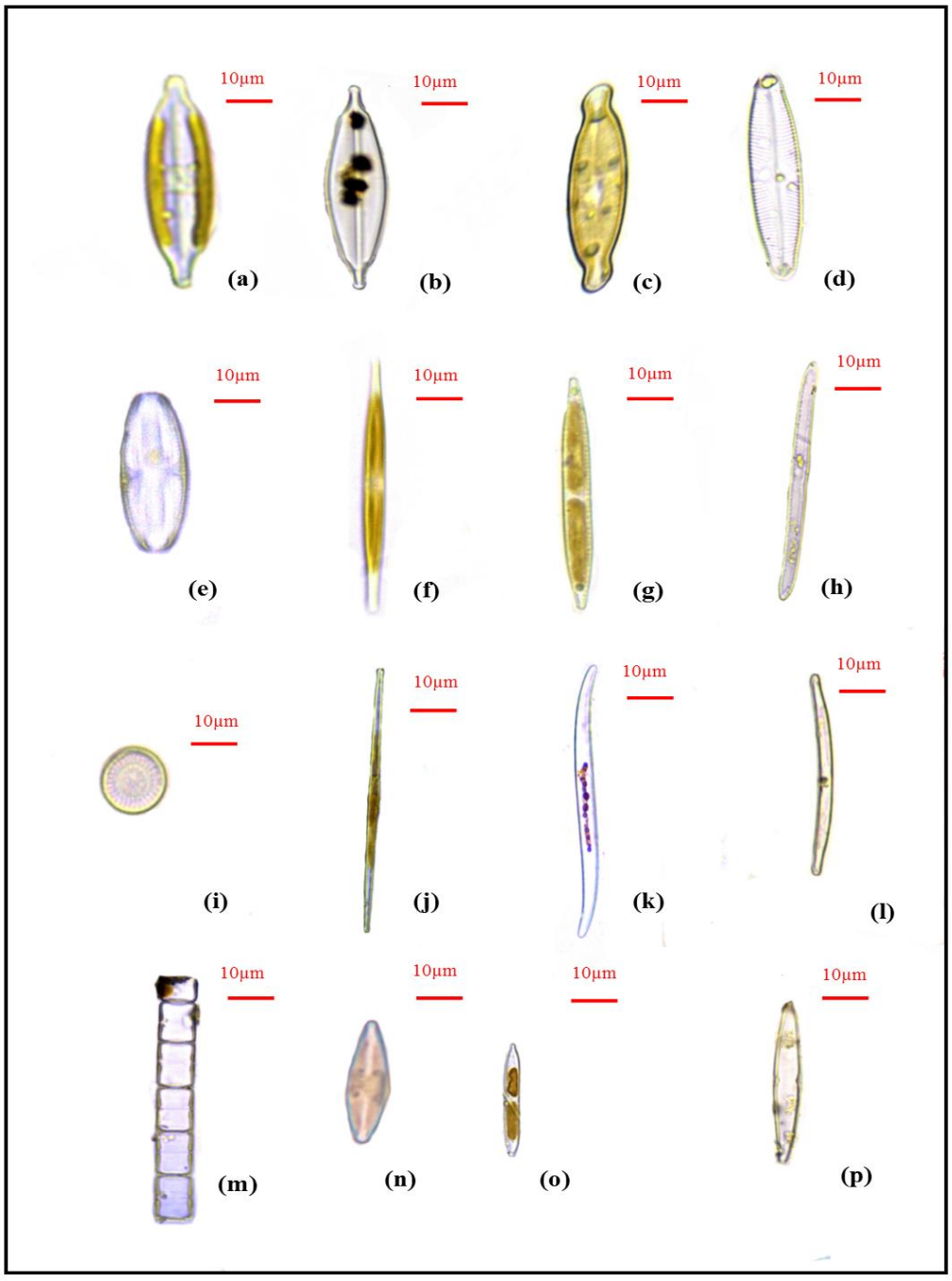

Plate 4.9 Photomicrographs of some of the Bacillariophyceae species
a- *Anomoeoneis brachysira* v. *thermalis*, b- *Navicula halophila*, c- *Pinnularia lundi*, d- *P. eburnean*, e- *Fragillaria sp.*, f- *Synedra tabulata*, g- *Nitzschia intermedia*, h- *Nitzschia obtuse*, i- *Cyclotella meneghiniana.*, j-*Synedra acus*, k- *Gyrosigma sp.*, l- *Eunotia sp.*, m- *Melosira juergensii*, n-*Gomphonema mexicanum*, o- *Nitzschia apiculata* , p- *Nitzschia palea*

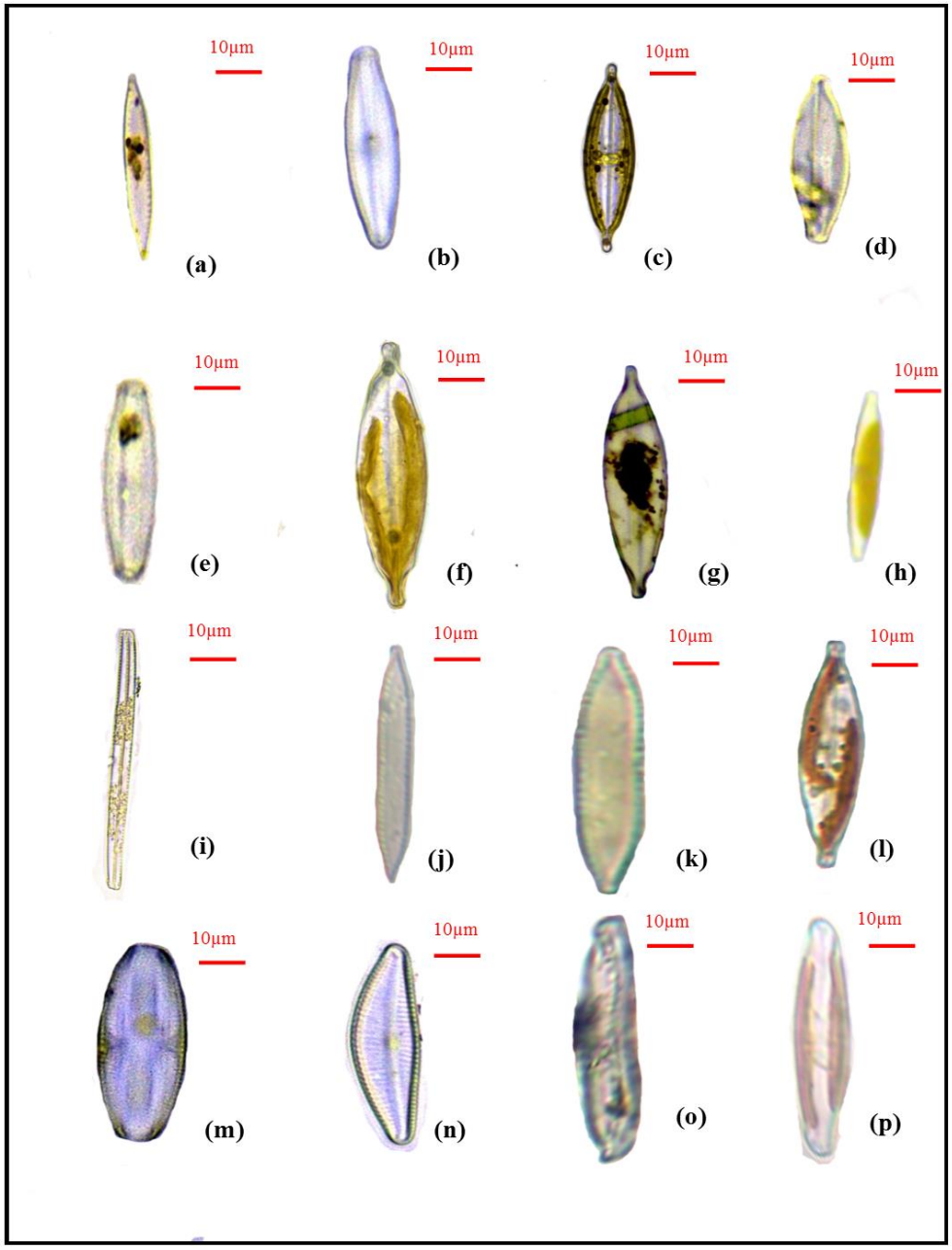

Plate 4.10 Photomicrographs of some of the Bacillariophyceae species
a- *Nitzschia heufleriana* , b-*Navicula viridula*, c- *Navicula cuspidata*, d- *Navicula salinarum*, e- *Navicula subdapaliformis* , f- *Navicula subrhynchocephala*, g- *Navicula grimii*, h-*Navicula pahalgarhensis*, i- *Nitzchia vermicularis*, j- *Fragilaria ungeriana*, k- *Hantschia amphioxys*, l- *Mastogloia recta*, m- *Amphora normani*, n- *Cymbella hungarica*, o- *Hantschia amphioxys* v. *densestriata*, p- *Navicula pupula*

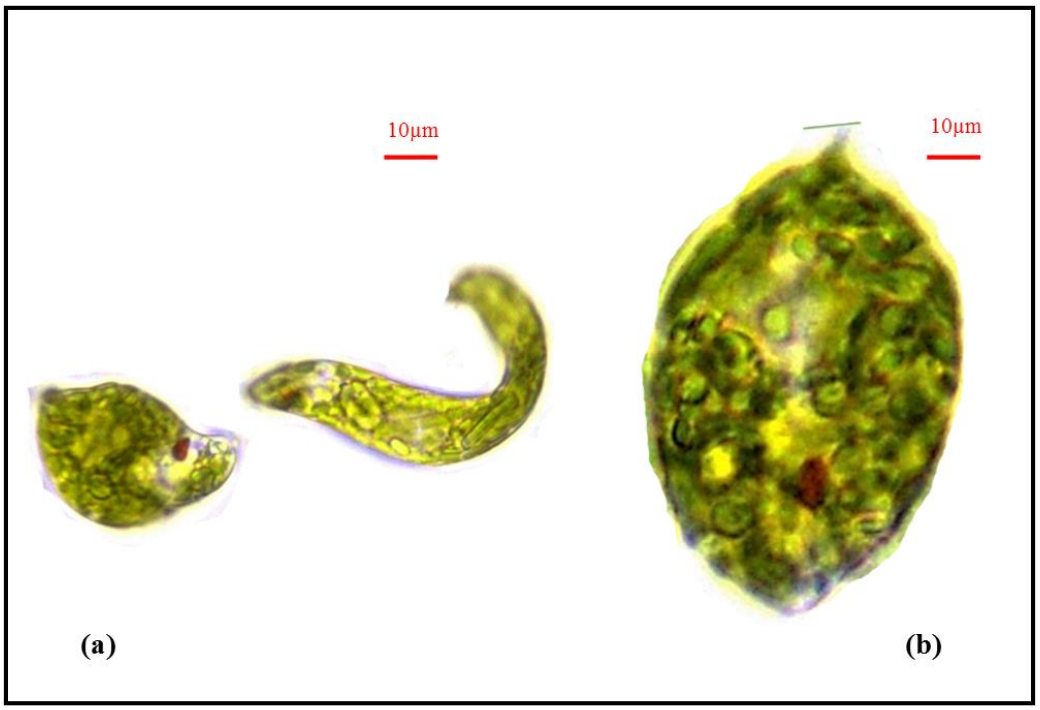

Plate 4.11 Photomicrograph of Euglenophyceae a. *Phacus* sp., b. *Euglena tuba*

* The abbreviations, PM, M, PO and W as mentioned in Fig.4.1-4.36 corresponds different seasons, respectively (p.73-85)

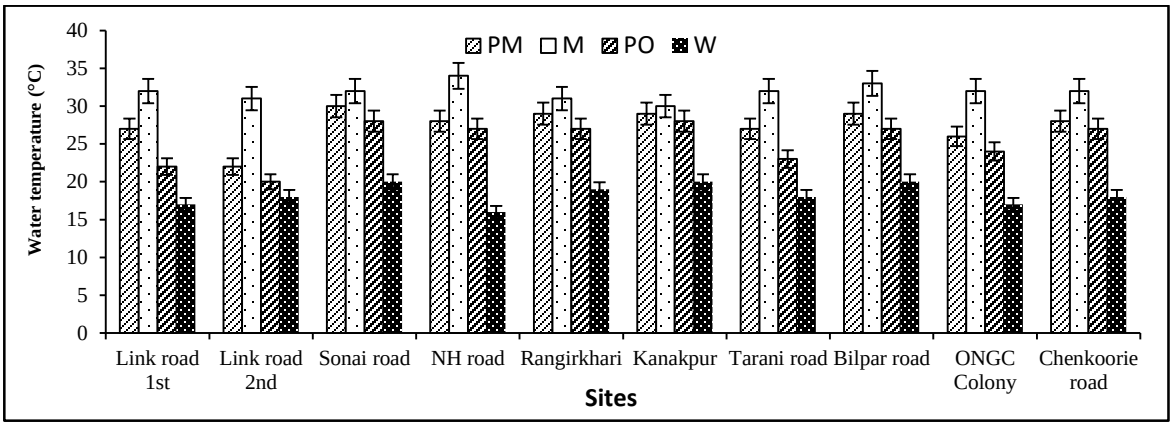

Fig. 4.1: Seasonal variation of temperature of water at the study sites

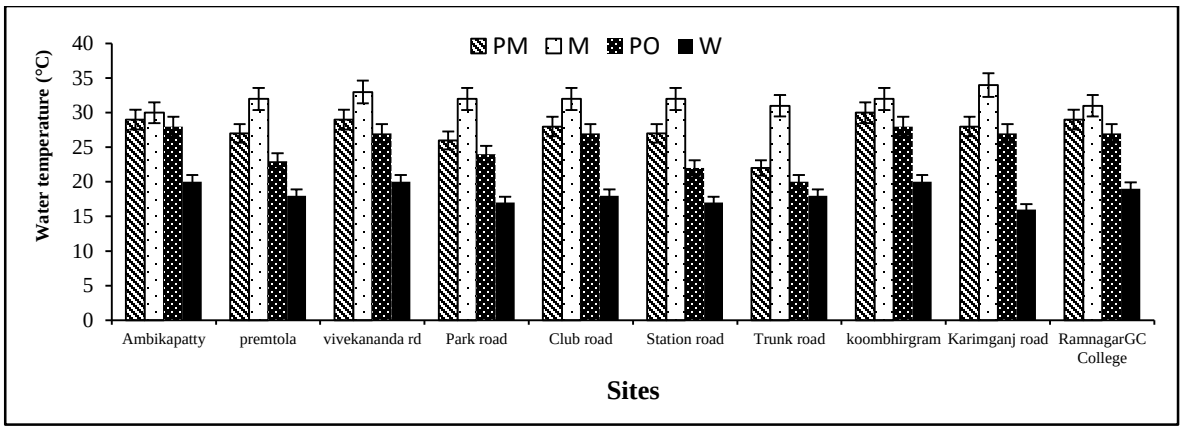

Fig. 4.2: Seasonal variation of temperature of water at the study sites

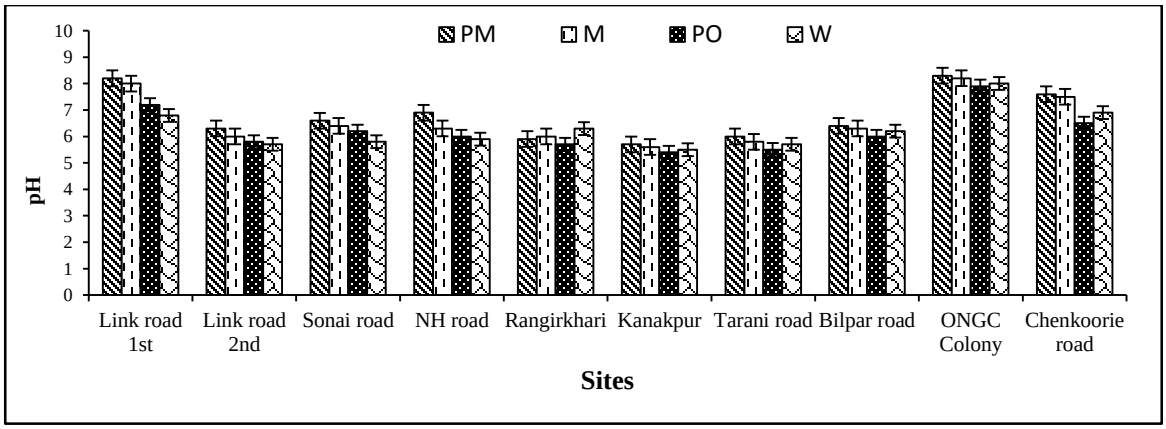

Fig. 4.3: Seasonal variation of pH of water at the study sites

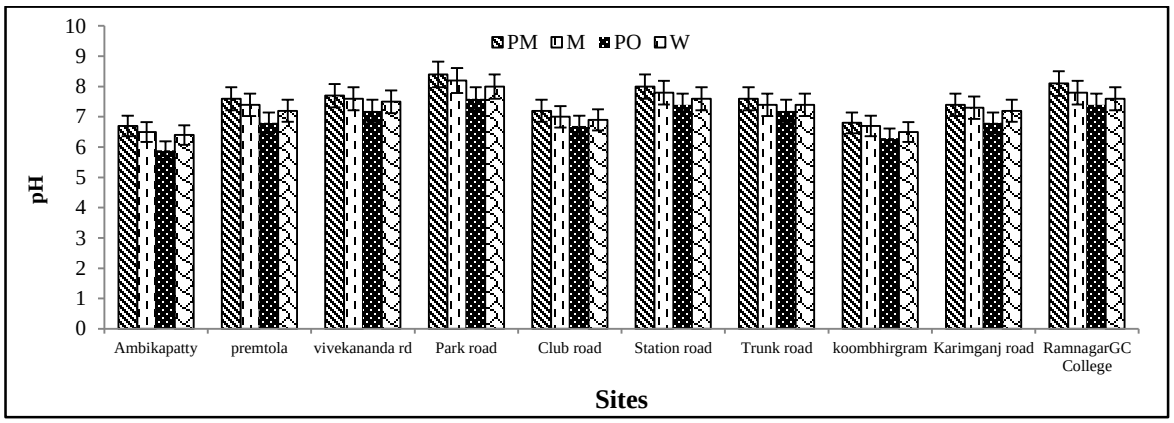

Fig. 4.4: Seasonal variation of pH of water at the study sites

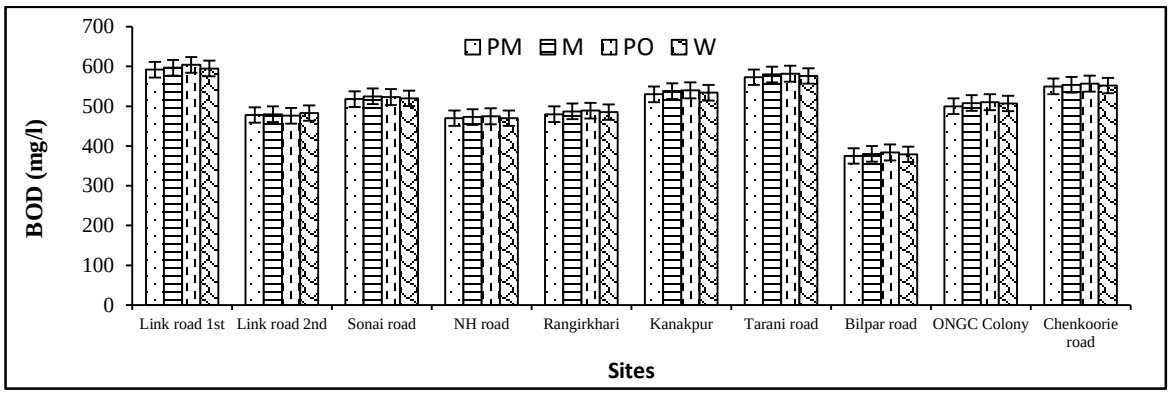

Fig. 4.5: Seasonal variation of BOD of water at the study sites

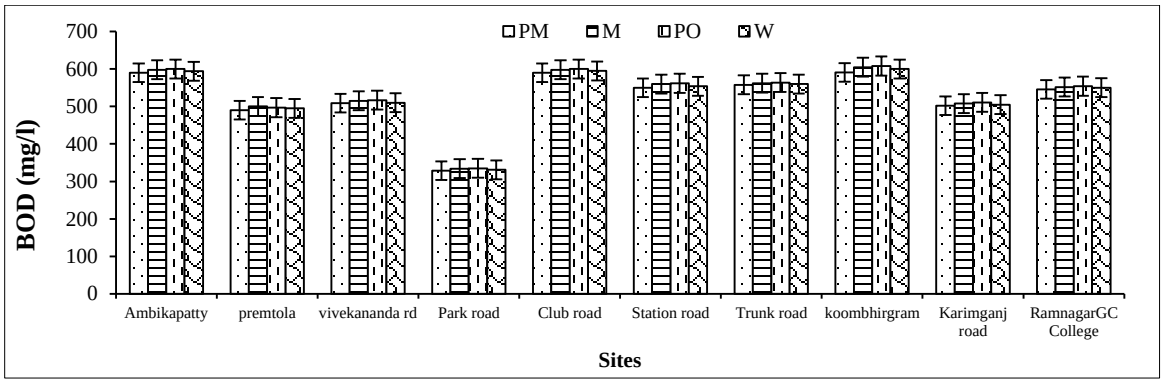

Fig. 4.6: Seasonal variation of BOD of water at the study sites

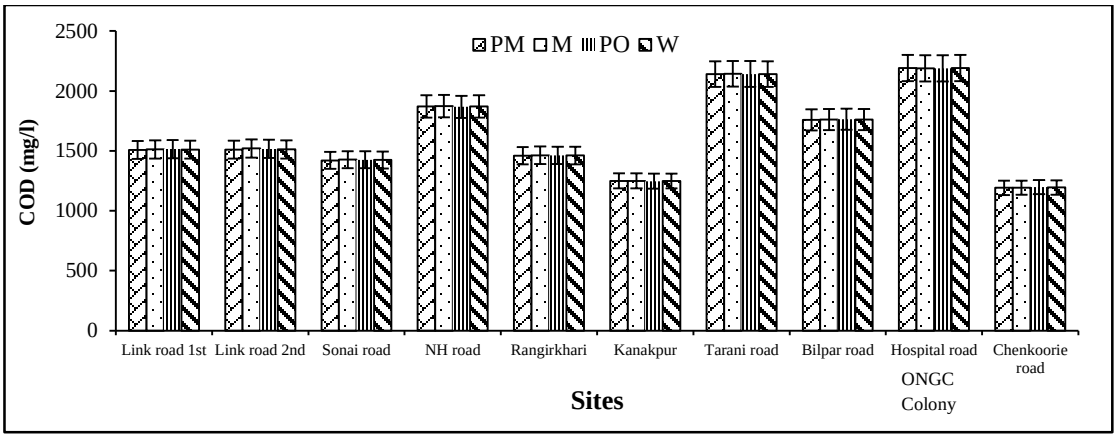

Fig. 4.7: Seasonal variation of COD of water at the study sites

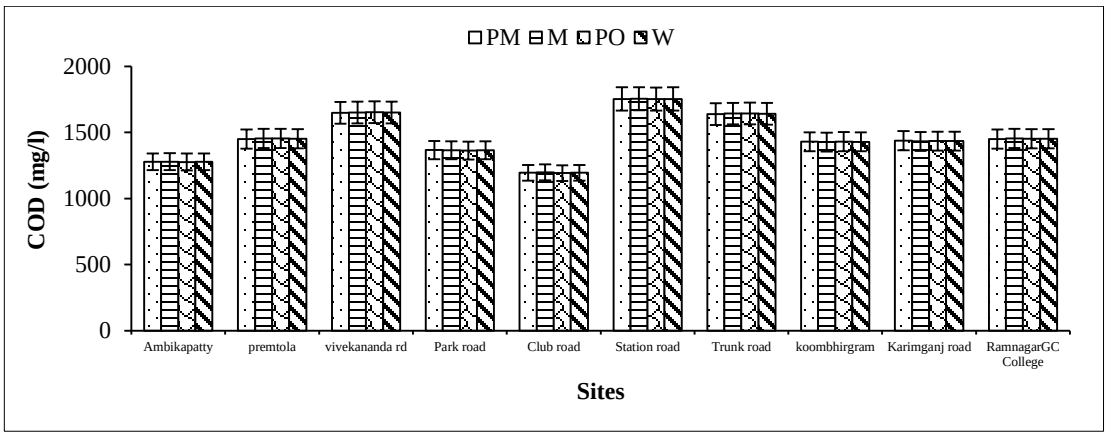

Fig. 4.8: Seasonal variation of COD of water at the study sites

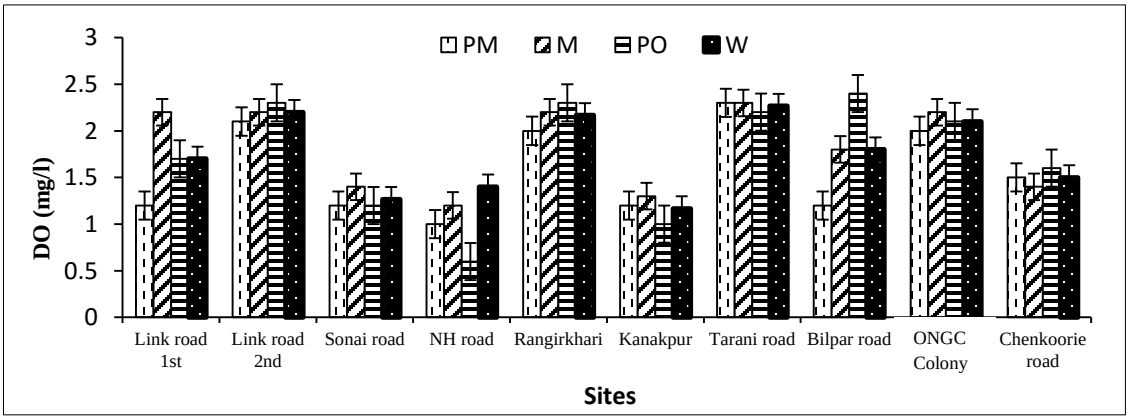

Fig. 4.9: Seasonal variation of DO of water at the study sites

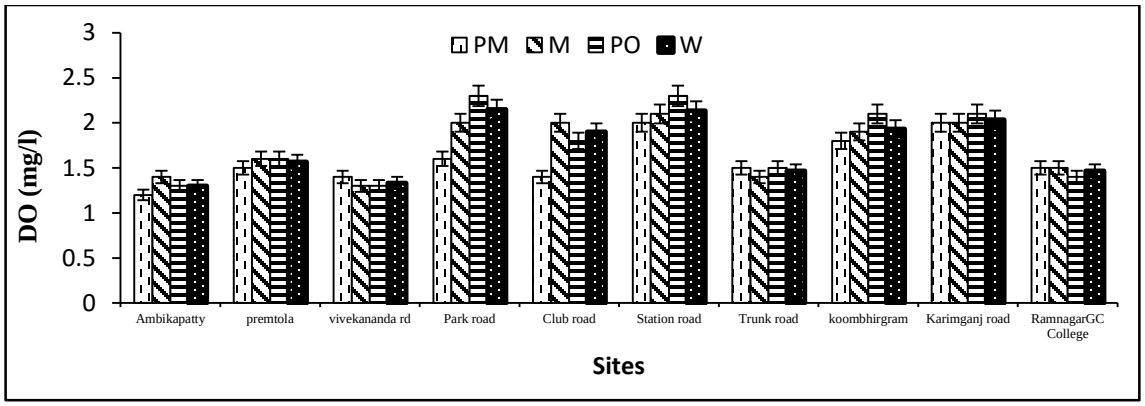

Fig. 4.10: Seasonal variation of DO of water at the study sites

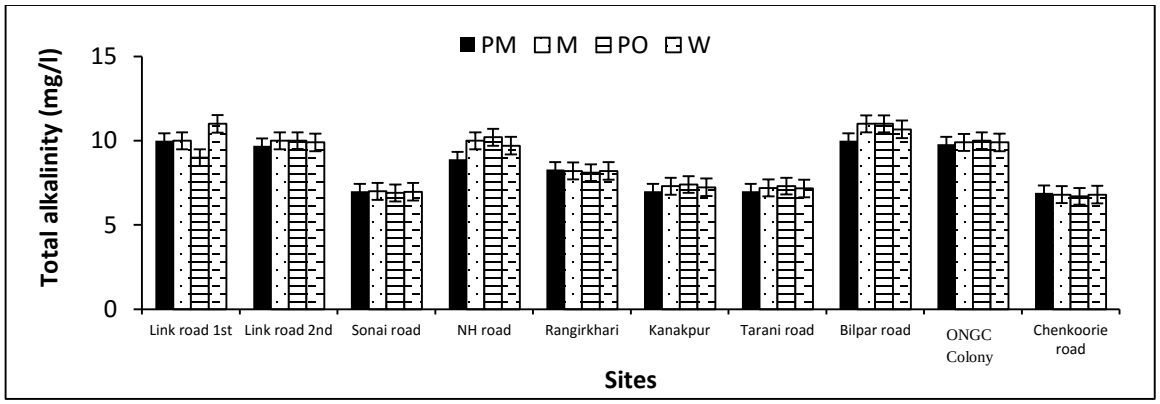

Fig. 4.11: Seasonal variation of total alkalinity of water at the study sites

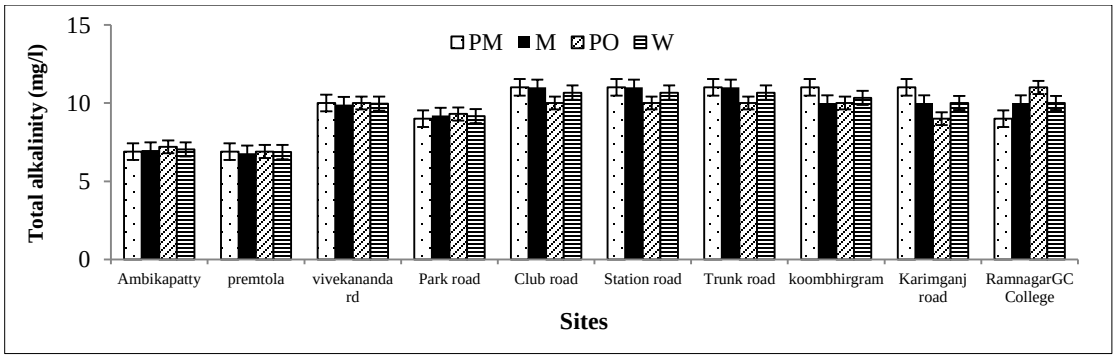

Fig. 4.12: Seasonal variation of total alkalinity of water at the study sites

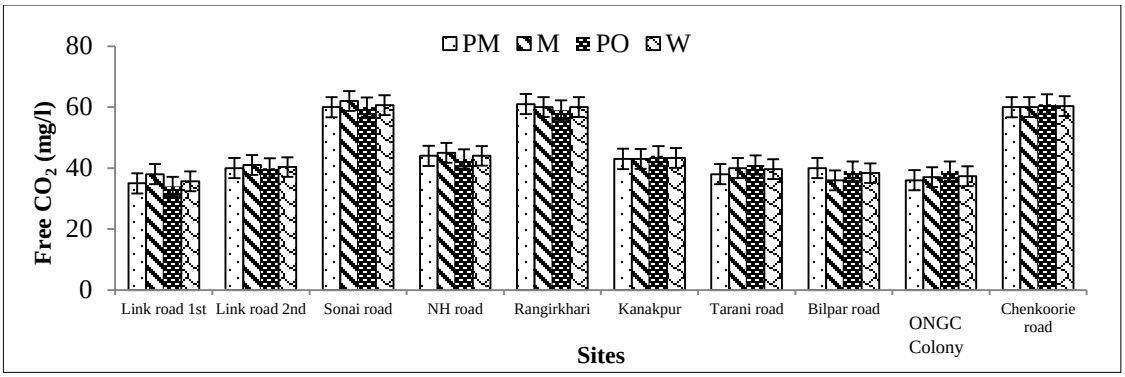

Fig. 4.13: Seasonal variation of free CO_2 of water at the study sites

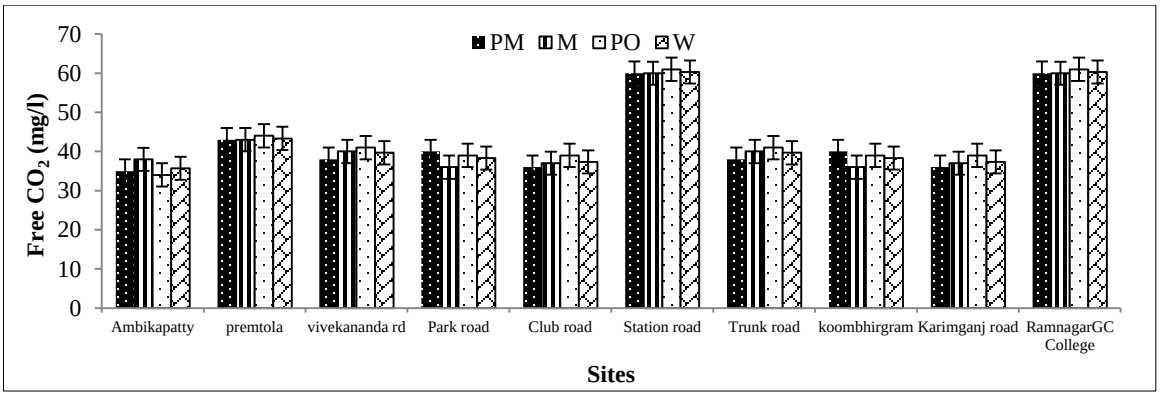

Fig. 4.14: Seasonal variation of free CO_2 of water at the study sites

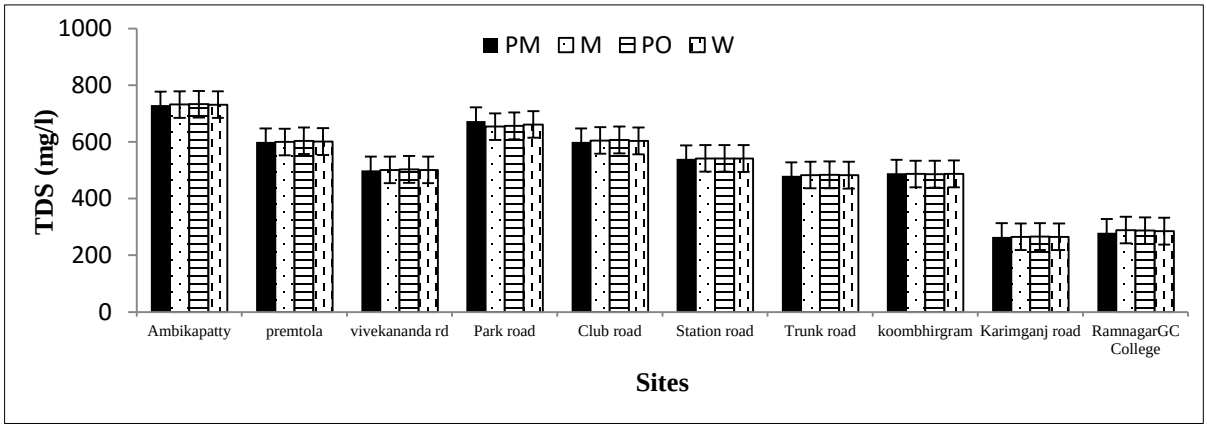

Fig. 4.15: Seasonal variation of total dissolved solid of water at the study sites

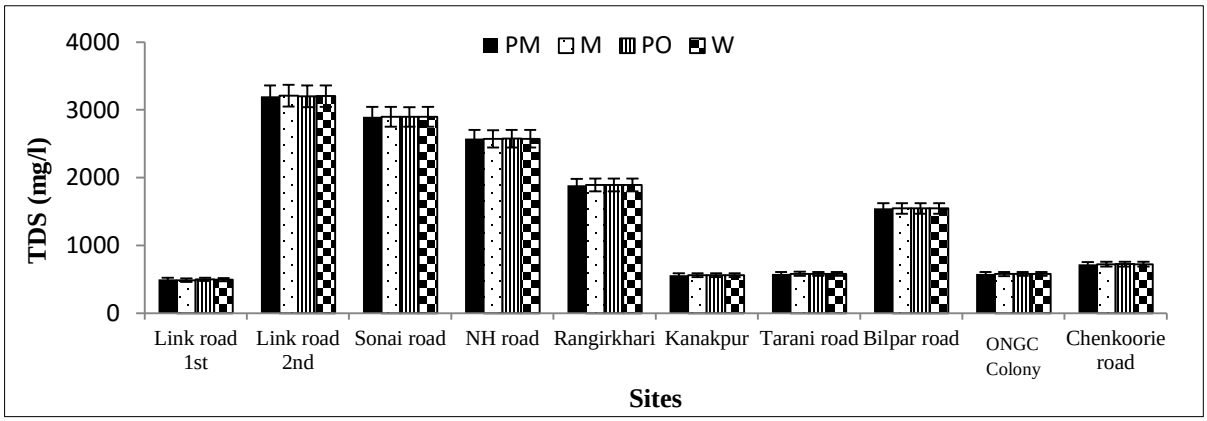

Fig. 4.16: Seasonal variation of total dissolved solid of water at the study sites

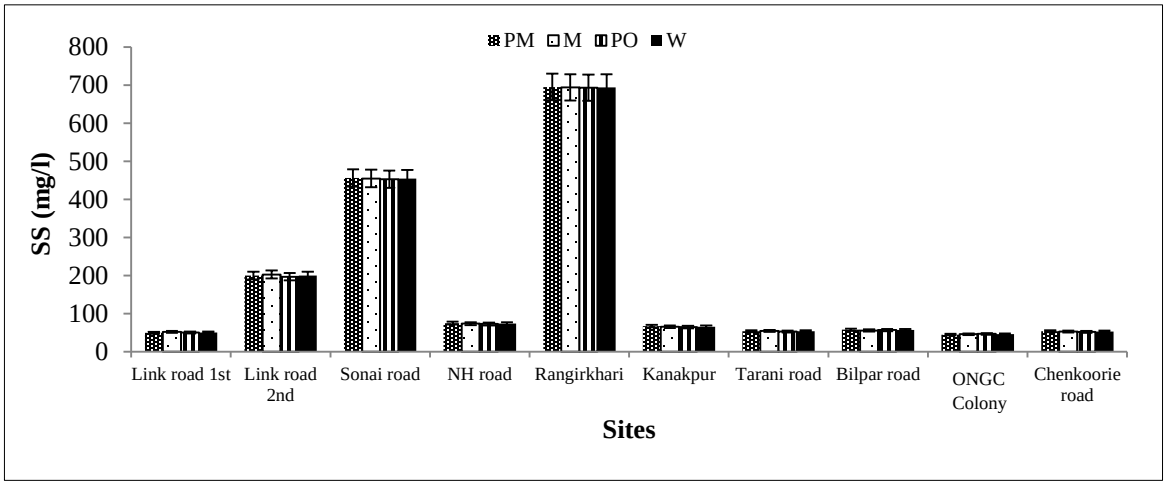

Fig. 4.17: Seasonal variation of suspended solid of water at the study sites

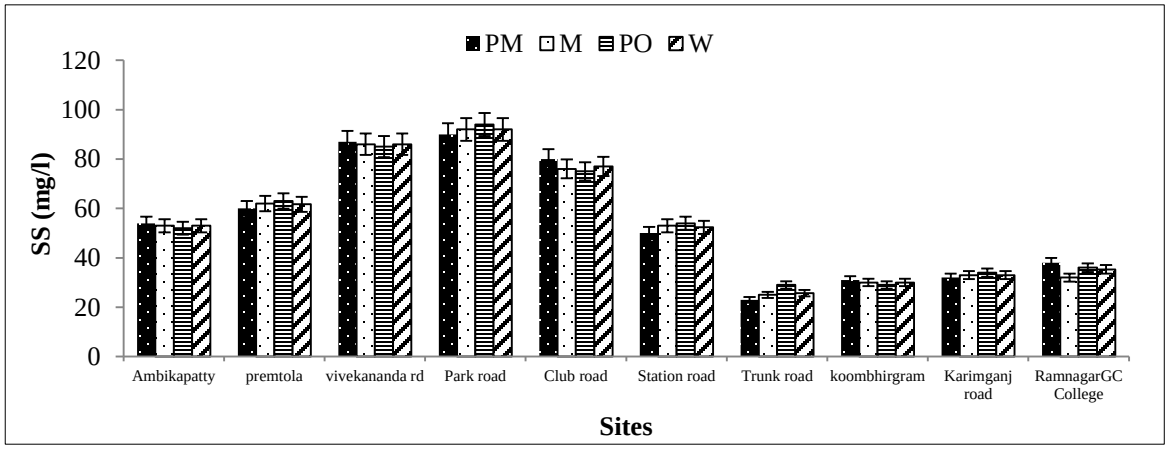

Fig. 4.18: Seasonal variation of suspended solid of water at the study sites

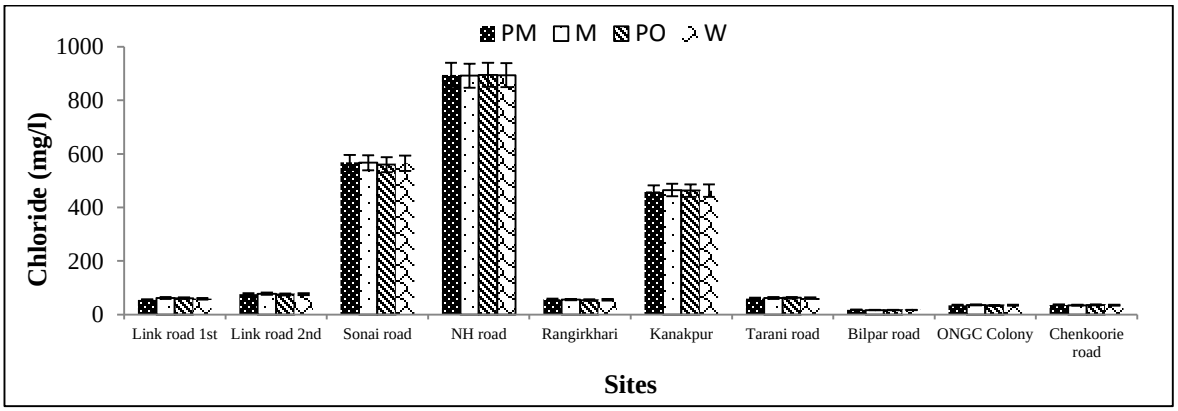

Fig. 4.19: Seasonal variation of chloride concentration of water at the study sites

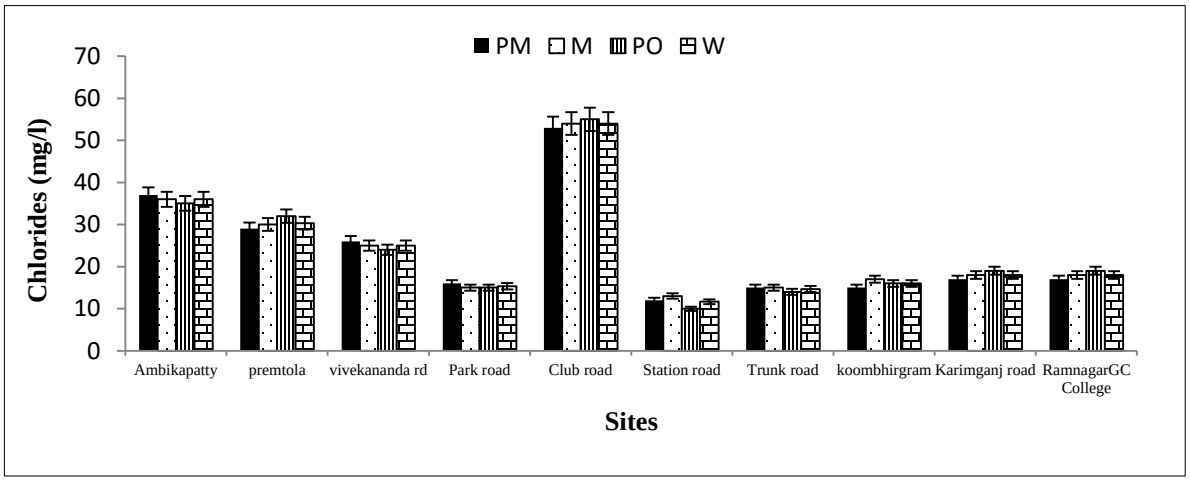

Fig. 4.20: Seasonal variation of chloride concentration of water at the study sites

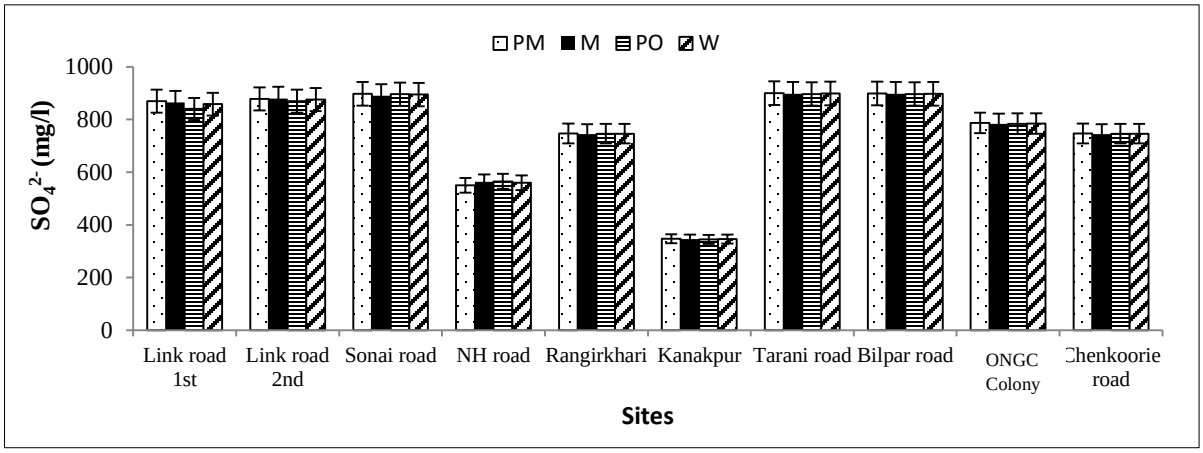

Fig. 4.21: Seasonal variation of sulphate concentration of water at the study sites

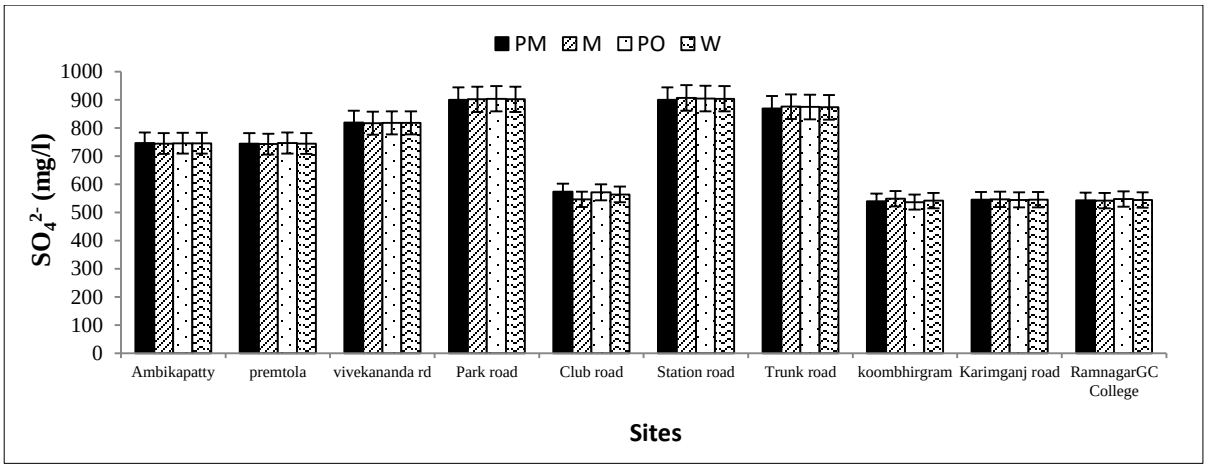

Fig. 4.22: Seasonal variation of concentration of sulphate of water at the study sites

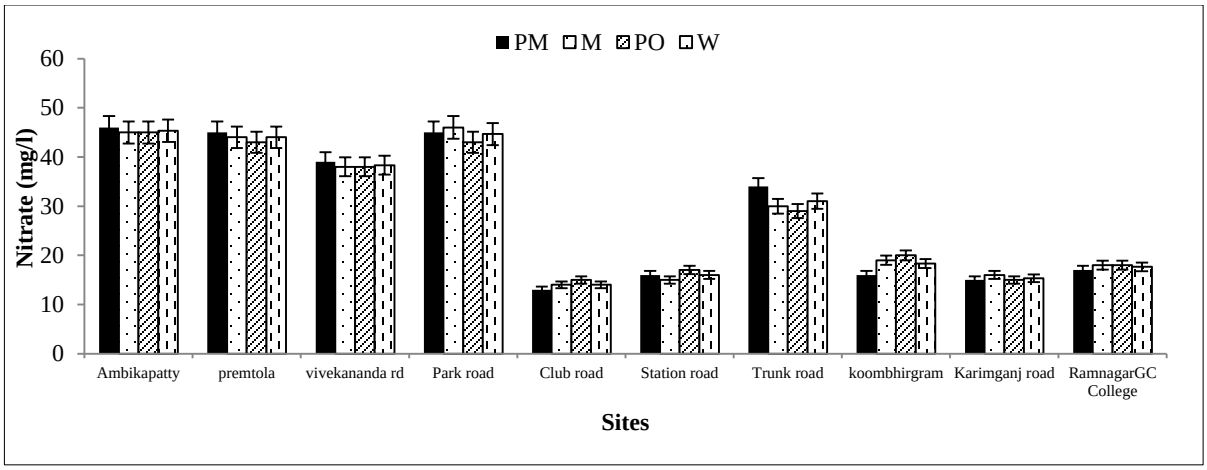

Fig. 4.23: Seasonal variation of nitrate of water at the study sites

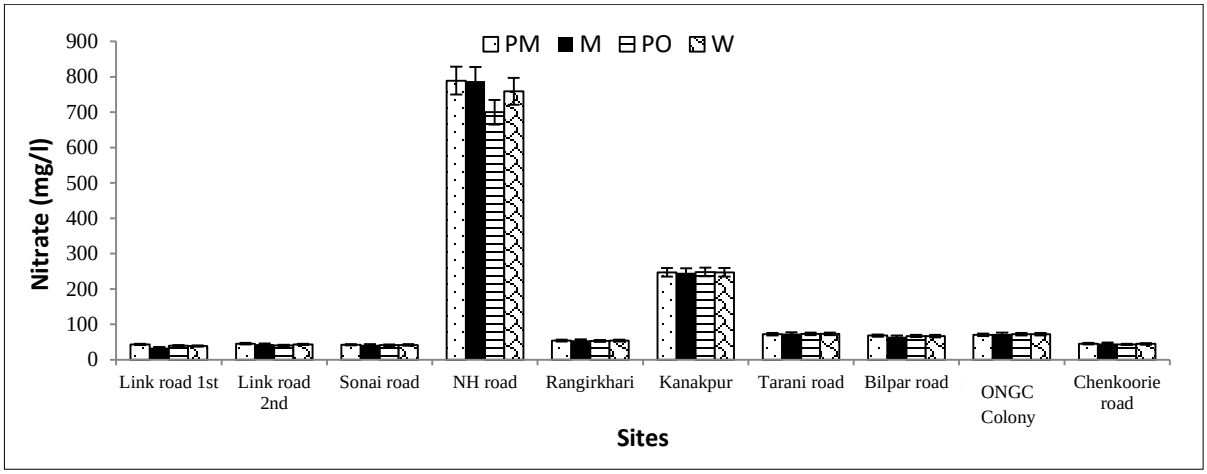

Fig. 4.24: Seasonal variation of nitrate of water at the study sites

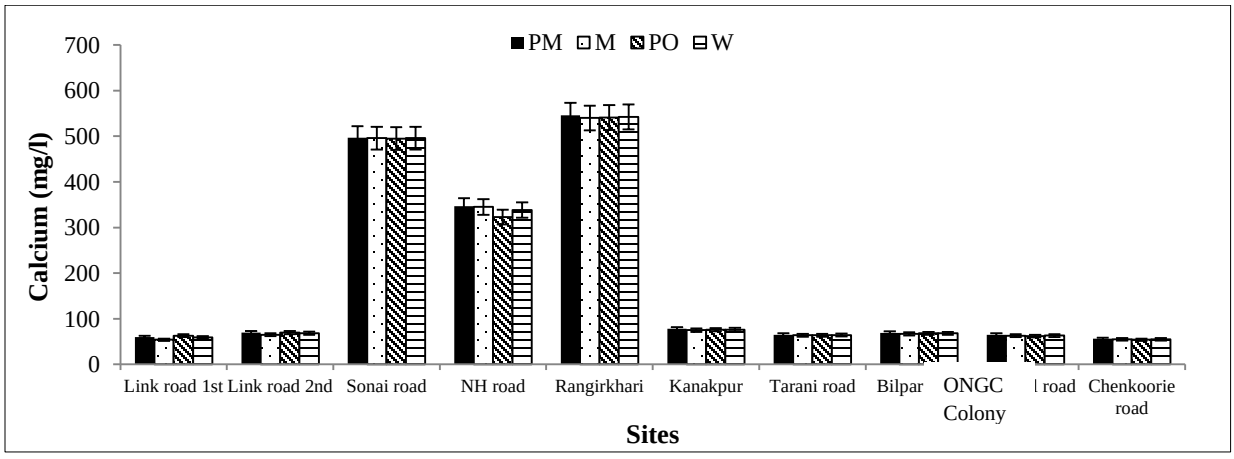

Fig. 4.25: Seasonal variation of calcium of water at the study sites

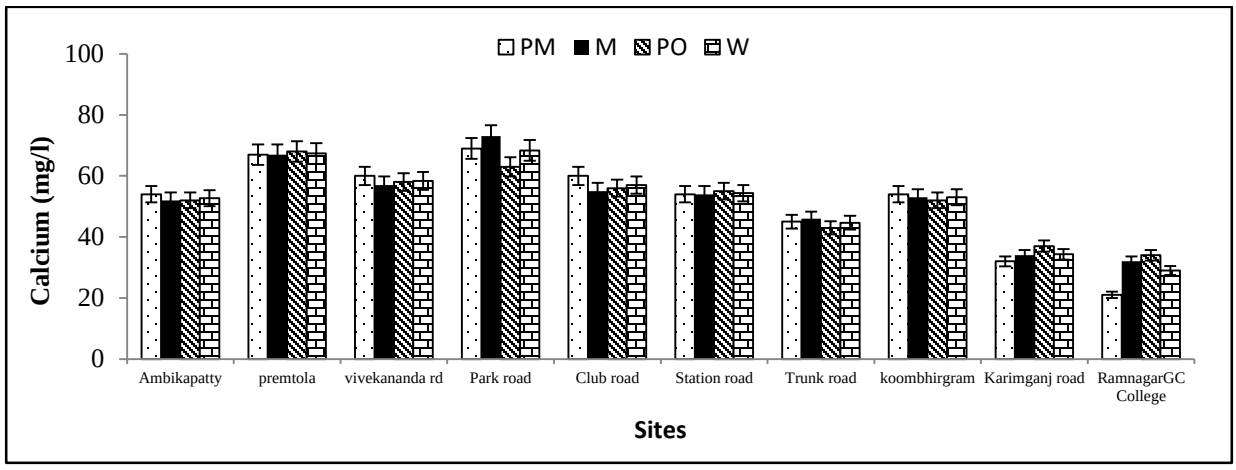

Fig. 4.26: Seasonal variation of calcium of water at the study sites

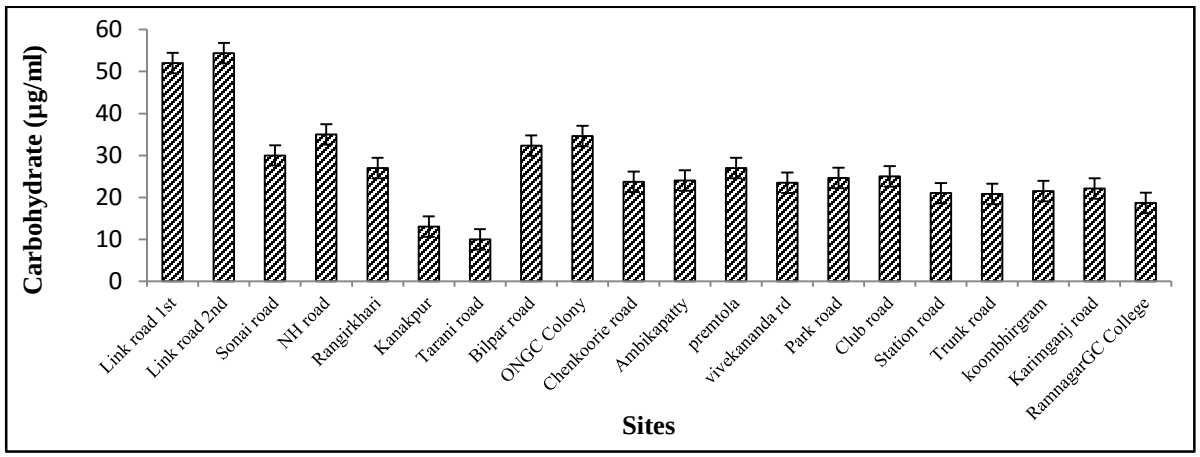

Fig. 4.27: Carbohydrate content in domestic sewage water at the study sites

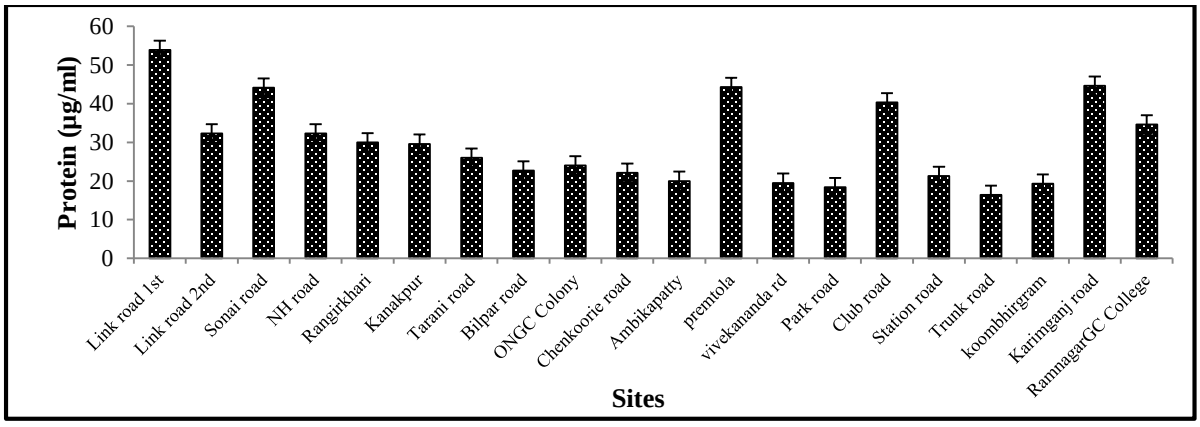

Fig. 4.28: Protein content in domestic sewage water at the study sites

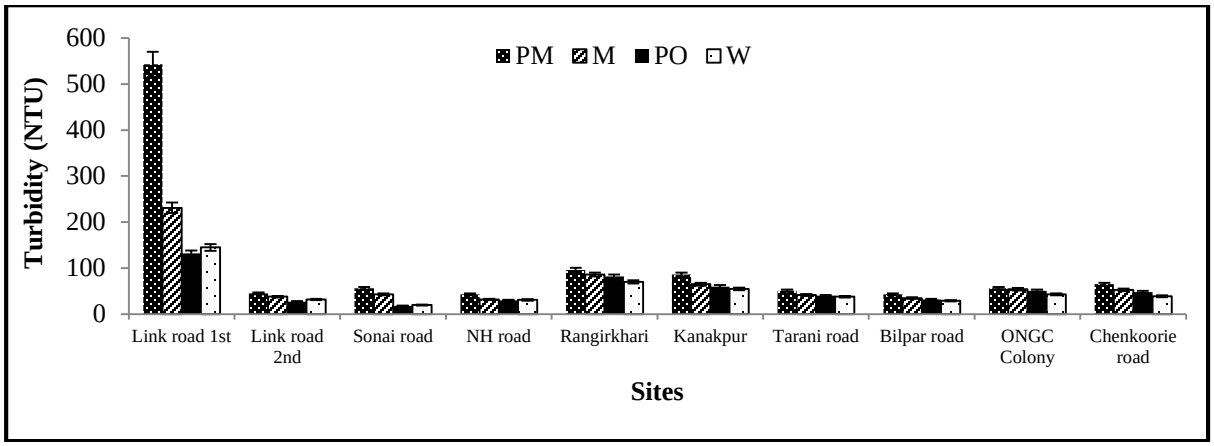

Fig. 4.29: Seasonal variation of turbidity of water at the study sites

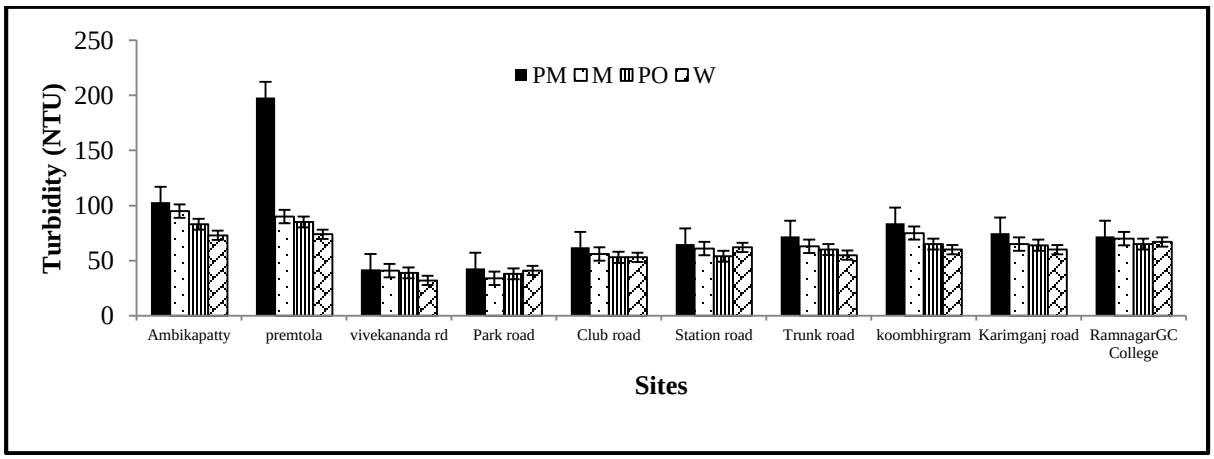

Fig. 4.30: Seasonal variation of turbidity of water at the study sites

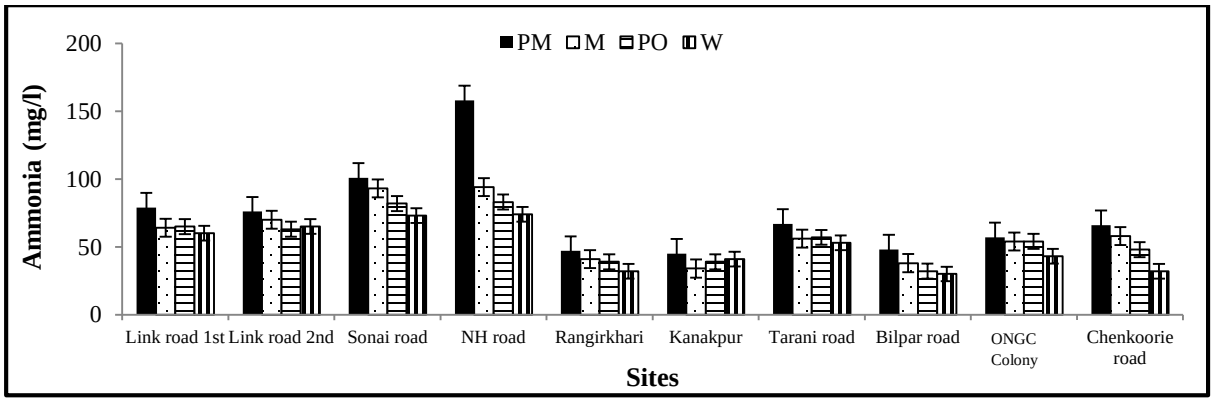

Fig. 4.31: Seasonal variation of ammonia of water at the study sites

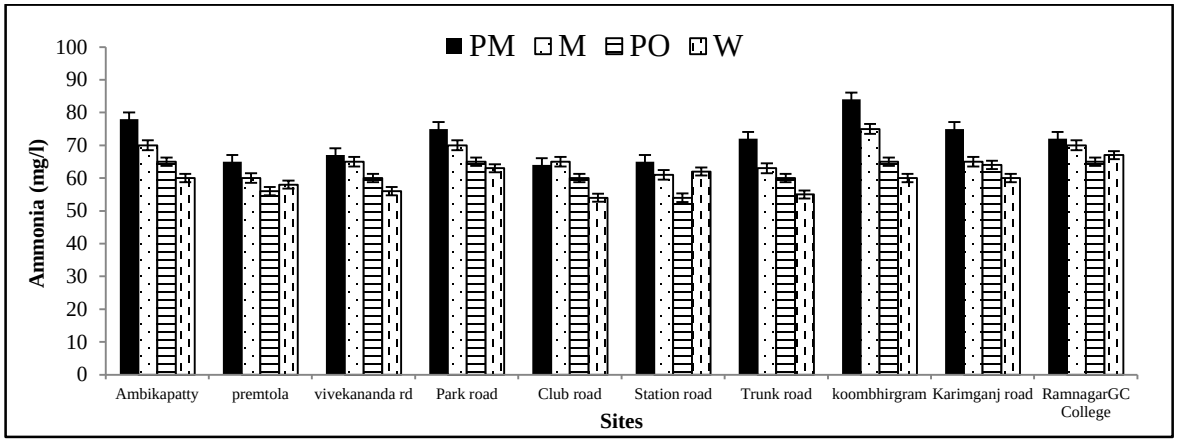

Fig. 4.32: Seasonal variation of ammonia of water at the study sites

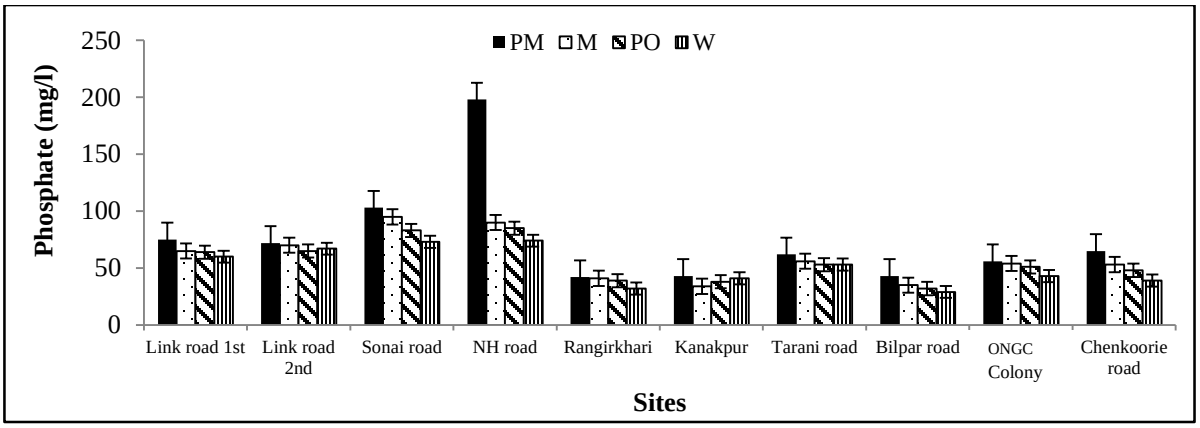

Fig. 4.33: Seasonal variation of phosphate of water at the study sites

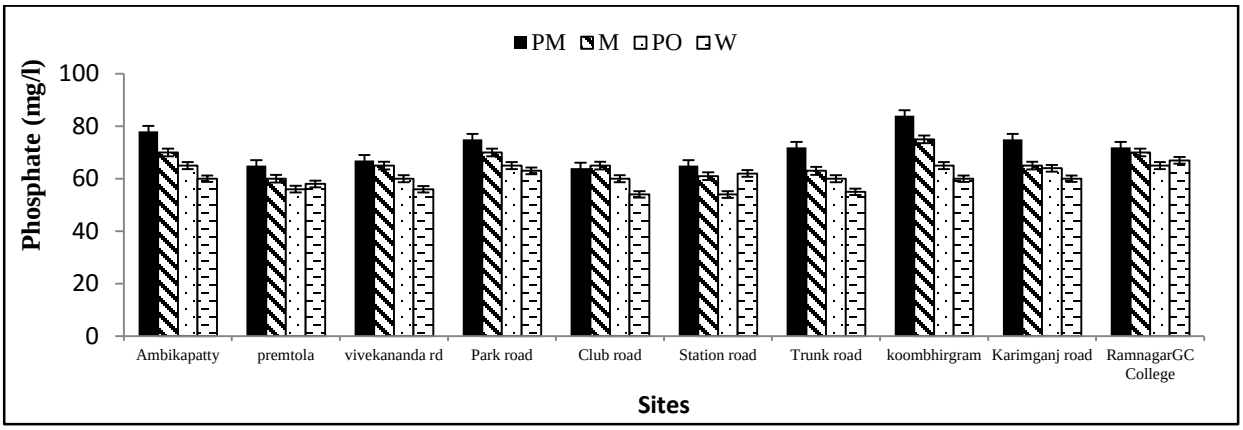

Fig. 4.34: Seasonal variation of phosphate of water at the study sites

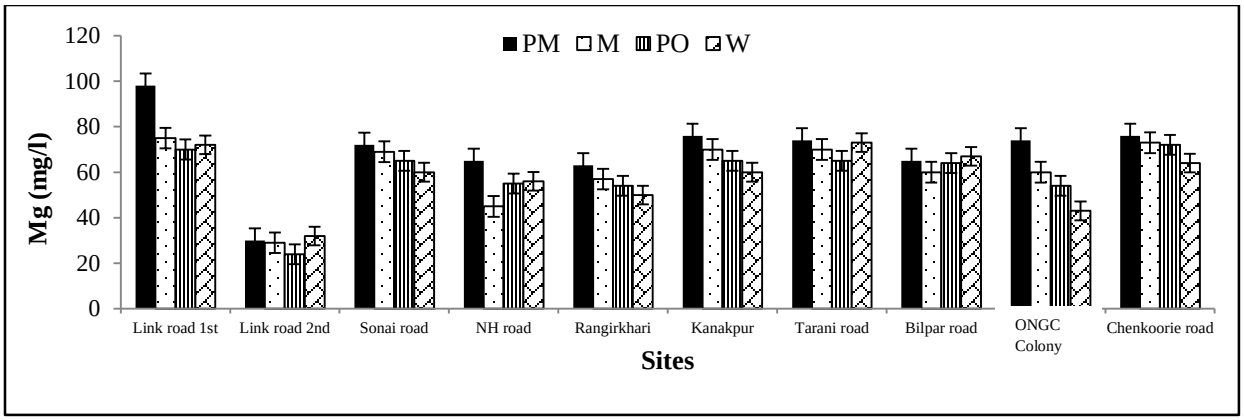

Fig. 4.35: Seasonal variation of magnesium of water at the study sites

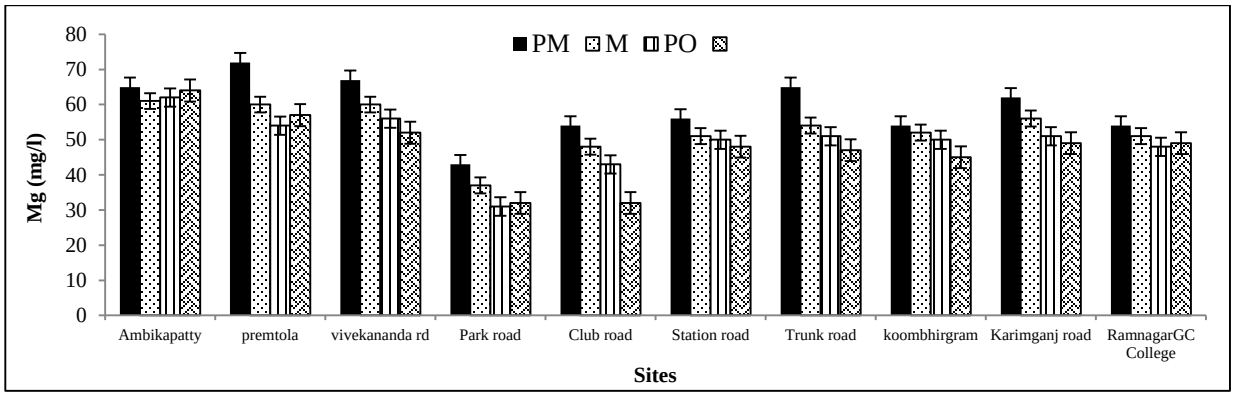

Fig. 4.36: Seasonal variation of magnesium of water at the study sites

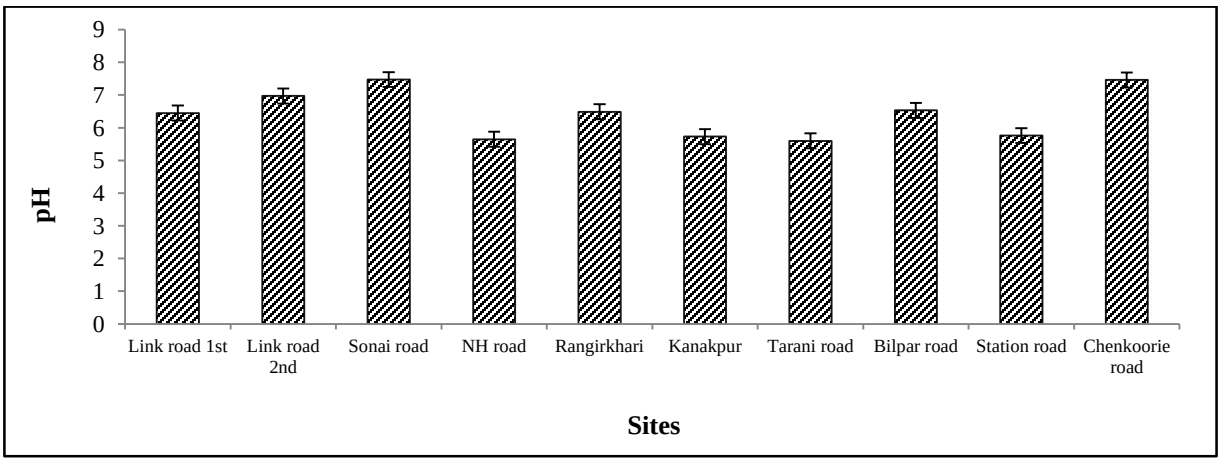

Fig. 4.37: Variation of pH of soil at the study sites

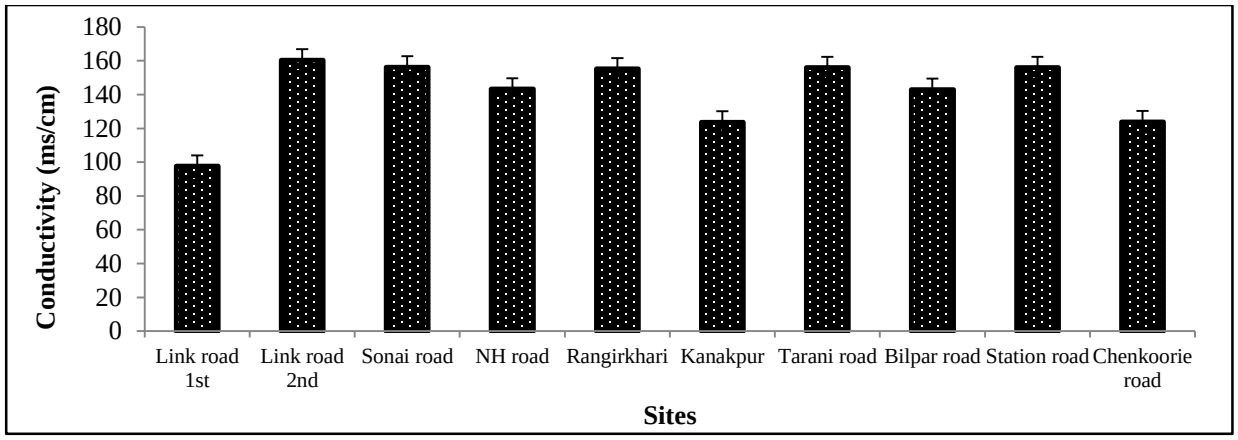

Fig. 4.38: Variation of conductivity of soil at the study sites

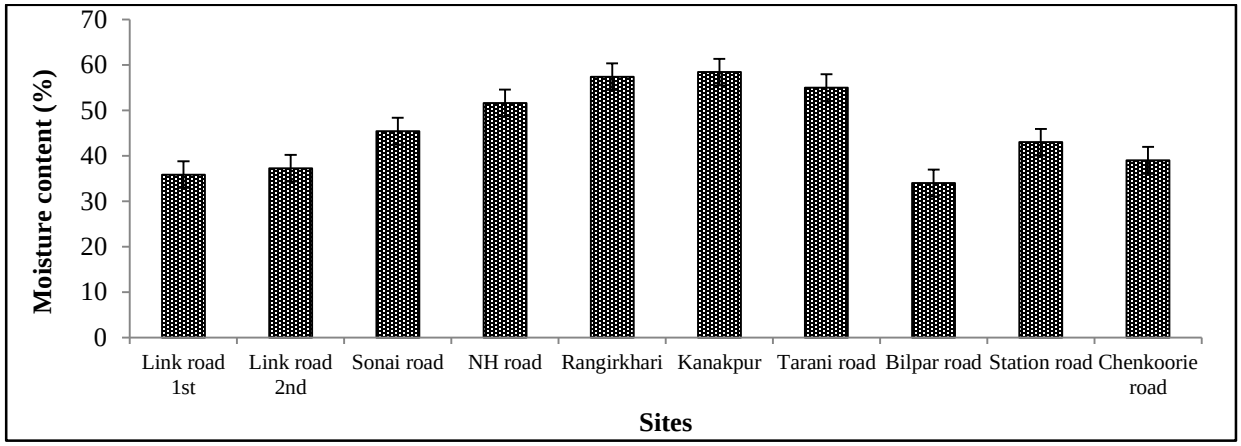

Fig. 4.39: Variation of moisture content of soil at the study sites

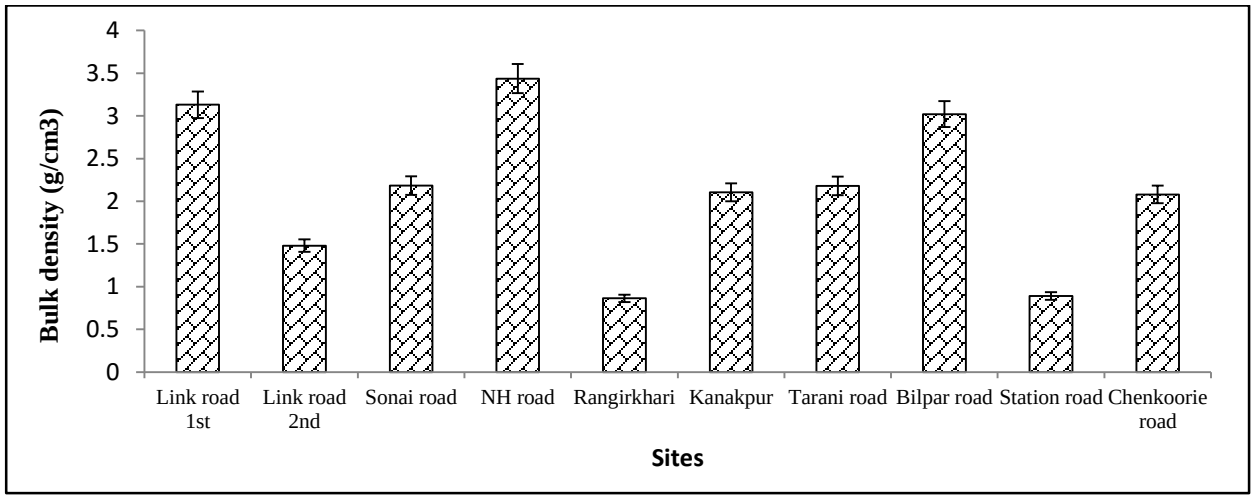

Fig. 4.40: Variation of bulk density of soil at the study sites

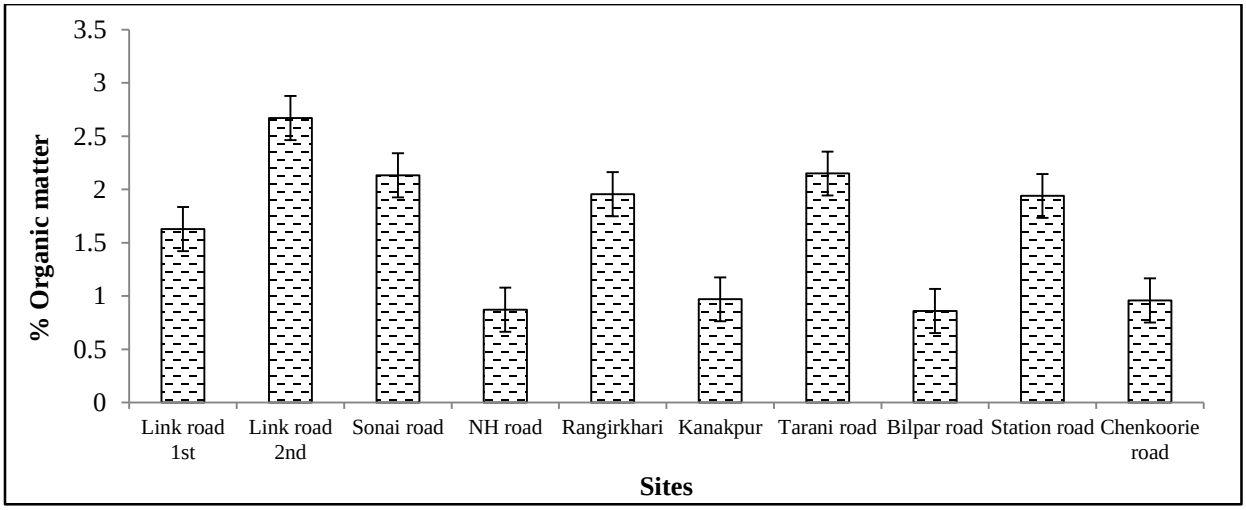

Fig. 4.41: Variation of percentage of organic matter of soil at the study sites

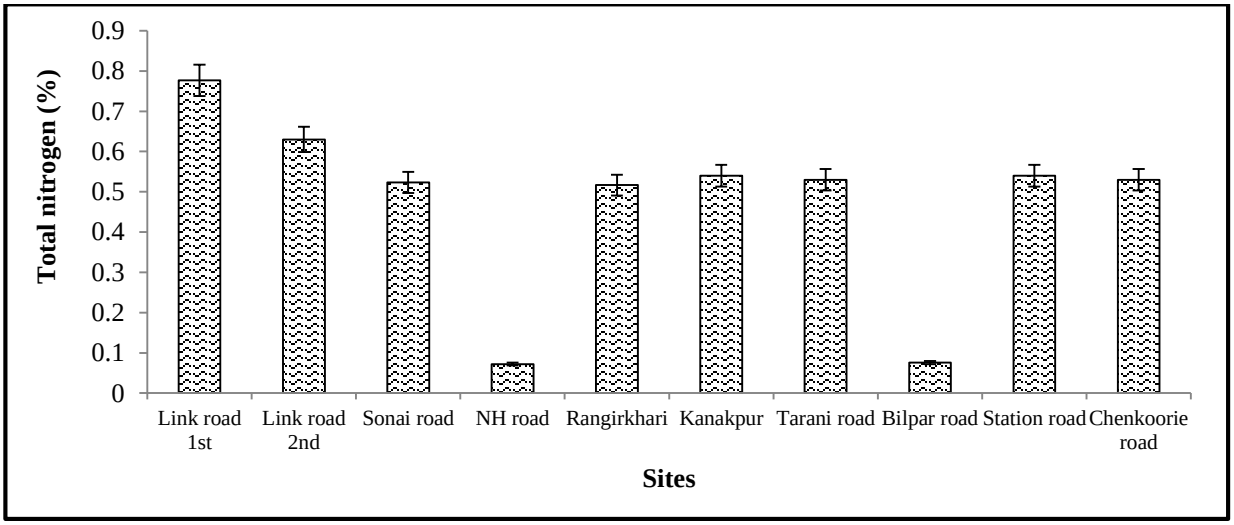

Fig. 4.42: Variation of total nitrogen of soil at the study sites

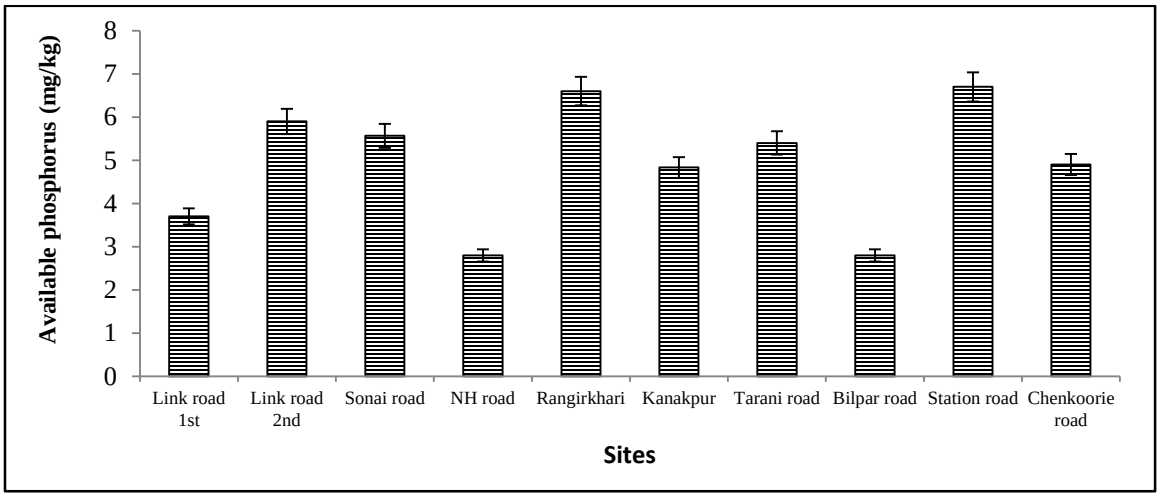

Fig. 4.43: Variation of available phosphorus of soil at the study sites

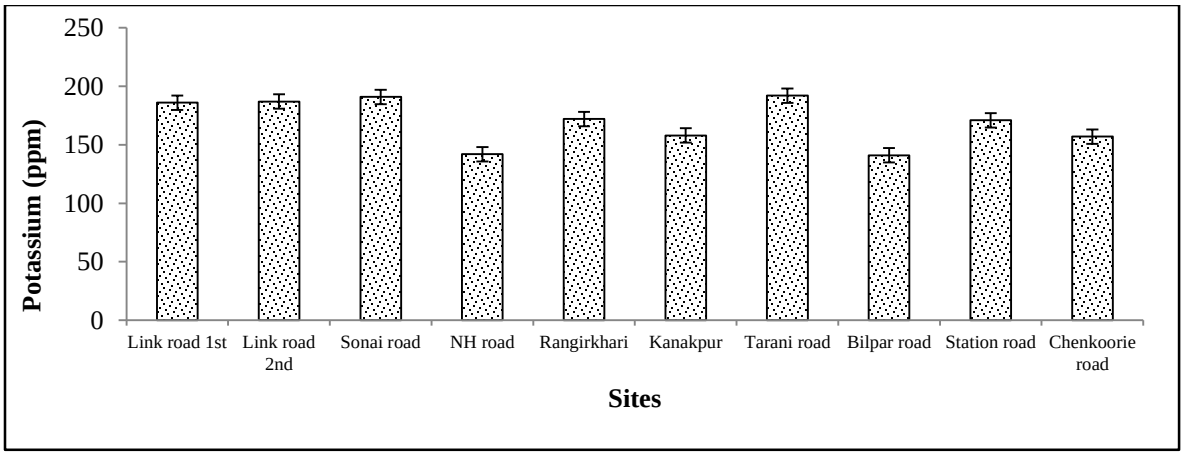

Fig. 4.44: Variation of potassium of soil at the study sites

Table 4.4 List of algae encountered in the present study

Algal species	Sl. no.	Name of the Species	Species code	Species	Genus
Cyanophyceae	1	*Anabaena doliolum* Bharadwaja (after Bharadwaja)	**C**1	57	13
	2	*Anabaena orientalis* Dixit (after Dixit)	**C**2		
	3	*Anabaena spiroides* v. *crassa (*after Smith, G.M.)	**C3**		
	4	*Anabaena variabilis* Kütz. (after Frémy)	**C**4		
	5	*Aphanothece microscopica* Nag. (after Fremy	**C5**		
	6	*Aphanothece naegelii* Wartm (after Skuja)	**C6**		
	7	*Arthospira plantensis* Gomont	**C7**		
	8	*Calothrix parietana Näg. Thuret (after Frémy)*	**C8**		
	9	*Calothrix elenkinii* Kossink (after poljansky)	**C9**		
	10	*Calothrix fusca (*Kütz.) Born. et Flah. forma	**C**10		
	11	*Calothrix marchica Lemm.*(after Frémy)	**C11**		
	12	*Calothrix linearis* Gardner	**C**12		
	13	*Chroococcus* sp.	**C13**		
	14	*Cylindrospermum muscicola* Kütz (after Frémy)	**C14**		
	15	*Cylindrospermum licheniformae* (Bory) Kütz (after Fremy)	**C15**		
	16	*Hydrocoleum* sp.	**C16**		
	17	*Lyngbya diguetii* Gom. (after Frémy)	**C17**		
	18	*Lyngbya nordgardhii* Wille	**C18**		
	19	*Nostoc linckia* v. (Roth) Born. et Flah. (after Frémy)	**C19**		
	20	*Nostoc muscorum* (after Bornet and Thuret)	**C20**		
	21	*Nostoc carneum* Ag. (after Frémy)	**C2**1		
	22	*Nostoc punciformae* Hariot	**C22**		
	23	*Oscillatoria acuminata* Gom.(after Frémy)	**C2**3		
	24	*Oscillatoria amphibia* Ag.(orig.)	**C24**		
	25	*Oscillatoria chalybea* Marrens (after Gomont)	**C25**		
	26	*Oscillatoria chlorina* Kütz (after Frémy)	**C26**		
	27	*Oscillatoria curviceps* Ag.(after Gomont)	**C27**		
	28	*Oscillatoria earlei* Gardner (after Gardner)	**C28**		
	29	*Oscillatoria formosa* Bory (after Frémy)	**C29**		
	30	*Oscillatoria geitleriana* (Frémy) Elenkin (after Frémy)	**C30**		
	31	*Oscillatoria homogenea* Elenkin (after Frémy)	**C31**		
	32	*Oscillatoria laetevirens* v.minimus Biswas (after Biswas)	**C32**		
	33	*Oscillatoria limnetica* Lemm.(Orig.)	**C3**3		
	34	*Oscillatoria limosa* Ag.(after Gomont)	**C34**		
	35	*Oscillatoria nigroviridis* Thwaites (after Gomont)	**C35**		
	36	*Oscillatoria okeni* Ag.(after Gomont),	**C3**6		
	37	*Oscillatoria ornata var.crassa* Rao (after Rao, C.B.)	**C37**		
	38	*Oscillatoria ornate* Kütz (after Frémy)	**C38**		
	39	*Oscillatoria peronata* f.attenuata Skuja (after Skuja)	**C39**		
	40	*Oscillatoria princeps* Vaucher (after Frémy)	**C40**		

	41	*Oscillatoria quandripunctulata* Bruhl & Biswas (orig.)	**C41**		
	42	*Oscillatoria raoi* De Toni (after Rao,C.B.)	**C42**		
	43	*Oscillatoria rubescens* f.forma (orig.)	**C43**		
	44	*Oscillatoria salina* biswas f.major f.n. (orig.)	**C44**		
	45	*Oscillatoria sancta* (Kütz Gom. (after Gomont)	**C45**		
	46	*Oscillatoria splendida* Grev. (after Gomont)	**C46**		
	47	*Oscillatoria subbrevis* Schmidle (orig.)	**C47**		
	48	*Oscillatoria tenuis* Ag.(after Gomont)	**C48**		
	49	*Oscillatoria terebiformis* Ag.(after Gomont)	**C49**		
	50	*Oscillatoria vizagapatensis* Rao (after Rao, C.B.)	**C50**		
	51	*Oscillatoria willei* Gardner em.Drouet (after Gomont)	**C51**		
	52	*Phormidium calcicola* Gardner	**C52**		
	53	*Phormidium lucidum* Kütz.	**C53**		
	54	Spirullina major Kutz. (after Skuja)	**C54**		
	55	*Spirulina platensis* (Gomont) Geitler	**C55**		
	56	*Spiruluna tenuissima* Kützing	**C56**		
	57	*Westiellopsis prolifica Janet*	**C57**		
Chlorophyceae	58	*Ankistrodesmus falcatus* (Corda) Ralfs.	**Ch58**	25	15
	59	*Chlorococcus* sp.	**Ch59**		
	60	*Chlorella ellipsoidea* Gerneck	**Ch60**		
	61	*Cladophora pellucida* Kützing	**Ch61**		
	62	*Closterium acerosum* Ehrenberg ex Ralfs	**Ch62**		
	63	*Closterium costatum* Corda ex Ralfs	**Ch63**		
	64	*Closterium lanceolatum* Kuetzing	**Ch64**		
	65	*Cosmarium constrictum* Delponte	**Ch65**		
	66	*Cosmarium formosulum* Hoffman	**Ch66**		
	67	*Endorina* sp.	**Ch67**		
	68	*Hyalotheca dissiliens* Brébisson ex Ralfs	**Ch68**		
	69	*Micrasterias denticulata* Brébisson ex Ralfs	**Ch69**		
	70	*Mougeotia* sp.	**Ch70**		
	71	*Oedogonium* sp.	**Ch71**		
	72	*Pitophora kewensis* Wittrock	**Ch72**		
	73	*Pitophora* sp.	**Ch73**		
	74	*Pediastrum integrum* Naegelii	**Ch74**		
	75	*Pediastrum tetras* (Ehrenb.) Ralfs	**Ch75**		
	76	*Scenedesmus denticulatus* Lagerheim	**Ch76**		
	77	*Scenedesmus quadricauda* var. *Westii* G.M. Smith	**Ch77**		
	78	*Spirogyra crassa* Kuetzing	**Ch78**		
	79	*Spirogyra punctiformis* Transeau	**Ch79**		
	80	*Stigoclonium* tenue (Agardh) Kuetzing	**Ch80**		
	81	*Stigeoclonium* sp.	**Ch81**		
	82	*Zygnema* sp.	**Ch82**		
Bacillariophyceae	83	*Amphora normani* Rabenhorst	**B83**	38	11
	84	*Amphora ovlis* Kuetz. v. *pediculus* Kuetz.	**B84**		
	85	*Anomoeoneis brachysira* v. *thermalis*	**B85**		
	86	*Cyclotella meneghiniana.*	**B86**		
	87	*Cymbella hungarica* (Grun.) Pant. v. *signata* (Pant.) A.Cl.	**B87**		
	88	*Eunotia* sp.	**B88**		
	89	*Fragilaria ungeriana* Grun.	**B89**		
	90	*Gomphonema mexicanum* Grunow	**B90**		
	91	*Gomphonema* sp.	**B91**		

	92	*Gomphonema sphaerophorum* Ehr	**B92**		
	93	*Gyrosigma baikalensis* Skv.	**B93**		
	94	*Hantschia amphioxys* (Ehr .) Grun.	**B94**		
	95	*Hantschia amphioxys* (Ehr .) Grun v. *densestriata* (Font.) A. Cl.	**B95**		
	96	*Mastogloia recta* Hustedt	**B96**		
	97	*Melosira juergensii* Agarth	**B97**		
	98	*Navicula cuspidata Kuetz. V. ambigua (Ehr.) Cleve*	**B98**		
	99	*Navicula grimii* Krasske	**B99**		
	100	*Navicula laterostrata* Hustedt	**B100**		
	101	*Navicula halophile* (Grun.) Cleve f. *subcapitata* Ostrup	**B101**		
	102	*Navicula pahalgarhensis* Gandhi	**B102**		
	103	*Navicula pupula* Kuetz.	**B103**		
	104	*Navicula salinarum* Grun.	**B104**		
	105	*Navicula subdapaliformis* Gandhi	**B105**		
	106	*Navicula subrhynchocephala* Hustedt	**B106**		
	107	*Navicula viridula* Kuetz.	**B107**		
	108	*Navicula radiosa* Kuetz.	**B108**		
	109	*Nitzschia apiculata* (Greg.) Grun.	**B109**		
	110	*Nitzschia intermedia* Hantzsch	**B110**		
	111	*Nitzschia palea* (Kuetz.) W. Smith	**B111**		
	112	*Nitzschia vermicularis* (Kuetzing) Huntzsch	**B112**		
	113	*Nitzschia heufleriana* Grun.	**B113**		
	114	*Nitzschia obtuse* W. Smith	**B114**		
	115	*Pinnularia lundi* Hustedt	**B115**		
	116	*Pinnularia lonavlensis* Gandhi	**B116**		
	117	*Pinnularia viridis* (Nitz.) Ehr. v. *fallax* Cleve	**B117**		
	118	*Pinnularia eburnean* (Carlson) Zanon	**B118**		
	119	*Synedra acus Kuetz*	**B119**		
	120	*Synedra tabulata* (Agardh) Kuetzing	**B120**		
Euglenophyceae	121	*Euglena tuba* Carter	**E121**	2	2
	122	*Phacus* sp.	**E122**		

Table 4.5 Distribution (presence and absence) of algae encountered in the present study

	Group(Species)		**Study sites**																		
Sl. no.	**Species**	**Code**	**S 1**	**S 2**	**S 3**	**S 4**	**S 5**	**S 6**	**S 7**	**S 8**	**S 9**	**S10**	**S11**	**S12**	**S13**	**S14**	**S15**	**S16**	**S17**	**S18**	**S19**	**S20**
1	*Anabaena doliolum*	**C**1	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	+	-	-
2	*Anabaena orientalis*	**C**2	-	-	-	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-
3	*Anabaena spiroides*	**C3**	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	+	+
4	*Anabaena variabilis*	**C**4	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
5	*Aphanothece microscopica*	**C5**	-	-	-	+	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-
6	*Aphanothece naegelii*	**C6**	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
7	*Arthospira plantensis*	**C7**	+	+	+	+	-	+	-	+	-	-	-	-	-	-	+	+	+	-	-	-
8	*Calothrix parietana*	**C8**	+	-	-	+	-	-	+	-	-	-	-	-	+	+	-	+	-	+	-	-
9	*Calothrix elenkinii*	**C9**	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-
10	*Calothrix fusca*	**C1**0	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	*Calothrix marchica*	**C11**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
12	*Calothrix linearis*	**C1**2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
13	*Chroococcus* sp.	**C13**	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
14	*Cylindrospermum muscicola*	**C14**	-	-	-	-	-	+	-	+	+	+	+	+	-	+	+	-	+	-	+	-
15	*Cylindrospermum licheniformae*	**C15**	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
16	*Hydrocoleum* sp.	**C16**	-	-	-	+	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-
17	*Lyngbya diguetii*	**C17**	+	-	-	-	+	+	+	+	+	+	+	-	+	+	-	-	+	-	-	+
18	*Lyngbya nordgardhii*	**C18**	-	+	+	-	-	-	-	-	+	+	-	-	+	-	+	-	-	-	-	+
19	*Nostoc linckia*	**C19**	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	*Nostoc muscorum*	**C2**0	-	-	+	-	+	-	+	-	-	-	+	-	+	-	-	+	-	-	+	-
21	*Nostoc carneum*	**C2**1	+	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	-	-	-
22	*Nostoc punciformae*	**C22**	-	+	-	-	+	-	-	+	-	-	-	+	-	-	-	+	-		+	-

23	*Oscillatoria acuminata*	**C23**	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+
24	*Oscillatoria amphibia*	**C24**	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
25	*Oscillatoria chalybea*	**C25**	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
26	*Oscillatoria chlorina*	**C26**	-	-	-	+	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-
27	*Oscillatoria curviceps*	**C27**	-	-	+	+	-	-	-	-	+	-	-	+	-	-	-	-	-	+	+	-
28	*Oscillatoria earlei*	**C28**	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
29	*Oscillatoria formosa*	**C29**	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-
30	*Oscillatoria geitleriana*	**C30**	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-
31	*Oscillatoria homogenea*	**C31**	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
32	*Oscillatoria laetevirens* v.minimus	**C32**	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
33	*Oscillatoria limnetica*	**C33**	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
34	*Oscillatoria limosa*	**C34**	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
35	*Oscillatoria nigroviridis*	**C35**	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
36	*Oscillatoria okeni*	**C36**	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
37	*Oscillatoria ornata var.crassa*	**C37**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
38	*Oscillatoria ornate*	**C38**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
39	*Oscillatoria peronata* f.attenuata	**C39**	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-
40	*Oscillatoria princeps*	**C40**	-	-	-	+	+	+	-	-	-	+	-	-	+	-	-	+	-	-	-	-
41	*Oscillatoria quandripunctulata*	**C41**	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
42	*Oscillatoria raoi*	**C42**	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-
43	*Oscillatoria rubescens* f.forma	**C43**	+	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	+	-	-
44	*Oscillatoria salina* biswas f.major	**C44**	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
45	*Oscillatoria sancta*	**C45**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
46	*Oscillatoria splendida*	**C46**	-	-	-	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-
47	*Oscillatoria subbrevis*	**C47**	+	+	-	-	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+
48	*Oscillatoria tenuis*	**C48**	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
49	*Oscillatoria terebiformis*	**C49**	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-

50	*Oscillatoria vizagapatensis*	**C50**	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
51	*Oscillatoria willei*	**C51**	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
52	*Phormidium calcicola*	**C52**	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-	-
53	*Phormidium lucidum*	**C53**	+	-	-	-	+	+	-	+	+	-	-	-	+	-	-	-	+	+	-	-
54	*Spirulina major*	**C54**	+	+	-	+	+	+	-	+	+	+	-	-	+	+	-	+	-	+	-	-
55	*Spirulina platensis*	**C55**	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	+
56	*Spiruluna tenuissima*	**C56**	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+
57	*Westiellopsis prolifica*	**C57**	-	+	-	-	-	+	-	+	-	+	-	-	-	-	-	+	+	-	-	+
58	*Ankistrodesmus falcatus*	**Ch58**	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
59	*Chlorococcus* sp.	**Ch59**	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
60	*Chlorella ellipsoidea*	**Ch60**	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-
61	*Cladophora pellucida*	**Ch61**	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
62	*Closterium acerosum*	**Ch62**	-	-	+	-	-	-	+	-	+	+	-	-	-	-	-		-	-	-	-
63	*Closterium costatum*	**Ch63**	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
64	*Closterium lanceolatum*	**Ch64**	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
65	*Cosmarium constrictum*	**Ch65**	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
66	*Cosmarium formosulum*	**Ch66**	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
67	*Endorina sp.*	**Ch67**	+	+	+	-	-	-		+	-	+	-	+	-	-	-	-	-	+	-	-
68	*Hyalotheca dissiliens*	**Ch68**	-	-	+	-	-	-	+	+	-	-	-	-	-	-	+	+	-	-	-	+
69	*Micrasterias denticulata*	**Ch69**	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-
70	*Mougeotia* sp.	**Ch70**	+	+	+	-	-	-	-	-	-	+	+	-	-	+	-	-	-	+	+	+
71	*Oedogonium sp*	**Ch71**	-	-	-	-	+	+	-	-	+	+	-	+	-	+	-	-	-	+	+	+
72	*Pitophora kewensis*	**Ch72**	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
73	*Pitophora* sp.	**Ch73**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
74	*Pediastrum integrum*	**Ch74**	-	-	-	-	+	+	-	-	-	+	-	+	-	-	-	-	-	-	+	+
75	*Pediastrum tetras*	**Ch75**	-	-	+	-	-	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-
76	*Scenedesmus denticulatus*	**Ch7**6	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-
77	*Scenedesmus quadricauda* var. *Westii*	**Ch77**	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-

78	*Spirogyra crassa*	**Ch78**	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+
79	*Spirogyra punctiformis*	**Ch79**	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+	+	+	+	-
80	*Stigoclonium* tenue	**Ch80**	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-
81	*Stigeoclonium* sp.	**B81**	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
82	*Zygnema* sp.	**B82**	-	+	-	-	-	-	-	-	-	+	+	+	-	+	+	+	-	-	-	+
83	*Amphora normani*	**B83**	-	+	-	-	-	+	-	-	-	-	+	+	+	+	-	-	-	-	-	
84	*Amphora ovlis* v. *pediculus*	**B84**	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	-	-	-	-
85	*Anomoeoneis brachysira v. thermalis*	**B85**	-	-	-	-	-	+	-	+	-	+	+	-	-	-	-	-	+	-	+	-
86	*Cyclotella meneghiniana*	**B86**	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	+	+
87	*Cymbella hungarica*	**B87**		+	-	+	-	-	+	-	-	-	-	-	+	+	-	+	-	-	-	-
88	*Eunotia* sp.	**B88**	+	-	-	-	-	+	-	-	-	+	+	+	-	-	-	-	-	+	+	-
89	*Fragilaria ungeriana*	**B89**	-	-	-	+	+	+	-	+	-	-	-		+	+	+	+		-	-	-
90	*Gomphonema mexicanum*	**B90**	+	-	-	-	+	+	+	-	-	-	-	-	+	+	+	+	-	-	+	-
91	*Gomphonema* sp.	**B91**	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-
92	*Gomphonema sphaerophorum*	**B92**	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
93	*Gyrosigma baikalensis* Skv.	**B93**	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
94	*Hantschia amphioxys*	**B94**	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-
95	*Hantschia amphioxys v. densestriata*	**B95**	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
96	*Mastogloia recta*	**B96**	-	-	-	-	-	+	-	-	-	+	+	+	-	-	-	-	+	-	+	+
97	*Melosira juergensii*	**B97**	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
98	*Navicula cuspidata* v. *ambigua*	**B98**	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
99	*Navicula grimii*	**B99**	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
100	*Navicula laterostrata Hustedt*	**B100**	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
101	*Navicula halophile*	**B101**	+	+	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	+
102	*Navicula pahalgarhensis*	**B104**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+

103	*Navicula pupula*	**B105**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
104	*Navicula salinarum*	**B106**	-	-	+	-	-	-	-	-	-	-	-			-	-	-	-	+	-	-
105	*Navicula subdapaliformis*	**B102**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
106	*Navicula subrhynchocephala*	**B103**	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-
107	*Navicula viridula*	**B107**	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
108	*Navicula recta*	**B108**	-	-	-	+	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-
109	*Nitzschia apiculata*	**B109**	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+
110	*Nitzschia intermedia*	**B110**	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
111	*Nitzschia palea*	**B111**	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
112	*Nitzschia vermicularis*	**B112**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
113	*Nitzschia heufleriana*	**B113**	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+
114	*Nitzschia obtuse*	**B114**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
115	*Pinnularia lundi*	**B115**	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
116	*Pinnularia lonavlensis*	**B116**	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+
117	*Pinnularia viridis*	**B117**	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
118	*Pinnularia eburnean*	**B118**	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
119	*Synedra acus*	**B119**	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
120	*Synedra tabulata*	**B120**	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	+
121	*Euglena tuba*	**E121**	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
122	*Phacus* sp.	**E122**	+	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-

Table 4.4 Seasonal variation of algal density, abundance and frequency in Link Road 1st

	LINK ROAD 1st	**Density (ind × $10^2 cm^{-2}$)**				**Abundance (ind × $10^2 cm^{-2}$)**				**Frequency (%)**			
SI.No.	Algal species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Arthospira plantensis*	-	-	2.93	-	-	-	5.27	-	-	-	66.67	-
2	*Calothrix parietana*	-	-	2.93	-	-	-	5.27	-	-	-	66.67	-
3	*Calothrix fusca*	-	-	1.76	-	-	-	5.27	-	-	-	33.33	-
4	*Calothrix linearis*	14.23	1.56	-	-	6.32	2.45	-	-	33.33	50.00	-	-
5	*Chroococcus* sp.	-	-	2.34	-	-	-	7.03	-	-	-	33.33	-

6	*Lyngbya diguetii*	20.66	-	-	15.67	29.3	-	-	21.50	100.00	-	-	66.66
7	*Nostoc linckia*	5.83	-	-	-	1.75	-	-	-	33.33	-	-	-
8	*Nostoc carneum*	2.38	-	-	-	7.03	-	-	-	33.33	-	-	-
9	*Oscillatoria acuminata*	2.93	-	-	-	2.93	-	-	-	100.00	-	-	-
10	*Oscillatoria limosa*	3.67	-	-	10.57	3.23	-	-	13.73	83.33	-	-	75.00
11	*Oscillatoria okeni*	16.71	11.54	75.95		10.5	1.71	10.85		58.33	56.66	50.00	-
12	*Oscillatoria princeps*	5.3	-	-	5.56	8.67	-	-	6.25	50.00	-	-	67
13	*Oscillatoria subbrevis*	4.52	16.6	96.7	22.45	8.16	49.65	2.45	18.92	66.67	33.33		50.00
14	*Oscillatoria terebiformis*	8.68	5.77	28.2	21	12.3	8.66	5.53	31.34	75.00	66.67	50	50.00
15	*Oscillatoria willei*	4.3	-	-	-	2.93	-	-	-	66.67	-	-	-
16	*Phormidium lucidum*	10.58	-	-	-	17.8	-	-	-	41.67	-	-	-
17	*Spirullina major*	3.24	-	-	-	9.87	-	-	-	66.67	-	-	-
18	*Spirullina tenuissima*	-	-	3.91	-	-	-	0.58	-	-	-	22.22	-
	Chlorophyceae	-	-		-	-	-	-	-	-	-	-	-
19	*Chlorella ellipsoidea*	2.17	-	1.79	8.36	2.74	-	4.22	24.8	68.33	-	41.67	46.33
20	*Cosmarium constrictum*	-	-	-	4.16	-	-	-	7.37		-		42.33
21	*Endorina* sp.	-	-	-	4.62	-	-	-	5.82		-		50.00
22	*Mougeotia* sp.	5.45	-	3.39		8.26	-	9.24		71.9		44.44	
23	*Spirogyra crassa*	1.57	30.71	0.66	6.12	24.8	4.41	1.99	11.5	66.66	74.33	33.33	58.33
	Bacillariophyceae												
24	*Fragilaria ungeriana*	12.04	3.26	-	6.24	9.65	4.41	-	7.13	66.7	71.33	-	66.66
25	*Gomphonema mexicanum*	3.68	-	1.6	1.8	9.37	-	3.26	4.33	41.66	-	41.67	41.33
26	*Gomphonema sp.*	-	-	1.56	1.5	-	-	3.42	3.62	-	-	41.58	45.33
27	*Gyrosigma baikalensis*	1.15	-	1.84	-	3.38	-	2.93	-	66.67	-	55.56	-
28	*Navicula laterostrata*	70.6	21.9	-	5.9	7.5	4.15	-	8.12	50.00	33.33	-	62.33
29	*Navicula grimii*	44.84	8.23	-	-	4.84	9	-	-	75.00	100.00	-	-

30	*Navicula pahalgarhensis*	-	-	1.53	4.82	-	-	3.83	7.91	-	-	50.00	58.33
31	*Navicula pupula*	-	6.78	-	-	-	13.55	-	-	-	50.00	-	-
32	*Nitzschia apiculata*	-	-	3.72	-	-	-	11.13	-	-	-	33.33	-
33	*Nitzschia palea*	30.53	-	-	6.83	30.5	-	-	27.23	100.00	-	-	25.00
34	*Pinnularia lonavlensis*	-	13.7	0.34	-	-	2.46	2.54	-	-	42.33	41.71	-
35	*Pinnularia lundi*	-	5.55	4.52	7.92	-	11.12	13.55	15.7	-	50.00	33.33	50.00
	Euglenophyceae												
36	*Phacus* sp.	139.6	-	0.17	0.43	3.27	-	0.35	6.63	59.22	-	52.81	56.33

Table 4.5 Seasonal variation of algal density, abundance and frequency in Link Road 2nd

	LINK ROAD 2nd	**Density (ind × 10^2cm^{-2})**				**Abundance (ind × 10^2cm^{-2})**				**Frequency (%)**			
SI.No.	Algal species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Arthospira plantensis*	1.55	0.38	-	-	6.83	4.16	-	-	66.67	33.33	-	-
2	*Calothrix fusca*	-	-	3.38	1.70	-	-	10.38	5.11	-	-	100.00	33.33
3	*Chroococcus* sp.	6.18	-	-	7.43	1.78	-	-	11.15	66.67	-	-	66.67
4	*Lyngbya nordgardhii*	-	1.06	-	3.18	-	1.59	-	22.77	-	66.67	-	66.67
5	*Nostoc punciformae*	13.82	-	-	-	11.48	-	-	-	33.33	-	-	-
6	*Oscillatoria chalybea*	16.21	-	1.02	-	16.20	-	1.02	-	100.00	-	100.00	-
7	*Oscillatoria geitleriana*	3.32	13.94	1.07	8.44	4.99	20.91	3.22	1.34	66.67	66.67	33.33	33.33
8	*Oscillatoria laetevirens*	-	6.67	-	10.75	-	1.01	-	10.75	-	66.67	-	100.00
9	*Oscillatoria limnetica*	-	6.22	-	2.96	-	6.64	-	8.88	-	83.50	-	33.33
10	*Oscillatoria nigroviridis*	4.08	-	-	-	12.25	-	-	-	33.33	-	-	-
11	*Oscillatoria peronata*	5.18	-	2.24	-	10.70	-	6.20	-	50.00	-	77.67	-

12	*Oscillatoria subbrevis*		1.64	1.34	11.99	-	4.94	9.34	11.99	-	33.00	100.00	100.00
13	*Oscillatoria willei*	8.66	9.34	-	7.47	12.99	9.34	-	22.42	66.67	100.00	-	33.33
14	*Phormidium calcicola*	11.73	-	15.18	21.15	5.19	-	22.77	21.15	33.33	-	66.67	100.00
15	*Spirulina major*	3.32	-	-	0.67	4.99	-	-	2.03	66.67	-	-	33.33
16	*Westiellopsis prolifica*	3.32	13.94	1.07	8.44	4.99	20.91	3.22	1.34	66.67	66.67	33.33	33.33
	Chlorophyceae												
17	*Chlorella ellipsoidea*	-	1.06	-	3.18	-	1.59	-	22.77	-	66.67	-	66.67
18	*Endorina* sp.	0.34	-	0.24	-	1.03	-	0.72		33.33	-	33.33	-
19	*Mougeotia* sp.	11.73	-	15.18	21.15	5.19	-	22.77	21.15	33.33	-	66.67	100.00
20	*Spirogyra crassa*	16.21	-	1.02	-	16.20		1.02	-	100.00	-	100.00	-
21	*Zygnema* sp.	7.94	3.94	-	-	23.84	5.91	-	-	33.33	66.67	-	-
	Bacillariophyceae												
22	*Amphora normani*	13.82	-	-	-	11.48	-	-	-	33.33	-	-	-
23	*Cymbella hungarica*	10.74	-	*3.23*	-	10.58	-	3.87	-	67.00	-	70.28	-
24	*Navicula laterostrata*	11.39	1.16	-	-	5.83	2.13	-	-	75.13	41.50	-	-
25	*Navicula halophile*	1.55	0.38	-	-	6.83	4.16	-	-	66.67	33.33	-	-
26	*Nitzschia intermedia*	10.61	-	1.79	4.29	19.82	-	3.91	7.28	66.50	-	56.79	50.00
27	*Nitzschia heufleriana*	5.10	-	-	1.53	10.20	-	-	6.10	50.00	-	-	25.00
28	*Pinnularia lundi*	3.82	2.36	-		5.73	4.92	-	-	67.00	50.00	-	
29	*Pinnularia lonavlensis*	3.23	-	-	4.69	3.23	-	-	9.38	100.00	-	-	50.00
30	*Pinnularia viridis*	-	-	1.72	12.00	-	-	4.58	12.00		-	50.00	100.00
31	*Pinnularia eburnea*	8.33	2.98	-	6.31	12.48	6.22	-	11.22	74.92	50.86	-	58.13
32	*Synedra acus*	4.68	-	-	3.24	9.35	-	-	12.95	50.00	-	-	25.00
33	*Synedra tabulata*	7.33	-	-	4.20	0.99	-	-	12.62	33.33	-	-	33.33
	Euglenophyceae												
34	*Euglena tuba*	-	-	7.15	10.38	-	-	21.15	10.38	-	-	100.00	100.00

Table 4.6 Seasonal variation of algal density, abundance and frequency in Sonai Road

	SONAI ROAD	**Density (ind × $10^2 cm^{-2}$)**				**Abundance (ind × $10^2 cm^{-2}$)**				**Frequency (%)**			
SI.No.	Algal species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Arthospira plantensis*	4.48	8.23	-	-	4.84	9.00		-	79.25	88.89	-	-
2	*Calothrix elenkinni*	1.14	-	1.84	-	3.38	-	2.93	-	62.44	-	55.56	-
3	*Calothrix fusca*	2.73	-	-	4.33	5.45	-	-	17.30	50.00	-	-	25.00
4	*Calothrix parietana*	7.06	2.18	-	5.90	7.50	4.15	-	8.03	50.00	39.78	-	62.50
5	*Lyngbya nordgardhii*	-	-	-	4.58		-		5.82	-	-	-	50.00
6	*Nostoc muscorum*	3.69	-	1.60	1.80	5.49	-	3.26	4.33	47.83	-	41.67	*41.67*
7	*Oscillatoria amphibia*	30.11	-	-	10.18	32.38	-	-	13.71	83.33	-	-	79.17
8	*Oscillatoria curviceps*	-	-	-	4.05	-	-	-	7.36	-	-	-	41.67
9	*Oscillatoria earlei*	2.19	-	0.17	8.31	2.74	-	0.42	24.76	68.44	-	41.67	45.83
10	*Oscillatoria limnetica*	16.71	1.09	7.59		44.55	1.71	10.85		58.33	55.56	66.67	-
11	*Oscillatoria limosa*	8.68	5.77	2.80	4.60	12.34	8.66	5.53	7.63	75.00	66.67	50.00	58.33
12	*Oscillatoria nigroviridis*	45.94	16.55	9.60	21.55	81.04	49.65	24.56	18.94	62.50	33.00	58.33	49.83
13	*Oscillatoria salina*	5.34	-	-	5.48	8.67	-	-	6.20	59.33	-	-	66.67
14	*Oscillatoria subbrevis*	20.66	-	-	14.77	29.34	-	-	21.53	72.22	-	-	66.67
15	*Oscillatoria tenuis*	10.58	-	-		17.75	-	-	-	54.17	-	-	-
16	*Oscillatoria vizagapatensis*	44.80	17.88	11.10	2.78	44.80	17.88	33.30	11.10	100.00	100.00	33.33	25.00
17	*Phormidium calcicola*	15.70	3.07	0.66	8.39	24.82	4.41	1.99	12.61	*63.50*	74.44	33.00	58.33
18	*Spirulina major*	12.04	3.26	-	6.20	9.65	4.44	-	7.19	66.72	70.88	-	66.67
	Chlorophyceae												

19	*Chlorella ellipsoidea*	1.39	-	0.17	0.43	3.27	-	0.35	0.66	59.22	-	52.81	55.55
20	*Closterium acerosum*	13.82	-	-	-	11.48	-		-	33.33	-		-
21	*Endorina sp*	0.34	-	0.24	-	1.03	-	0.72	-	33.33	-	33.33	-
22	*Mougeotia* sp.	7.94	3.94		-	23.84	5.91		-	33.33	66.67		-
23	*Pediastrum tetras*	11.73	-	15.18	21.15	5.19	-	22.77	21.15	33.33	-	66.67	100.00
24	*Zygnema sp*	-	-	7.15	10.38	-	-	21.15	10.38	-	-	100.00	100.00
	Bacillariophyceae												
25	*Hantschia amphioxys*	-	-	3.38	1.70	-	-	10.38	5.11	-	-	100.00	33.33
26	*Melosira juergensii*	3.32	-	-	0.67	4.99	-	-	2.03	66.67	-	-	33.33
27	*Navicula salinarum*	7.33	-	-	4.20	0.99	-	-	12.62	33.33	-	-	33.33
28	*Nitzchia intermedia*	0.88		0.38		1.75		1.15	-	50	-	33.33	-
29	*Pinnularia lundi*	1.55	0.38			6.83	4.16	-	-	66.67	33.33	-	-
30	*Pinnularia eburnea*	-	-	-	3.32	-	-	-	2.93		-	-	33.33
31	*Synedra acus*	0.98	0.58	0.77	0.25	1.95-	1.15	2.30	1.00	50.00	50.00	33.33	25.00

Table 4.7 Seasonal variation of algal density, abundance and frequency in Kanakpur Road

	Kanakpur Road	**Density (ind × $10^2 cm^{-2}$)**				**Abundance (ind × $10^2 cm^{-2}$)**				**Frequency (%)**			
Sl.No	**Species**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Anabaena orientalis*	0.78	0.38	-	-	1.55	0.75	-	-	50.00	50.00	-	-
2	*Anabaena variabilis*	3.24	1.66	-	-	4.85	3.29	-	-	66.67	49.75	-	-
3	*Aphanothece naegelii*	10.74	-	3.23	-	10.58		3.87	-	67.00	-	70.28	-
4	*Aphanothece microscopica*	10.31	-	-	5.44	15.36	-	-	9.03	71.13	-	-	55.50
5	*Arthospira plantensis*	-	1.69	-	6.84	-	4.26	-	9.98	-	47.17	-	63.92
6	*Calothrix parietana*	11.39	1.16	-	-	5.83	2.13	-	-	75.13	41.50	-	-
7	*Chroococcus* sp	-	3.28	3.22	7.24	-	7.15	6.67	9.68	-	38.67	69.42	69.47
8	*Hydrocoleum* sp.	8.33	2.98	-	6.31	12.48	6.22	-	11.22	74.92	50.86	-	58.13
9	*Oscillatoria chlorina*	12.03	5.55	9.42	3.33	14.76	6.28	13.48	8.60	83.50	83.00	83.42	41.25
10	*Oscillatoria curviceps*	7.82	-	2.63	9.96	8.46	-	7.12	13.75	340.96	-	41.50	69.67
11	*Oscillatoria earlei*	5.18	-	2.24		10.70	-	6.20		50.00	-	77.67	-
12	*Oscillatoria okeni*	10.20	-		2.55	13.31	-	-	7.65	79.38	-	-	33.00
13	*Oscillatoria princeps*	-	-	-	10.47	-	-	-	15.87	-	-	-	50.00
14	*Spirulina crassa*	10.61	-	1.79	4.29	19.82	-	3.91	7.28	66.50	-	56.79	50.00
	Chlorophyceae												
15	*Chlorella ellipsoidea*	4.76	1.41	-	-	7.14	3.19	-	7.66	67.00	66.50	-	-
16	*Cladophora pellucida*	3.82	2.36	-	-	5.73	4.92	-	-	67.00	50.00	-	-
17	*Pithophora kewensis*	-	-	1.72	12.00	-	-	4.58	12.00	-	-	50.00	100.00
	Bacillariophyceae												
18	*Cymbella sp*	-	-	3.49	-	-	-	6.99	-	-	-	45.83	-

19	*Fragilaria ungeriana*	7.33	-	6.06	-	10.70	-	10.15	-	63.92	-	70.75	-
20	*Gomphonema mexicanum*	3.82	2.36	-	-	5.73	4.92	-	-	67.00	50.00	-	-
21	*Gomphonema sphaerophorum*	-	-	1.72	12.00	-	-	4.58	12.00	-	-	50.00	100.00
22	*Navicula cuspidata*	1.45	1.13	0.70	-	2.90	2.25	1.05	-	50.00	50.00	66.67	-
23	*Navicula viridula*	0.78	0.38	-	-	1.55	0.75	-	-	50.00	50	66.67	-
24	*Navicula radiosa*	3.43	34.6	21.22	24.13	3.43	34.6	21.22	24.13	100.00	100.00	100.00	100.00

Table 4.8 Seasonal variation of algal density, abundance and frequency in Tarani Road

	TARANI ROAD	**Density (ind × $10^2 cm^{-2}$)**				**Abundance (ind × $10^2 cm^{-2}$)**				**Frequency (%)**			
Sl.No	Algal species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Calothrix fusca*	-	-	1.63	1.11	-	-	4.62	3.87	-	-	29.49	27.73
3	*Lyngbya cinerescens*	2.74	5.45	9.76	9.14	5.63	19.01	29.07	31.12	56.80	35.86	31.06	43.09
4	*Nostoc linckia*	5.30	1.27	3.57	2.81	12.00	6.04	10.25	5.44	46.31	20.04	27.40	50.00
5	*Nostoc muscorum*		1.82	1.38	-	-	4.88	4.73	-	-	42.79	32.58	
6	*Nostoc punciformae*	-	-	1.63	1.11	-	-	4.62	3.87	-	-	29.49	27.73
7	*Oscillatoria amphibia*	4.27	10.16	-	3.71	8.75	37.50		10.45	47.50	26.42	-	40.42
8	*Oscillatoria laetevirens v.minimus*	-	7.01	8.84	-	-	16.79	24.22	-	-	41.33	37.36	-
9	*Oscillatoria princeps*	0.90	8.16	3.02	-	4.54	17.57	12.22	-	20.00	43.23	23.27	-
10	*Oscillatoria raoi*	7.28	22.04	28.47	12.26	14.14	48.00	92.15	25.16	54.38	50.42	32.53	49.09
11	*Oscillatoria subbrevis*	-	12.56	19.61	4.96		31.70	37.16	8.16		43.16	55.09	58.51
12	*Phormidium lucidum*	1.67	8.67	2.98		4.11	16.97	7.71		43.39	41.54	37.12	-
13	*Spirullina major*	-	-	10.12	-	-	-	37.28	-	-	-	35.48	-
	Chlorophyceae												
14	*Chlorella ellipsoidea*	29.28	-	4.62	0.19	29.3		6.93	0.75	100.00		66.67	25.00
15	*Chlorococcus sp*	-	-	0.75	-	-	-	2.25	-	-	-	33.33	-

16	*Closterium lanceolatum*	0.38	-	-	-	0.75	-	-	-	50.00	-	-	-
17	*Hyalotheca burmensis*	0.78	0.38			1.55	0.75			50.00	50.00	-	-
18	*Pithophora kewensis*	-	-	2.78	-	-	-	8.53	-	-	-	29.38	-
	Bacillariophyceae												
19	*Cymbella sp*	-	0.58	1.57	-	-	1.86	8.10	-	-	32.39	27.84	-
20	*Fragilaria ungeriana*	0.28	1.90		2.19	0.73	5.69	-	8.95	38.75	29.21	-	21.59
21	*Gomphonema mexicanum*	10.31	-	-	5.44	15.36	-	-	9.03	71.13	-	-	55.50

Table 4.9 Seasonal variation of algal density, abundance and frequency in Chenkoorie Road

	CHENKOORIE ROAD	**Density (ind × $10^2 cm^{-2}$)**				**Abundance (ind × $10^2 cm^{-2}$)**				**Frequency (%)**			
SI.No.	Algal species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Anabaena spiroides*	0.78	1.41	1.43	-	2.36	3.73	6.62	-	40.12	34.61	23.97	-
2	*Anabaena variabilis*	1.62	-	-	0.19	2.43	-	-	0.75	66.67	-	-	25.00
3	*Aphanothece microscopica*	-	2.70	4.46	6.90	-	15.48	11.37	2.04	-	26.12	35.57	35.25
4	*Arthospira sp1*	0.13	5.29	2.59	-	0.34	14.64	7.77	-	40.18	32.39	33.40	-
5	*Calothrix parietana*	0.34	0.68	1.31	-	1.18	2.51	6.90	-	33.04	28.22	24.22	-
6	*Chroococcus* sp.												
7	*Cylindrospermum musicola*	1.11	0.80	2.95	-	8.49	2.50	9.00	-	13.39	24.53	32.62	-
10	*Nostoc punciformae*	-	1.39	0.85	0.16	-	5.10	2.25	0.73	-	27.27	26.99	26.19
11	*Oscillatoria acuminata*	3.79	14.45	5.29	6.93	7.51	40.45	11.00	19.22	51.96	46.33	47.01	69.57
12	*Oscillatoria princeps*	-	9.45	3.70	-	-	32.23	10.34	-	-	41.48	37.94	-
13	*Oscillatoria splendida*	10.32	-	13.30	-	30.43	-	34.60	-	50.04	-	39.25	-
14	*Oscillatoria subbrevis*	3.95	14.37	25.01	9.07	7.71	28.49	84.86	13.47	44.20	42.69	47.98	60.02
15	*Oscillatoria tenuis*	-	2.56	2.83	-	-	7.88	61.75	-	-	47.41	41.71	-

16	*Phormidium lucidum*	-	4.27	5.83	0.97	-	10.89	23.21	4.67	-	33.29	41.71	41.10
17	*Spirullina major*	-	16.13	-	-	-	16.13	-	-	-	66.67	-	-
18	*Westiellopsis prolifica*	-	26.18	-	-	-	52.35	-	-	-	33.33	-	-
	Chlorophyceae												
19	*Cladophora pellucida*		0.88				2.65				31.72		
20	*Micrasterias denticulata*	8.93	-	-	-	8.93	-	-	-	100	-	-	-
21	*Oedogonium sp*	20.00	22.30	18.02	1.98	20	22.30	27.03	2.63	100	100.00	66.67	75
22	*Pediastrum integrum*	0.28			2.19	0.73	5.69		8.95	38.75	29.21		21.59
23	*Pitophora kewensis*		1.25	-	-	-	4.70				32.28		
24	*Scenedesmus denticulatus*	1.13	0.55	-	-	2.25	1.10	-	-	50	50.00	-	-
	Bacillariophyceae												
25	*Amphora normani*	2.55	-	-	4.43	5.10	-	-	6.65	50.00	-	-	66.67
26	*Anomoeoneis brachysira v. thermalis*	0.85	-	-	5.70	1.70	-	-	17.1	50.00	-	-	33.33
27	*Eunotia sp.*	0.63	2.72	2.52	0.91	1.25	5.45	3.78	1.83	50.00	50.00	66.67	50.00
28	*Fragilaria ungeriana*	0.87	1.76	-	-	4.16	4.79	-	-	20.21	36.93	-	-
29	*Gomphonena sphaerophorum*	0.58	1.57	-	-	1.86	8.10	-	-	32.39	27.84	-	-
30	*Gomphonema mexicanum*	1.90		2.19	0.73	5.69		8.95	38.75	29.21	-	21.59	-
31	*Gomphonema sp.*	-	-	1.63	1.11	-	-	4.62	3.87	-	-	29.49	27.73
32	*Mastogloia recta*	8.93	2.15	2.2	0.74	8.93	4.3	6.6	1.48	100.00	50	33.33	50

Table 4.10 Seasonal variation of algal density, abundance and frequency in Bilpar Road

	BILPAR ROAD	**Density (ind × 10^2cm^{-2})**				**Abundance (ind × 10^2cm^{-2})**				**Frequency (%)**			
Sl.No	Algal Species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Anabaena doliolum*	-	6.80	2.36	-	-	12.19	16.27	-	-	45.54	19.67	
2	*Aphanothece microscopica*	-	-	25.20	13.62	-	-	98.13	23.29	-	-	27.46	28.22
3	*Arthospira sp1*	-	11.13	-	29.48	-	34.50	26.79	63.57	-	40.83	29.42	58.84
4	*Cylindrospermum muscicola*	10.00	10.47	31.27	8.64	19.65	22.74	107.78	77.74	50.47	51.50	37.41	14.00
6	*Hydrocoleum* sp.	0.78	-	5.86	-	1.77	8.30	12.53	-	50.32	30.05	40.44	-
7	*Lyngbya diguetii*	-	57.84	42.55	-	-	172.03	169.53	-	-	40.17	32.39	-
8	*Nostoc linckia*	17.88		2.06	1.40	26.33	-	10.37	8.01	52.67	-	19.70	25.00
9	*Oscillatoria formosa*	-	3.86	6.77	-	-	14.07	30.53	-	-	31.88	27.30	-
10	*Oscillatoria vizagapatensis*	6.32	14.07	21.53	17.18	16.85	8.55	58.24	35.38	38.00	41.67	38.62	45.36
	Chlorophyceae												
11	*Closterium acerosum*	-	3.18	8.53		-	19.92	24.10	-	-	24.14	33.33	-
12	*Hyalotheca burmensis*	6.57	-	-	2.77	14.33	-	-	9.49	28.44	-	-	30.51
13	*Scenedesmus quadricauda*	4.96	-	36.23	27.22	10.48	-	147.88	90.91	45.33	-	24.03	44.03
14	*Spirogyra punctiformis*		1.22	2.22	-	-	5.25	10.89	-	-	28.53	18.69	-
15	*Stigeoclonium* sp.		0.26	14.66	-	-	0.81	55.14	-	-	23.71	20.45	-
	Bacillariophyceae												
16	*Cymbella* sp.	20	22.3			20	22.3			50	50.00	-	-

17	*Gomphonema mexicanum*		5.89	2.56	-	-	56.33	9.40	-	-	23.50	27.00	-
18	*Gomphonema* sp.	-	-	11.45	-	-	-	41.33	-	-	-	28.00	-
19	*Navicula subrhynchocephala*	1.17	0.78		0.56	7.80	2.57		2.57	20.89	27.31	-	-
20	*Nitzchia intermedia*	3.33	-	9.6	-	6.65	-	28.8	-	50	-	33.33	-
21	*Pinnularia eburnea*	25.58	-	-	7.09	51.15	-	-	28.35	50	-	-	100

Table 4.12 Seasonal variation of algal density, abundance and frequency in Rangirkhari

	Rangirkhari	**Density (ind × $10^2 cm^{-2}$)**				**Abundance (ind × $10^2 cm^{-2}$)**				**Frequency (%)**			
SI.No.	Algal species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Arthospira plantensis*	-	2.56	2.83	-	-	7.88	61.75	-	-	47.41	41.71	-
2	*Cylindrospermum musicola*	1.42	13.24		21.07	5.05	48.98		63.20	33.74	462.74		33.33
4	*Lyngbya diguetii*	-	-	1.42	2.98	-	-	3.78	12.29	-	-	38.00	50.00
5	*Nostoc punciformae*	-	4.27	5.83	0.97	-	10.89	12.65	3.56	-	33.29	41.71	41.10
6	*Oscillatoria acuminata*	-	10.47	-	-	-	31.41	-	-	-	33.00	-	-
7	*Oscillatoria amphibia*	-	-	1.21	-	-	-	4.45	-	-	-	27.27	21.31
8	*Oscillatoria chalybea*	-	-	9.76	0.69	-	-	26.13	2.46	-	-	33.74	27.80
9	*Oscillatoria geitleriana*	-	2.08	0.45	0.30	-	6.38	2.69	1.13	-	68.00	17.80	25.21
10	*Phormidium lucidum*	-	-	5.13	6.39			20.30	44.60	-	-	26.00	23.50
11	*Spirullina major*	**14.93**	6.80	-	**5.08**	14.93	13.60	-	10.15	100.00	50.00	-	50.00
12	*Westiellopsis prolifica*	0.78	1.41	1.43	-	2.36	3.73	6.62	-	40.12	34.61	23.97	-
	Chlorophyceae	-	2.70	4.46	6.90	-	15.48	11.37	2.04	-	26.12	35.57	35.25
13	*Ankistrodesmus falcatus*	0.34	0.68	1.31	-	1.18	2.51	6.90	-	33.04	28.22	24.22	-
14	*Chlorococcus sp*	2.55	-	4.43	-	5.10	-	6.65	-	50.00	-	-	66.67
15	*Endorina sp*	-	1.62	1.89	-	-	7.25	4.57	-	-	24.13	36.62	-
16	*Hyalotheca sp*	1.11	0.80	2.95	-	8.49	2.50	9.00	-	13.39	24.53	32.62	-
	Bacillariophyceae												
17	*Anomoeoneis brachysira v. thermalis*	-	-	9.76	0.69	-	-	26.13	2.46	-	-	33.74	27.80
18	*Cymbella hungarica*	1.17	0.78		0.56	7.80	2.57		2.57	20.89	27.31	-	-
19	*Fragilaria ungeriana*	-	-	1.21		-	-	4.45		-	-	27.27	21.31

20	*Navicula recta*	-	2.08	0.45	0.30	-	6.38	2.69	1.13	-	68.00	17.80	25.21
21	*Nitzschia heufleriana*	-	-	11.45	-	-		41.33	-	-	-	28.00	-
22	*Pinnularia lonavlensis*	-	5.89	2.56	-	-	56.33	9.40	-	-	23.50	27.00	-
	Euglenophyceae												
23	*Phacus* sp	-	-	4.73	3.58	-	-	13.74	8.99	-	-	29.87	28.71

Table 4.13 Seasonal variation of algal density, abundance and frequency in Vivekananda Road

	VIVEKANANDA ROAD	**Density (ind × 10^2cm^{-2})**				**Abundance (ind × 10^2cm^{-2})**				**Frequency (%)**			
SI.No.	Algal species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Anabaena orientalis*	5.45	0.27	0.33	-	8.26	-	0.92	-	71.88	-	44.44	
2	*Anabaena spiroides*	-	-	-	4.58	-	-	-	5.82	-	-	-	50.00
3	*Chroococcus sp.*	5.34	-	-	5.48	8.67	-	-	6.20	59.33	-	-	66.67
4	*Cylindrospermum musicola*	15.70	3.07	0.66	8.39	24.82	4.41	1.99	12.61	*63.50*	74.44	33.00	58.33
5	*Hydrocoleum* sp.	30.11	-	-	10.18	32.38	-	-	13.71	83.33	-	-	79.17
6	*Lynbgya diguetii*	23.21	-	-	11.32	24.34	-	-	12.43	75.00	-	-	25.00
7	*Lyngbya nordgardhii*	2.19	-	0.17	8.31	2.74	-	0.42	24.76	68.44	-	41.67	45.83
8	*Oscillatoria chlorina*	8.68	5.77	2.80	4.60	12.34	8.66	5.53	7.63	75.00	66.67	50.00	58.33
9	*Oscillatoria homogenea*	16.71	1.09	7.59	-	44.55	1.71	10.85	-	58.33	55.56	66.67	-
10	*Oscillatoria nigroviridis*	20.66	-	-	14.77	29.34	-	-	21.53	72.22	-	-	66.67
11	*Oscillatoria quandripunctulata*	45.94	16.55	9.60	21.55	81.04	49.65	24.56	18.94	62.50	33.00	58.33	49.83
12	*Phormidium calcicola*	-	-	-	4.05	-	-	-	7.36	-	-	-	41.67
13	*Phormidium lucidum*	10.58	-	-	-	17.75	-	-	-	54.17	-	-	-
14	*Spirullina major*	30.53	-	-	6.79	30.53	-	-	27.15	100.00	-	-	25.00
15	*Spirullina plantensis*	3.69	-	1.60	1.80	5.49	-	3.26	4.33	47.83	-	41.67	*41.67*
16	*Westiellopsis prolifica*	-	0.13	0.34	-	-	0.24	0.25	-	-	42.33	41.71	-
	Chlorophyceae												
17	*Closterium acerosum*	7.06	2.18	-	5.90	7.50	4.15	-	8.03	50.00	39.78	-	62.50
18	*Closterium lanceolatum*	12.04	3.26	-	6.20	9.65	4.44	-	7.19	66.72	70.88	-	66.67
19	*Cosmarium constrictum*	-	-	24.43	13.57	-	-	48.85	40.70	-	-	50.00	33.33
20	*Cosmarium formosulum*	1.39	-	0.17	0.43	3.27	-	0.35	0.66	59.22		52.81	55.55
21	*Oedogonium* sp.	0.85	-	-	-	1.70	-	-	-	33.33	-	-	-

22	*Pediastrum integrum*	16.13	-	-	-	16.13	-	-	-	100.00	-	-	-
23	*Pediastrum tetras*	4.48	8.23	-	-	4.84	9.00	-	-	79.25	88.89	-	-
24	*Spirogyra punctiformis*	-	-	1.55	1.54	-	-	3.42	3.66	-	-	41.58	45.83
25	*Stigeoclonium tenue*	1.14	-	1.84		3.38	-	2.93	-	62.44	-	55.56	-
	Bacillariophyceae												
26	*Gyrosigma baikalensis*	2.23	-	1.34	-	1.36	-	1.65	-	33.33	25	-	-
27	*Pinnularia viridis*	-	-	1.53	4.79	-	-	3.83	7.99	-	-	44.44	58.33
28	*Synedra tabulata*	1.23	-	1.45	-	1.45	-	1.34	-	25	50	-	-

Table 4.14 Seasonal variation of algal density, abundance and frequency in Park Road

	PARK ROAD	**Density (ind × $10^2 cm^{-2}$)**				**Abundance (ind × $10^2 cm^{-2}$)**				**Frequency (%)**			
SI.No.	Algal species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Anabaena orientalis*	2.84	-	-	12.37	5.54	-	-	12.37	33.33	-	-	100.00
2	*Anabaena variabilis*	-	-	3.80	-	-	-	3.38	-	-	-	100.00	-
3	*Calothrix elenkinii*	4.08				12.25	-	-	-	33.33	-	-	-
4	*Cylindrospermum musicola*	1.39	-	2.05	9.34	11.78	-	3.07	9.34	33.33	-	66.67	100.00
6	*Lyngbya diguetii*	8.66	9.34	-	7.47	12.99	9.34	-	22.42	66.67	100.00	-	33.33
7	*Lyngbya nordgardhii*	12.40		1.06		8.45	-	6.06	-	100.00	-	100.00	-
8	*Oscillatoria okeni*	-	1.64	1.34	11.99	-	4.94	9.34	11.99	-	33.00	100.00	100.00
9	*Oscillatoria princeps*	6.18	-	-	7.43	1.78	-	-	11.15	66.67	-	-	66.67
10	*Spirullina major*	4.51	-	-	-	4.53	-	-	-	33.33	-	-	-
11	*Westiellopsis prolifica*	3.32	13.94	1.07	8.44	4.99	20.91	3.22	1.34	66.67	66.67	33.33	33.33
	Chlorophyceae												
12	*Closterium acerosum*	16.21	-	1.02	-	16.20	-	1.02	-	100.00	-	100.00	-
13	*Endorina sp*	-	6.67	-	10.75	-	1.01	-	10.75	-	66.67	-	100.00
14	*Mougeotia*	0.34	-	0.24	-	1.03	-	0.72	-	33.33	-	33.33	-
15	*Oedogonium sp*	-	-	-	26.22	-	-	-	26.22	-	-	-	100.00
16	*Pediastrum integrum*	-	1.06	-	3.18	-	1.59	-	22.77	-	66.67	-	66.67
17	*Zygnema sp*	11.73	-	15.18	21.15	5.19	-	22.77	21.15	33.33	-	66.67	100.00
	Bacillariophyceae												
18	*Anomoeoneis brachysira v. thermalis*	2.74	5.45	9.76	9.14	5.63	19.01	29.07	31.12	56.80	35.86	31.06	43.09
19	*Cyclotella meneghiniana*	-	-	3.44	2.84	-	-	8.34	8.34	-	-	40.34	37.22
20	*Eunotia sp.*	3.95	14.37	25.01	9.07	7.71	28.49	84.86	13.47	44.20	42.69	47.98	60.02
21	*Hantschia amphioxys*	-	9.45	3.70	-	-	32.23	10.34	-	-	41.48	37.94	-

22	*Mastogloia recta*	3.79	14.45	5.29	6.93	7.51	40.45	11.00	19.22	51.96	46.33	47.01	69.57
24	*Nitzchia apiculata*	1.67	8.67	2.98	-	4.11	16.97	7.71	-	43.39	41.54	37.12	-

Table 4.15 Seasonal variation of algal density, abundance and frequency in Club Road

	CLUB ROAD	**Density (ind × 10^2cm^{-2})**				**Abundance (ind × 10^2cm^{-2})**				**Frequency (%)**			
SI.No.	Algal species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Cylindrospermum musicola*	7.82	-	2.63	9.96	8.46	-	7.12	13.75	340.96	-	41.50	69.67
2	*Lyngbya diguetii*	10.20	-	-	2.55	13.31	-	-	7.65	79.38			33.00
3	*Nostoc muscorum*	12.03	5.55	9.42	3.33	14.76	6.28	13.48	8.60	83.50	83.00	83.42	41.25
4	*Oscillatoria rubescens*	-	-	-	10.47	-	-	-	15.87	-	-	-	50.00
5	*Oscillatoria splendida*	4.76	1.41	-	5.89	7.14	3.19	-	7.66	67.00	66.50	-	75.25
	Chlorophyceae	5.18	-	2.24		10.70	-	6.20		50.00	-	77.67	-
6	*Closterium lanceolatum*	11.39	1.16	-	-	5.83	2.13	-	-	75.13	41.50	-	-
7	*Cosmarium constrictum*	-	3.28	3.22	7.24	-	7.15	6.67	9.68	-	38.67	69.42	69.47
8	*Mougeotia* sp.	-	-	-	31.12	-	-	-	46.68	-	-	-	66.67
9	*Pediastrum tetras*	3.24	1.66	-	-	4.85	3.29	-	-	66.67	49.75	-	-
10	*Zygnema* sp.	10.74	-	*3.23*	-	10.58	-	3.87	-	67.00	-	70.28	-
	Bacillariophyceae												
11	*Amphora normani*	10.61	-	1.79	4.29	19.82		3.91	7.28	66.50		56.79	50.00
12	*Amphora ovlis*	3.82	2.36	-	-	5.73	4.92	-	-	67.00	50.00	-	-
13	*Anomoeoneis brachysira v. thermalis*	10.31	-	-	5.44	15.36	-	-	9.03	71.13	-	-	55.50
14	*Cyclotella meneghiniana*	-	1.69	-	6.84	-	4.26	-	9.98	-	47.17	-	63.92
15	*Eunotia* sp.	2.56	1.32	-	-	2.23	1.21	-	-	66.67	25.00	-	-
16	*Hantschia amphioxys*	2.64	1.45		4.17	5.49	3.39	-	10.23	49.92	44.33		41.42
17	*Mastogloia recta*	-	-	3.49	-	-	-	6.99	-	-	-	45.83	-
	Euglenophyceae												
19	*Euglena tuba*	8.33	2.98	-	6.31	12.48	6.22	-	11.22	74.92	50.86	-	58.13
20	*Phacus sp.*	15.28	-	-	21.80	30.55	-	-	87.20	50.00	-	-	25.00

Table 4.16 Seasonal variation of algal density, abundance and frequency in Trunk Road

	TRUNK ROAD	**Density (ind × $10^2 cm^{-2}$)**				**Abundance (ind × $10^2 cm^{-2}$)**				**Frequency (%)**			
SI.No.	Algal species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Anabaena orientalis*	1.67	8.67	2.98	-	4.11	16.97	7.71	-	43.39	41.54	37.12	-
2	*Cylindrospermum muscicola*	2.74	5.45	9.76	9.14	5.63	19.01	29.07	31.12	56.80	35.86	31.06	43.09
3	*Lyngbya diguetii*	4.27	10.16	-	3.71	8.75	37.50		10.45	47.50	26.42	-	40.42
4	*Nostoc carneum*	7.28	22.04	28.47	12.26	14.14	48.00	92.15	25.16	54.38	50.42	32.53	49.09
5	*Nostoc punciformae*	-	-	10.12	-	-	-	37.28	-	-	-	35.48	-
6	*Oscillatoria chlorina*	0.90	8.16	3.02	-	4.54	17.57	12.22	-	20.00	43.23	23.27	-
7	*Oscillatoria curviceps*	-	12.56	19.61	4.96	-	31.70	37.16	8.16	-	43.16	55.09	58.51
8	*Oscillatoria peronata*	-	7.01	8.84	-	-	16.79	24.22	-	-	41.33	37.36	-
9	*Spirullina tenuissima*	-	-	3.44	2.84	-	-	8.34	8.34	-	-	40.34	37.22
	Chlorophyceae												
10	*Endorina* sp.	5.30	1.27	3.57	2.81	12.00	6.04	10.25	5.44	46.31	20.04	27.40	50.00
11	*Micrasterias denticulata*	-	0.88	0.68	-	-	2.65	2.89	-	-	31.72	28.41	-
12	*Mougeotia* sp.	-	-	-		-	-	-		-	-	-	
13	*Oedogonium* sp.	2.55	0.98	3.41	3.42	5.32	6.06	22.80	10.01	44.12	17.77	28.03	36.09
14	*Pediastrum integrum*	-	3.16	5.60	-	-	9.83	22.02	-	-	36.01	27.27	
15	*Pediastrum tetras*	-	1.82	1.38	-	-	4.88	4.73	-	-	42.79	32.58	-
16	*Zygnema* sp.	0.39	1.25	2.06	0.81	1.21	4.70	6.99	2.62	40.00	32.28	35.02	29.35
	Bacillariophyceae												
17	*Amphora normani*	0.28	1.90	-	2.19	0.73	5.69	-	8.95	38.75	29.21	-	21.59

18	*Cyclotella meneghiniana*	-	0.58	1.57	-	-	1.86	8.10	-	-	32.39	27.84	-
19	*Eunotia* sp.	-	0.87	1.76	-	-	4.16	4.79	-	-	20.21	36.93	-
20	*Mastogloia recta*	-	-	1.63	1.11	-	-	4.62	3.87	-	-	29.49	27.73

Table 4.17 Seasonal variation of algal density, abundance and frequency in Station Road

	STATION ROAD	**Density (ind × 10^2cm^{-2})**				**Abundance (ind × 10^2cm^{-2})**				**Frequency (%)**			
SI.No.	Algal species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Anabaena doliolum*	-	2.56	2.83	-	-	7.88	61.75	-	-	47.41	41.71	-
2	*Aphanothece naegelii*	10.32	-	13.30	-	30.43	-	34.60	-	50.04	-	39.25	-
3	*Arthospira plantensis*	-	2.70	4.46	6.90	-	15.48	11.37	2.04	-	26.12	35.57	35.25
4	*Calothrix parietana*	0.78	1.41	1.43	-	2.36	3.73	6.62	-	40.12	34.61	23.97	-
5	*Lyngbya diguetii*	7.33	-	-	4.20	0.99		-	12.62	33.33		-	33.33
6	*Lyngbya nordgardhii*	1.55	0.38	-	-	6.83	4.16	-		66.67	33.33	-	
7	*Nostoc carneum*	3.32	-	-	0.67	4.99	-	-	2.03	66.67	-	-	33.33
8	*Nostoc muscorum*	-	-	-	12.20	-	-	-	24.40	-	-	-	50.00
9	*Oscillatoria princeps*	-	-	7.15	10.38			21.15	10.38	-	-	100.00	100.00
10	*Oscillatoria subbrevis*	7.94	3.94	-	-	23.84	5.91	-	-	33.33	66.67	-	-
11	*Oscillatoria tenuis*	-	-	3.38	1.70	-	-	10.38	5.11	-	-	100.00	33.33
12	*Oscillatoria willei*	13.82	-	-	-	11.48	-	-	-	33.33	-	-	-
13	*Phormidium lucidum*	85.48	-	-	89.58	170.95	-	-	22.30	50.00	-	-	50.00

14	*Spirullina major*	-	4.27	5.83	0.97	-	10.89	+	+	-	33.29	41.71	41.10
15	*Westiellopsis prolifica*	0.34	0.68	1.31	-	1.18	2.51	6.90	-	33.04	28.22	24.22	-
	Chlorophyceae												
16	*Chlorella ellipsoidea*	-	1.62	1.89	-	-	7.25	4.57	-	-	24.13	36.62	-
17	*Pithophora kewensis*	1.11	0.80	2.95	-	8.49	2.50	9.00	-	13.39	24.53	32.62	-
	Bacillariophyceae												
18	*Amphora normani*	1.42	13.24	-	21.07	5.05	48.98	-	63.20	33.74	462.74	-	33.33
19	*Cymbella hungarica*	-	-	4.73	3.58	-	-	13.74	8.99	-	-	29.87	28.71
20	*Fragilatia ungeriana*	0.13	5.29	2.59	-	0.34	14.64	7.77	-	40.18	32.39	33.40	-
21	*Gomphonema mexicanum*	-	1.39	0.85	0.16	-	5.10	2.25	0.73	-	27.27	26.99	26.19
22	*Melosira juergensii*	-	-	5.13	6.39	-	-	20.30	44.60	-	-	26.00	23.50
23	*Navicula grimii*	-	-	1.42	2.98	-	-	3.78	12.29	-	-	38.00	50.00

Table 4.18 Seasonal variation of algal density, abundance and frequency in Karimganj Road

	KARIMGANJ ROAD	**Density (ind $\times 10^2 cm^{-2}$)**				**Abundance (ind $\times 10^2 cm^{-2}$)**				**Frequency (%)**			
SI.No.	Algal species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Anabaena beckii*			3.49				6.99				45.83	
2	*Aphanothece microscopica*	2.64	1.45	-	4.17	5.49	3.39	-	10.23	49.92	44.33	-	41.42
3	*Arthospira plantensis*	10.74	-	3.23	-	10.58	-	3.87	-	67.00	-	70.28	-
4	*Cylindrospermum musicola*	8.33	2.98	-	6.31	12.48	6.22	-	11.22	74.92	50.86	-	58.13
5	*Lyngbya cinerescens*	-	3.28	3.22	7.24	-	7.15	6.67	9.68	-	38.67	69.42	69.47

6	*Oscillatoria chlorina*	5.18	-	2.24	-	10.70	-	6.20		50.00	-	77.67	-
7	*Oscillatoria earlei*	4.76	1.41	-	5.89	7.14	3.19	-	7.66	67.00	66.50	-	75.25
8	*Oscillatoria formosa*	3.24	1.66	-	-	4.85	3.29			66.67	49.75		-
9	*Oscillatoria limosa*	-	-	-	10.47		-	-	15.87	-	-	-	50.00
10	*Oscillatoria rubescens*	7.82	-	2.63	9.96	8.46	-	7.12	13.75	340.96	-	41.50	69.67
11	*Oscillatoria subbrevis*	10.20	-	-	2.55	13.31	-	-	7.65	79.38	-	-	33.00
12	*Oscillatoria terebiformis*	12.03	5.55	9.42	3.33	14.76	6.28	13.48	8.60	83.50	83.00	83.42	41.25
13	*Spirullina major*	11.39	1.16	-	-	5.83	2.13			75.13	41.50		-
	Chlorophyceae												
14	*Chlorella ellipsoidea*	3.16	5.60	-	-	9.83	22.02	-	-	36.01	27.27	-	-
15	*Cladophora pellucida*	-	-	1.72	12.00	-	-	4.58	12.00	-	-	50.00	100.00
16	*Mougeotia* sp	10.61	-	1.79	4.29	19.82	-	3.91	7.28	66.50		56.79	50.00
17	*Oedogonium sp*	-	1.69	-	6.84		4.26		9.98		47.17		63.92
18	*Pediastrum tetras*	10.31	-	-	5.44	15.36	-	-	9.03	71.13	-	-	55.50
19	*Spirogyra crassa*	7.33	-	6.06		10.70		10.15		63.92		70.75	
20	*Zygnema sp*	3.82	2.36			5.73	4.92			67.00	50.00		
	Bacillariophyceae												
21	*Amphora normani*	-	0.88	0.68	-	-	2.65	2.89			31.72	28.41	
22	*Amphora ovlis*	-	-	1.63	1.11	-		4.62	3.87	-	-	29.49	27.73
23	*Cymbella* sp.	-	-	2.78	-	-	-	8.53	-	-	-	29.38	-
24	*Fragilatia* sp.	-	0.87	1.76	-	-	4.16	4.79	-	-	20.21	36.93	-
25	*Gomphonema mexicanum*	-	0.58	1.57	-	-	1.86	8.10	-	-	32.39	27.84	-
26	*Eunotia* sp.	0.39	1.25	2.06	0.81	1.21	4.70	6.99	2.62	40.00	32.28	35.02	29.35

Table 4.19 Seasonal variation of algal density, abundance and frequency in Koombhirgram Road

	KOOMBHIRGRAM ROAD	**Density (ind × $10^2 cm^{-2}$)**				**Abundance (ind × $10^2 cm^{-2}$)**				**Frequency (%)**			
SI.No.	Algal species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Arthospira platensis*	1.39	-	2.05	9.34	11.78	-	3.07	9.34	33.33	-	66.67	100.00
2	*Cylindrospermum muscicola*	-	6.67	-	10.75	-	1.01	-	10.75	-	66.67	-	100.00
3	*Hydrocoleum* sp.	4.08	-	-	-	12.25	-	-	-	33.33	-	-	-
4	*Lyngbya nordgardhii*	-	6.22	-	2.96		6.64	-	8.88		83.50		33.33
5	*Nostoc muscorum*	3.32	13.94	1.07	8.44	4.99	20.91	3.22	1.34	66.67	66.67	33.33	33.33
6	*Nostoc carneum*	-	-	-	0.75	-	-	-	2.25	-	-	-	33.33
7	*Nostoc punciformae*	16.21		1.02		16.20		1.02		100.00		100.00	
8	*Oscillatoria laetevirens* v. *minimus*	4.51	-	-	-	4.53	-	-	-	33.33	-	-	-
9	*Oscillatoria ornate*	-	-	3.80	-	-	-	3.38	-	-	-	100.00	-
10	*Oscillatoria amphibia*	6.18	-	-	7.43	1.78	-	-	11.15	66.67	-	-	66.67
11	*Oscillatoria peronata*	-	1.64	1.34	11.99	-	4.94	9.34	11.99	-	33.00	100.00	100.00
12	*Oscillatoria splendida*	12.40	-	1.06	-	8.45	-	6.06		100.00		100.00	
13	*Oscillatoria terebiformis*	8.66	9.34	-	7.47	12.99	9.34	-	22.42	66.67	100.00		33.33
14	*Spirullina platensis*	2.84	-		12.37	5.54	-		12.37	33.33	-	-	100.00
	Chlorophyceae												
15	*Chlorella ellipsoidea*	13.82	-	-	-	11.48	-	-	-	33.33	-	-	-
16	*Cladophora pellucida*	-	1.06	-	3.18	-	1.59	-	22.77	-	66.67	-	66.67
17	*Hyalotheca* sp.	11.73	-	15.18	21.15	5.19	-	22.77	21.15	33.33	-	66.67	100.00
18	*Scenedesmus denticulatus*	0.34	-	0.24	-	1.03	-	0.72	-	33.33	-	33.33	-
19	*Stigeoclonium tenue*	7.94	3.94	-	-	23.84	5.91			33.33	66.67		

20	*Zygnema sp.*	-	-	7.15	10.38	-	-	21.15	10.38	-	-	100.00	100.00
21	**Bacillariophyceae**												
22	*Amphora ovlis*	7.33	-	-	4.20	0.99	-	-	12.62	33.33	-	-	33.33
23	*Cymbella hungarica*	1.55	0.38	-	-	6.83	4.16			66.67	33.33	-	-
24	*Fragilatia* sp.	0.58	0.30	-	-	1.15	0.90	-	-	50.00	33.33	-	-
25	*Gomphonema mexicanum*	3.32	-	-	0.67	4.99	-	-	2.03	66.67	-	-	33.33
26	*Synedra tabulata*	-	-	3.38	1.70	-	-	10.38	5.11	-	-	100.00	33.33

Table 4.20 Seasonal variation of algal density, abundance and frequency in Ambikapatty

	AMBIKAPATTY	**Density (ind × $10^2 cm^{-2}$)**				**Abundance (ind × $10^2 cm^{-2}$)**				**Frequency (%)**			
SI.No.	Algal species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Arthospira plantensis*	8.66	9.34	-	7.47	12.99	9.34	-	22.42	66.67	100.00	-	33.33
2	*Calothrix parietana*	12.40	-	1.06	-	8.45	-	6.06	-	100.00	-	100.00	-
3	*Cylindrospermum musicola*	4.08	-	-	-	12.25	-	-	-	33.33	-	-	-
4	*Lyngbya nordgardhii*	-	-	3.80	-	-	-	3.38	-	-	-	100.00	-
5	*Nostoc muscorum*	1.39	-	2.05	9.34	11.78	-	3.07	9.34	33.33		66.67	100.00
6	*Nostoc carneum*	2.84	-	-	12.37	5.54	-		12.37	33.33			100.00
7	*Nostoc punciformae*	-	6.22	-	2.96	-	6.64	-	8.88	-	83.50	-	33.33
8	*Oscillatoria geitleriana*	6.18	-	-	7.43	1.78	-	-	11.15	66.67	-	-	66.67
9	*Oscillatoria princeps*	-	1.64	1.34	11.99	-	4.94	9.34	11.99	-	33.00	100.00	100.00
10	*Spirullina major*	4.51	-	-	-	4.53	-	-	-	33.33	-	-	-
	Chlorophyceae			-				-	-		-	-	-
11	*Chlorella ellipsoidea*	0.34		0.24		1.03		0.72	-	33.33		33.33	-
12	*Hyalotheca burmensis*	-	-	-		-	-	-		-	-	-	-
13	*Spirogyra crassa*	3.32	13.94	1.07	8.44	4.99	20.91	3.22	1.34	66.67	66.67	33.33	33.33
14	*Spirogyra punctiformis*	16.21		1.02		16.20		1.02		100.00		100.00	
15	*Stigeoclonium tenue*	-	6.67	-	10.75	-	1.01	-	10.75	-	66.67	-	100.00
16	*Zygnema sp.*	-	1.06	-	3.18	-	1.59	-	22.77	-	66.67		66.67
	Bacillariophyceae								-				-
17	*Amphora ovlis* v. *pediculus*	7.94	3.94	-	-	23.84	5.91	-	-	33.33	66.67		-
18	*Cymbella hungarica* v. *signata*		-	7.15	10.38		-	21.15	10.38		-	100.00	100.00

19	*Fragilaria ungeriana*	13.82	-			11.48	-			33.33	-		
20	*Gomphonema mexicanum*		-	3.38	1.70		-	10.38	5.11		-	100.00	33.33
21	*Gomphonema* sp.	7.33			4.20	0.99			12.62	33.33	-	-	33.33
22	*Pinnularia lonavlensis*	1.55	0.38	-	-	6.83	4.16	-	-	66.67	33.33	-	-
23	*Pinnularia lundi*												
24	*Synedra tabulata*	11.73	-	15.18	21.15	5.19	-	22.77	21.15	33.33	-	66.67	100.00

Table 4.21 Seasonal variation of algal density, abundance and frequency in Premtola

	PREMTOLA	**Density (ind × $10^2 cm^{-2}$)**				**Abundance (ind ×$10^2 cm^{-2}$)**				**Frequency (%)**			
Sl.No	Algal Species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Arthospira plantensis*	5.30	1.27	3.57	2.81	12.00	6.04	10.25	5.44	46.31	20.04	27.40	50.00
2	*Calothrix fusca*	-	-	10.12	-	-		37.28	-	-	-	35.48	-
3	*Calothrix marchica*	4.27	10.16		3.71	8.75	37.50		10.45	47.50	26.42	-	40.42
4	*Calothrix parietana*		1.82	1.38	-	-	4.88	4.73	-	-	42.79	32.58	-
5	*Chroococcus* sp.		3.16	5.60	-	-	9.83	22.02	-	-	36.01	27.27	-
6	*Cylindrospermum* sp.	0.39	1.25	2.06	0.81	1.21	4.70	6.99	2.62	40.00	32.28	35.02	29.35
7	*Lyngbya cinerescens*	2.74	5.45	9.76	9.14	5.63	19.01	29.07	31.12	56.80	35.86	31.06	43.09
8	*Nostoc carneum*	1.67	8.67	2.98	-	4.11	16.97	7.71	-	43.39	41.54	37.12	-
9	*Oscillatoria chalybea*	0.90	8.16	3.02		4.54	17.57	12.22		20.00	43.23	23.27	-
10	*Oscillatoria peronata*		7.01	8.84	-	-	16.79	24.22	-	-	41.33	37.36	-
11	*Oscillatoria rubescens*	-	12.56	19.61	4.96	-	31.70	37.16	8.16	-	43.16	55.09	58.51
13	*Phormidium lucidum*	7.	22.04	28.47	12.26	14.14	48.00	92.15	25.16	54.38	50.42	32.53	49.09
12	*Phormidiumautumnale*	-	-	3.44	2.84	-	-	8.34	8.34	-	-	40.34	37.22
14	*Spirulina platensis*	2.55	0.98	3.41	3.42	5.32	6.06	22.80	10.01	44.12	17.77	28.03	36.09
15	*Westiellopsis* sp.	0.58	1.57	-	-	1.86	8.10			32.39	27.84		-
	Chlorophyceae												
17	*Chlorella ellipsoidea*	-	-	2.78		-	-	8.53	-	-	-	29.38	-
16	*Pithophora kewensis*	-	-	1.63	1.11	-	-	4.62	3.87	-	-	29.49	27.73
18	*Spirogyra punctiformis*		0.87	1.76	-	-	4.16	4.79			20.21	36.93	-
	Bacillariophyceae												
19	*Anomoeoneis brachysira v. thermalis*	0.28	1.90	-	2.19	0.73	5.69	-	8.95	38.75	29.21	-	21.59

20	*Gomphonema* sp.	-	-	3.38	1.70	-	-	10.38	5.11	-	-	100.00	33.33
21	*Hantschia amphioxys*	7.33	-	-	4.20	0.99	-	-	12.62	33.33	-	-	-
22	*Mastogloia recta*	1.55	0.38	-	-	6.83	4.16	-	-	66.67	33.33	-	-

Table 4.22 Seasonal variation of algal density, abundance and frequency in ONGC Colony

	ONGC COLONY	**Density (ind×10²cm⁻²)**				**Abundance (ind× 10²cm⁻²)**				**Frequency (%)**			
SI.No.	Algal species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Anabaena doliolum*	1.39	-	2.05	9.34	11.78	-	3.07	9.34	33.33	-	66.67	100.00
2	*Anabaena spiroides*	-	6.22	-	2.96		6.64		8.88		83.50		33.33
3	*Calothrix parietana*	-	-	3.80	-	-	-	3.38	-	-	-	100.00	-
4	*Calothrix fusca*	2.84	-	-	12.37	5.54	-	-	12.37	33.33	-	-	100.00
5	*Cylindrospermum musicola*	4.08	-	-	-	12.25				33.33			
6	*Oscillatoria curviceps*	6.18	-	-	7.43	1.78	-	-	11.15	66.67	-	-	66.67
7	*Oscillatoria formosa*	-	1.64	1.34	11.99	-	4.94	9.34	11.99	-	33.00	100.00	100.00
8	*Oscillatoria raoi*	8.66	9.34	-	7.47	12.99	9.34		22.42	66.67	100.00	-	33.33
9	*Phormidium calcicola*	4.51	-	-	-	4.53	-	-	-	33.33	-	-	-
10	*Phormidium lucidum*	12.40	-	1.06	-	8.45	-	6.06	-	100.00	-	100.00	-
11	*Spirullina major*	3.32	13.94	1.07	8.44	4.99	20.91	3.22	1.34	66.67	66.67	33.33	33.33
	Chlorophyceae												
12	*Endorina sp*	-	-	7.15	10.38	-	-	21.15	10.38	-	-	100.00	100.00
13	*Mougeotia*	0.34	-	0.24		1.03	-	0.72		33.33	-	33.33	
14	*Oedogonium* sp.	11.73	-	15.18	21.15	5.19	-	22.77	21.15	33.33	-	66.67	100.00
15	*Pediastrum tetras*	7.94	3.94			23.84	5.91			33.33	66.67		
16	*Pithophora* sp.	-	6.67	-	10.75	-	1.01	-	10.75	-	66.67	-	100.00
17	*Spirogyra punctiformis*	-	1.06	-	3.18	-	1.59	-	22.77	-	66.67	-	66.67
	Bacillariophyceae												
18	*Eunotia* sp.	2.74	5.45	9.76	9.14	5.63	19.01	29.07	31.12	56.80	35.86	31.06	43.09

20	*Gomphonema mexicanum*	-	-	3.38	1.70	-	-	10.38	5.11	-	-	100.00	33.33
21	*Gomphonema* sp.	7.33			4.20	0.99			12.62	33.33			33.33
22	*hantschia amphioxys v. densestriata*	8.93	-	-	-	8.93	-	-	-	100.00	-	-	-
23	*Navicula cuspidata*	13.82	-	-	-	11.48	-	-	-	33.33	-	-	-
24	*Navicula pahalgarhensis*	16.21	-	1.02	-	16.20	-	1.02	-	100.00	-	100.00	-
25	*Navicula salinarum*	0.38	-	-	-	0.75	-	-	-	50.00	-	-	-
26	*Navicula subrhynchocephala*	22.30	-	5.7	-	22.30	-	17.1	-	100.00	-	33.33	-
27	*Navicula pupula*	8.63	-	-	10.18	8.63	-	-	40.7	100.00	-	-	25
28	*Nitzchia obtuse*	16.10	-	-	9	16.1	-	-	18	100.00	-	-	50
29	*Nitzchia vermicularis*	-	-	-	0.57	-	-	-	1.7	-	-	-	33.33

Table 4.23 Seasonal variation of algal density, abundance and frequency in Ramnagar GC College road

	Ramnagar GC College road	**Density (ind × 10^2cm^{-2})**				**Abundance (ind × 10^2cm^{-2})**				**Frequency (%)**			
SI.No.	Algal species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Anabaena spiroides*	2.20	23.20	9.62	-	4.41	48.32	22.58	-	50.00	45.75	58.50	-
2	*Cylindrospermum musicola*	4.78	3.86	12.43	4.09	7.32	9.39	47.17	9.91	63.58	56.25	58.50	50.00
3	*Nostoc muscorum*	-	38.40		14.46	-	106.52		28.92	-	62.28	-	50.00
4	*Nostoc punciformae*	-	-	22.88	5.94	-		33.52	8.60	-	-	64.25	62.50
5	*Oscillatoria curviceps*	3.75	11.18	22.52	2.47	-	25.89	46.02	4.94	-	56.50	44.62	50.00
6	*Oscillatoria earlei*	5.30	1.27	3.57	2.81	12.00	6.04	10.25	5.44	46.31	20.04	27.40	50.00
7	*Oscillatoria geitleriana*	-	-	10.29	2.01	-	-	18.53	4.00	-	-	50.00	50.00
8	*Oscillatoria ornate* var. *crassa*	2.72	5.56	47.70	-	3.50	22.23	82.98		56.25	33.00	40.33	-
9	*Oscillatoria sancta*	-	43.38	13.66	-	-	152.53	22.08	-	-	46.22	50.00	-
10	*Westiellopsis prolifica*	-	31.71	37.54	6.21	-	62.66	20.53	10.39	-	54.12	50.00	60.42
	Chlorophyceae												
11	*Mougeotia*	-	14.62	41.36	15.75	-	34.33	92.16	58.26	-	49.25	47.33	44.44
12	*Oedogonium sp*	0.82	0.83	0.93	-	1.45	2.37	3.49	-	59.72	51.33	37.50	-
13	*Pediastrum integrum*	-	0.85	1.77	-		1.73	4.43	-		54.33	34.38	-
14	*Spirogyra punctiformis*		4.03	13.54	1.81		7.60	22.46	4.97		66.88	50.06	50.00
	Bacillariophyceae												
15	*Anomoeoneis brachysira v. thermalis*	-	0.88	0.68	-	-	2.65	2.89	-	-	31.72	28.41	-
16	*Cyclotella meneghiniana*	0.39	1.25	2.06	0.81	1.21	4.70	6.99	2.62	40.00	32.28	35.02	29.35
17	*Eunotia* sp.	0.47	1.79	5.28	0.96	0.86	3.43	9.69	1.85	53.33	64.67	52.83	48.61
18	*Gomphonema mexicanum*	-	-	2.18	4.55	-	-	4.56	14.27	-	-	45.83	41.67

19	*Gomphonema sp.*	-	2.27	1.80	-	-	6.77	4.69	-	-	56.25	34.38	-
20	*Hantschia amphioxys v. densestriata*	2.55	0.98	3.41	3.42	5.32	6.06	22.80	10.01	44.12	17.77	28.03	36.09
21	*Mastogloia recta*	-	3.16	5.60	-	-	9.83	22.02	-	-	36.01	27.27	-
22	*Melosira juergensii*	-	1.82	1.38	-	-	4.88	4.73	-	-	42.79	32.58	-
23	*Pinnularia eburnea*	0.88	-	-	0.38	1.75	-	-	1.15	50	-	-	33.33
24	*Synedra tabulata*	0.77	-	-	-	2.3	-	-	-	33.33	-	-	-
25	*Synedra acus*	0.98	-	-	0.25	1.15	-	-	0.98	50	-	-	25

Table 4.24 Seasonal variation of algal density, abundance and frequency in National Highway Road

	National Highway Road	**Density (ind × $10^2 cm^{-2}$)**				**Abundance (ind × $10^2 cm^{-2}$)**				**Frequency (%)**			
SI.No.	Algal species	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**	**PM**	**M**	**PO**	**W**
	Cyanophyceae												
1	*Anabaena spiroides*	10.75	2.43	-	5.85	10.75	3.46	-	6.02	100.00	72.22	-	25
2	*Chroococcus sp*	15.47	-	-	-	7.21	-	-	-	62.38	-	-	25.00
3	*Calothrix linearis*	14.23	1.56	-	-	6.32	2.45	-	-	33.33	50.00	-	-
4	*Lyngbya diguetii*	22.30	-	-	5.70	22.30	-	-	-	100.00	-	-	33.33
5	*Lyngbya nordgardhii*	5.77	2.54	-	9.18	9.25	5.31	-	2.58	58.25	50.00	-	66.67
6	*Oscillatoria acuminata*	14.22	-	-	5.94	16.94	-	-	7.05	66.75	-	-	-
7	*Oscillatoria limnetica*	-	-	3.72	13.82	-	-	5.75	6.63	-	-	83.38	-
8	*Oscillatoria salina*	9.42	2.85	4.94	5.17	10.11	2.85	5.80	13.11	79.17	100.00	83.50	25.00
9	*Oscillatoria subbrevis*	10.03	-	1.66	3.83	11.85	-	2.49	5.02	58.25	-	67.00	75.00
10	*Spirullina platensis*	0.80	-	-	-	3.04	-	-	-	50.00	-	-	33.33
11	*Spirullina tenuissima*	-	-	2.10	-	-	-	3.15	-	-	-	67.00	25.00
12	*Westiellopsis prolifica*	7.80	46.80	6.72	10.69	15.60	46.80	10.08	21.38	50.00	100.00	66.67	50.00
	Chlorophyceae												
13	*Hyalotheca burmensis*	11.07	3.05	5.34		14.60	6.76	8.26		66.67	54.13	64.92	
14	*Mougeotia* sp.	5.19	-	-	-	8.34	-	-	-	58.25	-	-	-
15	*Oedogonium* sp.	-	0.54	3.56	5.58		9.31	9.12	6.66		58.25	44.33	-
16	*Pediastrum integrum*	14.63	-	-	3.59	6.98	-	-	4.68	58.25	-	-	-
17	*Spirogyra crassa*	1.49	-	0.10	0.57	10.02	-	4.08	4.18	67.64	-	54.13	75.00
18	*Zygnema* sp.	19.28	-	2.79	10.75	9.76	-	8.37	15.07	91.75	-	33.00	25
	Bacillariophyceae												
19	*Cyclotella meneghiniana*	1.71	-	3.00	-	5.12	-	5.46	-	33.00	-	66.67	-
20	*Mastogloia recta*	1.55	0.38	-	-	6.83	4.16	-	-	66.67	33.33	-	-

21	*Navicula grimii*	4.21	2.89	4.25	3.88	12.63	4.37	6.38	6.65	33.00	75.13	67.00	-
22	*Navicula subdapaliformis*	-	2.27	1.80	-		6.77	4.69	-	-	56.25	34.38	-
23	*Navicula halophile*	16.13	-	-	-	16.13	-	-	-	100	-	-	-
24	*Navicula pupula*	-	2.31	2.57	-	-	4.62	3.43	-	-	67.00	75.00	-
25	*Navicula viridula*	4.86	0.99	-	2.22	13.52	2.96	-	6.66	41.50	33.00	-	55
26	*Nitzschia apiculata*	7.33	-	-	4.20	0.99	-	-	12.62	33.33	-	-	33.33
27	*Nitzschia palea*			-	-			-	-			-	-
28	*Nitzschia obtuse*	0.28	1.90		2.19	0.73	5.69	-	8.95	38.75	29.21	-	21.59
29	*Nitzschia heufleriana*		0.58	1.57			1.86	8.10			32.39	27.84	
30	*Pinnularia lundi*	0.74	-	-	-	1.13	-	-	-	25.00	-	-	50
31	*Pinnularia lonavlensis*	3.32	-	-	0.67	4.99	-	-	2.03	66.67	-	-	33.33
32	*Pinnularia eburnea*	8.73	-	-	-	17.45	-	-	-	50	-	-	-
33	*Synedra tabulata*	7.80	-	-	-	15.69	-	-	-	50	-	-	-

Fig. 4.45: Relative abundance (%) of different algal groups at the study sites (S1-S10) in different seasons

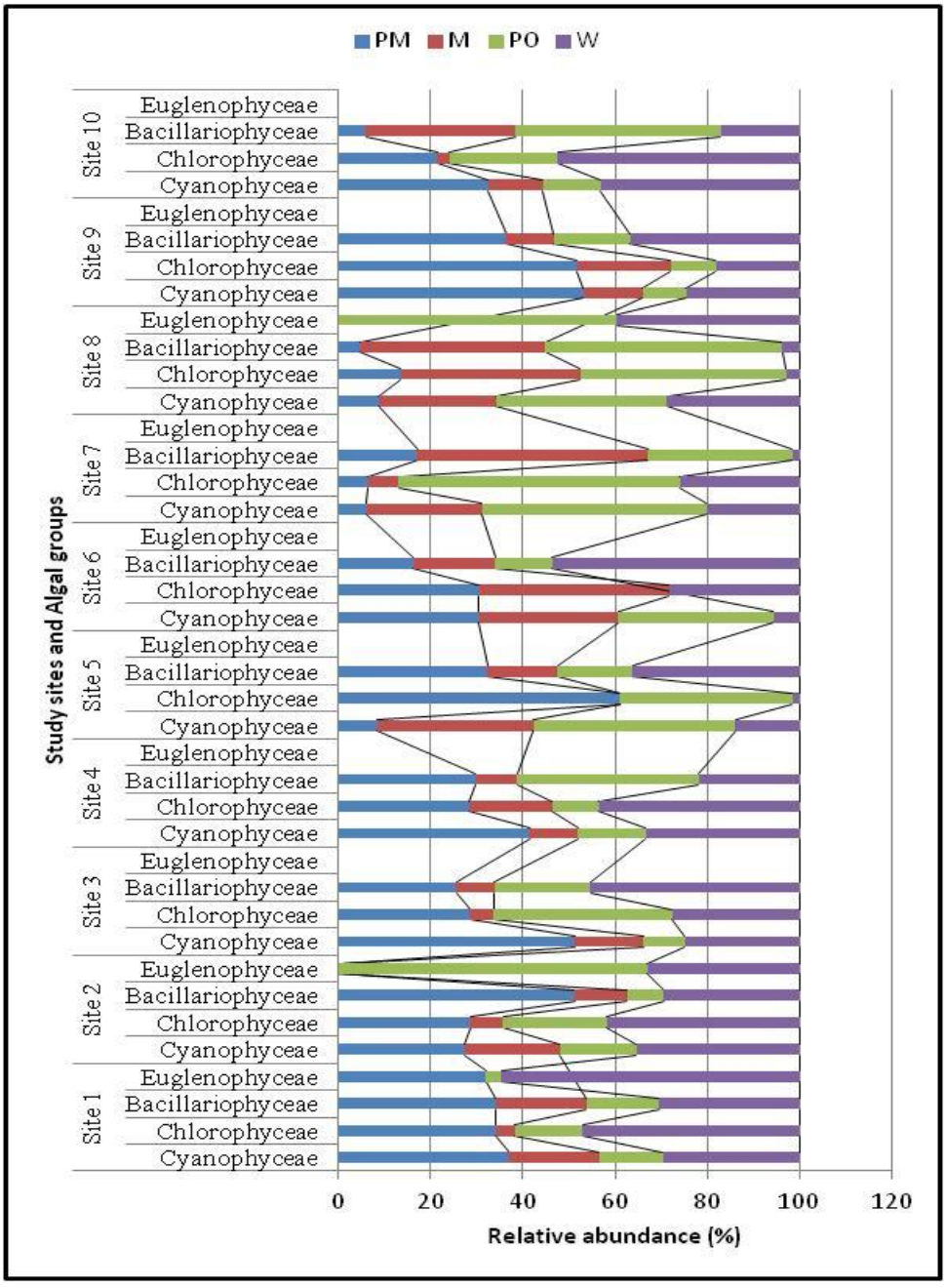

Fig. **4.46**: Relative abundance (%) of different algal groups at the study sites (S11-S20) in different seasons

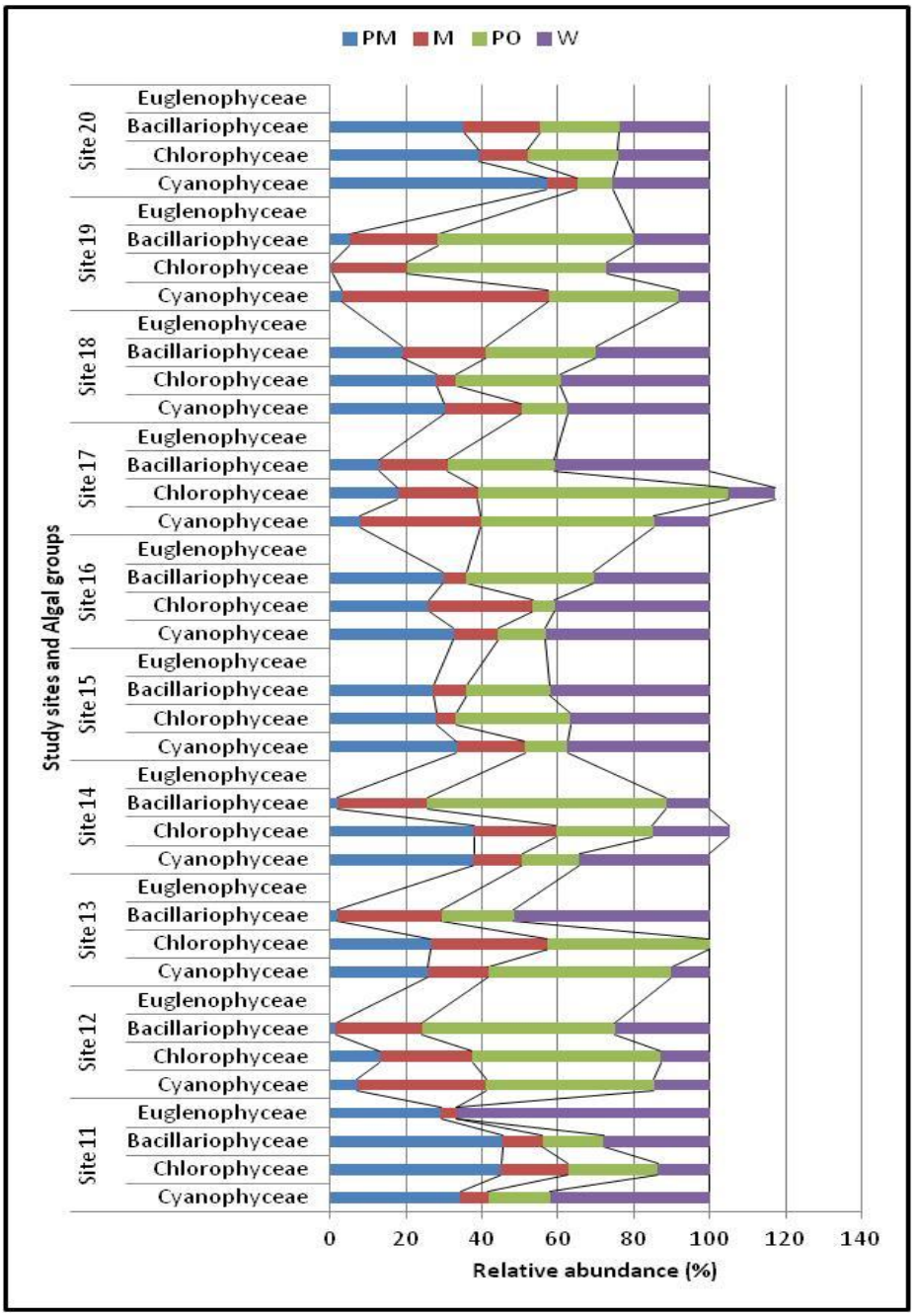

Fig. **4.47:** Relative abundance of Cyanophyceae at different study site in different seasons

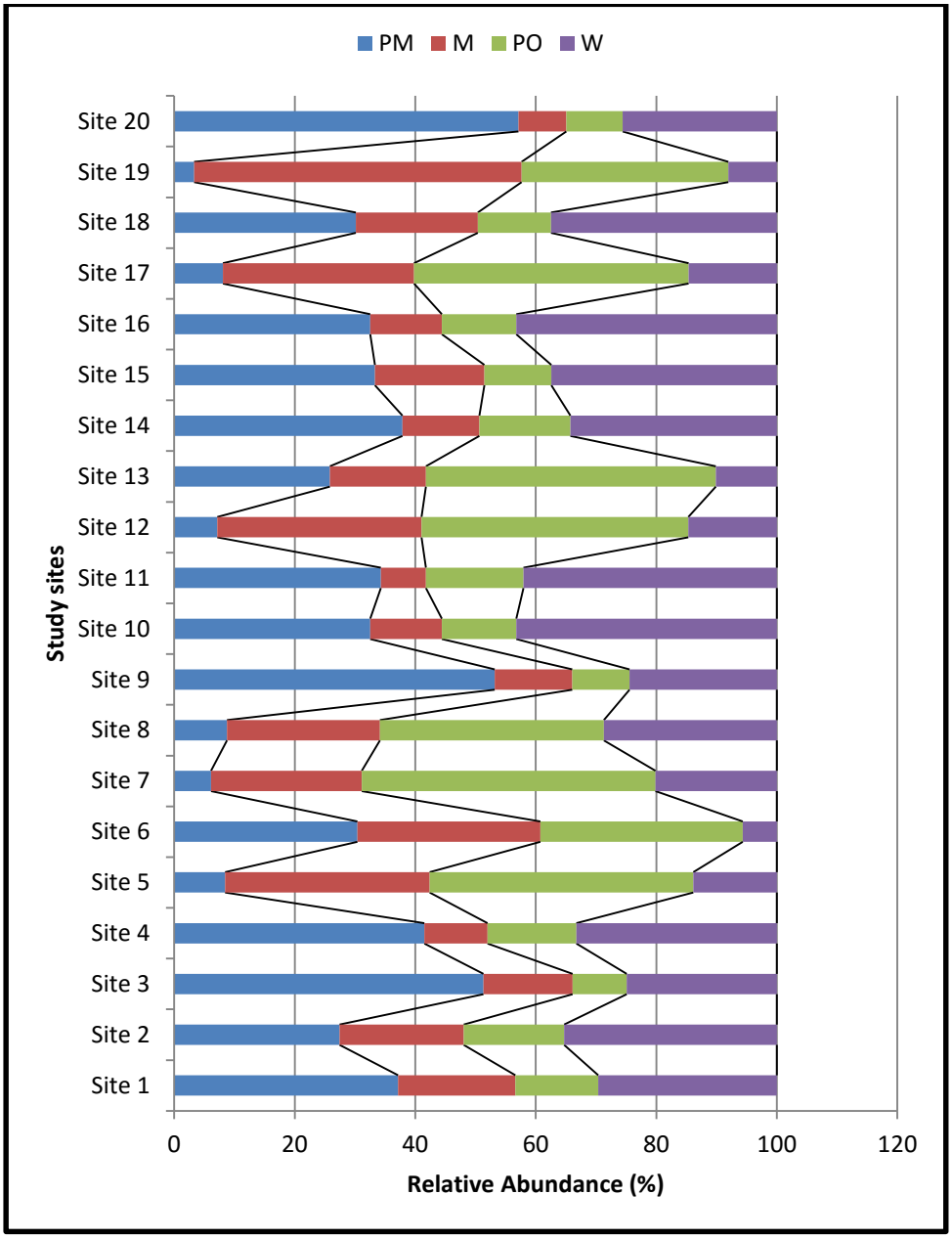

Fig 4.48: Relative abundance of Chlorophyceae at different study site in different seasons

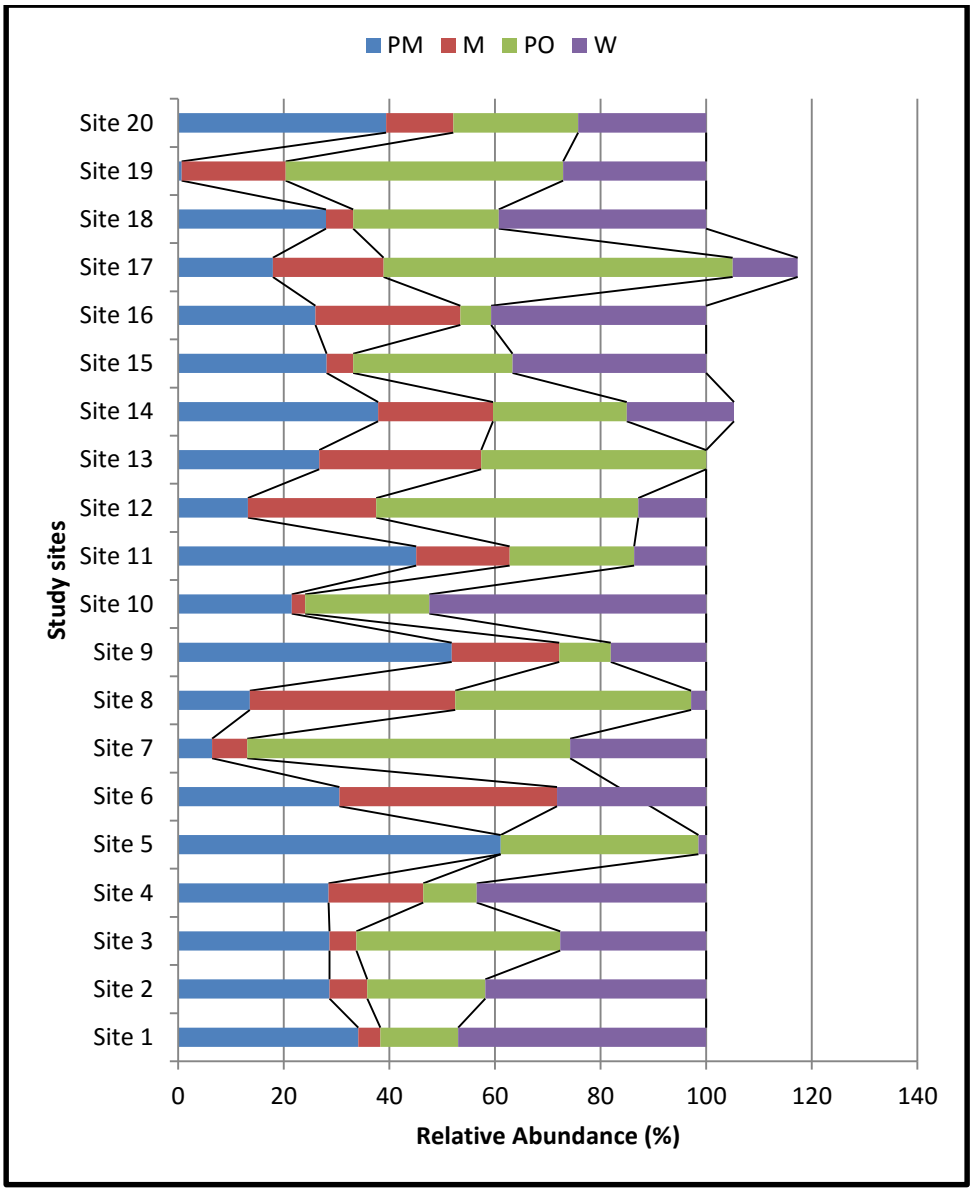

Fig 4.49: Relative abundance of Bacillariophyceae at different study site in different seasons

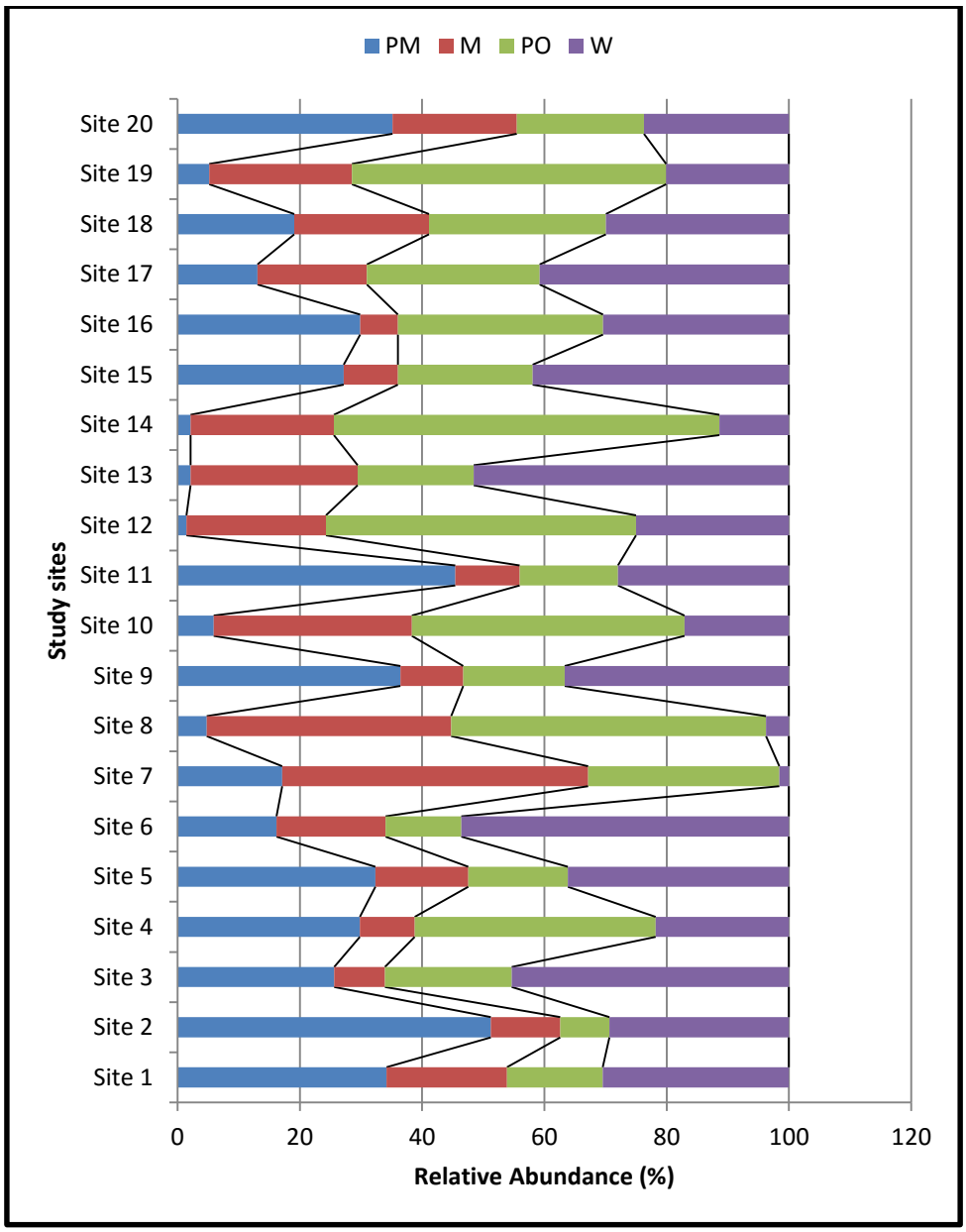

Fig 4.50: Relative abundance of Euglanophyceae at different study site in different seasons

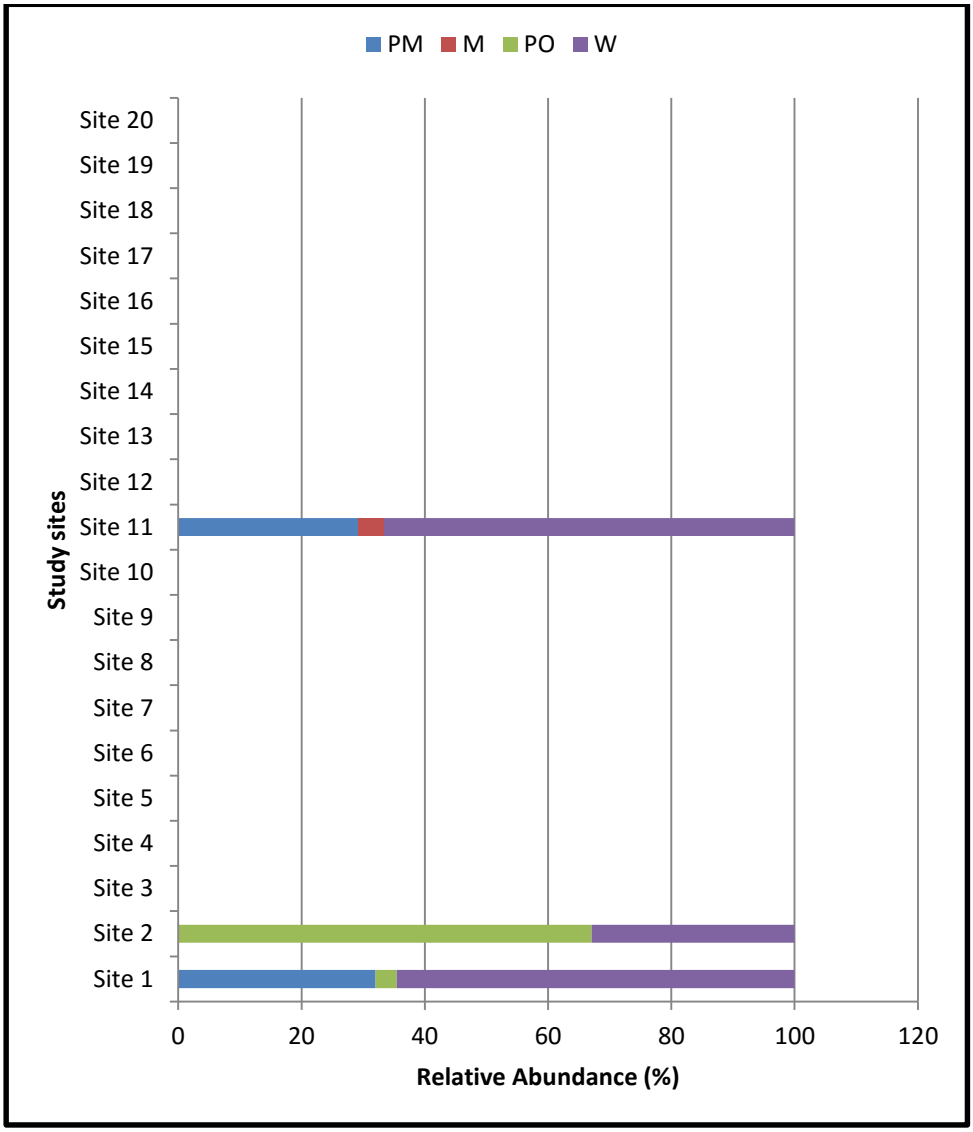

Table 4.26: Variation of Shannon-Wiener Diversity Index (H) at the study sites

Diversity index	Sites	Pre monsoon	Monsoon	Post monsoon	Winter
Shannon-Wiener Diversity Index (H)	**Site 1**	2.81±0.15	2.39±0.07	2.38±0.03	2.41±0.24
	Site 2	1.72±0.24	1.43±0.11	1.44±0.12	1.49±0.44
	Site 3	1.63±0.44	2.71±0.34	2.08±0.07	2.29±0.25
	Site 4	1.38±0.41	0.95±0.005	2.04±0.06	1.39±0.34
	Site 5	2.66±0.71	2.21±0.13	2.37±0.13	2.21±0.62
	Site 6	2.71±0.16	2.18±0.07	2.32±0.05	2.13±0.20
	Site 7	1.35±0.16	1.18±0.14	1.2±0.19	1.1±0.06
	Site 8	1.74±0.14	1.32±0.19	1.0±0.06	1.52±0.14
	Site 9	2.27±0.03	2.24±0.04	2.13±0.02	2.15±0.03
	Site 10	2.39±0.38	2.35±0.02	2.26±0.34	2.26±0.15
	Site 11	1.55±0.14	2.27±0.19	1.01±0.14	2.14±0.38
	Site 12	2.60±0.11	2.16±0.06	2.27±0.24	2.16±0.10
	Site 13	2.36±0.03	2.11±0.34	2.19±0.33	2.37±0.12
	Site 14	2.44±0.14	2.61±0.11	2.01±0.34	2.11±0.05
	Site 15	1.04±0.26	1.08±0.06	0.98±0.14	1.28±0.03
	Site 16	2.46±0.13	2.18±0.15	1.99±0.20	2.16±0.06
	Site 17	2.56±0.38	2.16±0.10	2.05±0.22	2.25±0.24
	Site 18	2.48±0.14	2.14±0.13	2.16±0.62	2.25±0.14
	Site 19	2.55±0.08	2.02±0.07	2.06±0.16	2.11±0.18
	Site 20	2.17±0.14	2.26±0.03	2.18±0.03	2.14±0.13

Table 4.27: Variation of Simpson's dominance index (D) at the study sites

Diversity index	Sites	Pre monsoon	Monsoon	Post monsoon	Winter
Simpson's dominance index (D)	**Site 1**	0.035±0.14	0.02±0.18	0.039±0.10	0.01±0.14
	Site 2	0.065±0.09	0.051±0.12	0.051±0.10	0.064±0.12
	Site 3	0.076±0.10	0.077±0.34	0.048±0.14	0.06±0.10
	Site 4	0.11±0.04	0.42±0.10	0.16±0.10	0.31±0.005
	Site 5	0.073±0.10	0.11±0.12	0.09±0.12	0.09±0.12
	Site 6	0.098±0.03	0.045±0.07	0.068±0.34	0.046±0.34
	Site 7	0.37±0.03	0.38±0.10	0.40±0.14	0.39±0.10
	Site 8	0.19±0.07	0.28±0.08	0.45±0.10	0.25±0.005
	Site 9	0.11±0.03	0.11±0.10	0.12±0.12	0.12±0.14
	Site 10	0.097±0.07	0.10±0.12	0.10±0.03	0.06±0.12
	Site 11	0.087±0.005	0.066±0.10	0.042±0.12	0.09±0.21
	Site 12	0.081±0.38	0.091±0.14	0.10±0.21	0.06±0.10
	Site 13	0.10±0.16	0.099±0.18	0.07±0.10	0.06±0.18
	Site 14	0.094±0.08	0.077±0.12	0.16±0.12	0.12±0.20
	Site 15	0.54±0.21	0.66±0.12	0.70±0.14	0.67±0.14
	Site 16	0.091±0.04	0.066±0.10	0.16±0.12	0.085±0.10
	Site 17	0.083±0.26	0.066±0.14	0.13±0.14	0.11±0.08
	Site 18	0.089±0.08	0.066±0.10	0.10±0.10	0.11±0.21
	Site 19	0.087±0.13	0.091±0.08	0.11±0.18	0.10±0.18
	Site 20	0.12±0.20	0.091±0.10	0.083±0.14	0.07±0.01

Table 4.28: Variation of Pielou's evenness index (J) at the study sites

Diversity index	Sites	Pre monsoon	Monsoon	Post monsoon	Winter
Pielou's evenness index (J)	**Site 1**	0.98±0.01	0.92±0.05	0.89±0.01	0.93±0.13
	Site 2	0.72±0.34	0.87±0.56	0.86±0.05	0.93±0.45
	Site 3	0.82±0.10	0.84±0.13	0.75±0.01	0.86±0.11
	Site 4	0.88±0.14	0.61±0.34	0.86±0.04	0.78±0.01
	Site 5	0.94±0.38	0.84±0.01	0.81±0.11	0.90±0.04
	Site 6	0.74±0.38	0.69±0.16	0.59± 0.67	0.46±0.34
	Site 7	0.85±0.22	0.68±0.04	0.71±0.04	0.64±0.11
	Site 8	0.89±0.12	0.90±0.38	0.75±0.62	0.88±0.08
	Site 9	0.94±0.14	0.92±0.05	0.87±0.38	0.91±0.34
	Site 10	0.86±0.03	0.94±0.10	0.75±0.08	0.90±0.10
	Site 11	0.84±0.38	0.93±0.14	0.67±0.10	0.55±0.12
	Site 12	0.76±0.07	0.86±0.01	0.72±0.44	0.65±0.14
	Site 13	0.85±0.10	0.92±0.12	0.67±0.62	0.67±0.34
	Site 14	0.85±0.11	0.91±0.03	0.31±0.04	0.76±0.13
	Site 15	0.72±0.14	0.76±0.07	0.26±0.33	0.66±0.44
	Site 16	0.86±0.07	0.86±0.11	0.70±0.34	0.64±0.08
	Site 17	0.84±0.04	0.76±0.13	0.68±0.41	0.54±0.03
	Site 18	0.76±0.34	0.86±0.38	0.55±0.08	0.44±0.34
	Site 19	0.86±0.11	0.72±0.08	0.64±0.35	0.51±0.38
	Site 20	0.84±0.04	0.66±0.10	0.55±0.03	0.35±0.33

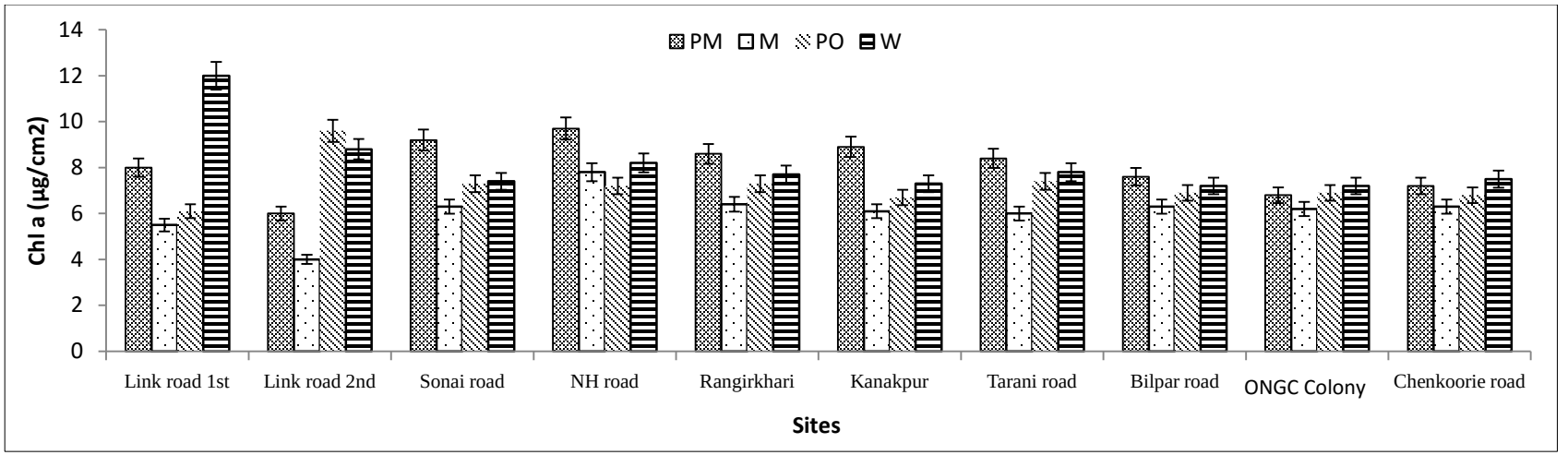

Fig. 4.51: Variation of chlorophyll *a* concentration at the study sites

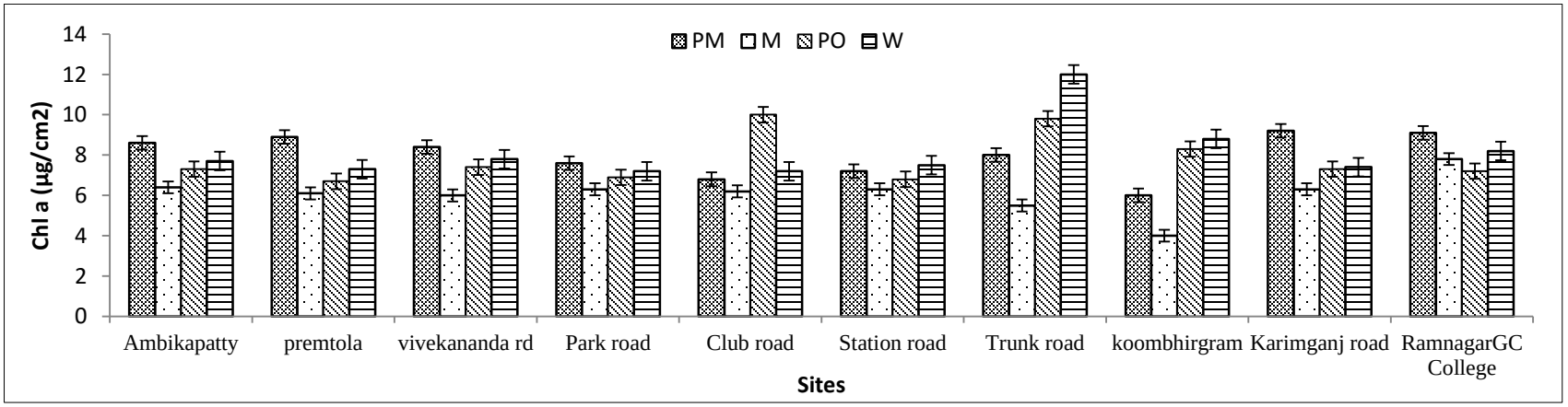

Fig. 4.52: Variation of chlorophyll *a* concentration at the study sites

Table 4.28 Bivariate correlation analysis of the physico-chemical and biological parameters using Pearson correlation coefficients

	Temp	pH	BOD	COD	DO	TK	SS	Cl	SO_4^{2-}	Nitrate	Ca	TDS	$FreeCO_2$	TCS	TCHS	TBS
Temp	1															
pH	**.997****	1														
BOD	.134	**.265***	1													
COD	.204	**-.331****	**.516****	1												
DO	.068	**.768****	.203	**-.261***	1											
TK	-.078	**.997****	**.269***	**-.325****	**.810****	1										
SS	-.064	**.979****	**.369****	**-.293****	**.775****	**.975****	1									
Cl	-.155	-.029	**-.671****	**-.883****	-.011	-.031	-.073	1								
SO_4^{2-}	-.051	**.614****	-.073	**-.551****	**.519****	**.610****	**.615****	**.383****	1							
Nitrate	-.032	**.462****	.051	**-.386****	**.336****	**.455****	**.442****	**.265***	**.362****	1						
Ca	-.134	**.498****	-.141	**-.522****	**.387****	**.495****	**.457****	**.307****	**.245***	**.779****	1					
TDS	**.994****	.099	**.420****	**.532****	.079	.094	.169	**-.575****	-.117	**-.483****	**-.550****	1				
Free_CO2	-.092	**.651****	-.168	**-.684****	**.483****	**.639****	**.638****	**.468****	**.925****	**.527****	**.510****	**-.305****	1			
TCS	.583**	**.942****	**.311****	**-.281***	**.656****	**.923****	**.960****	-.084	**.585****	**.446****	**.440****	.131	**.629****	1		
TCHS	.311**	**-.992****	**-.226***	**.367****	**-.832****	**-.995****	**-.974****	-.012	**-.636****	**-.475****	**-.516****	-.065	**-.670****	**-.930****	1	
TBS	**-.656****	**-.959****	-.170	**.393****	**-.626****	**-.938****	**-.941****	-.045	**-.639****	**-.493****	**-.505****	-.035	**-.704****	**-.972****	**.945****	1

*TCS=Total cyanobacterial species,TCHS=Total chlorophyceae species,TBS=Total Bacillariophyceae

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.30 Component loading scores for PCA with eigenvalue and percentage of variance

PC	Eigen value	% variance
pH	988279	79.567
BOD	102295	10.341
COD	97270.9	17.611
DO	34774.8	12.428
Alkalinity	12087.7	10.185
Free CO_2	5034.96	4.2453
TDS	2549.48	2.1079
SS	579.067	1.113
Chloride	349.368	0.18693
Sulphate	296.164	0.099626
Nitrate	219.636	0.068067
Ca	104.79	0.040907
Cyanophyceae	26.0831	0.0047652
Chlorophyceae	1.45611	0.002021
Bacillariophyceae	0.363537	7.06E-20
Euglenophyceae	0.0498	3.98E-06

Table 4.31 Component loading scores for PCA with the two dimensions

	Axis 1	Axis 2
pH	0.031584	0.47017
BOD	-0.07321	-0.48877
COD	-0.35463	-0.051877
DO	-0.066793	0.42965
Alkalinity	0.41552	0.15855
Free CO_2	0.25366	-0.38808
TDS	-0.25415	0.14976
SS	0.32027	0.018275
Chloride	0.089887	-0.12436
Sulphate	0.42363	0.16211
Nitrate	-0.30481	0.080387
Ca	0.34055	-0.16024
Cyanophyceae	0.15883	-0.050791
Chlorophyceae	0.13711	-0.19721
Bacillariophycea	-0.13711	-0.19721
Euglenophyceae	-0.000372	-0.0056

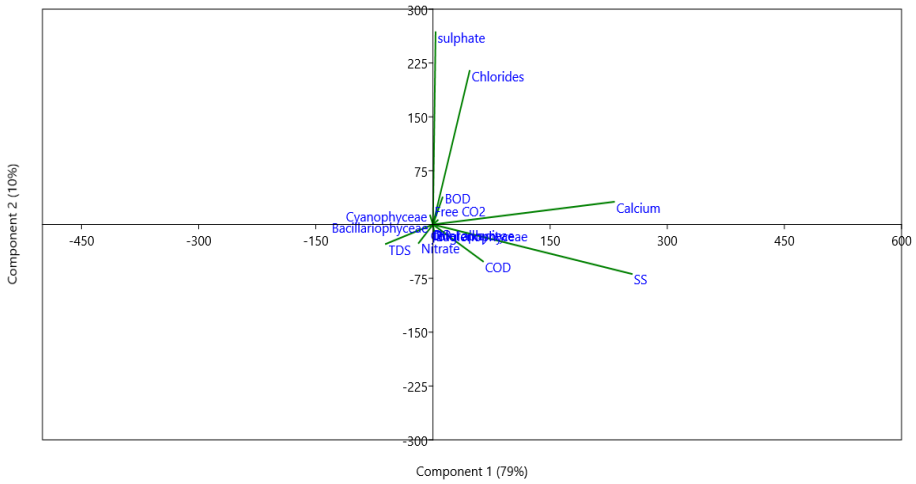

Fig. 4.53: Principal component analysis of water quality and distribution of algal groups from polythene surface in twenty domestic sewage water sites

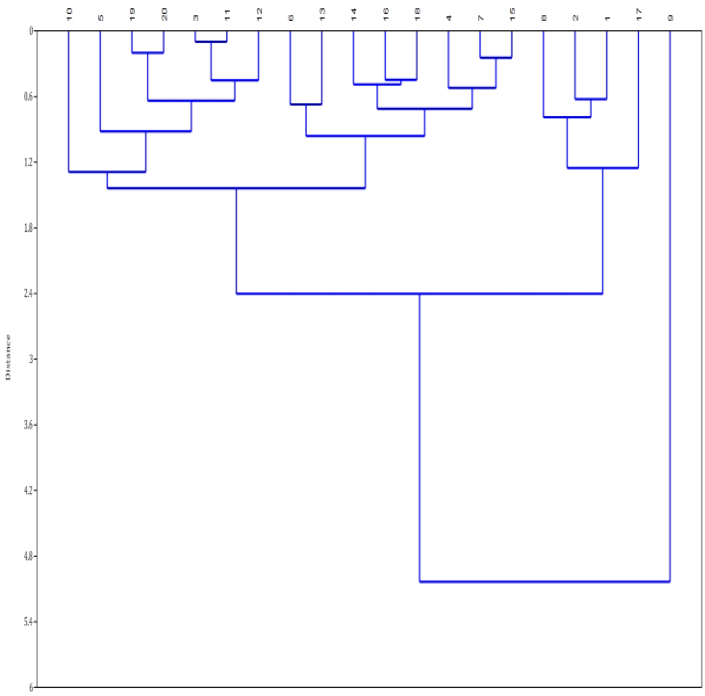

Fig. 4.54: Dendrogram showing the clusters of relative abundance among the selected study sites

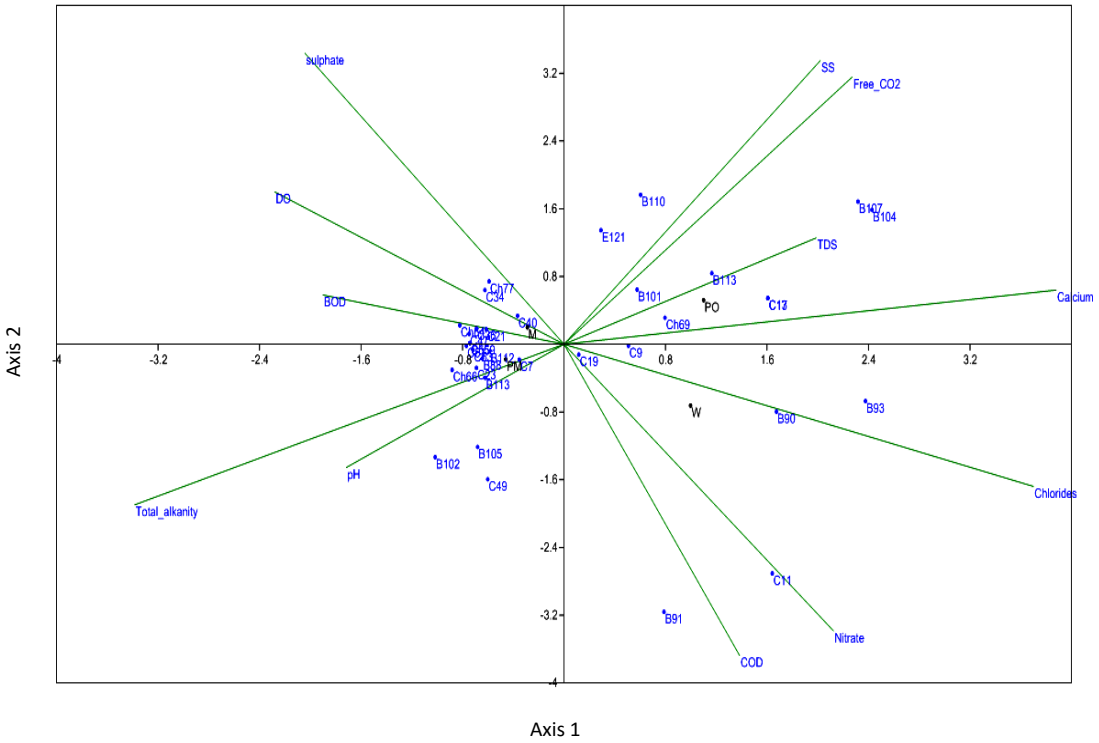

Fig. 4.55: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for Link Road 1st

Fig. 4.55-4.74, abundances of each taxa (blue dots) are indicated with the species code (table 4.3) and physico-chemical parameters of water (green lines) as pH, BOD, COD, DO, total alkalinity, free CO_2, TDS, SS, Chloride, sulphate, nitrate, calcium, ammonia, phosphate and magnesium (black dots) as PM-pre monsoon, M-monsoon, PO-post monsoon, W- winter.

Canonical correspondence analysis is an ecological tool to infer of species-environment relation from the community compositon data and associated habitat characteristics (Ter Braak, 1986).Such canonical correspondence analysis data sets consists of two sets, one is occurrence or abundance of species and other is physio-chemical charateristics of habitats.In canonical correspondence analysis ordination diagram, a particular species with habitat characteristics can

be inferred. Canonical Correspondence Analysis of environmental data and abundance of different species for each site were conducted and the ordination diagrams are presented on **Fig. 4.55- 4.74.** CCA eigenvalues of the first two axes are given on **Table 4.32** and the CCA scores of environmental factors with the first two axes are given on **Table 4.33.** CCA ordination diagrams show that seasonal variations of algal communities were highly influenced by the nutrients like silica, nitrate and phosphate. For Link road 1st, CCA axes 1 and 2 explained 61.43 % and 21.08% of the variance in the species environment biplot (Fig. 4.55). The factors closely correlating with the composition of algal community were pH, total alkalinity, dissolved oxygen, chemical oxygen demand (COD), chlorides and nitrate. *Nostoc linckia* (C19), *Oscillatoria limosa* (C34), *Oscillatoria princeps* (C40), and *Oscillatoria terebiformis* (C49) thrive in water with high phosphate and pH. The taxa that prefer the inhabiting water with high nitrate, pH and low nitrate values were positioned on the right of the ordination plot. The species that have preferred to grow in high nitrate and alkalinity values were positioned on the left. For Link road 2nd, CCA axes 1 and 2 explained 54.48 % and 24% of the variance in the species environment biplot (Fig. 4.56). The factors closely correlating with the composition of algal community were pH, total alkalinity, TDS, sulphate, dissolved oxygen and calcium. *Chrococcus* sp. (C13), *Lyngbya nordgardhii* (C18), *Nostoc punctiformae* (C22) and *Oscillatoria peronata* (C39) thrive in water with low dissolved oxygen and pH. For Sonai Road, CCA axes 1 and 2 explained 49.83 % and 47% of the variance in the species environment biplot (Fig. 4.57).The factors closely correlating with the composition of algal community were dissolved oxygen, calcium and free CO_2. *Lyngbya nordgardhii* (C18), *Oscillatoria limnetica* (C33), *Oscillatoria subbrevis* (C47) and *Oscillatoria vizagapatensis* (C50) thrive in water with lowest DO, highest nitrate and calcium. For Kanakpur road, CCA axes 1 and 2 explained 71.99 % and 17.44% of the variance in the species environment biplot (Fig. 4.58). The factors closely correlating with the composition of algal community were nitrate, pH and BOD. *Anabaena variabilis* (C2), and *Aphanothece naegelii* (C6) are distributed in high nitrate and pH condition in domestic sewage water drains. For Tarani road, CCA axes 1 and 2 explained 50.64 % and 30.54% of the variance in the species environment biplot (Fig. 4.59). pH and BOD influecened the growth of *Lyngbya diguetii* (C17) and *Nostoc linckia* (C19). For Chenkoorie road, CCA axes 1 and 2 explained 43.82 % and 33.29 % of the variance in the

species environment biplot (Fig. 4.60). *Oscillatoria princeps* (C40) and *Oscillatoria sancta* (C45) are present in this environment. For Bilpar road, CCA axes 1 and 2 explained 50.63 % and 35.07% of the variance in the species environment biplot (Fig. 4.61).

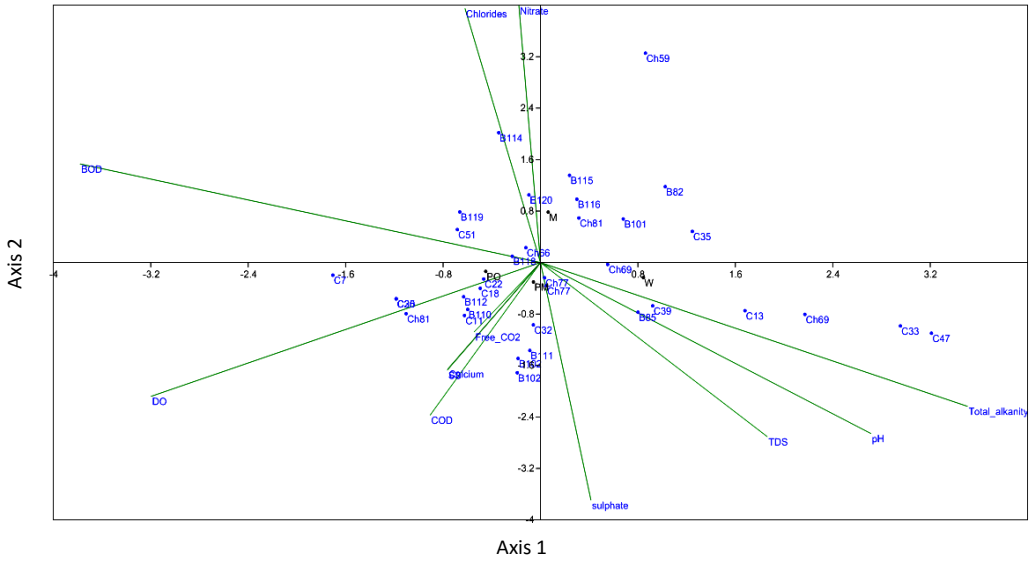

Fig. 4.56: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for Link Road 2nd

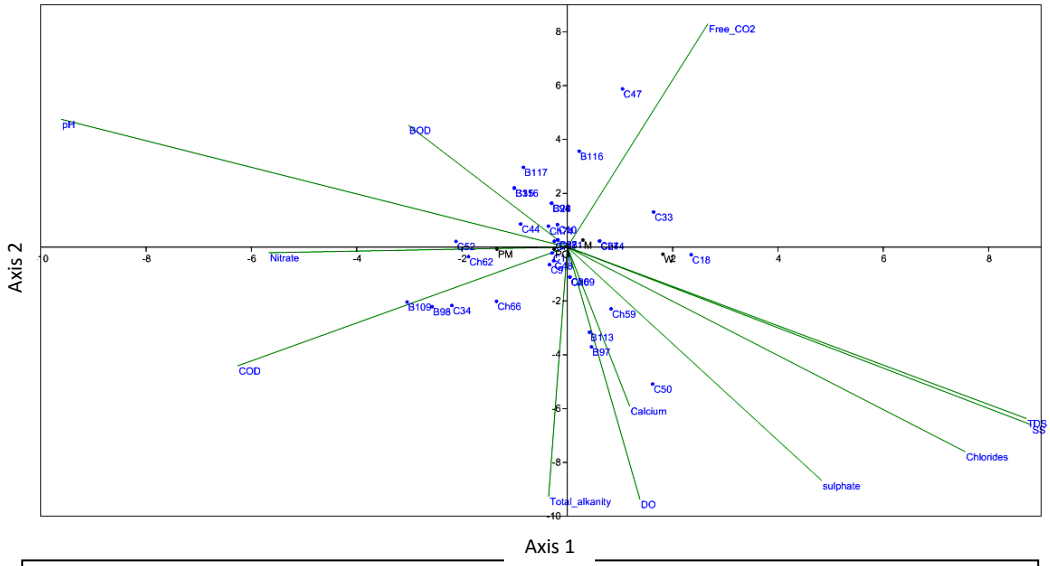

Fig. 4.57: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for Sonai Road

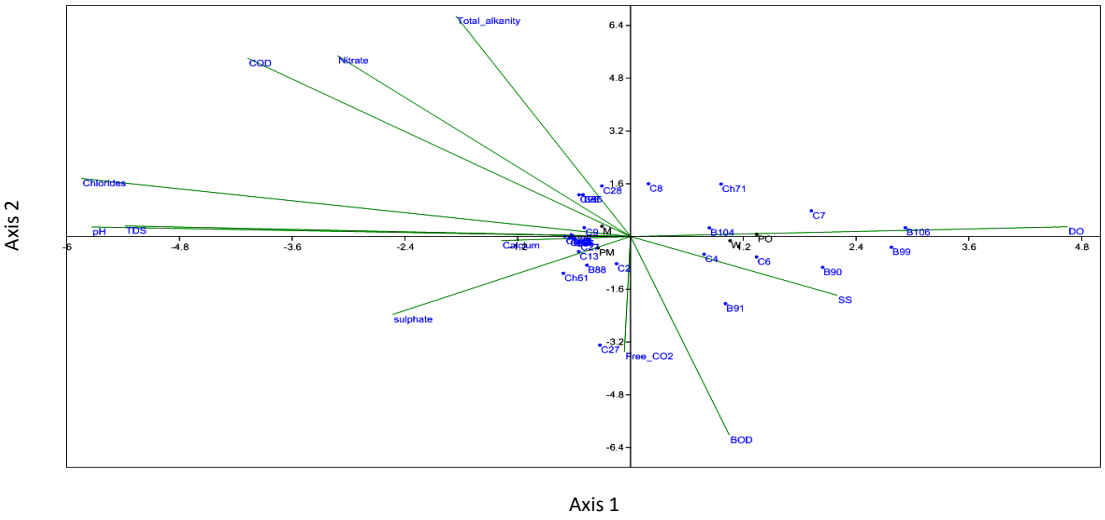

Fig. 4.58: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for Kanakpur Road

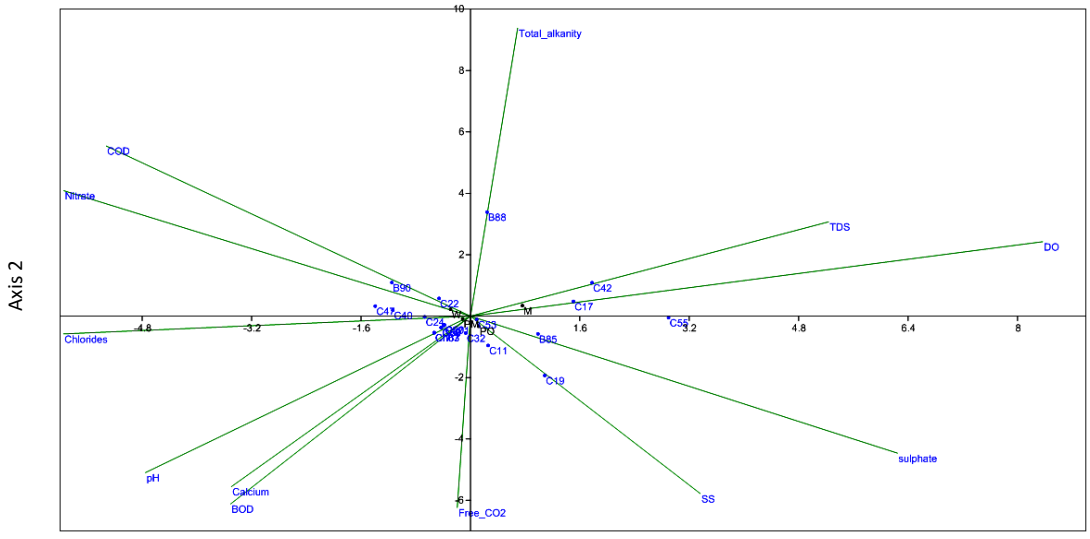

Fig. 4.59: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for Tarani Road

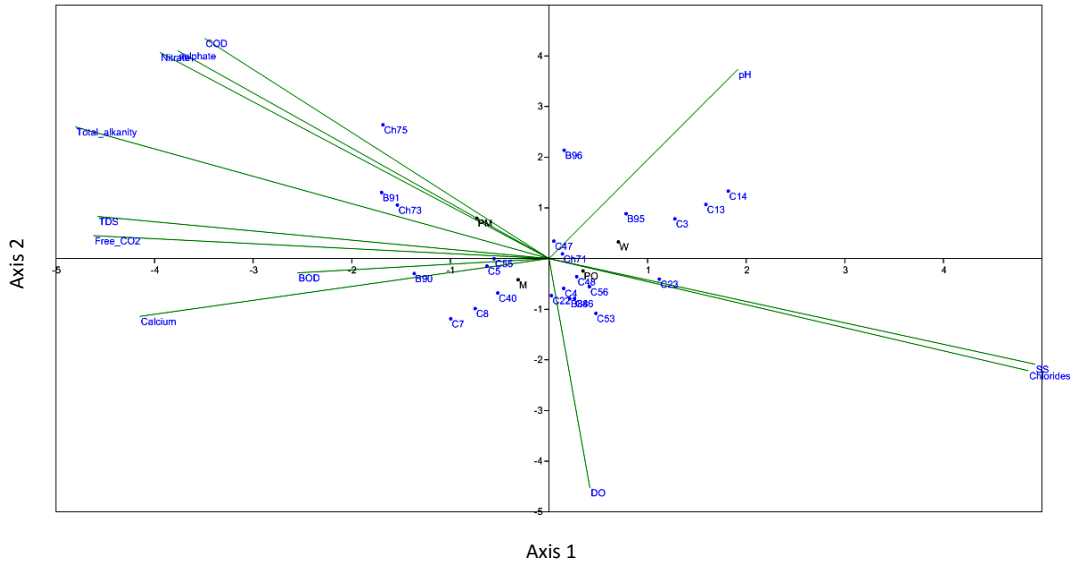

Fig. 4.60: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for Chenkoorie Road

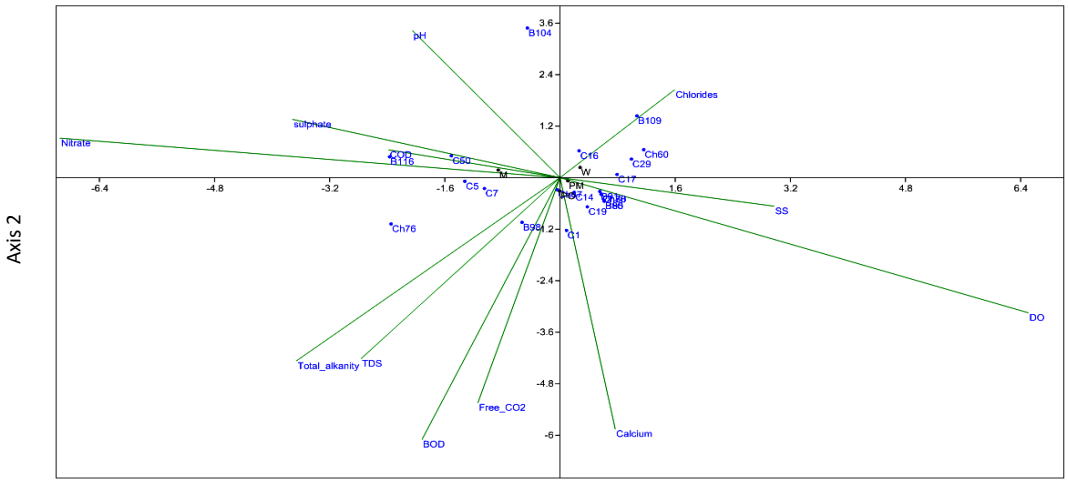

Fig. 4.61: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for Bilpar Road

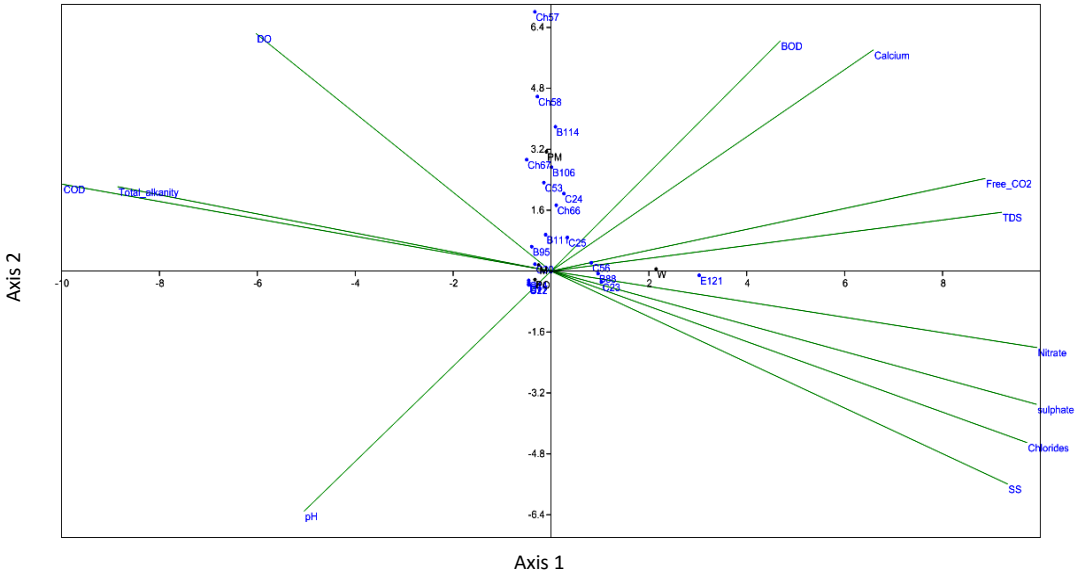

Fig. 4.62: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for ONGC Colony

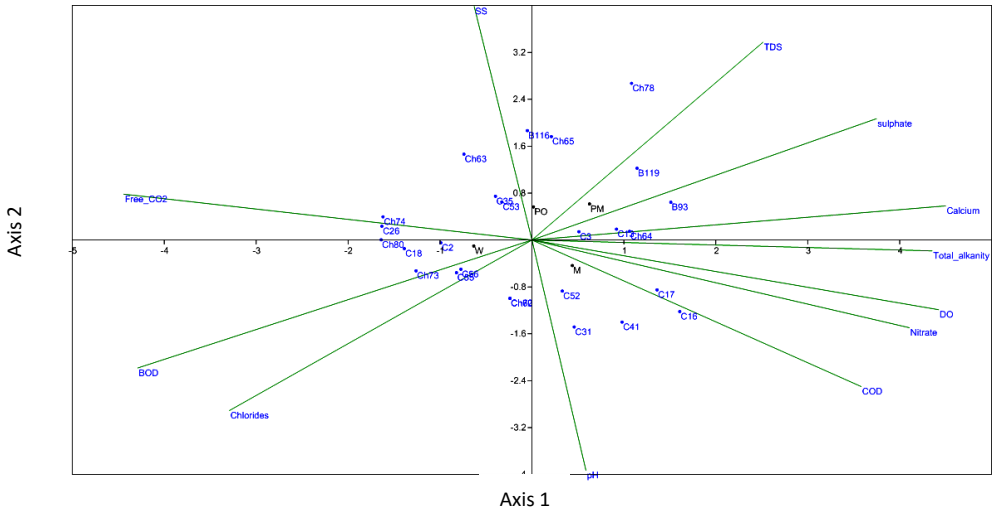

Fig. 4.63: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for Vivekananda Road

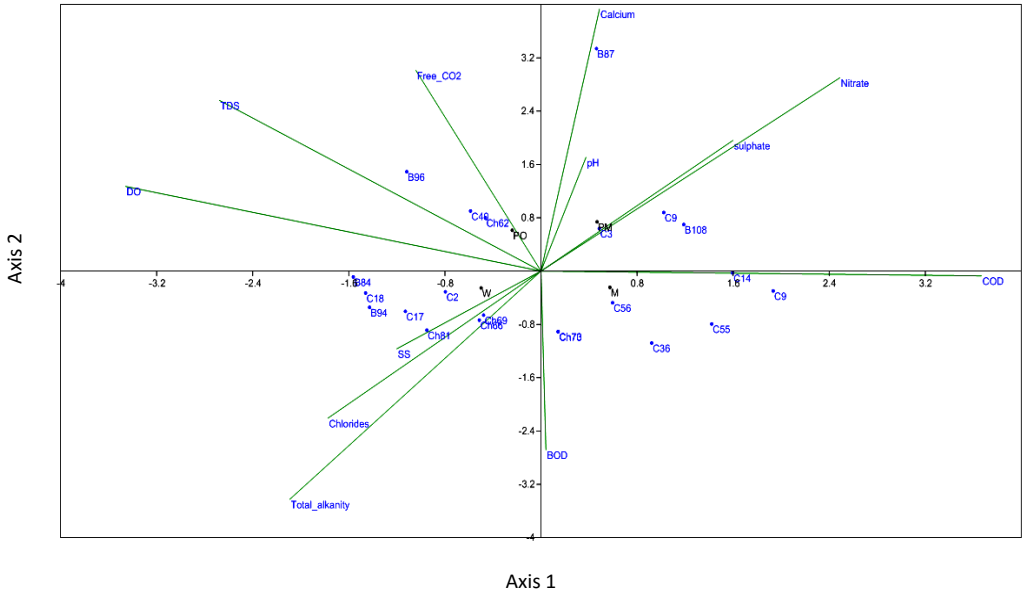

Fig. 4.64: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for Park Road

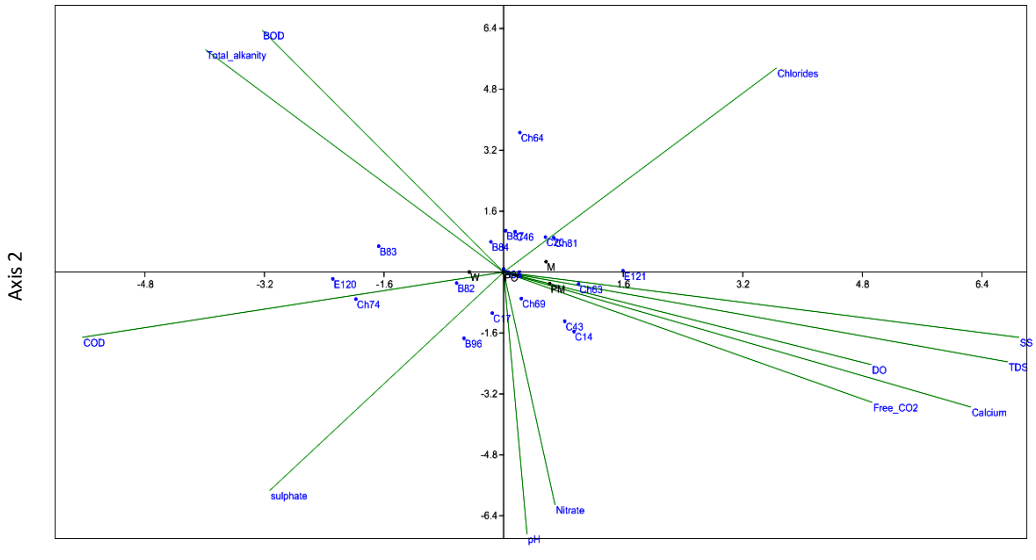

Fig. 4.65: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for Club Road

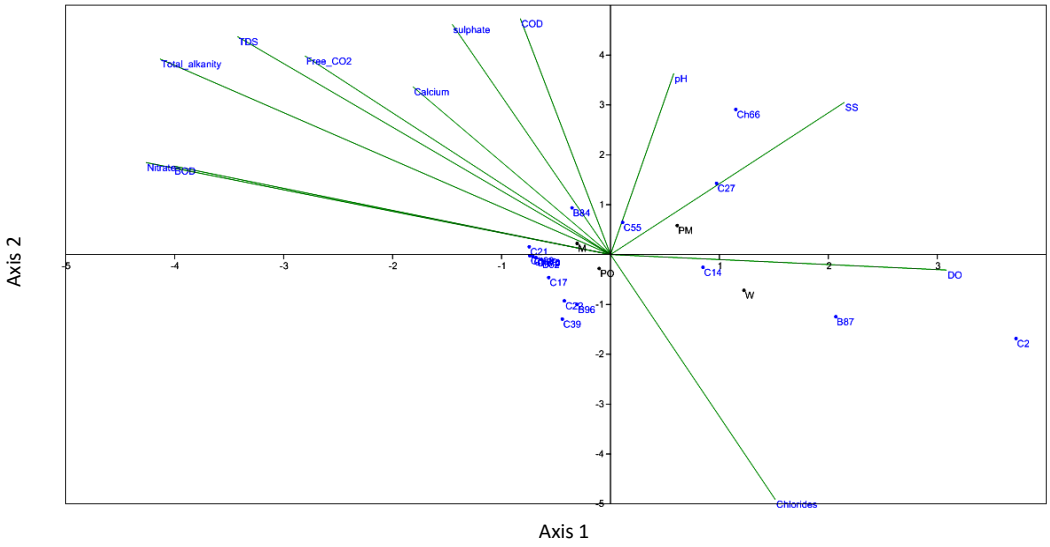

Fig. 4.66: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for Trunk Road

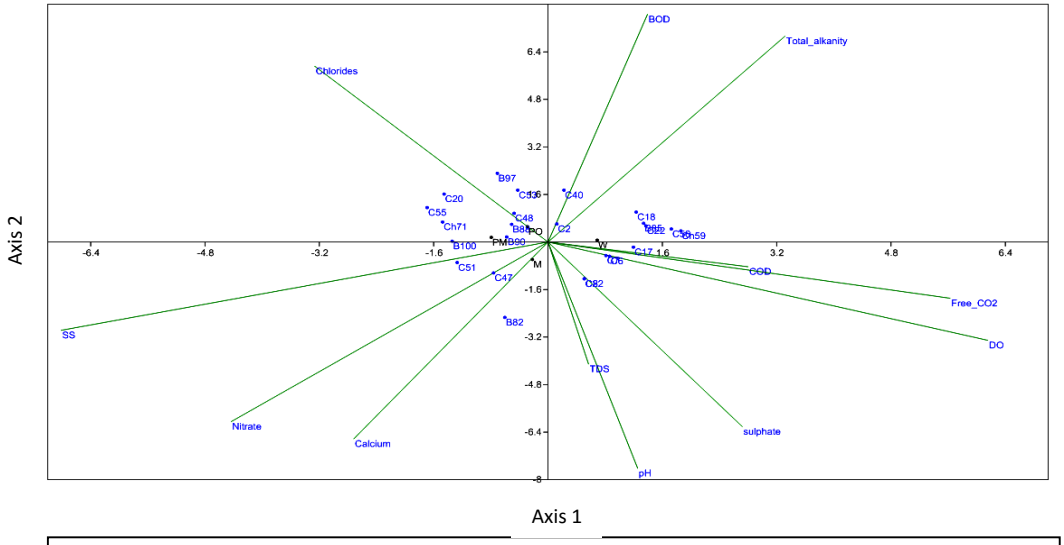

Fig. 4.67: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for Station Road

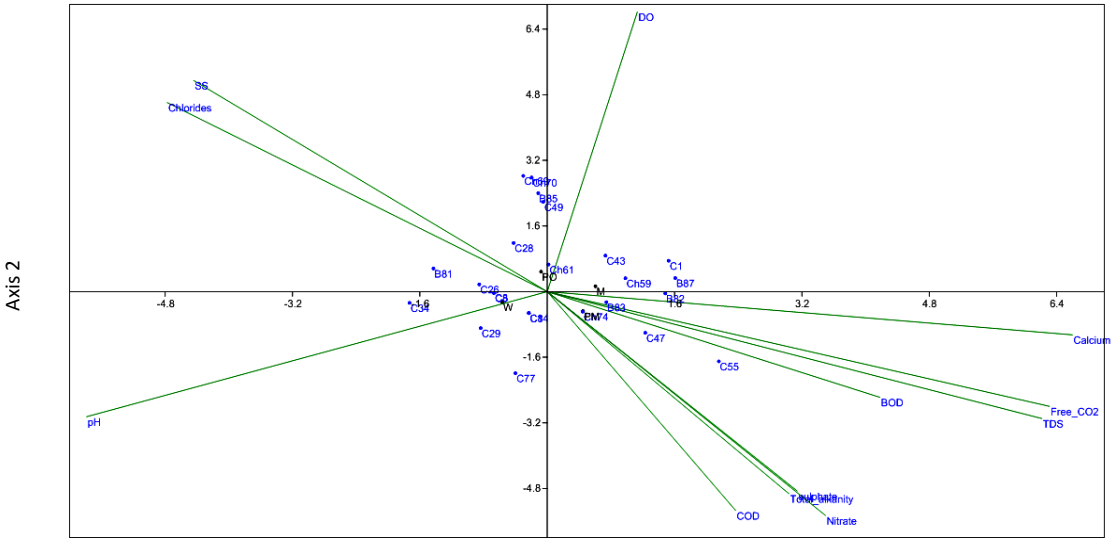

Fig. 4.68: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for Karimganj Road

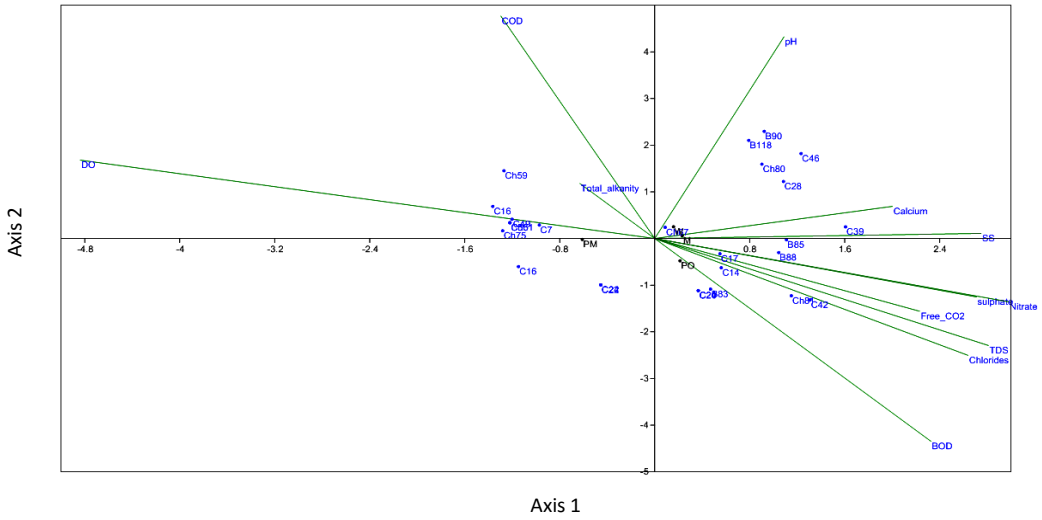

Fig. 4.69: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for Koombhirgram Road

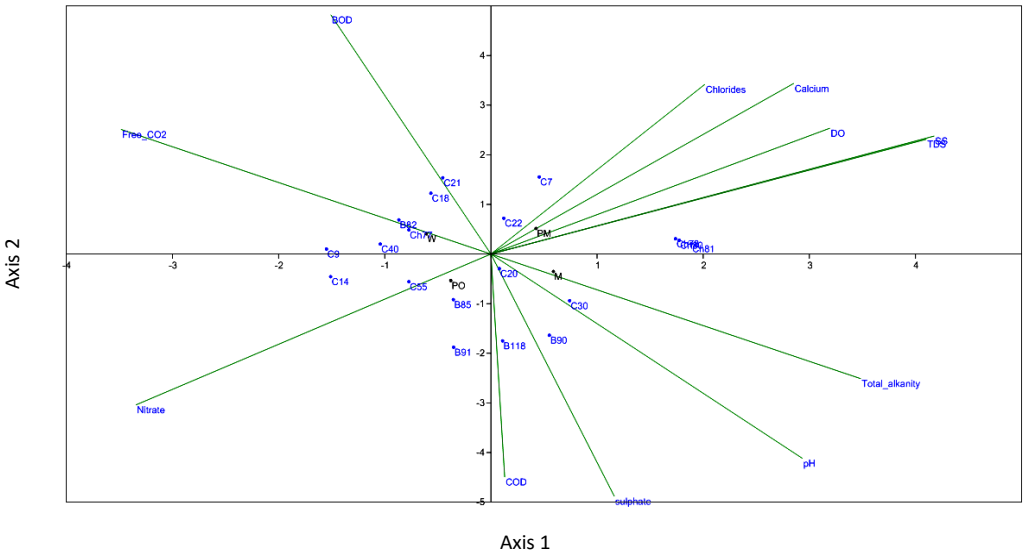

Fig. 4.70: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for Ambikapatty

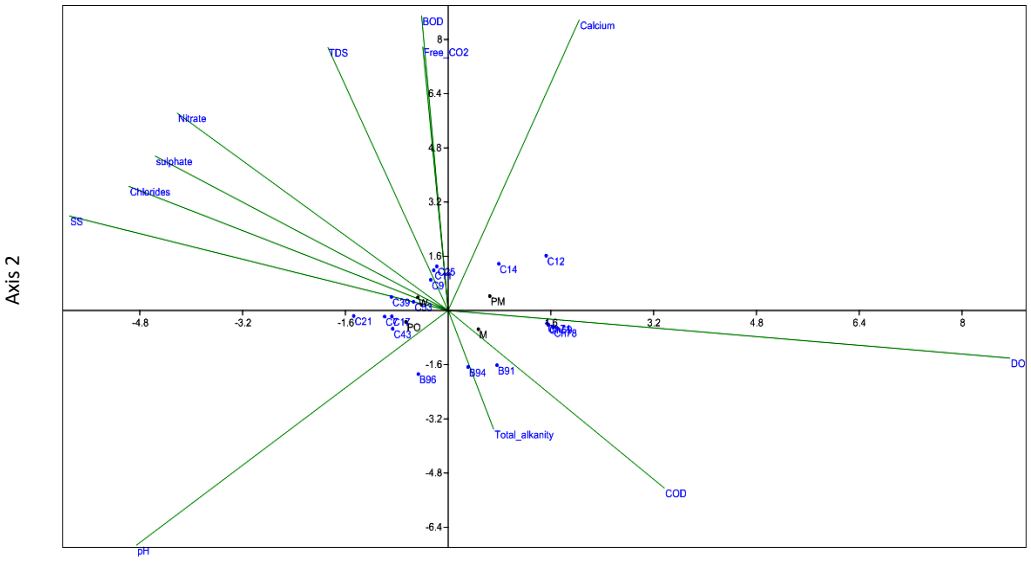

Fig. 4.71: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for Premtola

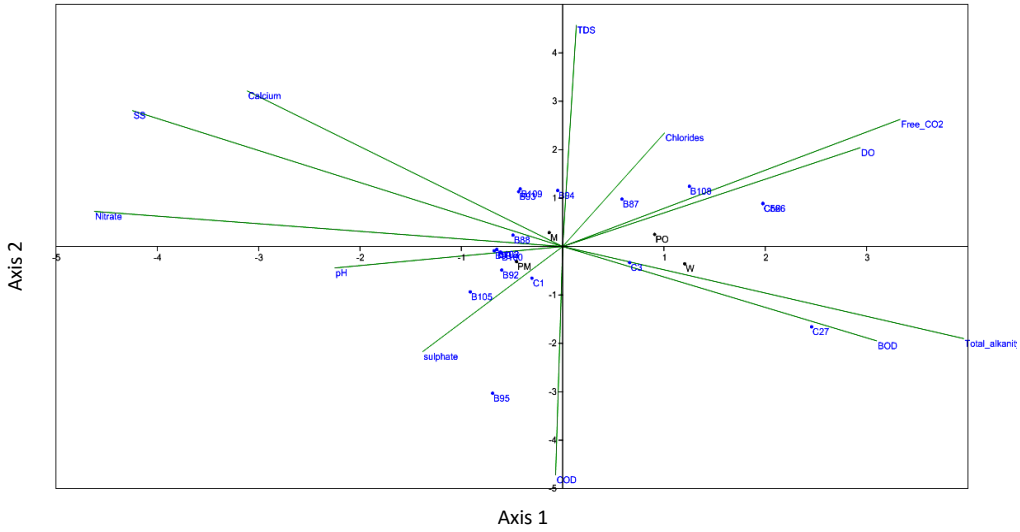

Fig. 4.72: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for Rangirkhari

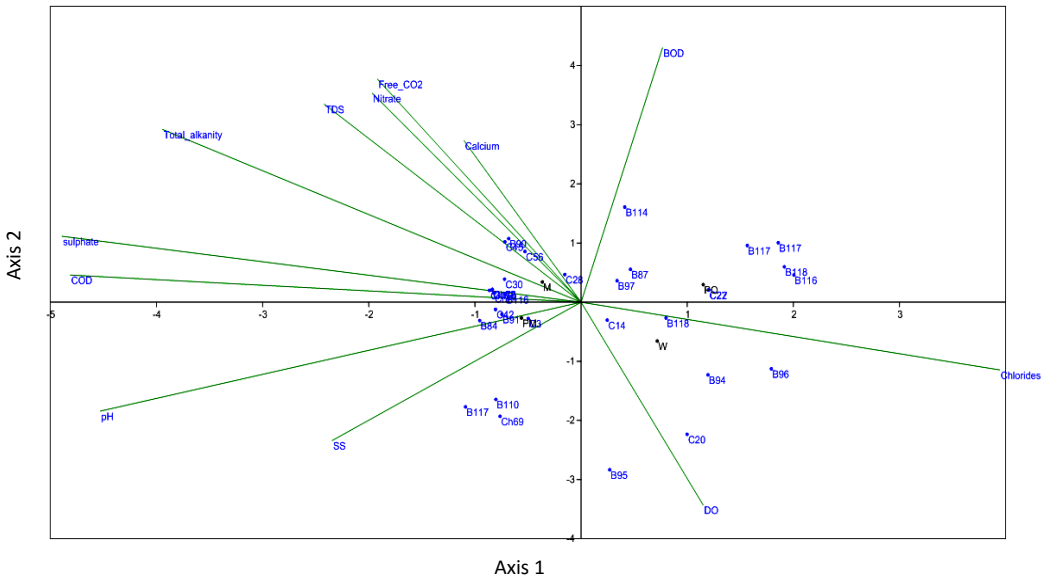

Fig. 4.73: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for Ramnagar GC College

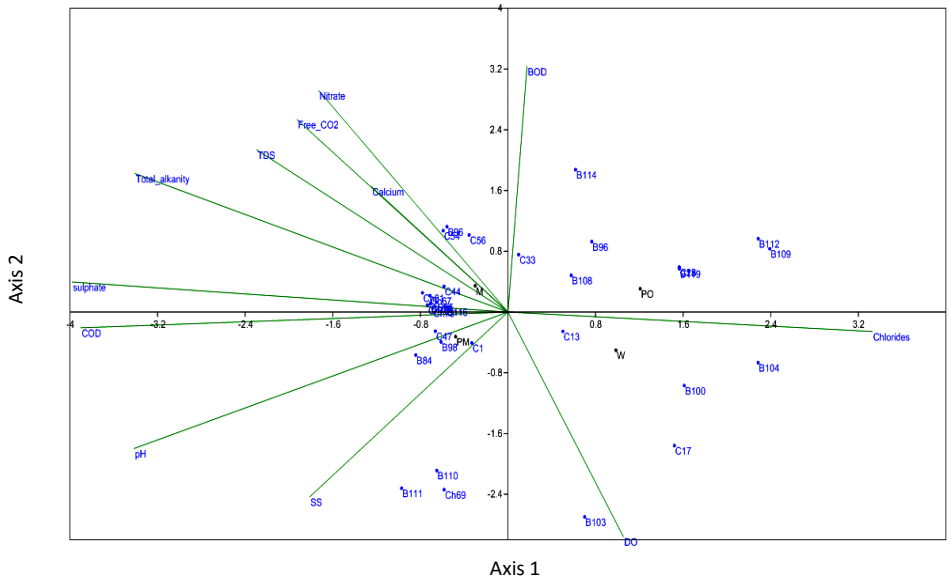

Fig. 4.74: Ordination diagram for Canonical Correspondence Analysis showing the seasonal distribution of algal species in relation to environmental variables for National Highway Road

Table 4.31 CCA eigen values of the first two axes

	Eigenvalue		% of the variance	
Axis	1	2	1	2
Link Road 1st	0.37095	0.12732	61.43	21.08
Link Road 2nd	0.43972	0.19367	54.48	24
Sonai Road	0.44307	0.41793	49.83	47
Kanakpur Road	0.35554	0.086117	71.99	17.44
Tarani Road	0.17352	0.10463	50.64	30.54
Chenkoorie Road	0.27314	0.20751	43.82	33.29
Bilpar Road	0.18676	0.12936	50.63	35.07
ONGC Colony	0.61718	0.28706	64.37	29.94
Vivekananda Road	0.24702	0.16885	47.29	32.32
Park Road	0.23628	0.15477	48.43	31.72
Club Road	0.22074	0.12289	61.16	34.05
Trunk Road	0.14991	0.12893	49.29	42.39
Station Road	0.30237	0.15093	54.8	27.36
Karimganj Road	0.20055	0.14487	44.07	31.83
Koombhirgram Road	0.28851	0.12118	61.16	25.69
Ambikapatty	0.31678	0.18872	54.79	32.64
Premtola	0.44667	0.14102	61.85	19.53
Rangirkhari	0.51015	0.092406	88	13.69
Ramnagar GC college	0.2947	0.09115	66.58	20.59
National Highway Road	0.40359	0.1279	63.09	19.99

Cylindrospermum muscicola (C14) and *Oscillatoria formosa* (C29) are present in that environment. For ONGC Colony, CCA axes 1 and 2 explained 64.37 % and 29.94 % of the variance in the species environment biplot (Fig. 4.62). *Euglena* sp (E121) and *Westiellopsis prolifica* (C57) are present. For Vivekananda Road, CCA axes 1 and 2 explained 47.29 % and 32.32 % of the variance in the species environment biplot (Fig. 4.63). *Hydrocoleum* sp. (C16) and *Oscillatoria homogenea* (C31) are present. For Park Road, CCA axes 1 and 2 explained 48.43 % and 31.72 % of the variance in the species environment biplot (Fig. 4.64). *Westiellopsis prolifica* (C56) and *Lyngbya diguetii* (C17) present in those environment. For Club Road, CCA axes1 and 2 explained 61.16 % and 34.05 % of the variance in the species environment biplot (Fig. 4.65). *Cylindrospermum muscicola* (C14) is tolerant to those environment. For Trunk Road, CCA axes1 and 2 explained 49.29 % and 42.39 % of the variance in the species environment

biplot (Fig. 4.66). *Oscillatoria peronata* (C39) and *Cylindrospermum muscicola* (C14) present in those environment. For Station Road, CCA axes1 and 2 explained 54.8 % and 27.36 % of the variance in the species environment biplot (Fig. 4.67).*Oscillatoria subbrevis* (C47) and *Oscillatoria willei* (C51) present in those environment. For Karimganj Road, CCA axes1 and 2 explained 44.07 % and 31.83 % of the variance in the species environment biplot (Fig. 4.68). *Oscillatoria subbrevis* (C47), and *Spirulina major* (C55) present in those environment.pH, nitrate, chlorides are the main physio-chemical parameters influenced the *Oscillatoria subbrevis* (C47), and *Spirulina major* (C55). For Koombhirgram Road, CCA axes1 and 2 explained 61.16 % and 25.69 % of the variance in the species environment biplot (Fig. 4.69).pH, nitrate, calcium influenced the *Cylindrospermum muscicola* (C14), *Oscillatoria raoi* (C42), and *Oscillatoria peronata* (C39). For Ambikapatty, CCA axes1 and 2 explained 54.79 % and 32.64 % of the variance in the species environment biplot (Fig. 4.70). pH, nitrate, total alkalinity influenced the *Oscillatoria geitleriana* (C30). For Premtola, CCA axes1 and 2 explained 61.85 % and 19.53 % of the variance in the species environment biplot (Fig. 4.71). pH, nitrate, total alkalinity influenced the *Oscillatoria peronata* (C39) and *Oscillatoria rubescens* (C43). For Rangirkhari, CCA axes1 and 2 explained 88 % and 13.69 % of the variance in the species environment biplot (Fig. 4.72). *Oscillatoria amphibia* (C27) and *Cymbella hungarica* (B87) are present abundantly in this habitat.For Ramnagar GC college, CCA axes1 and 2 explained 66.58 % and 20.59 % of the variance in the species environment biplot (Fig. 4.73). pH, nitrate,total alkanity influenced the *Oscillatoria geitleriana* (C30) distribution. For National Highway Road, CCA axes1 and 2 explained 66.58 % and 20.59 % of the variance in the species environment biplot (Fig. 4.74). pH, nitrate, total alkalinity influenced the *Oscillatoria salina* (C56) growth in this habitat.

Table 4.32 CCA scores of the environmental variables with the first two axes

		pH	BOD	COD	DO	TK	FCO_2	TDS	SS	Cl	SO_4^{2-}	NH_4-N	Ca
Link Road 1st	Axis 1	-0.42	-0.47	0.34	-0.57	-0.84	0.56	0.49	0.50	0.92	-0.51	0.53	0.96
	Axis 2	-0.36	0.14	-0.91	0.45	-0.47	0.78	0.31	0.83	-0.41	0.85	-0.84	0.16
Link Road 2nd	Axis 1	0.55	-0.03	0.72	-0.07	0.10	-0.94	-0.50	-0.88	-0.23	0.42	-0.07	-0.88
	Axis 2	0.67	-0.94	-0.22	-0.79	0.87	-0.13	0.46	-0.19	-0.14	0.10	-0.04	-0.19
Sonai Road	Axis 1	0.11	-0.76	0.85	0.09	0.29	-0.41	0.083	0.03	-0.3	-0.21	0.95	0.86
	Axis 2	-0.96	-0.30	-0.62	0.13	-0.03	0.26	0.87	0.88	0.75	0.48	-0.56	0.11
Kanakpur Road	Axis 1	-0.81	0.15	-0.58	0.66	-0.26	-0.009	-0.76	0.31	-0.83	-0.36	-0.44	-0.19
	Axis 2	0.04	-0.86	0.77	0.04	0.95	-0.49	0.045	-0.25	0.25	-0.33	0.78	-0.017
Tarani Road	Axis 1	0.79	0.76	-0.25	0.53	0.22	-0.77	-0.843	-0.60	-0.77	0.28	-0.42	-0.82
	Axis 2	-0.47	-0.35	-0.53	0.83	0.06	-0.019	0.523	0.33	-0.59	0.62	-0.59	-0.34
Chenkoorie Road	Axis 1	0.38	-0.50	-0.69	0.082	-0.96	-0.92	-0.91	0.98	0.97	-0.75	-0.78	-0.82
	Axis 2	0.74	-0.05	0.86	-0.90	0.51	0.09	0.16	-0.41	-0.44	0.81	0.81	-0.22
Bilpar Road	Axis 1	0.84	-0.085	0.92	0.32	0.35	0.53	0.54	0.92	-0.88	0.81	-0.12	0.52
	Axis 2	-0.29	-0.27	-0.34	0.92	-0.52	-0.16	-0.39	0.42	0.22	-0.53	-0.99	0.10
ONGC Colony	Axis 1	-0.50	0.46	-0.99	-0.60	-0.88	0.88	0.92	0.93	0.97	0.99	0.99	0.65
	Axis 2	-0.63	0.60	0.22	0.62	0.22	0.24	0.15	-0.55	-0.45	-0.34	-0.20	0.58
Vivekananda Road	Axis 1	0.11	-0.85	0.71	0.88	0.87	-0.88	0.50	-0.12	-0.65	0.74	0.82	0.89
	Axis 2	-0.78	-0.43	-0.49	-0.23	-0.03	0.15	0.67	0.79	-0.58	0.41	-0.29	0.11
Park Road	Axis 1	0.09	0.01	0.91	-0.86	-0.52	-0.26	-0.66	-0.30	-0.44	0.39	0.62	0.12
	Axis 2	0.42	-0.67	-0.01	0.31	-0.85	0.75	0.64	-0.29	-0.55	0.49	0.72	0.98
Club Road	Axis 1	-0.07	-0.35	-0.69	-0.37	-0.44	-0.28	0.34	0.29	0.34	-0.31	0.56	0.21
	Axis 2	0.04	-0.46	-0.80	0.70	-0.56	0.70	0.96	0.98	0.52	-0.44	0.09	0.89
Trunk Road	Axis 1	0.11	-0.80	-0.16	0.61	-0.82	-0.56	-0.68	0.42	0.30	-0.29	-0.85	-0.36

	Axis 2	0.72	0.35	0.94	-0.06	0.78	0.79	0.87	0.61	-0.98	0.92	0.36	0.67
Station Road	Axis 1	0.15	0.17	0.34	0.76	0.41	0.70	0.07	-0.85	-0.40	0.33	-0.55	-0.33
	Axis 2	-0.95	0.95	-0.10	-0.41	0.86	-0.23	-0.51	-0.37	0.73	-0.77	-0.75	-0.82
Karimganj Road	Axis 1	-0.82	0.59	0.33	0.16	0.43	0.90	0.88	-0.63	-0.68	0.44	0.49	0.94
	Axis 2	-0.43	-0.36	-0.76	0.97	-0.70	-0.39	-0.44	0.73	0.65	-0.69	-0.77	-0.15
Koombhirgram	Axis 1	-0.50	-0.50	-0.36	0.01	-0.98	0.88	0.77	0.82	0.77	0.84	0.80	0.87
	Axis 2	0.21	0.21	-0.25	-0.96	-0.12	0.44	0.56	0.54	0.52	0.54	0.59	0.40
Ambikapatty	Axis 1	0.58	-0.30	0.02	0.63	0.69	-0.69	0.82	0.83	0.40	0.23	-0.66	0.57
	Axis 2	-0.82	0.96	-0.89	0.50	-0.50	0.50	0.46	0.47	0.68	-0.97	-0.60	0.68
Premtola	Axis 1	-0.53	-0.04	0.37	0.97	0.07	-0.04	-0.20	-0.65	-0.55	-0.50	-0.46	0.22
	Axis 2	-0.76	0.96	-0.58	-0.15	-0.38	0.86	0.86	0.30	0.40	0.50	0.64	0.95
Rangirkhari	Axis 1	-0.90	0.15	-0.96	0.23	-0.78	-0.38	-0.48	-0.46	0.78	-0.97	-0.39	-0.22
	Axis 2	-0.36	0.86	0.09	-0.68	0.58	0.75	0.66	-0.46	-0.22	0.22	0.70	0.54
Ramnagar GC college	Axis 1	-0.44	0.62	-0.01	0.58	0.79	0.66	0.66	-0.84	0.20	-0.27	-0.92	-0.62
	Axis 2	-0.08	-0.38	-0.94	0.40	-0.38	0.52	0.52	0.56	0.46	-0.43	0.14	0.64
National Highway Road	Axis 1	-0.85	0.04	-0.97	0.26	-0.85	-0.48	-0.58	-0.45	0.83	-0.99	-0.43	-0.31
	Axis 2	-0.44	0.80	-0.05	-0.73	0.45	0.63	0.53	-0.60	-0.06	0.09	0.72	0.41

Chapter 5

ISOLATION AND MORPHOLOGICAL CHARACTERIZATION OF ALGAL ISOLATES

5.1 Introduction

Algae are the photosynthetic, prokaryotic or eukaryotic group of organisms which occur primarily in freshwater and marine water, but also in terrestrial ecosystems. Algae have a wide-ranging impact on natural ecosystems and many of them have both beneficial and harmful effects. To characterize the algal communities, morphology plays an important role. The characterization of the harmful algal communities remains challenging because the characterization of some algal genus has not yet been resolved (Cook *et al.,* 2004; Paerl *et al*., 2011). Of late, algal taxonomy is not based on morphological characters but also examined using a polyphasic approach by assessing morphological and molecular data combined with toxicological characters (Moustaka-Gouni *et al.,* 2006). Some of workers

have made important taxonomic assignments which helped in better understanding of algal taxonomy (Anagnostidis & Komárek, 1985, 1988, 1990; Komárek & Anagnostidis, 1986, 1989; Büdel & Kauff, 2012).

In the present study, the algal isolates were diverse in cultural characteristics. Here, we have taken into consideration all the cultural characters and ecological habitat and supported the comprehensive morphological characterization of the isolates. Morphological characters variations were observed in the present study due to different light, temperature and salinity conditions of the domestic sewage water and solid waste dumping site and they can survive under adverse conditions, deviations in morphology occurred, in those characteristics that have been traditionally accepted as useful in algal taxonomy (Stulp and Stam 1982, 1984). Species were identified based on their ecological habitat and taxonomical assignments (Geitler, 1932; Fritsch, 1949; Desikachary, 1959). However, Desikachary and Anand (1974) identified a number of species based on their geographical distribution cultural conditions and they emphasized the need for more cultural studies of the isolates. The current chapter describes the isolation, purification and taxonomic assignment of some algal strains based on their morphological characterization.

5.2 Methodology

The details of the isolation procedure and culture conditions were mentioned at **Chapter 3.** All morphological characteristics such as cell morphology, ultrastructure of cell, trichome morphology of the isolated algae in late exponential phase were observed under the microscope. The culture conditions of each algae were observed in both liquid and solid BG11 medium. Morphological characteristics i.e., cell morphology (cell colour, cell shape, sheath, gas vacuoles, heterocyst and akinete position), ultrastructure (cell wall and sheath structure), trichome type, tapped, straight, helical, septate, shape of terminal cell, branching (true or false), culture conditions were observed in late exponential phase. Photomicrographs were taken in stationary period of the growth curve. Morphological characteristics presented in Desikachary (1959), Prescott (1951) and Anagnostidis & Komárek, (1985, 1988, 1990); Komárek & Anagnostidis, (1986, 1989).

5.3 Results and discussion

Algal samples grown on polythene bags in domestic solid waste and domestic solid waste dumping sites were selected for isolation with the help of microscopic observation. The polythene bags were cut into $1cm^2$ size pieces with a sterilized blade. The algae samples from polythene surfaces were scrubbed with a sterilized brush and observed under microscope. The algal samples were homogenized in sterile water with glass beads, centrifuged at 3000 rpm for 10 minutes with repeated washing. The pellets were suspended in sterilized BG-11 medium and placed onto the agar petri plates by pour plate method. The plates were incubated for 15 days under continuous illumination (2000lux) at 24 ± 1^0C. A total of 33 algae samples were isolated by repeated streaking on the agar plates. The morphological characteristics of algal species revealed by microscopic analysis are given in the **Table 5.1.**.Algal isolates includes 32 cyanobacteria and one green algae.Out of 32 cyanobacteria,8 species were non-heterocystous and 24 species were heterocystous cyanobacteria. Of which 24 species of cyanobacteria, 5 species of *Nostoc*, 5 species of *Calothrix*, 5 species of *Anabaena*, 2 species of *Cylindrospermum*, one species of *Hapalosiphon*, two species of *Westiellopsis*. Out of 8 species of non-heterocystous cyanobacteria, one species of *Phormidium*, 4 species of *Oscillatoria*, two species of *Lyngbya* and one *Aphanothece*.

***Anabaena anomala* Fritsch** (**AUS/EC/JR/PSCYA258**) (Plate: 5.1)

This cyanobacterial isolate was screened from polythene surface in domestic solid waste dumping site of Link road 2nd, on 20 April, 2014. Cells are curved, olive green, ellipsoidal, 3-4µm long and 2.5-3µm breadth. Akinete square to ellipsoidal, 5-64µm long and 2.5-3µm breadth. Trichome bent. Filaments are arranged in parallel lines.

***Anabaena flos-aquae (Lyngb.)* De Brébisson** (**AUS/EC/JR/PSCYA259**) (Plate: 5.2)

The cyanobacterial isolate was screened from polythene surface in domestic solid waste dumping site of Link road 1st, on 10 July, 2014. In BG11 agar media, olive green colour filamentous colony appears, but in liquid medium, it shows planktonic, very flexuous and contorted trichome. Coiling in trichome appears in an irregular spiral fashion. Cells spherical to subcylindric, 4-5 µm in diameter, 5-6.5µm long, cell contents granular with

Table 5.1 Morphological characteristics of algal species revealed by microscopic analysis

Strain	Taxonomic assignment	Place of isolation & year	Germplasm deposit code	Cell (µm)		Heterocysts (µm)		Akinete (µm)		Terminal cell (µm)		Sheath	Any Special Characters
				L	B	L	B	L	B	L	B		
1	*Anabaena anomala* Fritsch	Link road 2nd, 20April, 2014	AUS/EC/JR/PSCYA 258	3	3	4.5	3	6	5	6	2	-	-
2	*Anabaena flos-aquae (Lyngb.)* De Brébisson	Link road 1st, 10July, 2014	AUS/EC/JR/PSCYA 259	6	4.5	3	3	-	-	3	3	-	-
3	*Anabaena oscillarioides Bory (after Frémy)*	Chenkoorie road, 4 March, 2014	AUS/EC/JR/PSCYA 291	3	3	6	4.5	10	6	6	2	-	-
4	*Anabaena variabilis* Kuetzing	Tarani Road, 5 February, 2014	AUS/EC/JR/PSCYA 292	7.5	5	10	6	12	7	-	-	-	-
5	*Anabaena wisconsinense* Prescott	Rangirkhari, 12 March, 2014	AUS/EC/JR/PSCYA 293	4	3	4.5	3	5	4	3	2	-	-
6	*Anabaenopsis arnoldii* Aptekarj v. indica Ramanathan	Karimganj Road, 11 April 2014	AUS/EC/JR/PSCYA 288	4	4	4	4	4	3	2	3	-	-
7	*Aphanothece microscopia* Näg.	Tarani Road, 17 February, 2014	AUS/EC/JR/PSCYA 261	7.5	6	-	-	-	-	-	-	-	-
8	*Calothrix elenkinii Kossink* (after poljansky)	Sonai Road, 17 March, 2014	AUS/EC/JR/PSCYA 265	7.5	4	8	5	-	-	-	-	Present	

9	*Calothrix fusca (Kütz.) Born. et Flah. forma*	Sonai Road, 12 August, 2014	AUS/EC/JR/PSCYA 263	3	3	3	3	-	-	-	-	Present	-
10	*Calothrix marchica Lemm.*(after Frémy)	Trunk Road, March, 2015	AUS/EC/JR/PSCYA 286	2	2	3	2	-	-	-	-	Present	-
11	*Calothrix parietina Näg.)* Thuret (after Frémy)	Link Road 1st, 27 January, 2015	AUS/EC/JR/PSCYA 262	6	3	6	7	-	-	-	-	Present	-
12	*Calothrix sp*	Vivekananda Road, 23 March, 2014	AUS/EC/JR/PSCYA 266	4	2	4	3	-	-	-	-	Present	-
13	*Chlorella ellipsoidea* Gerneck	Vivekananda Road, 12 May, 2014	AUS/EC/JR/PSGA2 94	9	7	-	-	-	-	-	-	-	-
14	*Cylindrospermum licheniforme* (Bory) Kütz (after Fremy)	Kanakpur Road, 12 February, 2014	AUS/EC/JR/PSCYA 267	2	1	5	4	-	-	-	-	-	Heterocyst terminal with akinete
15	*Cylindrospermum musicola* Kütz (after Fremy)	Ambikapatty, 13 May 2014	AUS/EC/JR/PSCYA 268	4	3	6	7	-	-	-	-	-	Heterocyst terminal with akinete
16	*Fischerella sp*	Rangirkhari, 11 March, 2014	AUS/EC/JR/PSCYA 270	4	3	3	2	-	-	-	-	-	Lateral branches distinct from main filament
17	*Hapalosiphon welwitschii W.et.Gs.West*	Chenkoorie Road, 11 March, 2015	AUS/EC/JR/PSCYA 269	4	3	4	2	-	-	-	-	-	Homogones present
18	*Lyngbya diguetii Gom. (after Frémy)*	NH Road, 12 July 2014	AUS/EC/JR/PSCYA 271	4	2	3	2	-	-	-	-	Present	Single trichome with sheath

19	*Lyngbya nordgardhii Wille*	Station road, 10 September, 2014	AUS/EC/JR/PSCYA 272	2	2	-	-	-	-	-	-	Present	Single monoliform cells trichome with sheath
20	*Nostoc carneum Ag. (after Frémy)*	Link road 1st, 17 March 2014	AUS/EC/JR/PSCYA 273	2	2	4	3	-	-	-	-	-	Thallus rounded with pellicle
21	*Nostoc commune Vaucher ex Born. et Flah*	Bilpar Road, 18 April, 2014	AUS/EC/JR/PSCYA 274	3	3	3	2	-	-	-	-	-	Thallus rounded with pellicle
22	*Nostoc linckia v. (Roth) Born. et Flah.* (after Frémy)	Rangirkhari, 12 October, 2014	AUS/EC/JR/PSCYA 276	2	1	2	2	-	-	-	-	-	Thallus rounded with pellicle
23	*Nostoc muscorum* (after Bornet and Thuret)	Club Road, 19 March 2014	AUS/EC/JR/PSCYA 277	7	4	6	3	-	-	-	-	-	Thallus rounded with pellicle
24	*Nostoc sp*	Link Road 2nd, 7 June, 2014	AUS/EC/JR/PSCYA 278	2	2	2	1	-	-	-	-	-	Thallus rounded with pellicle
25	*Oscillatoria acuminata* Gom. (after Gomont)	Link road 1st, 20 February, 2014	AUS/EC/JR/PSCYA 282	2	2	-	-	-	-	-	-	-	Trichome straight
26	*Oscillatoria curviceps* forma (after Rao, C.B.)	ONGC Colony, 21 January, 2015	AUS/EC/JR/PSCYA 279	4	3	-	-	-	-	-	-	-	Trichome straight
27	*Oscillatoria limosa* Ag. (after Gomont)	Rangirkhari, 22 June, 2014	AUS/EC/JR/PSCYA 281	4	2	-	-	-	-	-	-	-	Trichome straight
28	*Oscillatoria subbrevis Schmidle (orig.)*	Premtola, 12 March, 2014	AUS/EC/JR/PSCYA 280	4	3	-	-	-	-	-	-	-	Trichome straight
29	*Phormidium lucidum Kütz.*	Sonai Road, 13 May, 2014	AUS/EC/JR/PSCYA 283	4	2	-	-	-	-	-	-	Present	Sheath confluent

													with filament
30	*Westiellopsis prolifica Janet (after Janet)*	NH road, 23 March, 2014	AUS/EC/JR/PSCYA 290	4	3	9	3	-	-	-	-	-	Spores present
31	*Westiellopsis sp*	Link Road 2nd, 24 April, 2014	AUS/EC/JR/PSCYA 291	3	2	2	2	-	-	-	-	-	Spores present
32	*Unknown*	Link Road 2nd, 27 July, 2014	AUS/EC/JR/PSCYA 287	2	2	2	1	-	-	-	-	-	-

*L= Length, B= Breadth

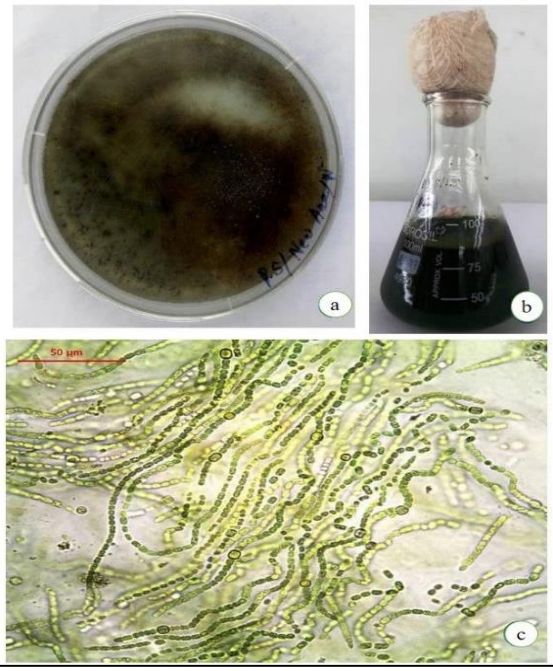

Plate 5.1: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Anabaena anomala* isolated from Link road 2nd.

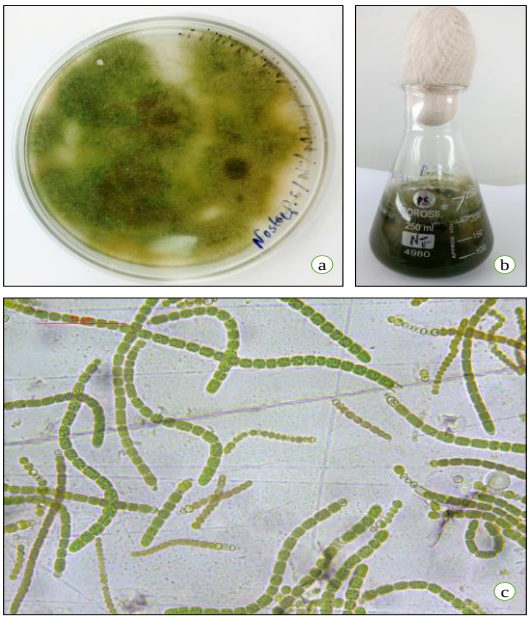

Plate 5.2: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Anabaena flos-aquae* isolated from Link road 1st

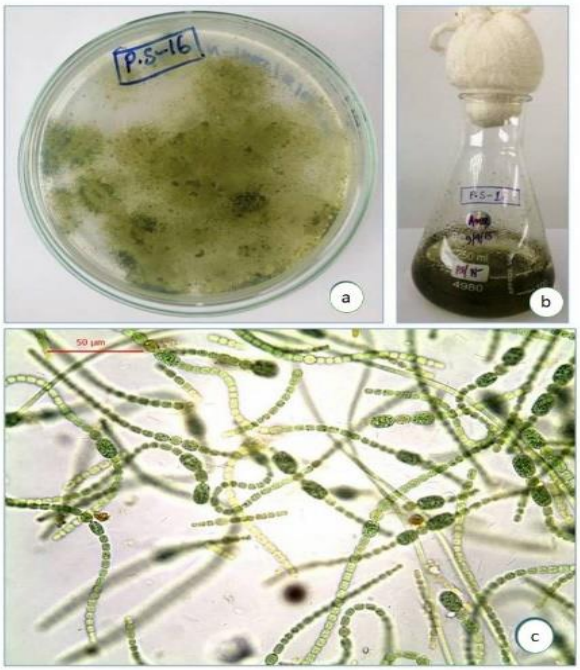

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Plate 5.3: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Anabaena oscillarioides* Bory (after Frémy) isolated from Link road 2nd.

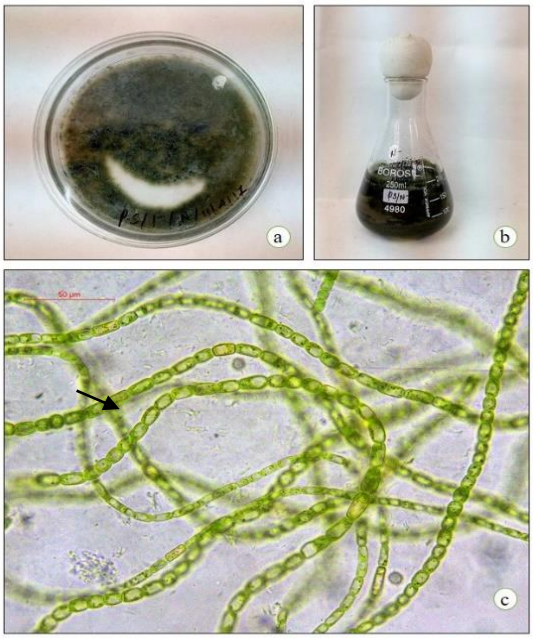


Plate 5.4: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Anabaena variabilis* isolated from Tarani road

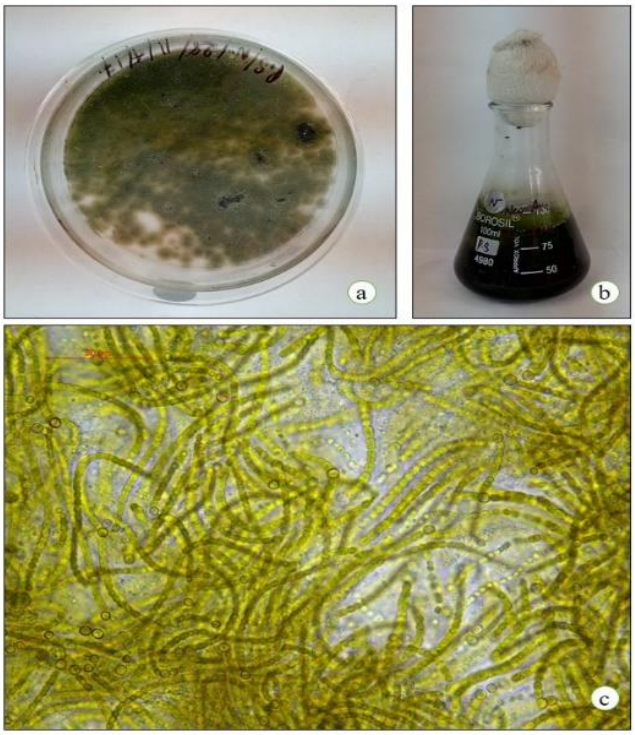


Plate 5.5: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Anabaena wisconsinense* isolated from Rangirkhari**.**

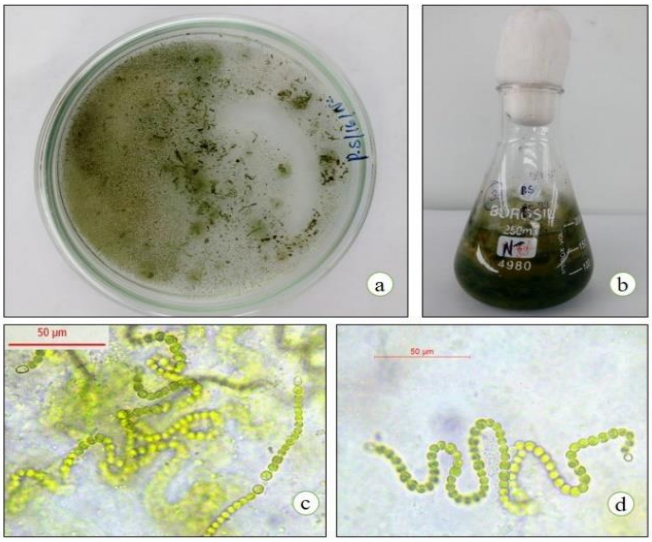


Plate 5.6: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Anabaenopsis arnoldii* v. indica Ramanathan Karimganj Road.

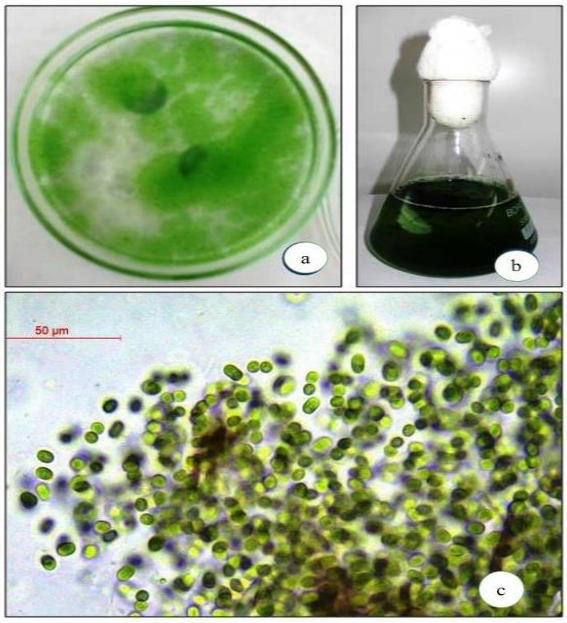


Plate 5.7: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Aphanothece microscopia* isolated from Tarani Road**.**

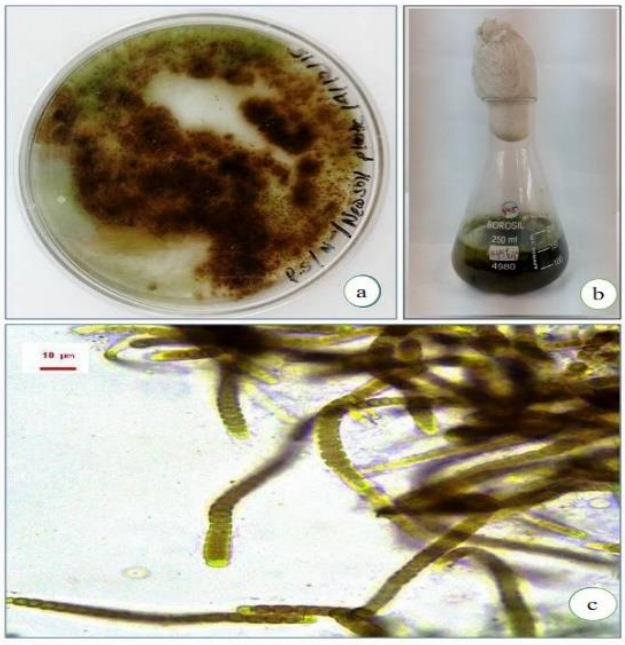


Plate 5.8: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Calothrix elenkinii* isolated from Sonai Road.

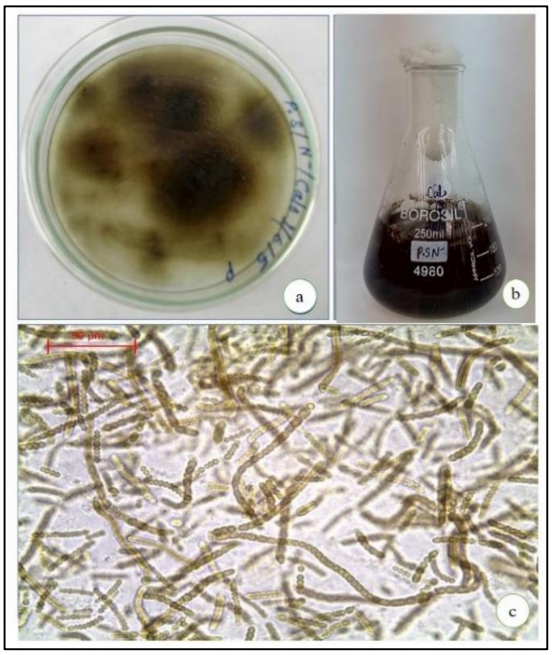


Plate 5.9: Colony development on agarized medium (a), growth behavior on liquid culture (b) and microphotographs (c) of *Calothrix fusca* isolated from Sonai Road.

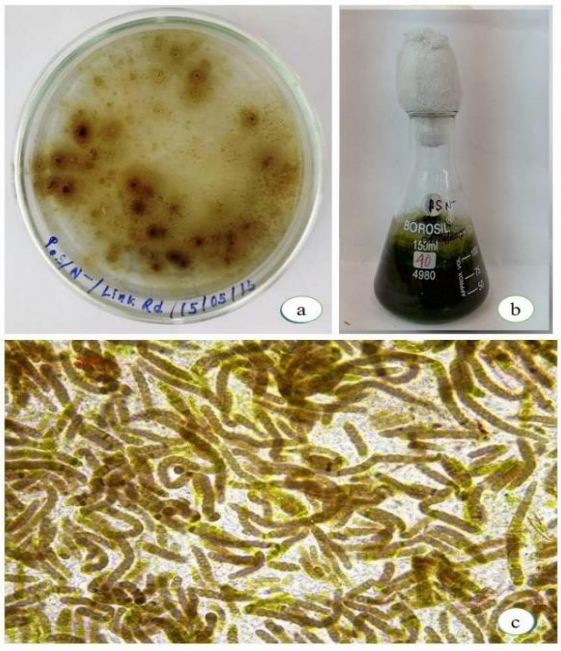


Plate 5.10: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Calothrix marchica* isolated from Trunk Road.

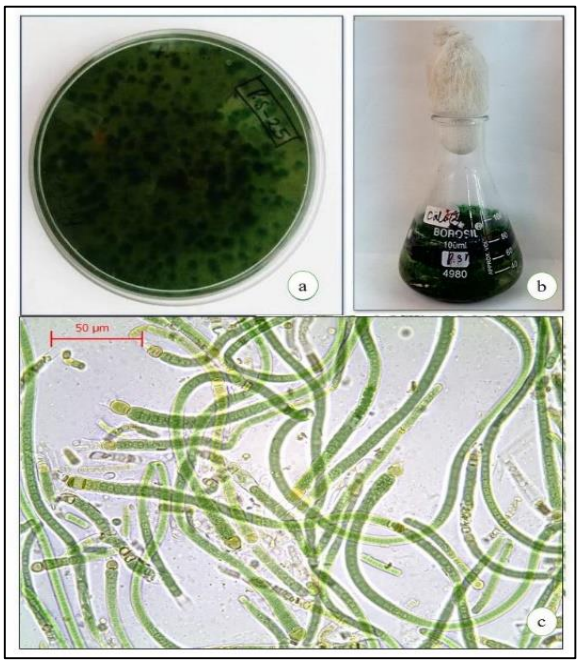


Plate 5.11: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Calothrix parietina* isolated from Link Road 1st.

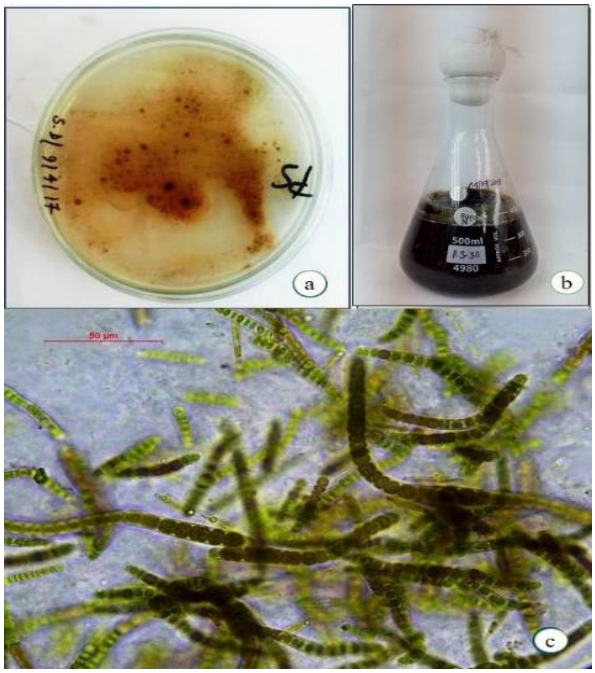


Plate 5.12: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Calothrix linearis* isolated from Vivekananda Road.

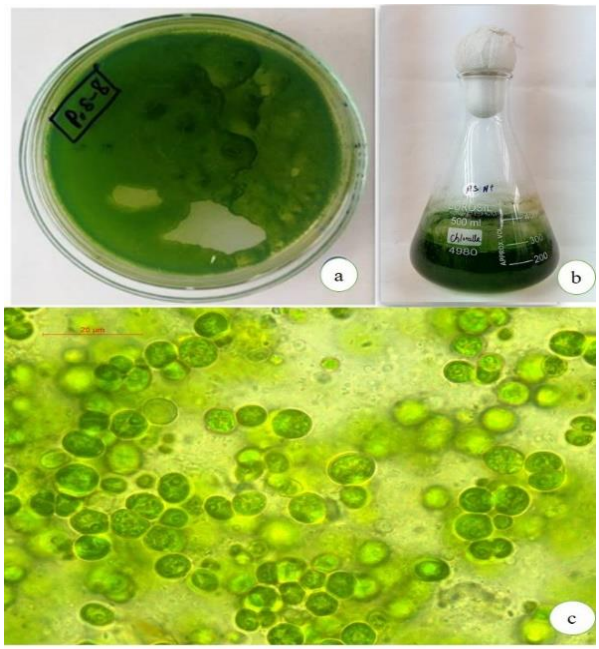


Plate 5.13: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Chlorella ellipsoidea* isolated from Vivekananda Road.

Plate 5.14: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Cylindrospermum licheniformae* isolated from Kanakpur Road.

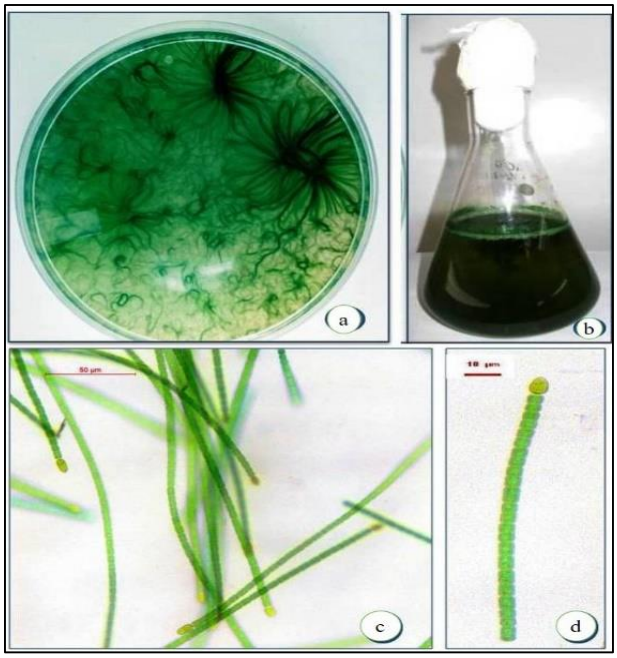


Plate 5.15: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Cylindrospermum muscicola* isolated from Ambikapatty.

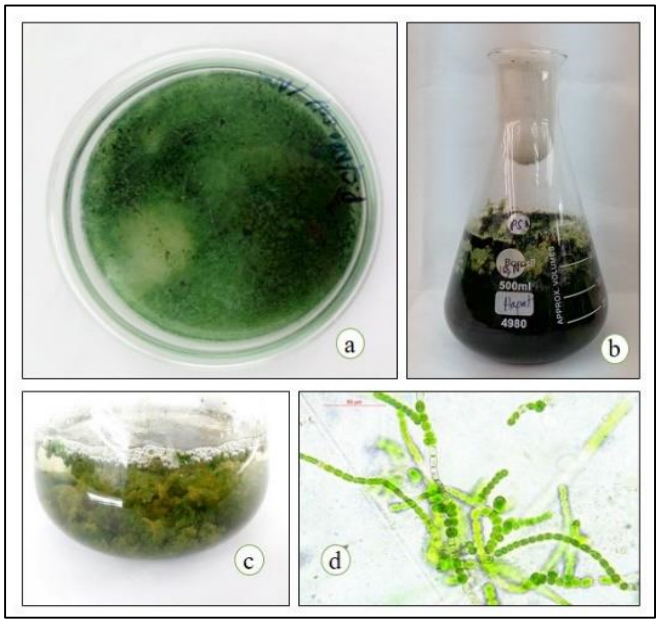


Plate 5.16: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Fischerella* sp. isolated from Rangirkhari.

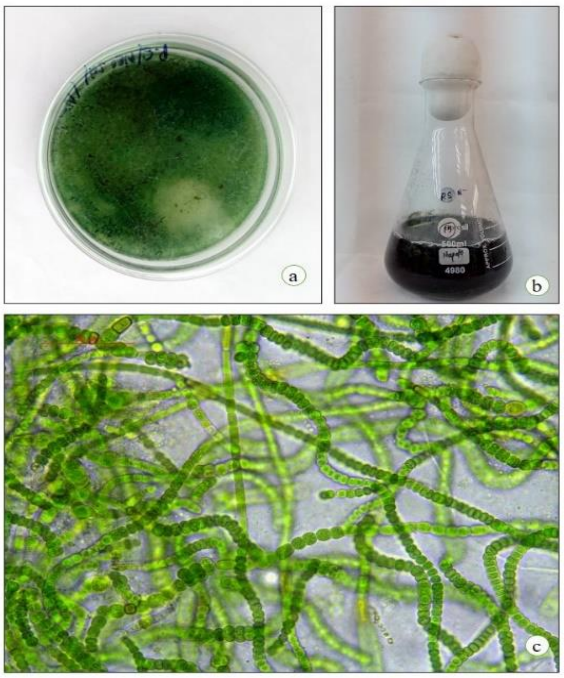


Plate 5.17: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Hapalosiphon welwitschii* isolated from Chenkoorie Road.

Plate 5.18: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Lyngbya diguetii* isolated from NH Road.

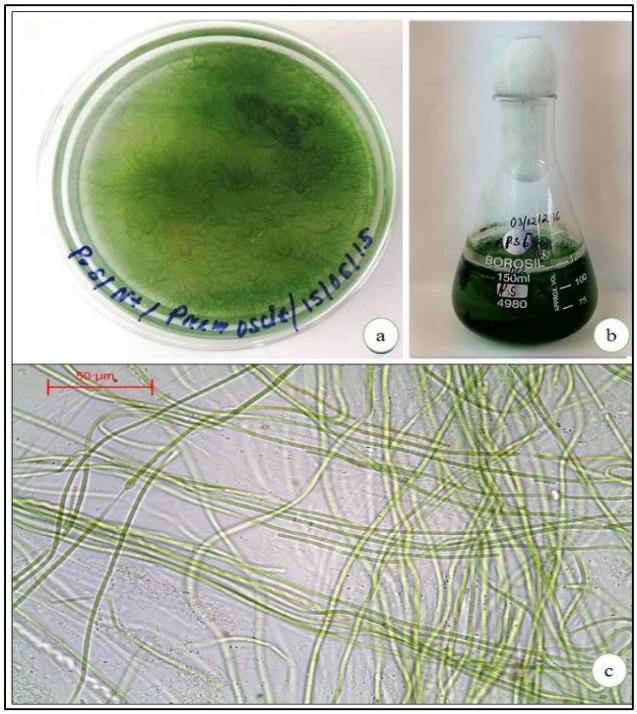


Plate 5.19: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Lyngbya nordgardhii* isolated from Station road.

Plate 5.20: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Nostoc carneum* isolated from Link road 1st.

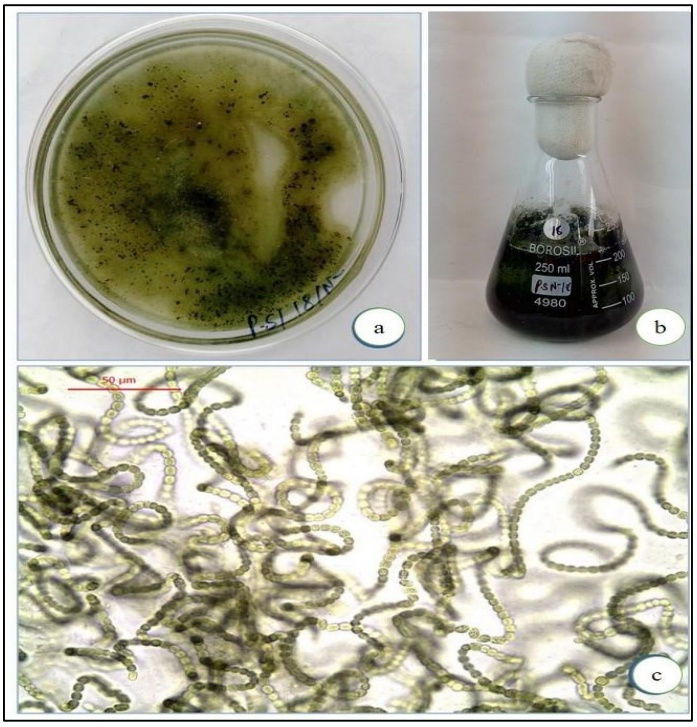


Plate 5.21: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Nostoc commune* isolated from Bilpar Road.

Plate 5.22: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Nostoc linckia* isolated from Rangirkhari.

Plate 5.23: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Nostoc muscorum* isolated from Club Road.

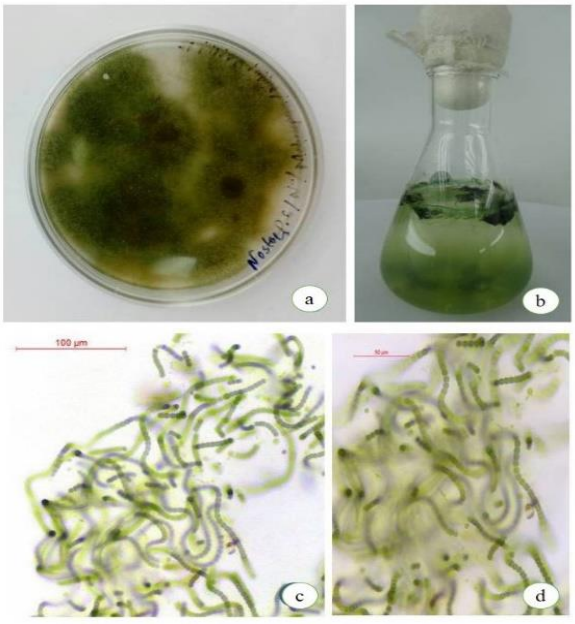


Plate 5.24: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Nostoc verrucosum* isolated from Link Road 2nd.

Plate 5.25: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Oscillatoria acuminata* isolated from Link road 1st.

Plate 5.26: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Oscillatoria curviceps* isolated from ONGC Colony.

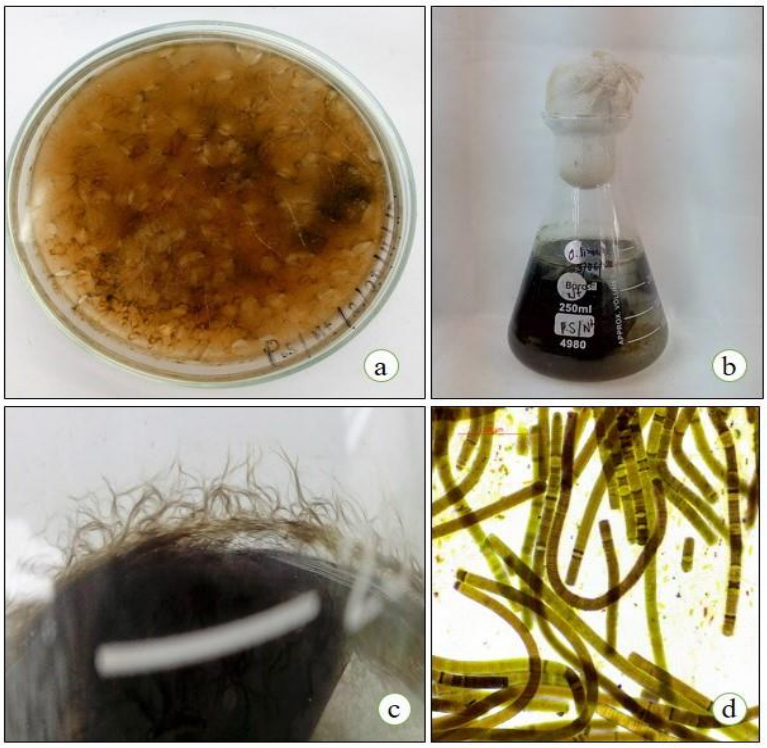


Plate 5.27: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Oscillatoria limosa* isolated from Rangirkhari.

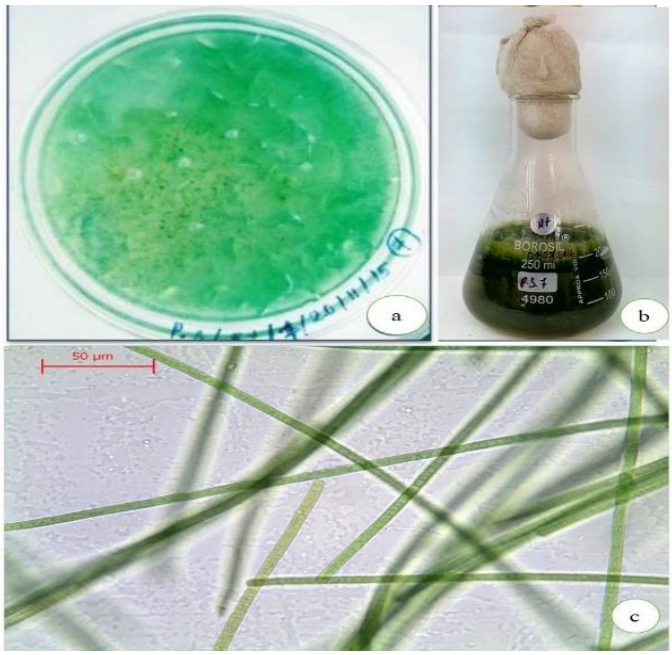


Plate 5.28: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Oscillatoria subbrevis* isolated from Premtola.

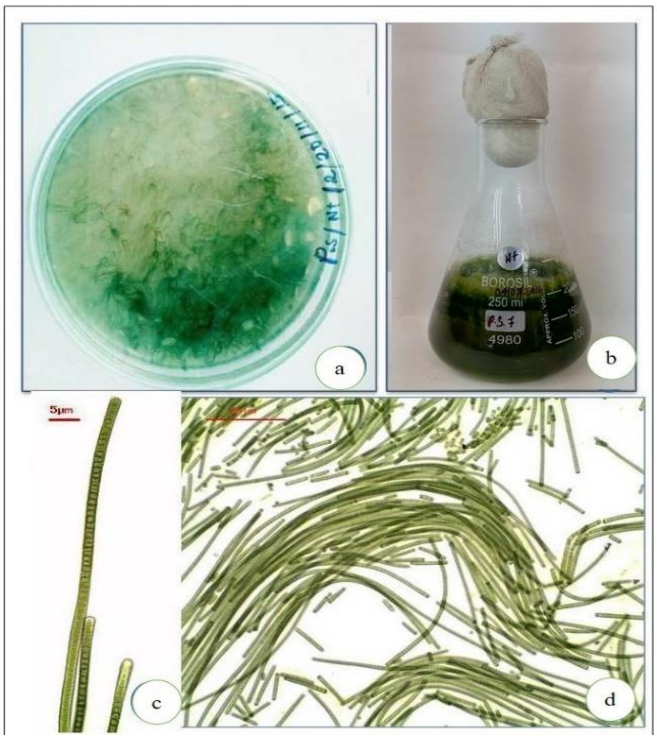


Plate 5.29: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Phormidium lucidum* isolated from Sonai Road.

Plate 5.30: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Westiellopsis prolifica* isolated from NH road.

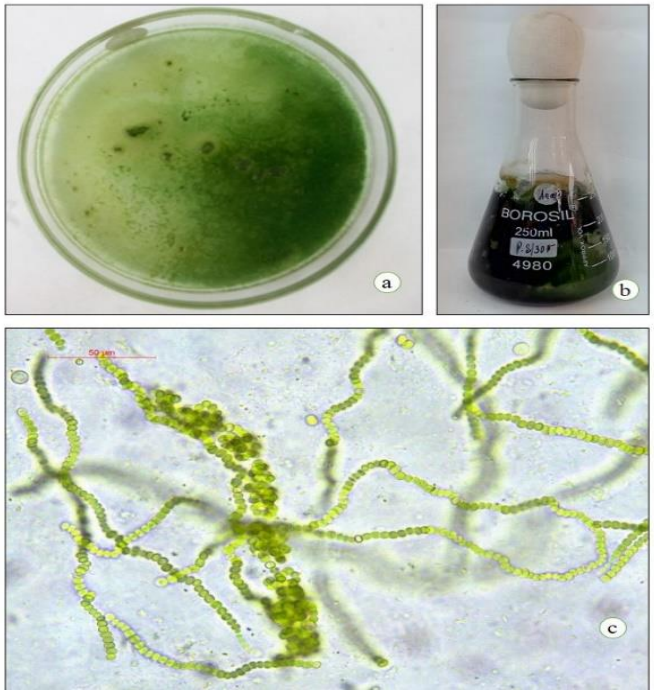


Plate 5.31: Colony development on agarized medium (a), growth behavior on liquid culture (b) and photomicrograph (c) of *Westiellopsis* sp. isolated from Link Road 2nd.

conspicuous pseudovacuoles. Heterocysts globose, 3-4.5µm long, 3µm breadth, gonidia cylindrical, arranged in regular series.

***Anabaena oscillarioides* Bory (after Frémy)** (**AUS/EC/JR/PSCYA291**) (Plate: 5.3)

The heterocystous cyanobacterial isolates was screened from polythene surface in domestic solid waste dumping site of Chenkoorie road, on March 4, 2014. Filaments are straight and olive green in colour. Cells are barrel shaped, 4-5µm long and 3µm breadth. Heterocysts are round, 5-6.5µm long and 4µm breadth. Gonidia cylindrical developing on both sides of the heterocysts.

***Anabaena variabilis* Kuetzing (AUS/EC/JR/PSCYA292)** (Plate: 5.4)

The cyanobacterial isolate was screened from polythene surface in domestic solid waste dumping site of Tarani road, on 5 February, 2014. Cells are ellipsoidal and yellowish green colour, 6-7.5µm long and 4.5-5µm breadth. Heterocyst ovate, 9-10.5µm long and 6µm breadth.

***Anabaena wisconsinense* Prescott (AUS/EC/JR/PSCYA293)** (Plate: 5.5)

The cyanobacterial isolate was screened from polythene surface in domestic solid waste dumping site of Rangirkhari, on 12 March, 2014. Trichome are planktonic in BG11 liquid medium and slightly flexuous in solid BG11 medium. Cells are quadrate to cylindrical, 6-7.5µm long and 5-6µm breadth. Heterocysts are spherical, 10µm long and 5-6µm breadth. Akinete are 5µm long and 4µm breadth. Terminal cell are 3µm long and 2µm breadth.

***Anabaenopsis arnoldii* Aptekarj v. indica Ramanathan** (**AUS/EC/JR/PSCYA288**) (Plate: 5.6)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of Karimganj road, on 11 April, 2014. Trichome are planktonic in liquid BG11 medium, short and coiled. Cells are round, 4µm long and 4µm breadth. Heterocysts are present in pair in intercalary position, 4µm long and 4µm breadth. Akinete are 4µm long and 3µm breadth. Terminal cell are 2µm long and 3µm breadth.

***Aphanothece microscopia* Näg**. (**AUS/EC/JR/PSCYA261**) (Plate: 5.7)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of Tarani road, on 17 February, 2014. Thallus gelatinous, small, blue green colour, cells cylindrical, 6-7.5µm long and 5-6µm breadth.

***Calothrix elenkinii* Kossink (after poljansky)** (**AUS/EC/JR/PSCYA265**) (Plate: 5.8)

The cyanobacterial isolate was screened from polythene surface in domestic solid waste dumping site of Sonai road, on 17 March, 2014. Filaments united in tufts, bent at the base, interlaced on each other, swollen at the base, and 6-8 µm broad. Sheath close to the trichome, thin not lamellated, colourless; trichomes olive green, at the base 5-7 µm broad, not constricted at the base, apical hair not formed, cells quadrate or somewhat shorter than; heterocysts basal, single 4.5-7 µm broad.

***Calothrix fusca* (Kütz.) Born. et Flah. f*orma* (AUS/EC/JR/PSCYA263)** (Plate: 5.9)

The cyanobacterial isolate was screened from polythene surface in domestic solid waste dumping site of Sonai road, on 12 August, 2014. Thallus filamentous, dark-green colored, planktonic in cultural behaviour having floccose type of growth pattern initially, later these were generally found attached to the walls and the bottom of the flasks in liquid medium; filaments bent and distinctly swollen (up to 15 µ) at the base; sheath colourless, diffluent

at theapices; cells often discoid, shorter than broad; heterocysts basal, single, hemispherical, smaller than the basal cell of the trichome.

***Calothrix linearis* Gardner (AUS/EC/JR/PSCYA266**) (Plate: 5.10)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of Vivekananda road, on 23 March, 2014. Filaments erect, for the major part straight, cylindrical, but swollen at the base and attenuated at the apex, mostly at the lower portions branched; sheath 2-2.5 µm thick, somewhat slimy, colourless, not lamellated, ending in a short hair, heterocysts basal, mostly hemispherical.

***Calothrix marchica* Lemm.(after Frémy) (AUS/EC/JR/PSCYA286**) (Plate: 5.11)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of Trunk road, on 27 March, 2014.` Thallus filamentous, blackish-brown in colour, planktonic in cultural behaviour having floccose type of growth pattern initially and later the filaments were generally found attached to the walls and the bottom of the flasks in liquid medium; filaments in groups, slightly bent, with a close thin colorless sheath at young stage, prominent sheath at old stage, without a terminal hair, end cell conical with rounded apex, vegetative cells quadrate or somewhat shorter than broad, heterocysts single, basal, spherical or subspherical.

***Calothrix parietina* Näg.) Thuret (after Frémy)** (**AUS/EC/JR/PSCYA262**) (Plate: 5.12)

The cyanobacterial isolate was screened from polythene surface in domestic solid waste dumping site of Link road 1st, on 11 January, 2014. Calonies olive-green, filaments up to 1 mm,at the basis 7-12 µ wide, often branched. Trichomes by the cross walls constricted or not constricted, in hair ended. Cell sizes at the basis of trichome 5-12 µ, in the middle zone 4-7µ. Heterocysts terminal, sometimes 2-3 together, with sizes 7-10×7-10µ.

***Chlorella ellipsoidea* Gerneck (AUS/EC/JR/PSGA294)** (Plate: 5.13)

The green algae isolate was screened from polythene surface in sewage water drains of Vivekananda road, on 12 May, 2014. Cells are ellipsoidal, 9µm long and 7µm breadth. During log period, they produced 32 autospores at a time.

***Cylindrospermum licheniforme* (Bory) Kütz (after Fremy) (AUS/EC/JR/PSCYA267)** (Plate: 5.14)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of Kanakpur road, on 12 February, 2014. Filaments entangled and forming an expanse of

macroscopic proportions, dark green in color. Cells short cylindric; constricted at the cross walls; 2.5-4.2 µ in diameter, 4-5µ long. Heterocysts elongate, 5-6µ in diameter, 7-12µ long. Gonidia solitary, elongate ellipsoid to oblong; the wall thick and smooth; 12-14µ in diameter, 20-38µ long.

***Cylindrospermum musicola* Kütz (after Fremy) (AUS/EC/JR/PSCYA268)** (Plate: 5.15)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of Ambikapatty, on 13 May, 2014. Thallus filamentous, colony mucilaginous and expanded, olive green in colour; filaments attached to the vertical glass surface in batch culture at young stage, later at the stage of spore development finger like protrude developed attachedto the bottom of the conical flask, cells quadrate to cylindrical with slight constriction at the cross walls, 3-4.5 µm wide, 3.5-5 µm long; heterocysts 4-5 µm wide, 5-7 µm long; akinate single, ovoid, 9-12 µm wide, 10-20 µm long, rounded at the ends, walls smooth.

***Fischerella* sp (AUS/EC/JR/PSCYA270)** (Plate: 5.16)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of Rangirkhari, on 11 March, 2014. Thallus bright green, filamentous, plant mass floccose; Main filaments creeping, torulose, flexous interwoven, initially submerged and later floating; thin membranous biomass, lateral branches almost erect with false branches, filaments elongated. Trichome uniseriate and 5 µm broad, heterocyst elongate, spherical and sometimes compressed with 7 µm broad.

***Hapalosiphon welwitschii* W.et.Gs.West (AUS/EC/JR/PSCYA269)** (Plate: 5.17)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of Chenkoorie road, on 11 March, 2014. Filaments tortuous and creeping in BG11 liquid medium. Curved branches are arises from both side of the filament. Cells are ellipsoidal, 4µm long and 3µm broad. Heterocysts are ellipsoidal, 4µm long and 2µm broad.

***Lyngbya diguetii* Gom. (after Frémy) (AUS/EC/JR/PSCYA271)** (Plate: 5.18)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of NH road, on 12 July, 2014. Plants solitary or entangled, sometimes forming bundles, frequently adhering to and growing out from filamentous algae which have non-mucilaginous walls; filaments up to 3.2µ in diameter, sheaths thin; trichomes 2.5-3 µ in diameter, cells 1.5-3.5µ long; apical cell convex, smooth.

***Lyngbya nordgardhii* Wille (AUS/EC/JR/PSCYA272)** (Plate: 5.19)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of Station road, on 10 September, 2014. Plants solitary or forming minute patches on the walls of larger filamentous algae,curved and vermiform,sometimes recurved from a basal attachment;trichomes gray-green,not tapering at the apices,1.2-2µ in diameter,about as long as wide or a little shorter,sheaths very thin and transparent.

***Nostoc carneum* Ag. (after Frémy) (AUS/EC/JR/PSCYA273)** (Plate: 5.20)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of Link road 1st, on 17 March, 2014. Colony at first spherical, later irregular and eventually spread out, soft and slimy; old colonies a range of colours,including blue-green,but often red-brown or pink or violet.Filamentous flexuous; sheath indistinct,colourless.Cells subsperical to barrel-shaped,3-4 µ wide,longer than wide. Heterocyst 5.5-6.5µ wide. Akinete ellipsoid, 5-6 µ wide, 8-10 µ long ; wall smooth, colourless.

***Nostoc commune* Vaucher ex Born. et Flah (AUS/EC/JR/PSCYA274)** (Plate: 5.21)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of Bilpar road, on 18 April, 2014. Thallus firm, expanding, undulated, membranous, brownish on the agarized medium. homogenized powdery sedimentation was observed in liquid medium; filaments flexous, closely entangled forming globose membranous structure at young stage, sheath mostly distinct only at the periphery; at old stage, filaments more or less straight, loosely entangled, cells spherical, epispore smooth colorless, trichome 3.9-4.3 µm broad. Cells short barrel shaped or nearly spherical, mostly shorter or a little longer than broad, 5µm long, heterocysts nearly spherical, about 4.3 µm broad.

***Nostoc linckia* v. (Roth) Born. et Flah. (after Frémy) (AUS/EC/JR/PSCYA276))** (Plate: 5.22)

The cyanobacterial isolate was screened from polythene surface in domestic solid waste dumping site of Rangirkhari, on 12 October, 2014. Thallus gelatinous, brownish green in color, varying in size, at first globose later irregularly expanding on agarized medium, sedimentation was seen in liquid medium; filaments densely entangled, flexuous or highly coiled; trichomes 4-5 µ broad, cells short barrel-shaped; heterocysts spherical.

***Nostoc muscorum* (after Bornet and Thuret) (AUS/EC/JR/PSCYA277)** (Plate: 5.23)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of Club road, on 19 March, 2014. The thallus were gelatinous, dark blue-green in color, colony wide round shaped on agarized medium, sedimentary cultural behavior in liquid culture were observed, filaments highly coiled and densely entangled, trichome 3-4µm broad, cells spherical to barrel shaped, heterocysts were nearly spherical and 4-7µm broad.

***Nostoc sp* (AUS/EC/JR/PSCYA278)** (Plate: 5.24)

The cyanobacterial isolate was screened from polythene surface in domestic solid waste dumping site of Link road 2nd, on 7 June, 2014. Cells are round, 2µm long and 2µm broad. Heterocysts are round to ovate, 2µm long and 1µm broad.

***Oscillatoria acuminata* Gom. (after Gomont) (AUS/EC/JR/PSCYA282)** (Plate: 5.25)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of Link road 1st, on 20 February, 2014. Trichomes blue-green,straight,not constricted at the cross-walls,cells 3-5 µm broad,5-8µm long;ends tapering pointed,bent and generally mucronate,calyptra absent.

***Oscillatoria curviceps* forma (after Rao, C.B.) (AUS/EC/JR/PSCYA279)** (Plate: 5.26)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of ONGC Colony, on 21 January, 2015. Trichomes forming blue-green and twisted, tapering to the apex. Apical cell broadly rounded. Cells 3-5 µm broad,5-8µm long and granulate.

***Oscillatoria limosa* Ag. (after Gomont) (AUS/EC/JR/PSCYA281)** (Plate: 5.27)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of Rangirkhari, on 22 June, 2014. Trichomes dark brownish, filaments straight. Apical cell rounded. Cells 4-58µm long and 2.5-3µm broad.

***Oscillatoria subbrevis* Schmidle (orig.) (AUS/EC/JR/PSCYA280)** (Plate: 5.28)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of Premtola, on 12 February, 2014. Filaments solitary. Apical cell rounded. Cells 4-5µm long and 2.5-3µm broad. Cell contents pale green

***Phormidium lucidum* Kütz. (AUS/EC/JR/PSCYA283)** (Plate: 5.29)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of Sonai road, on 13 May, 2014. Thallus pale blue-green colour,trichomes flexuous,7-8µ

broad,sheath diffluent,sometimes slightly attenuated at the apex; cross-wall constricted and granulated ; cells 2-2.5µ long,end cell with a calyptra.

***Westiellopsis prolifica* Janet (after Janet) (AUS/EC/JR/PSCYA290)** (Plate: 5.30)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of NH road, on 23 March, 2014. Thallus filamentous, green in color, plant mass are planktonic floccose, with true branching filaments of two kinds, primary filaments thicker and creeping, with short barrel-shaped cells, 5-16 µm broad, secondary filaments thinner and erect, not constricted at the cross walls, with elongate, cylindrical cells, 2-4 µm broad; filaments without a sheath and consisting of a single row of cells; heterocysts intercalary, oblong cylindrical, 2-6 µm broad and 9-15 µm long, sometimes round (5-8 µm diameter); the dilated terminal portion of secondary branches by profuse transverse and longitudinal divisions forming chains of rounded cells (pseudohormocysts), gonidia formed singly in each cell of the pseudohormocysts .

***Westiellopsis sp* (AUS/EC/JR/PSCYA291)** (Plate: 5.31)

The cyanobacterial isolate was screened from polythene surface in sewage water drains of Link road 2nd, on 24 April, 2014. Cells are short barrel-shaped cells, 2.5-3µm long and 2µm broad. Heterocysts are barrel shaped and 2µm long and 2µm broad.

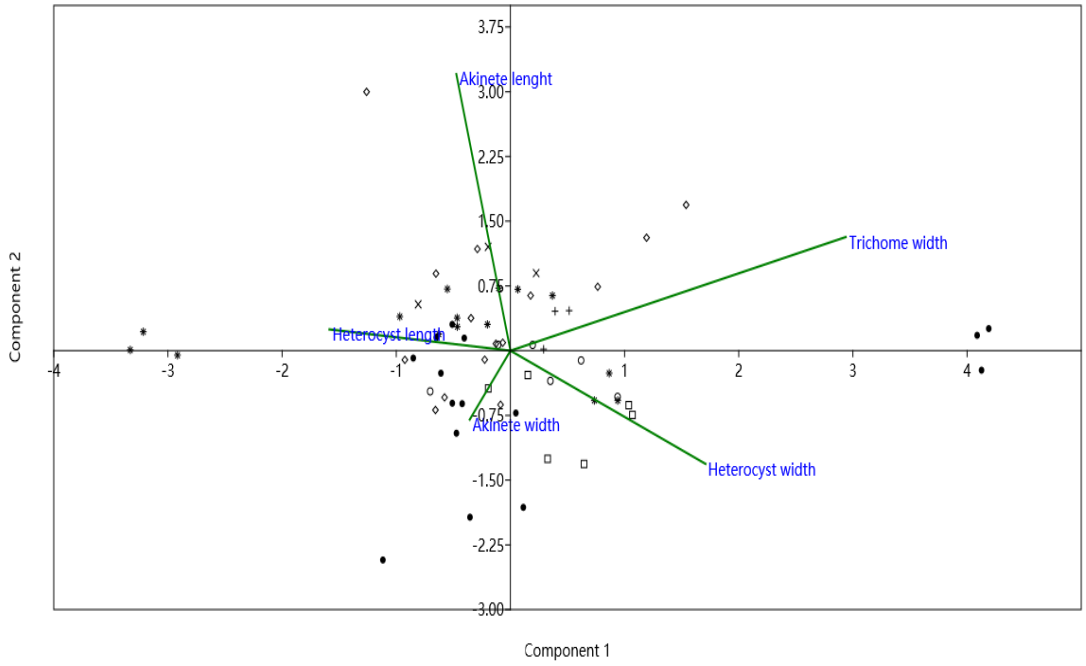


Fig. 5.1 PCA plot based on the morphological characteristics of studied heterocystous cyanobacterial isolates (*-*Anabaena*, x-*Anabaenopsis*, ·-*Calothrix*,□-*Cylindrospermum*, +-*Hapalosiphon*, o- *Fischerella*,◇-*Nostoc*).

Table 5.2 Component loading scores for PCA with the two dimensions

PC	Eigenvalue	% variance
Akinete width	9.16278	58.756
Akinete length	3.49776	22.429
Heterocyst width	1.67654	10.751
Heterocyst length	0.723648	4.6404
Trichome width	0.533896	3.4236

In the present study, results indicated that akinetes and heterocyst were important taxonomic characters for heterocystous cyanobacterial isolates. PCA of all morphological characters of heterocystous cyanobacterial isolates showed that total variance is attributable to variance in width and length of akinetes and heterocysts (**Fig. 5.1**). The component loading scores with the two dimensions are given at **Table 5.2**. In the present

study, akinete width, akinete length, heterocyst width, heterocyst length and trichome width were studied and they were found to be correlated. The contribution of five component on the total variance of the data (**Table 5.2**) explained that akinete width, akinete length, heterocyst width were important in morphological characterization of the isolates. The shape of the akinetes and heterocysts were found to quite stable in between the genera. Several workers found that position of heterocysts, position of akinetes, shape of terminal cell and width of vegetative cells useful in morphological characterization of heterocystous cyanobacteria (Stulp and Stam, 1982, 1985). In the present study, *Oscillatoria*, *Phormidium*, and *Lyngbya* were differentiated by sheath properties (Geitler 1932). Under different environmental and culture conditions, sheath production may show different morphology (Rippka *et al.*, 1979, Whitton 1992).

Conclusion

Morphological characteristics i.e., cell morphology (cell colour, cell shape, sheath, gas vacuoles, heterocyst and akinete position), ultrastructure (cell wall and sheath structure), trichome type, tapped, straight, helical, septate, shape of terminal cell, branching (true or false), culture conditions together with habitat ecology can provide a better characterization of algal strains. A total of 33 algal strains were isolated from polythene surfaces in domestic sewage water and domestic solid waste dumping site. The isolated algae from the polythene surfaces can provide the beneficial application towards promising candidates for polythene degradation, food and nutraceuticals importance, biofertilizers and domestic sewage water remediation. Further molecular and chemotaxomic characterisation of the isolated algae will be needful for better understanding of the algae colonised on polythene surfaces.

Chapter 6

GROWTH KINETICS AND BIOCHEMICAL EVALUATION OF ALGAL SPECIES

As discussed already, algae are known to occupy a broad range of habitats across all latitudes and are believed to be accummulate macromolecules such as protein, carbohydrates, and lipids through CO_2 fixation by means of photosynthesis (Fogg *et al.,* 1973; Borowitzka, 2013; Wijffels *et al.*, 2013). Algae are regarded as cell factory for polysaccharides (β-1,3-Glucan, gelling agents, starch and cellulose), lipids (polyunsaturated, fatty acids, and hydrocarbons), bioactive compounds (terpenes, various antibacterial, anti-viral and anti-fungal), biopolyesters (poly hydroxybutrate and lactic acid), proteins (amino acids, polypeptides and enzymes) and pigments (chlorophylls, carotenoids and phycobilins) (Koller *et al.,* 2014). Growth of algae is

defined as the increase in mass by the synthesis of macromolecules. In a batch culture, where food supply is limited, algae generally showed several different phases, such as adaption (lag phase), accelerating growth phase, exponential growth (log phase), decreasing log growth (linear growth), stationary phase, accelerated death and log death. Growth rate is generally determines in the log phase of an algal culture to know the state of a culture. The rate of exponential growth in an algal culture is expressed as generation time (G) and from the initial biomass in a fixed time, the doubling rate of algae can be calculated (Becker, 1994).

Different pigments present in algae are associated with the light harvesting, CO_2 fixation, and protection of cells from the environmental damage. Chlorophyll are present in algae, higher plants and cyanobacteria. Chlorophyll is the mostly used in the food pigmentation and dietary supplements (Koller *et al.,* 2014). Carotenoids are regarded as secondary light harvesting pigments in algae act as antioxidants that inactivate ROS formed by exposure to excessive solar radiation. Carotenoids such as β-carotene, astaxanthin, canthaxanthin, violaxanthin, fucoxanthin, zeaxanthin and lutein use as food additive, animal feed, pharmaceutical and cosmetics products (Matos, 2017). Phycobiliproteins (PBPs) are the secondary light harvesting pigment found in algae and they are unique among all photosynthetic pigments as they form protein-protein complexes (Koller *et al.,* 2014). The PBPs are believed to be a strong antioxidant which have antiviral, antitumor, anti-inflammatory and antifungal activities (Raj and Rajashekhar, 2015). The Vitamin A, C, K and B complex can be found in various algae. Total phenolic compounds in algae can act as electron donor in order to form stable radical intermediates (Jimenez-Escrig *et al.,* 2001). Algae generally considered as the potent source of antioxidants due to higher contents of ascorbic acid, phenols and flavonoids (Wu *et al.,* 2010).

The present study therefore addresses the screening of biochemical constituents of algae isolated from submerged polythene surface in domestic sewage water.Hence the present study addresses with the preliminary screening of the isolates based on their growth kinetics, pigment composition, biochemical constituents such as total carbohydrates, solubleproteins, acid, tannin, flavonoids, polyphenols, total phenolics content and phycobiliproteins content.

6.2 Methodology

Details of the methods of estimation of the growth curves and biochemical analysis were already described at **Chapter 3**. The abbreviations used for different strains of cyanobacteria are given in **Table 6.1**. All the experiments were replicated three times. The data obtained were subjected to one way ANOVA by Tukey multiple comparison of the means using SPSS V-19. Significant differences were indicated at $p< 0.05$.

Table 6.1 List of cyanobacterial strains along with abbreviation used for the biochemical analysis

Sl. No.	**Name of the strain**	Abbreviation
1	*Phormidium lucidum*	E1
2	*Oscillatoria subbrevis*	E2
3	*Oscillatoria acuminata*	E3
4	*Oscillatoria curviceps*	E4
5	*Oscillatoria limosa*	E5
6	*Lyngbya diguetii*	E6
7	*Lyngbya nordgardhii*	E7
8	*Aphanothece microscopia*	E8
9	*Chlorella ellipsoidea*	E9
10	*Nostoc carneum*	E10
11	*Nostoc commune*	E11
12	*Nostoc muscorum*	E12
13	*Nostoc linckia*	E13
14	*Nostoc sp*	E14
15	*Fischerella sp*	E15
16	*Calothrix fusca*	E16
17	*Calothrix parietana*	E17
18	*Calothrix marchica*	E18
19	*Calothrix elenkinii*	E19
20	*Calothrix sp*	E20
21	*Cylindrospermum musicola*	E21
22	*Anabaena variabilis*	E22
23	*Anabaena anomala*	E23
24	*Anabaena flos-aquae*	E24
25	*Westiellopsis prolifica*	E25
26	*Anabaena wisconsinense*	E26
27	*Westiellopsis*sp	E27
28	*Hapalosiphon flexuosus*	E28
29	*Anabaenopsis arnoldii*	E29
30	*Anabaena oscillatoriales*	E30

6.3 Results and discussion

6.3.1 Growth kinetics of the isolated strains

The growth curves of 30 algal species were presented in Fig. 6.1 to 6.5.During the growth period, the isolates represents the lag phase upto 5 days and this stage is regarded as the earliest and most poorly understood growth stage of the isolates. In this period, the isolates tries to adapt in BG11 media, this phase was short due to inoculum which added to freshly prepared BG11 was fresh culture.The log phase in BG11 media begins after 5 days and towards the late log phase, the cell division proceeds at a constant rate. Generation time (G) and specific growth rate (K) are evaluated in this period. The generation time and specific growth rate are shown in Table 6.2. The maximum growth rate (Table 2) has been shown by *Oscillatoria subbrevis* (E2) ($0.158\mu d^{-1}$) followed by *Anabaena oscillatoriales* (E30) ($0.157\mu d^{-1}$). The generation time was maximum in *Anabaena anomala* (E23) (277.86h) and minimum in *Calothrix* sp. (E20) (109.69h).

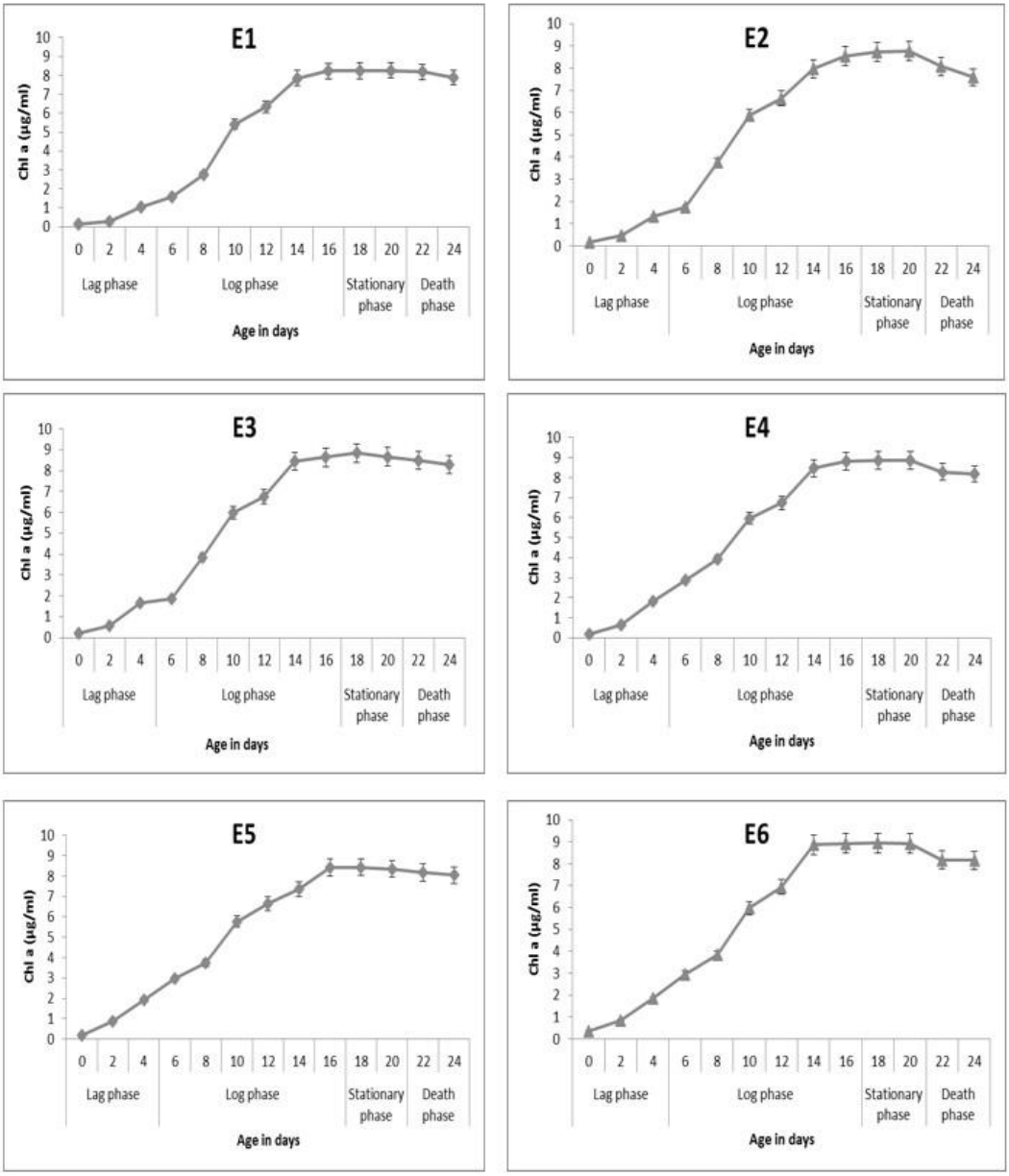


Fig. 6.1 Growth curves of the isolated cyanobacteria (E1-E6)

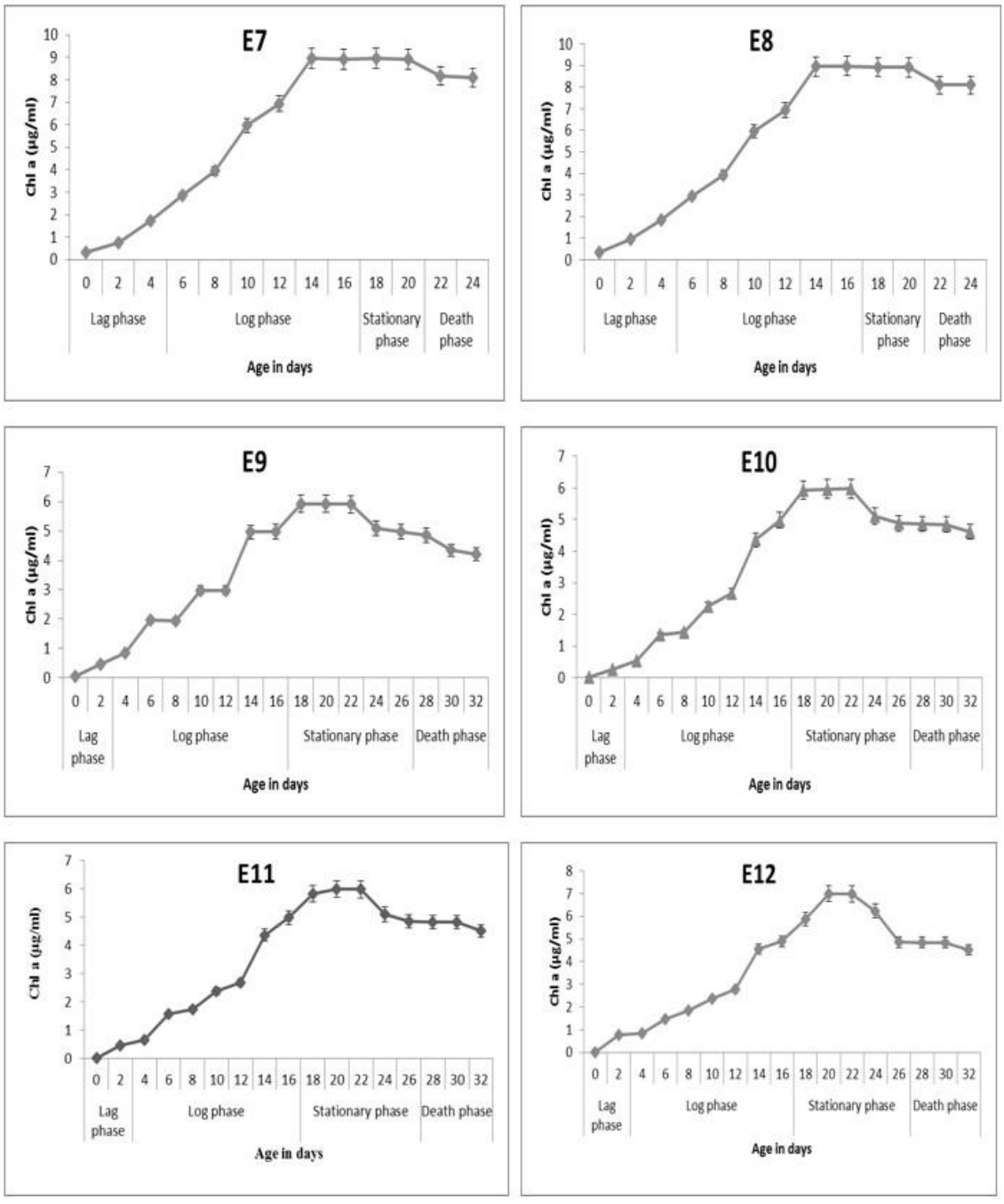


Fig. 6. 2 Growth curves of the isolated cyanobacteria (E7-E12)

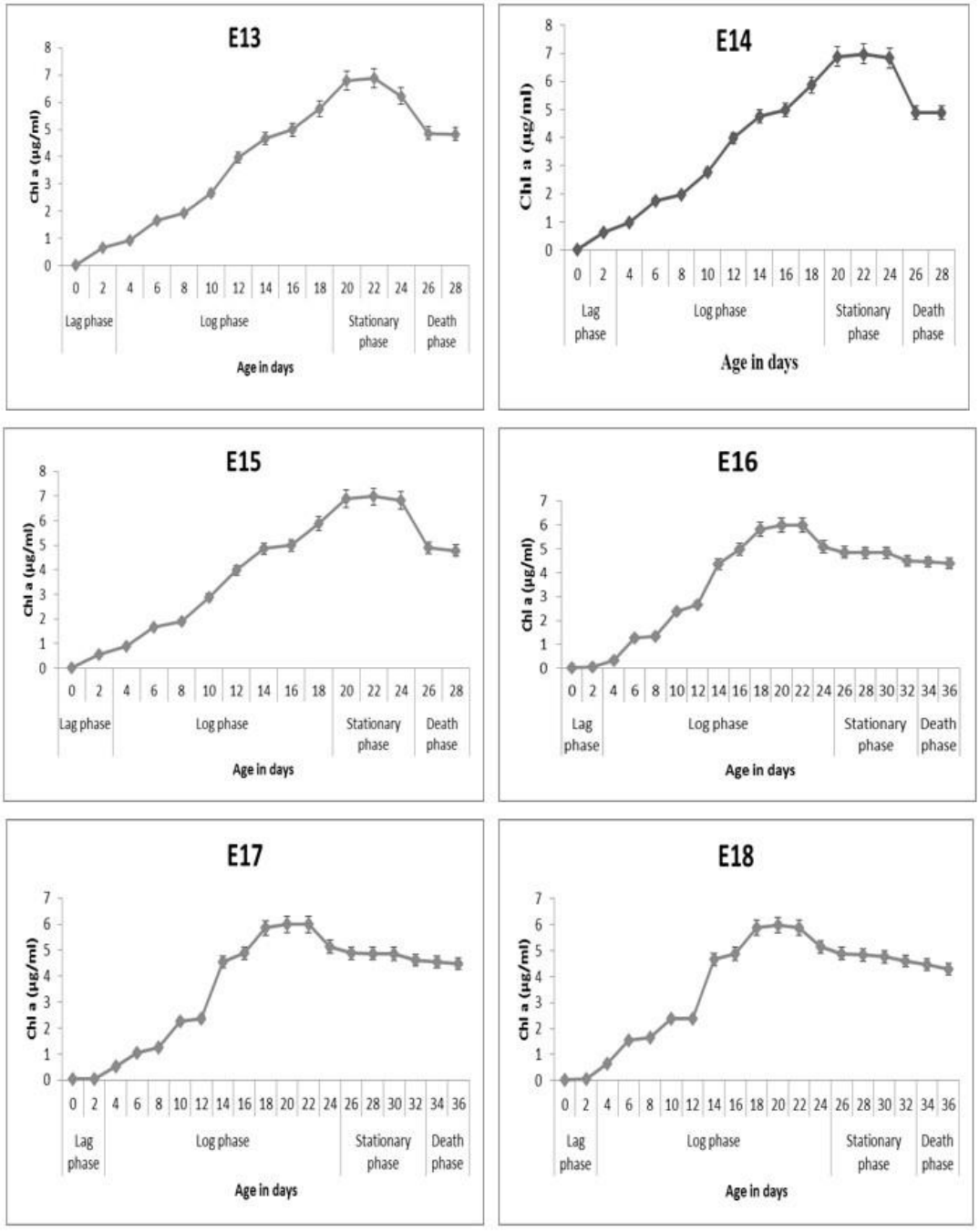


Fig. 6.3 Growth curves of the isolated cyanobacteria (E13-E18)

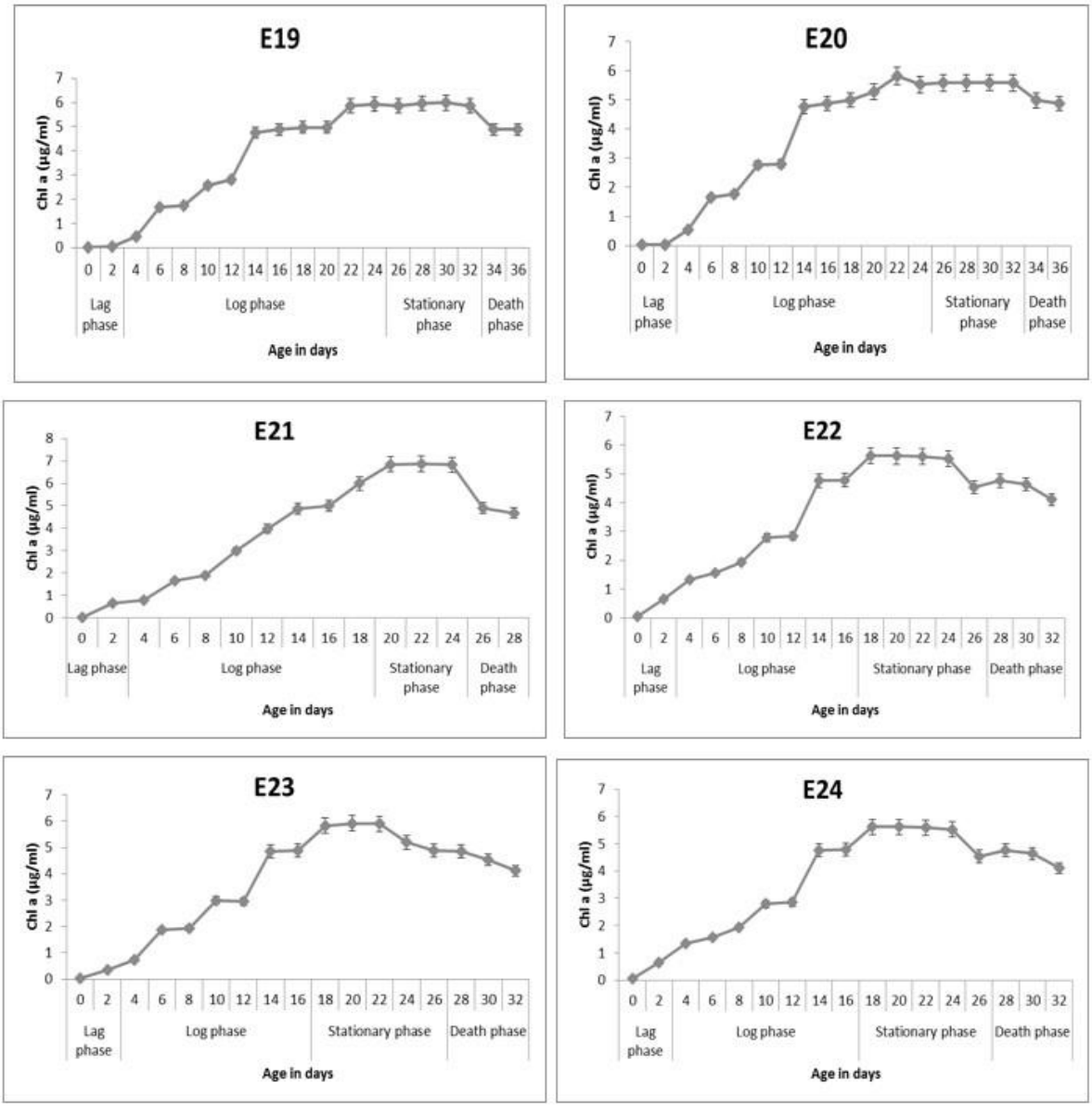


Fig. 6.4 Growth curves of the isolated cyanobacteria (E19-E24)

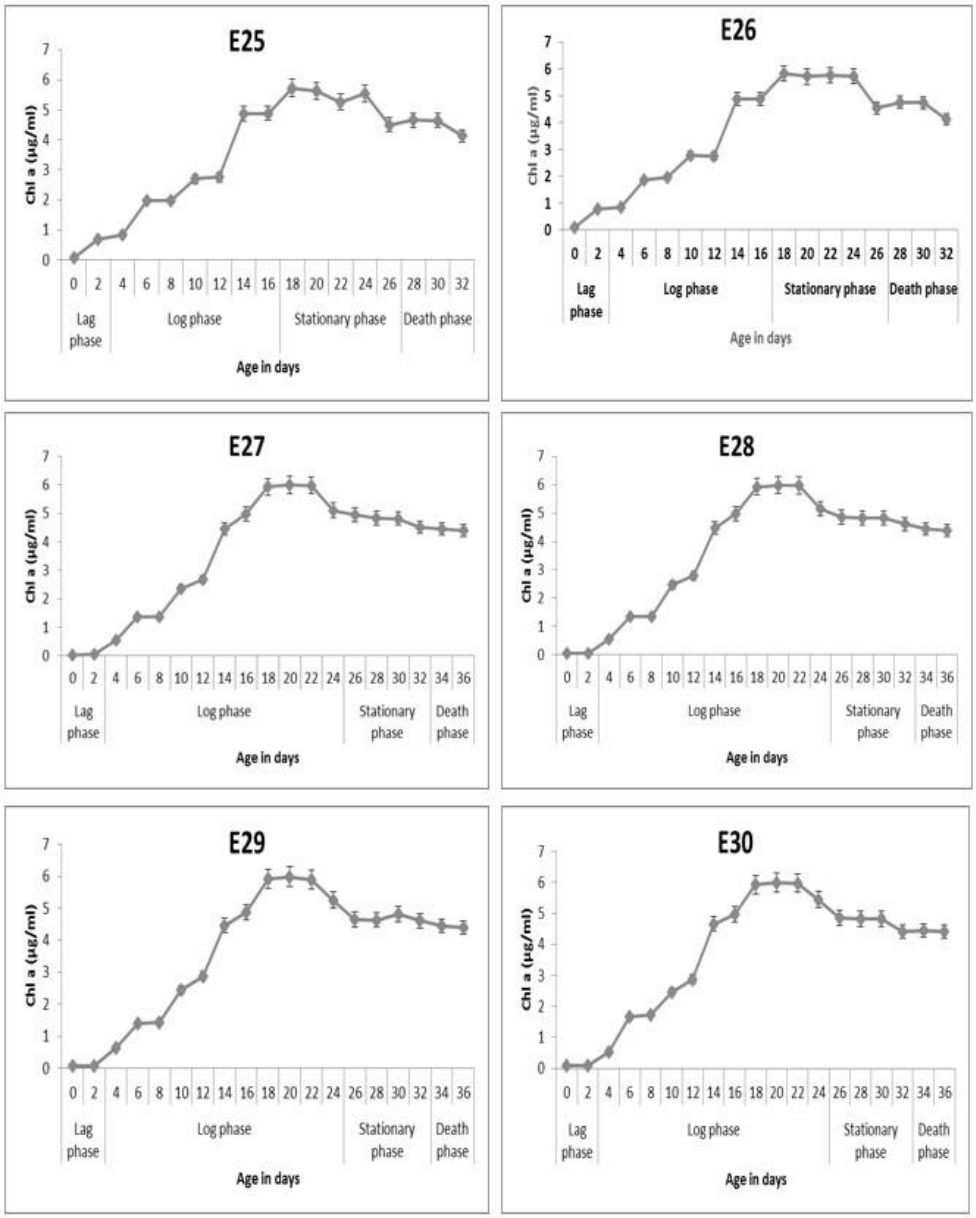


Fig. 6.5 Growth curves of the isolated cyanobacteria (E25-E30)

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

Serial no.	Strains	K(μd^{-1})	G(h)
1	**E1**	0.134 ± 0.002	178.25 ± 0.51
2	**E2**	0.158 ± 0.001	151.34 ± 0.32
3	**E3**	0.135 ± 0.002	151.56 ± 0.23
4	**E4**	0.103 ± 0.003	159.52 ± 0.31
5	**E5**	0.109 ± 0.001	218.22 ± 0.20
6	**E6**	0.138 ± 0.002	173.67 ± 0.42
7	**E7**	0.147 ± 0.004	162.64 ± 0.34
8	**E**8	0.132 ± 0.002	124.42 ± 0.32
9	**E**9	0.149 ± 0.002	161.03 ± 0.13
10	**E**10	0.152 ± 0.002	157.21 ± 0.14
11	**E**11	0.134 ± 0.003	178.83 ± 0.34
12	**E**12	0.132 ± 0.002	131.67 ± 0.31
13	**E**13	0.154 ± 0.001	155.74 ± 0.54
14	**E**14	0.127 ± 0.002	143.31 ± 0.43
15	**E**15	0.147 ± 0.001	163.16 ± 0.32
16	**E**16	0.155 ± 0.003	123.86 ± 0.43
17	**E**17	0.118 ± 0.001	157.38 ± 0.21
18	**E**18	0.128 ± 0.002	122.96 ± 0.14
19	**E**19	0.119 ± 0.001	200.25 ± 0.12
20	**E**20	0.118 ± 0.004	109.69 ± 0.26
21	**E**21	0.136 ± 0.002	153.89 ± 0.12
22	**E**22	0.13 ± 0.001	184.36 ± 0.23
23	**E**23	0.086 ± 0.001	277.86 ± 0.24
24	**E**24	0.19 ± 0.003	184.37 ± 0.20
25	**E**25	0.126 ± 0.004	125.53 ± 0.13
26	**E**26	0.143 ± 0.001	156.39 ± 0.11
27	**E**27	0.123 ± 0.002	194.86 ± 0.23
28	**E**28	0.106 ± 0.001	122.16 ± 0.10
29	**E**29	0.137 ± 0.002	174.92 ± 0.12
30	**E**30	0.157 ± 0.001	152.69 ± 0.21

6.3.2 Results and Discussion

The biochemical analysis of 30 species of algal species revealed the chlorophyll *a* to be in the range of 8.47-0.98µgml^{-1}. The maximum chlorophyll *a* present in E1 (*Phormidium lucidum*) and minimum in E24 (*Anabaena flos-aquae*) and E30 (*A. oscillatoriales*). The range of carotenoids was 3.44-0.78µgml^{-1}. The maximum carotenoids content was noted in E5 (*Oscillatoria limosa*) and minimum in E20 (*Calothrix linearis*). The range of carbohydrates content in algal isolates was 265.71-78µgml^{-1}. The maximum carbohydrate content was observed in E17 (*Calothrix parietana*) and minimum in E30 (*A. oscillatoriales*). The protein range was 378-79µgml^{-1}. The maximum protein content found in E5 (*Oscillatoria limosa*) and minimum in E30 (*A. oscillatoriales*). The range of phycobiliproteins content in algal isolates was 102.55-31.7µgml^{-1}. Phycocyanin (PC) content was maximum in E5 (*Oscillatoria limosa*) (60.7µgml^{-1}) and minimum in E26 (*Anabaena wisconsinense*) (12µgml^{-1}). Phycoerythrin (PE) content was maximum in E15 (*Calothrix fusca*) (42µgml^{-1}) and minimum in E20 (*Calothrix linearis*) (3.2µgml^{-1}). Allophycocyanin (APC) was maximum in E12 (*Nostoc muscorum*) (31µgml^{-1}) and minimum in E23 (*Anabaena anomala*) (7.6µgml^{-1}). The range of lipid content in algal isolates was 11.2-1.0µgml^{-1}. The maximum lipid content was noted in E9 (*Oscillatoria limosa*) (16.6µgml^{-1}) and minimum in E22 (*Anabaena variabilis*). EPS content in algal isolates was found to be 35.82-3.7µgml^{-1}. The maximum EPS was noted in E29 (*Anabaenopsis arnoldii)* (35.82µgml^{-1}) and minimum in E13 (*Nostoc linckia*). The polyphenol content in algal isolates was 7.8-1.2µgml^{-1}. The maximum polyphenol content was noted in E19 (*Calothrix elenkinii*) (7.8µgml^{-1}) and minimum in E7 (*Lyngbya nordgardhii*) and E14 (*Nostoc* sp) (1.2µgml^{-1}). The total phenolics content was found to be in the range of 32-18µgml^{-1}. The maximum total phenolics content was noted in E3 (*Oscillatoria acuminata*) and E4 (*Oscillatoria curviceps*) (32µgml^{-1}) and minimum in E5 (*Oscillatoria limosa*) (18µgml^{-1}). The range of flavonoids content in algal isolates was 9.7-1.2µgml^{-1}. The maximum flavonoids content was observed in E25 (*Westiellopsis prolifica*) (9.7µgml^{-1}) and minimum in E17 (*Calothrix parietina*) (1.2µgml^{-1}). The tannin range was 7.8-2.0µgml^{-1}. The maximum tannin content was noted in E1 (*Phormidium lucidum*) (7.8 µgml^{-1}) and minimum was found to be in E21 (*Cylindrospermum muscicola*) (2.0µgml^{-1}). The biochemical constituents of algal isolates screened from polythene surface submerged in domestic sewage water showed

that algal isolates contain high cellular constituents of chlorophyll *a*, carotenoids, total carbohydrate, protein, total phycobiliproteins, lipid, EPS, polyphenol, total phenolics, flavonoids and tannin. Significant differences were observed in biochemical constituents between the isolates. The biochemical characteristics of the species might be ascribed to the physico-chemical parameter of domestic sewage water. It is reported that the cellular composition of algal species depend on the nature of strains, physiological state of the isolates and the environment from where they have collected (Smith and Schindler, 2009; Vargas *et al.,* 1998; Subhashini *et al.,* 2003; Rosales *et al.,* 2005). The algal isolates were found well adapted to the oxygen depleted condition of the domestic sewage water and it was presumed that in the absence of additional nitrogen source. The carbon fixation pathway might be switched from the protein synthesis leading to higher accumulation of the constituents (Fogg, 1975). The extent of dissolved solid and suspended solid of domestic sewage water was quite different in all the twenty sites. Therefore, the sunlight penetration on submerged polythenes is anticipated to be different. Grossman *et al*., (1993) opined that environmental condition of species might alter the composition and abundance of phycobiliproteins. In the present study, the physico chemical parameters of the twenty sites were at variance. This, we believe, might have caused a variation in the total phycobiliproteins in the species studied. In a previous study, some of the algal species *Phormidium angustissimum, Lyngbya holdenii, Anabaena doliolum, Calothrix marchica* and *Fischerella muscicola* isolated from lime sludge waste of a paper mill showed higher accumulation of chl *a*, phycocyanin, carbohydrates and protein (Paul and Rout, 2017; Paul, 2017). The present study found conformity with the studies made by Paul (2016) and Paul and Rout (2017).

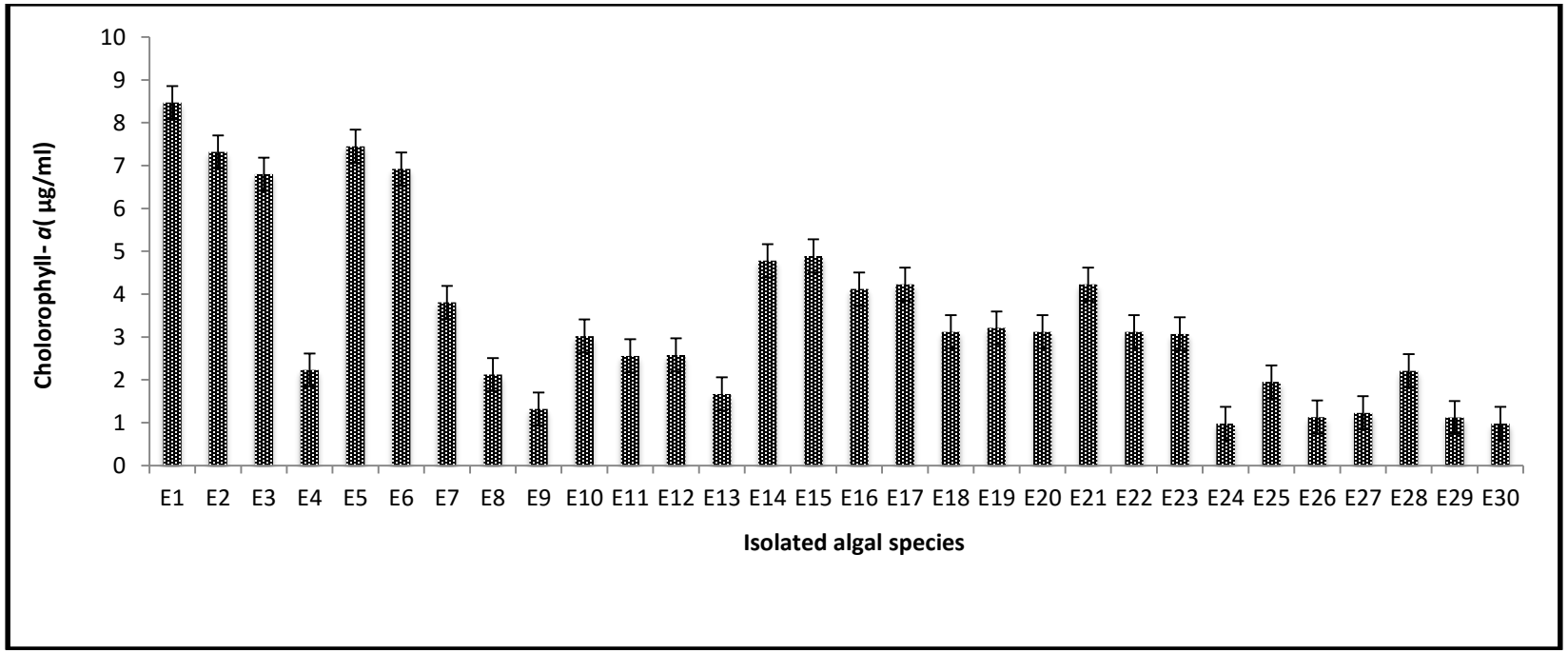


Fig. 6.6 Variation of chlorophyll-*a* contents in different isolates, P<0.05

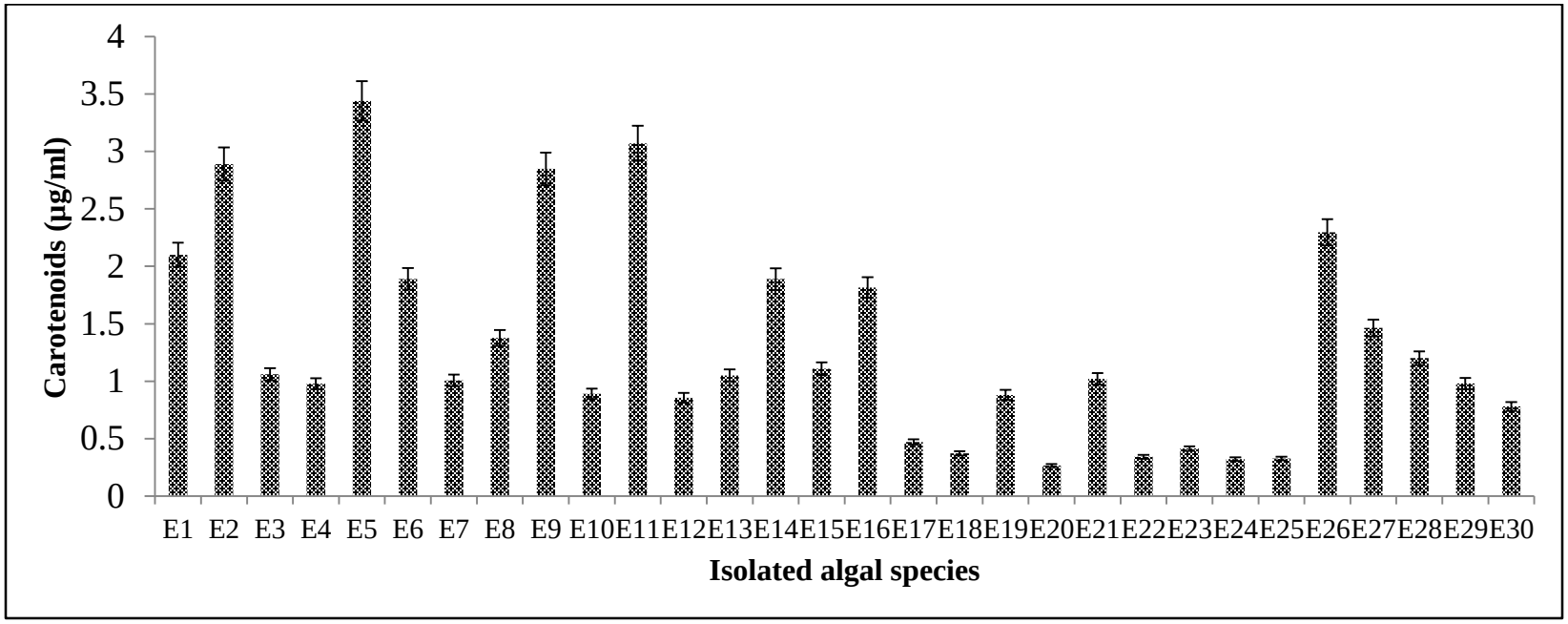


Fig. 6.7 Variation of carotenoids contents in different isolates, P<0.05

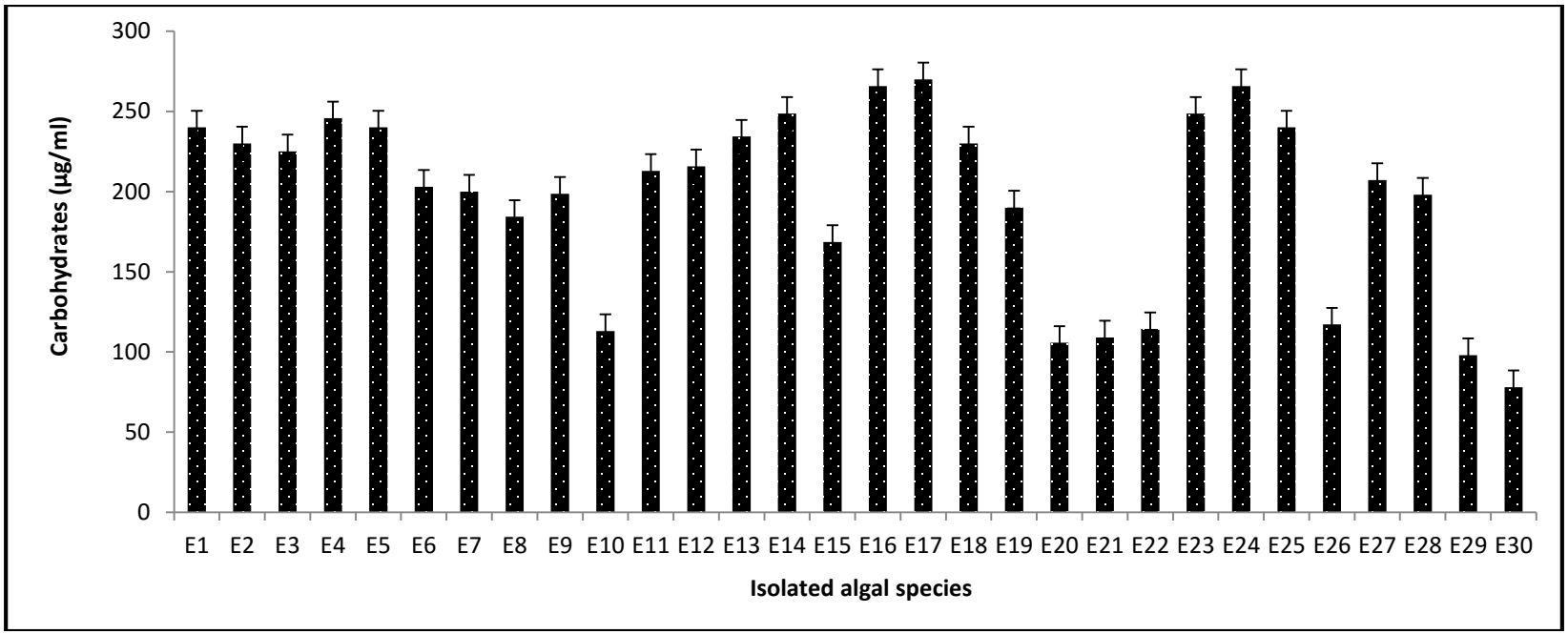


Fig. 6.8 Variation of total carbohydrates contents in different isolates, P<0.05

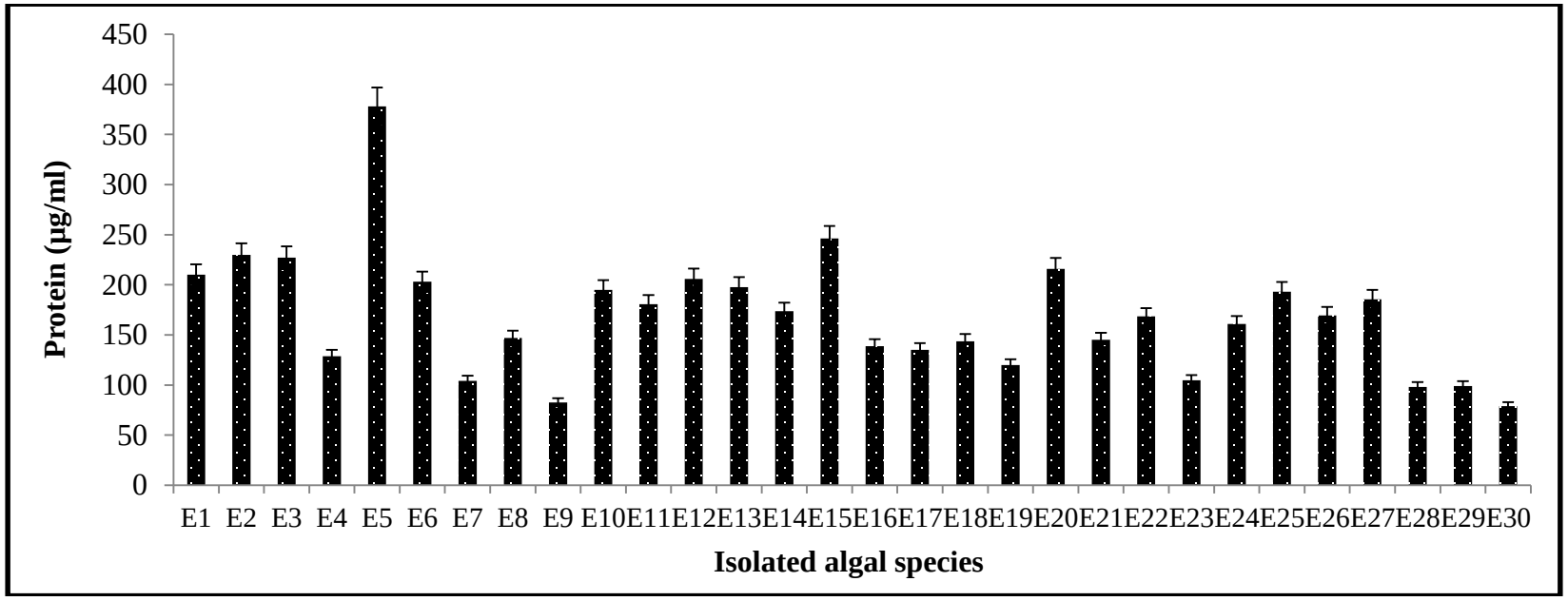


Fig. 6.9 Variation of protein contents in different isolates, P<0.05

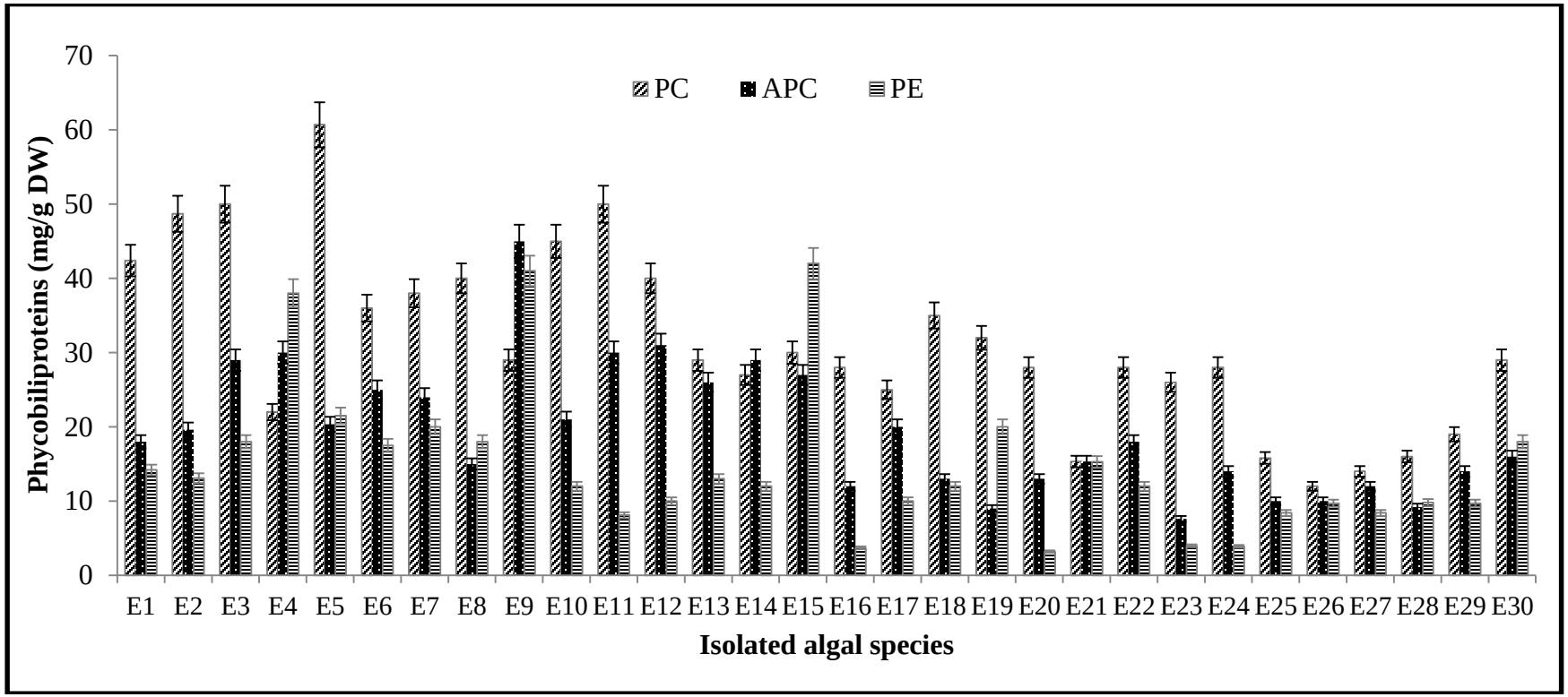


Fig. 6.10 Variation of phycobiliproteins contents in different isolates, P<0.05

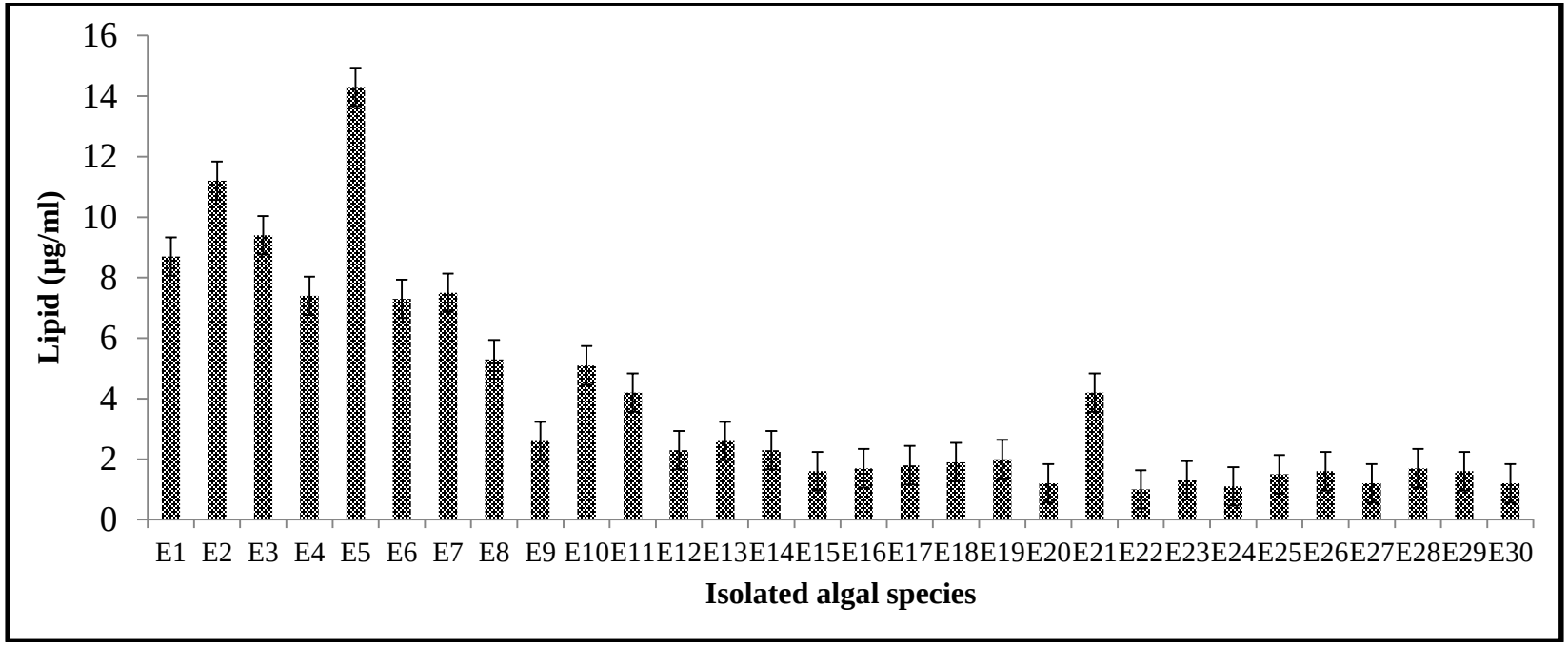


Fig. 6.11 Variation of lipid contents in different isolates, P<0.05

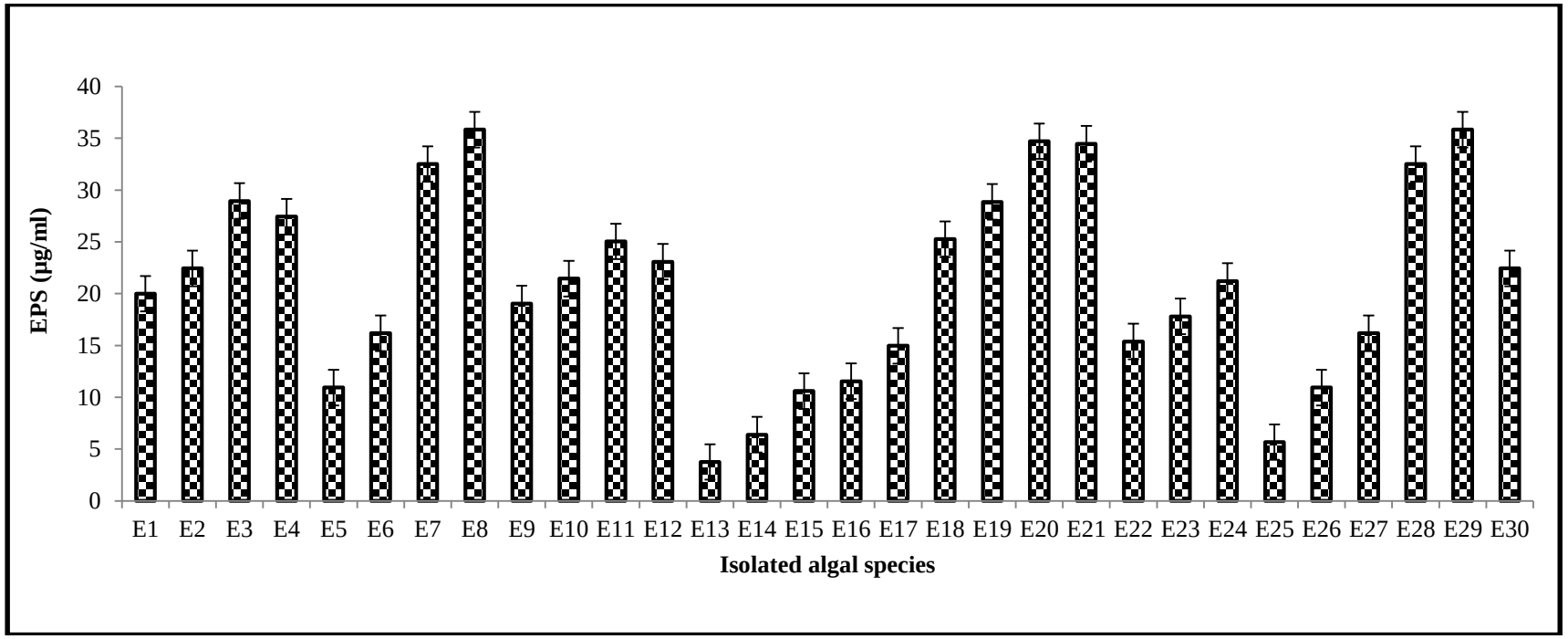


Fig. 6.12 Variation of EPS contents in different isolates, P<0.05

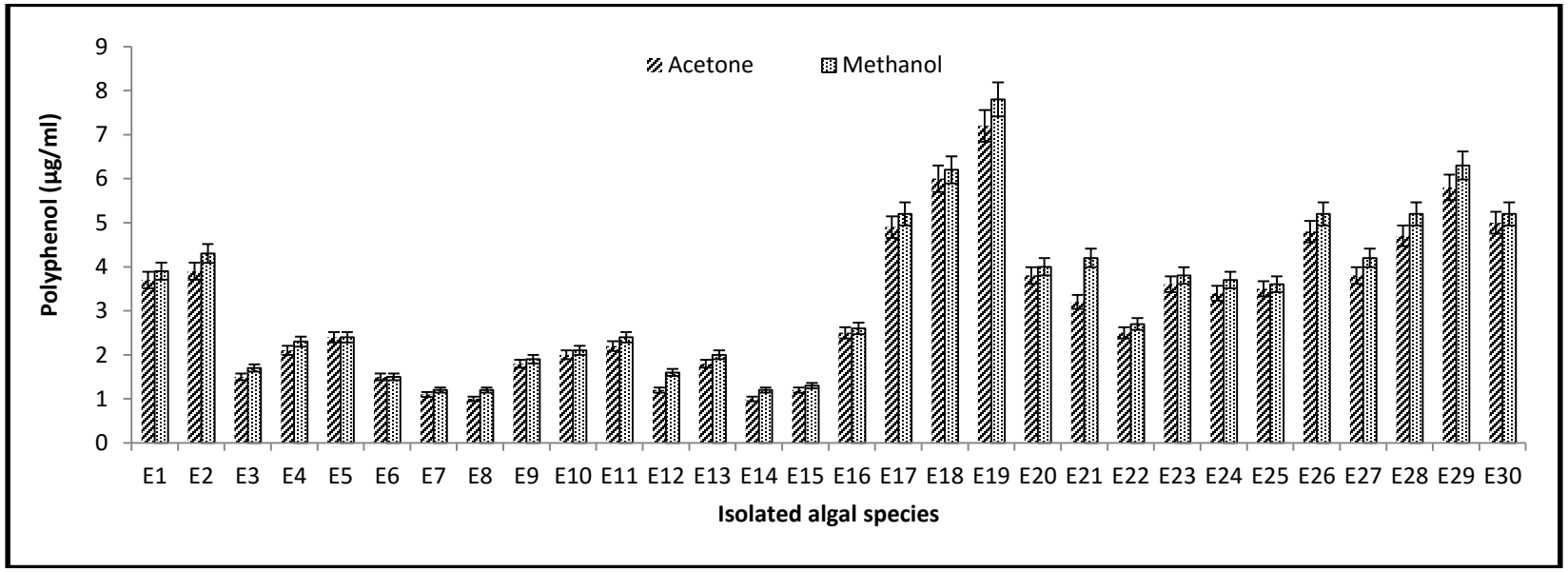


Fig. 6.13 Variation of polyphenol contents in different isolates, P<0.05

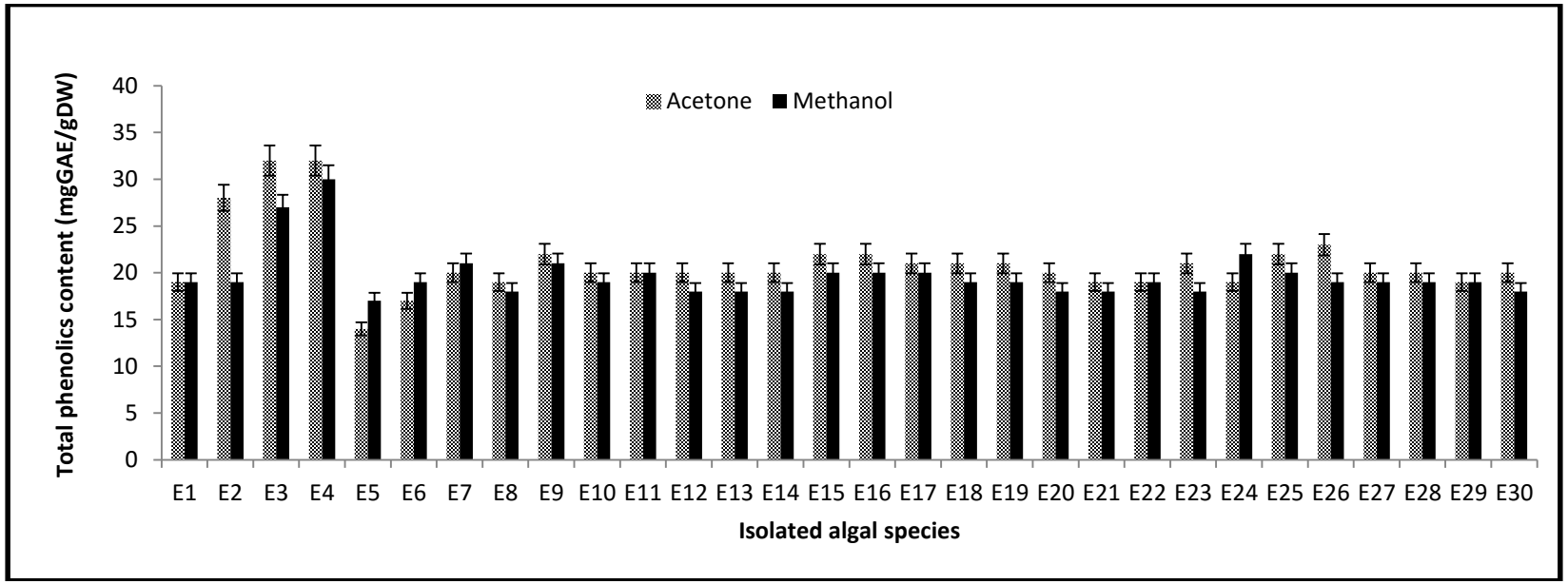


Fig. 6.14 Variation of total phenolicscontents in different isolates, P<0.05

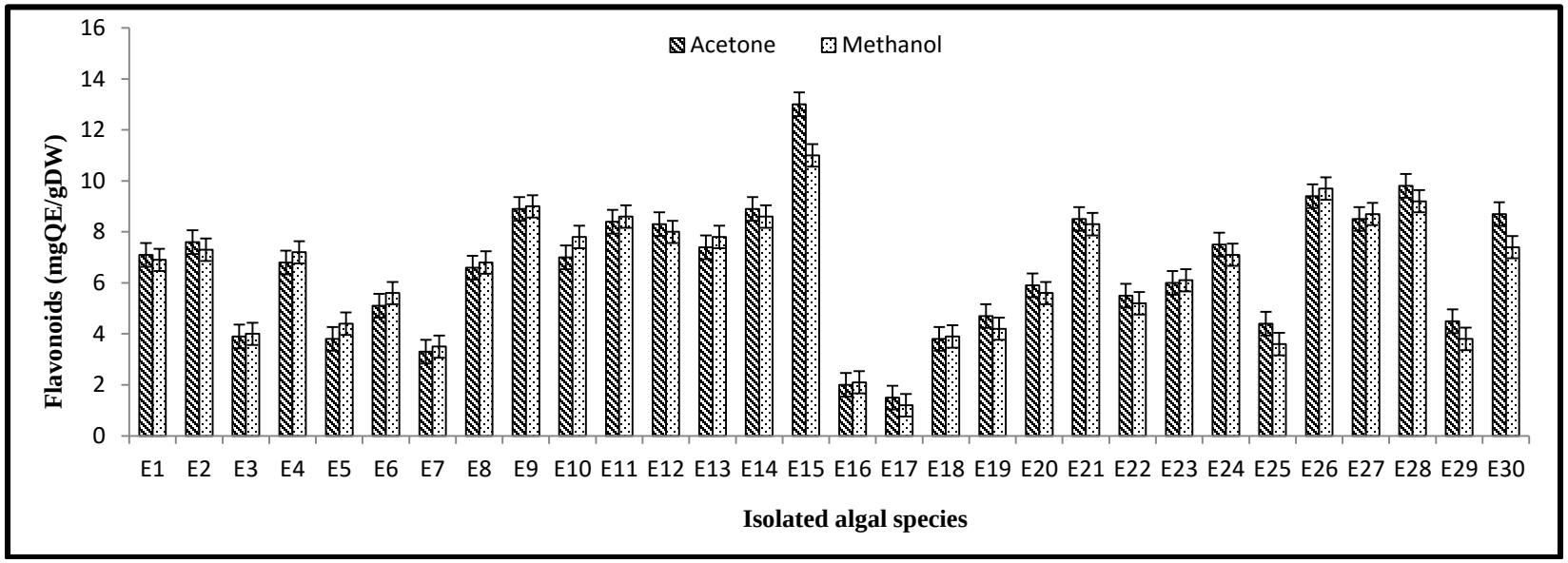


Fig. 6.15 Variation of flavonoids contents in different isolates, P<0.05

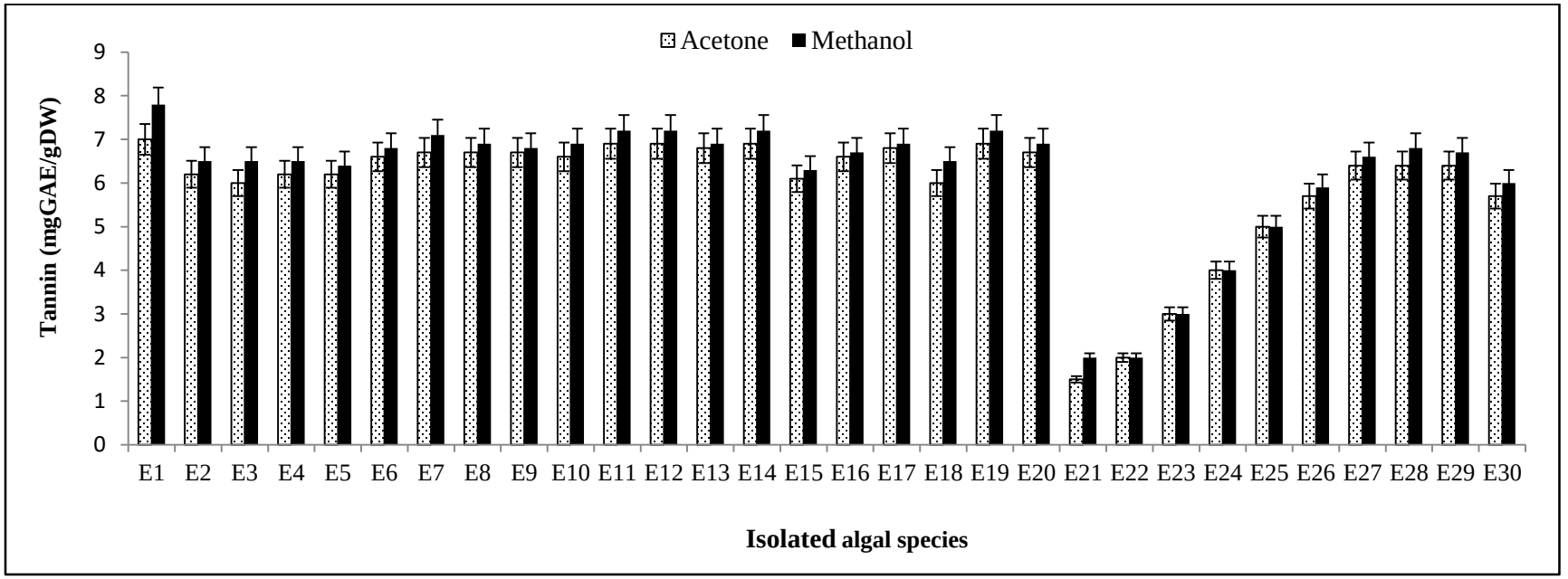


Fig. 6.16 Variation of tannin contents in different isolates, P<0.05

Vitamin C, a wide spectrum antioxidant not synthesized in the body is obtained from dietary sources (Rivas *et al.*, 2008). Algae with brighter thalli were reported to be rich in vitamin C (Sarojini and Sarma, 1999). In the present study, algal isolates in the field conditions were found to colonize with brighter blue-green and olive green colour thalli on polythene bags

Table 6.2 Component loading scores for PCA with the two dimensions

Principal components	Eigenvalue	% variance
1	4148.08	55.814
2	2761.22	37.153
3	451.306	6.0725
4	64.8876	0.87309
5	4.47737	0.060245
6	1.63249	0.021966
7	0.272951	0.0036727
8	0.0496628	0.00066823
9	0.0213537	0.00028732
10	0.00186488	2.5093E-05

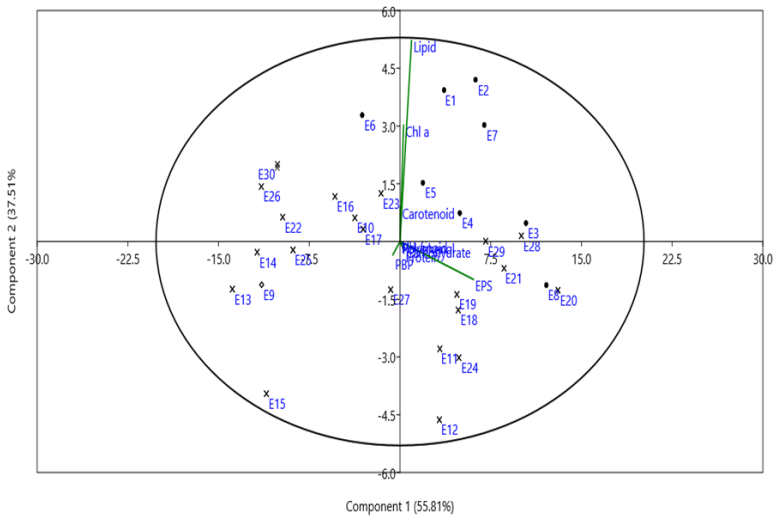


Fig. 6. 17 Bi-plot of algal isolates obtained after PCA analysis of biochemical profile (with first two principal components).The microalgae strains are indexed according to their number in the Table 6.1 (·-non-heterocystous cyanobacteria, ◇-green algae, x-heterocystous cyanobacteria).

The principal component analysis of biochemical profiles provided a clear distinction between non-heterocystous Cyanophyceae, heterocystous Cyanophyceae and Chlorophyceae; it showed a 93.32% variation, which reveals a strong correlation due to taxonomical implications. *Chlorella ellipsoidea* (E9) biochemical profile was showed correlation with the *Nostoc* (E13, E14 and E15) genus. Based on these observations, Chl *a*, carotenoids, carbohydrate, protein, EPS, polyphenol, and flavonoid can be considered as strong chemotaxonomic biomarkers for Cyanophyceae while phycobiliproteins can be indicative of Chlorophyceae phylum. The identification and classification of microalgae follows the same basic principles in PCA as well as CHEMTAX (Mackey *et al.*, 1996).

6.3.2 Conclusion

The algal isolates screened from submerged polythene surface in sewage water are demonstrated to be a rich source of chlorophyll *a*, carotenoids, carbohydrate, protein, phycobiliproteins, lipid, EPS, polyphenol, total phenolics, flavonoids and tannin. The results are anticipated to be of relevance to biodegradation of polythenes, aquaculture, pharmaceutical appilications and biofuel. Statistical analysis revealed clear distinction between Chlorophyceae and Cyanophyceae in line of biochemical profile of the algal isolates.

Chapter 7

ENZYMATIC AND NON-ENZYMATIC PROFILING OF ANTIOXIDANT ACTIVITY OF ALGAL SPECIES

7.1 Introduction

Algae are found to distribute in fresh, brackish and marine aquatic environments and in moist soil surfaces, owing to capacity to perform photosynthesis, fix nitrogen and grow in almost all types of extreme habitats including wastewater highly polluted environments and high salinity levels (Noctor and Foyer, 1998; Cuellar-Bermudez *et al.,* 2017; Singh and Thakur, 2015; Dubey *et al*., 2011; Akoijam *et al.,* 2015).

Algae are considered to acclimatize to extreme environmental conditions by producing different metabolites (Paliwal *et al.,* 2017). To combat with the extreme environment, algae developed a series of enzymatic and non-enzymatic antioxidants to protect the cells from

oxidative damage (Sairam and Tyagi, 2004).The enzymatic antioxidants Catalase (CAT), Ascorbate peroxidase (APOX) and Glutathione reductase (GR) serves as detoxification of super oxide and hydrogen peroxide (Mittler,2002). In a previous study, *Oscillatoria terebriformis* showed promising agent for enymatic and non-enymatic antioxidants which can be used as effective protecting agent against oxidative stress and various related diseases (Mukund *et al.,* 2014).

In the present study, enymatic and non-enymatic antioxidants of isolated algae from submerged polythene surface in domestic sewage water has been done. The algae were screened from submerged polythene surface in domestic sewage water and algae faces different types of abiotic stress in such environment.

7.2 Methodology

The detail methodology for profiling of enymatic and non-enymatic antioxidants of isolated algae from domestic sewage water shown in **Chapter 3.** The enzymatic antioxidants Catalase (CAT), Ascorbate peroxidase (APOX) and Glutathione reductase (GR) and the non-enymatic antioxidants Ascorbate for algal isolates obtained results are depicted in the tables, graphs and figures below**.**

7.3 Results and discussion

The Catalase activity of methanolic extract from algal isolates were estimated and were compared with the Butylated hydroxytoluene (BHT). **Figure 7.1-7.5** shows the activity of CAT of isolated algae from submerged polythene surface in domestic sewage water drains. The catalase activity of methanolic extract from E7 (*Lyngbya nordgardhii*) was found to be 106.3 ± 0.12% at for 100µg/ml. The methanol extract of E6 (*Lyngbya diguetii*) was found to be 103.6 ± 0.23% at for 100µg/ml. The methanolic extract of E4 (*Oscillatoria curviceps*) was found to be 102.5 ± 0.34% at for 100µg/ml. The methanolic extract of E28 (*Hapalosiphon flexuosus*) was found to be minimum (7.1 ± 0.21%) at for 100µg/ml.

The ascorbate peroxidase activity of methanolic extract from algal isolates were estimated and were compared with the Butylated hydroxytoluene (BHT). **Figure 7.6-7.10** shows the activity of ascorbate peroxidase of isolated algae from submerged polythene surface in domestic sewage water drains. The methanolic extract of E11 (*Nostoc commune*) and E13 (*Nostoc* sp) was found to be maximum (97 ± 0.14%) at for 100µg/ml. The methanolic

extract of E1 (*Phormidium lucidum*) was found to be minimum (36.96± 0.13%) at for 100µg/ml.

The glutathione reductase activity of methanolic extract from algal isolates were estimated and were compared with the Butylated hydroxytoluene (BHT). **Figure 7.11-7.15** shows the activity of glutathione reductase of isolated algae from submerged polythene surface in domestic sewage water drains. The methanolic extract of E6 (*Lyngbya diguetii*) was found to be maximum (56 ± 0.12%) and minimum (2.9 ± 0.15%) in E28 (*Hapalosiphon flexuosus*).

The non-enzymatic antioxidant ascorbic acid (vitamin-C) was found to be maximum in E14 (*Nostoc* sp.) and minimum in E22 (*Anabaena variabilis*) (**Figure 7.16)**.

DPPH radical scavenging activity of isolated algal strains from submerged polythene surface in sewage water were done and compared with the Butylated hydroxytoluene (BHT) (Figure 7.17-7.20). The percentage inhibition was found to be maximum in E2 (*Oscillatoria subbrevis*) (72.05 ± 0.12%) and minimum in E16 (*Calothrix parietina*) (52.65 ± 0.17%).

H_2O_2 radical scavenging activity of methanol extract of isolated algae were done and compared with the Butylated hydroxytoluene (BHT) (Figure 7.17-7.20). The percentage inhibition was found to be maximum in E23 (*Anabaena variabilis*) (77 ± 0.15%) and minimum in E25 (*Westiolopsis prolifica*) and E28 (*Hapalosiphon flexuosus*) (63 ± 0.17%).

Total antioxidant activity of methanol extract of isolated algae were done and compared with the Butylated hydroxytoluene (BHT) (Figure 7.17-7.20). The percentage inhibition was found to be maximum in E25 (*Westiolopsis prolifica*) (69.65 ± 0.11%) and minimum in E1 (*Phormidium lucidum*) (58.11 ± 0.13%).

The presence of enzymatic and non-enzymatic antioxidants in isolated algae from submerged polythene surface in domestic sewage water clearly demonstrated its role against oxidant and other free radicals. The occurrence of enzymes viz. catalase, peroxidase and glutathione reductase in isolated algae are key factors to its adaptation to

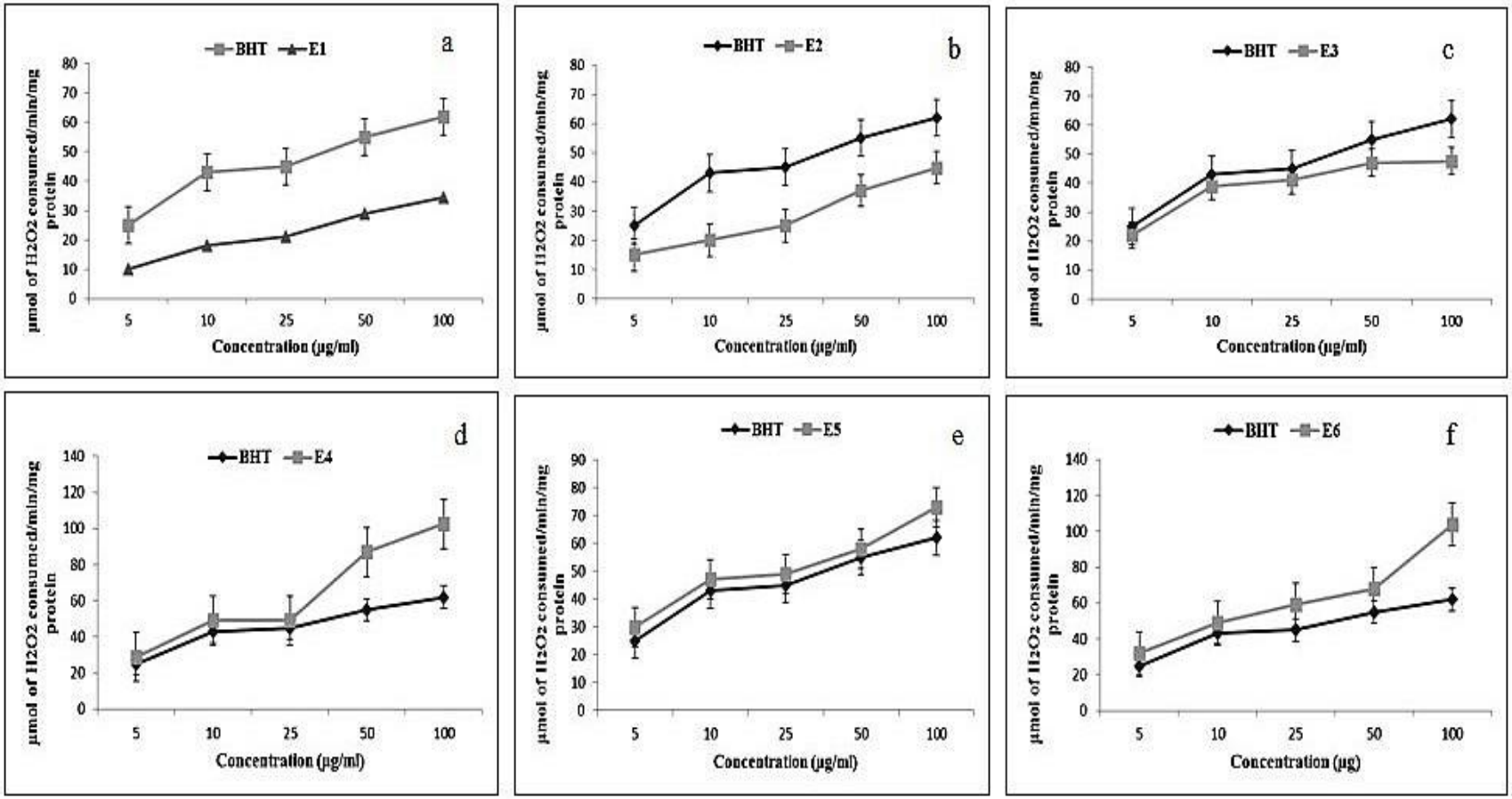


Fig.**7.1** Variation of Catalase (CAT) activity in E1-E6

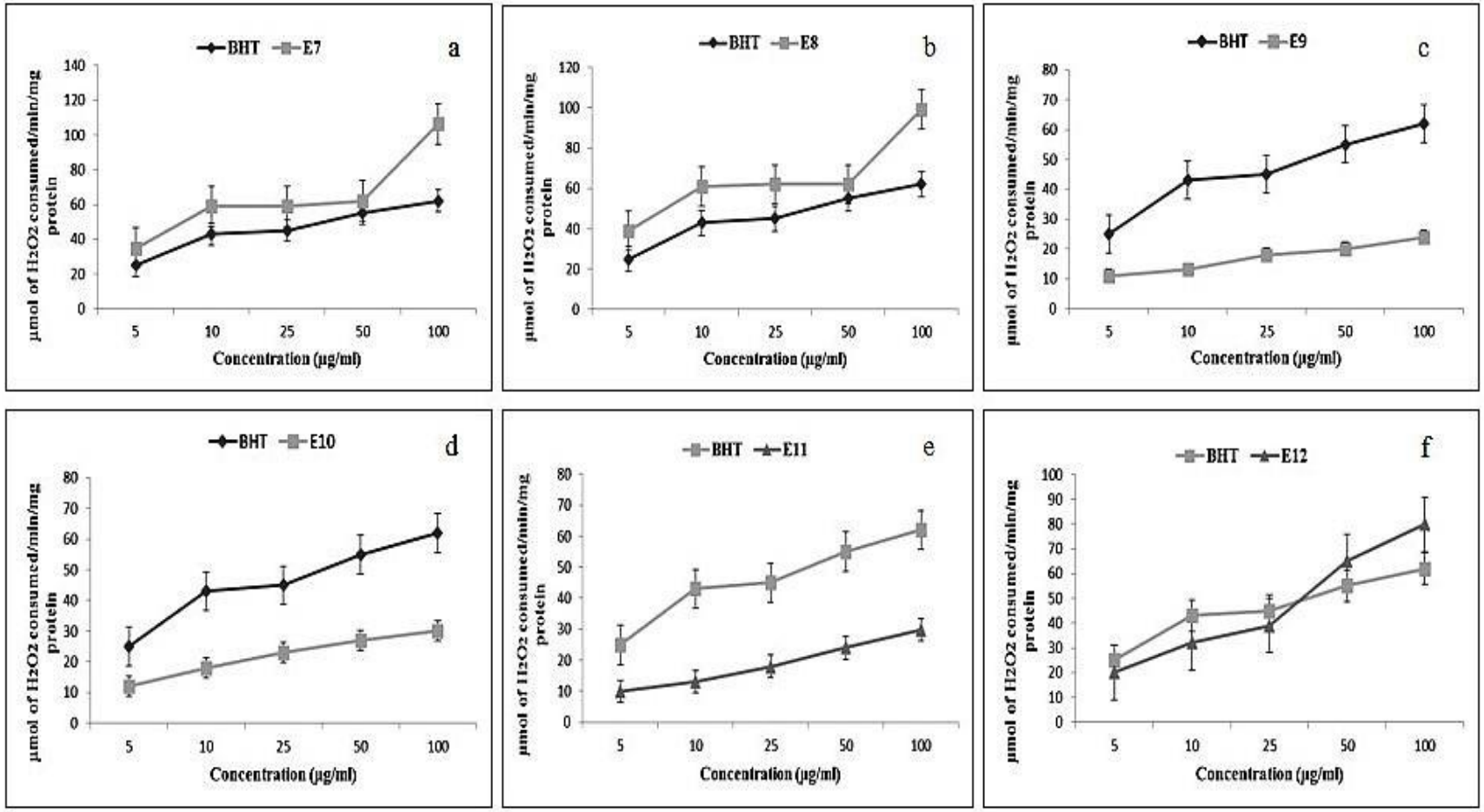


Fig.7.2 Variation of Catalase (CAT) activity in E7-E12

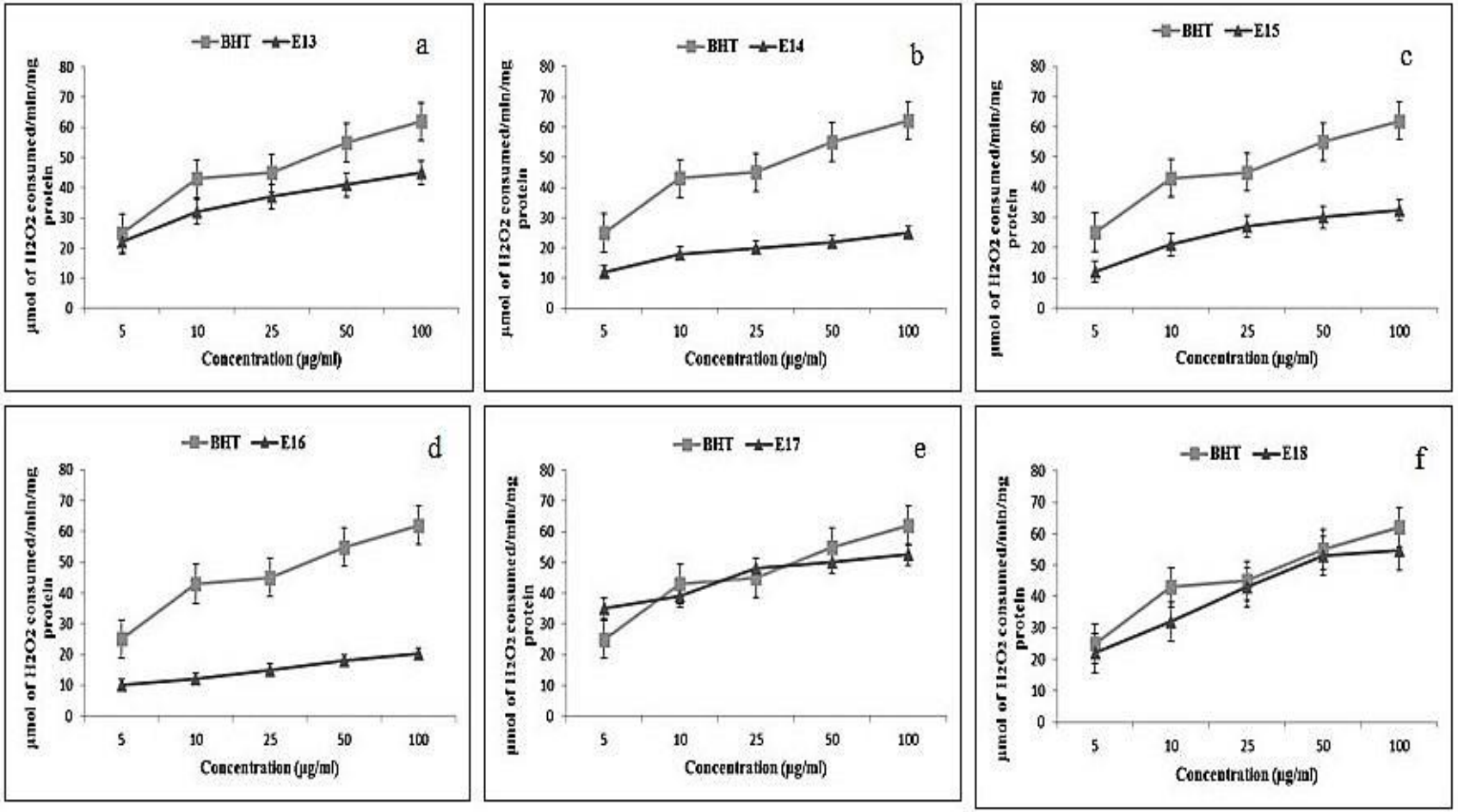


Fig.7.3 Variation of Catalase (CAT) activity in E13-E18

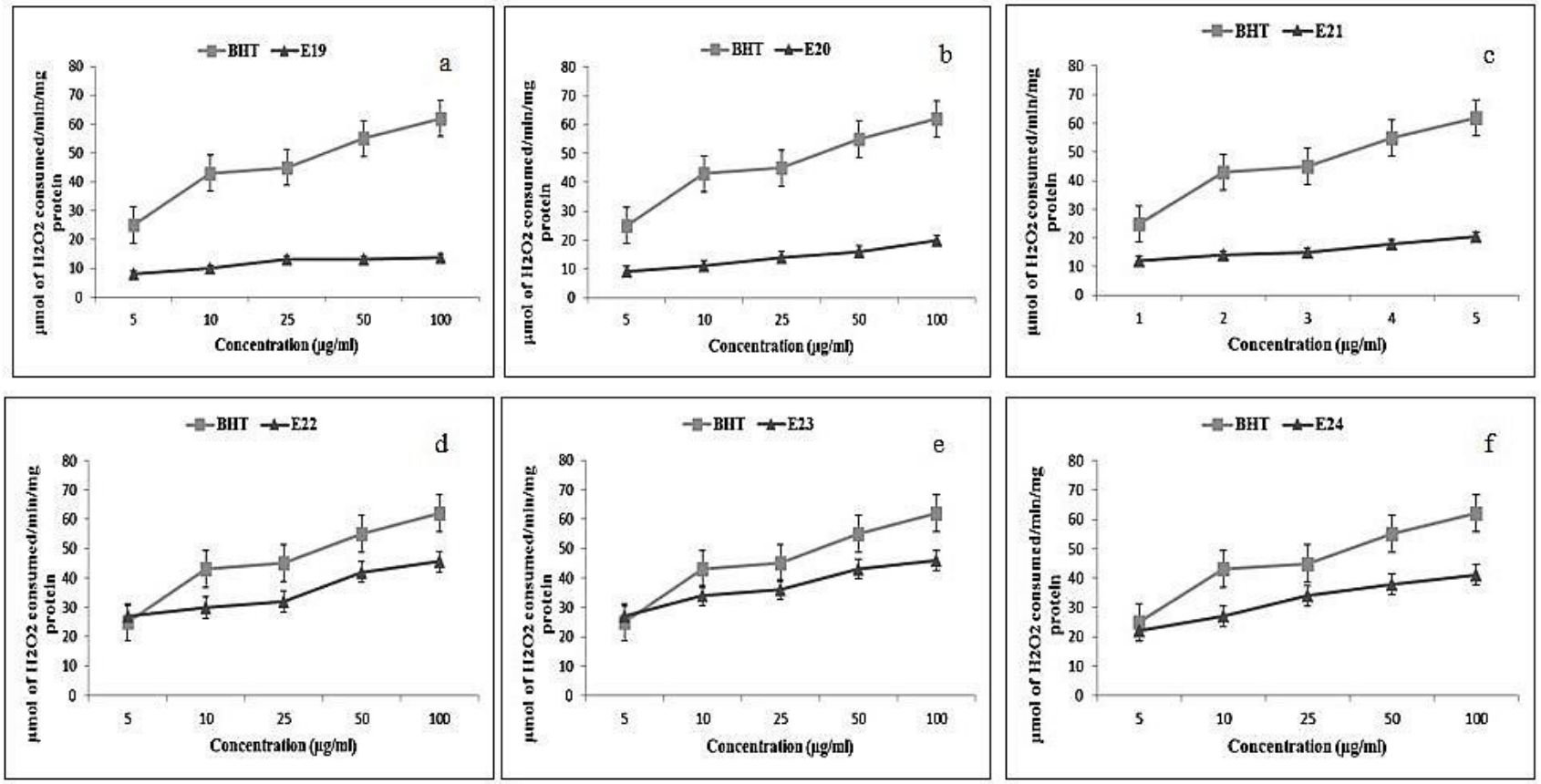


Fig.7.4 Variation of Catalase (CAT) activity in E19-E24

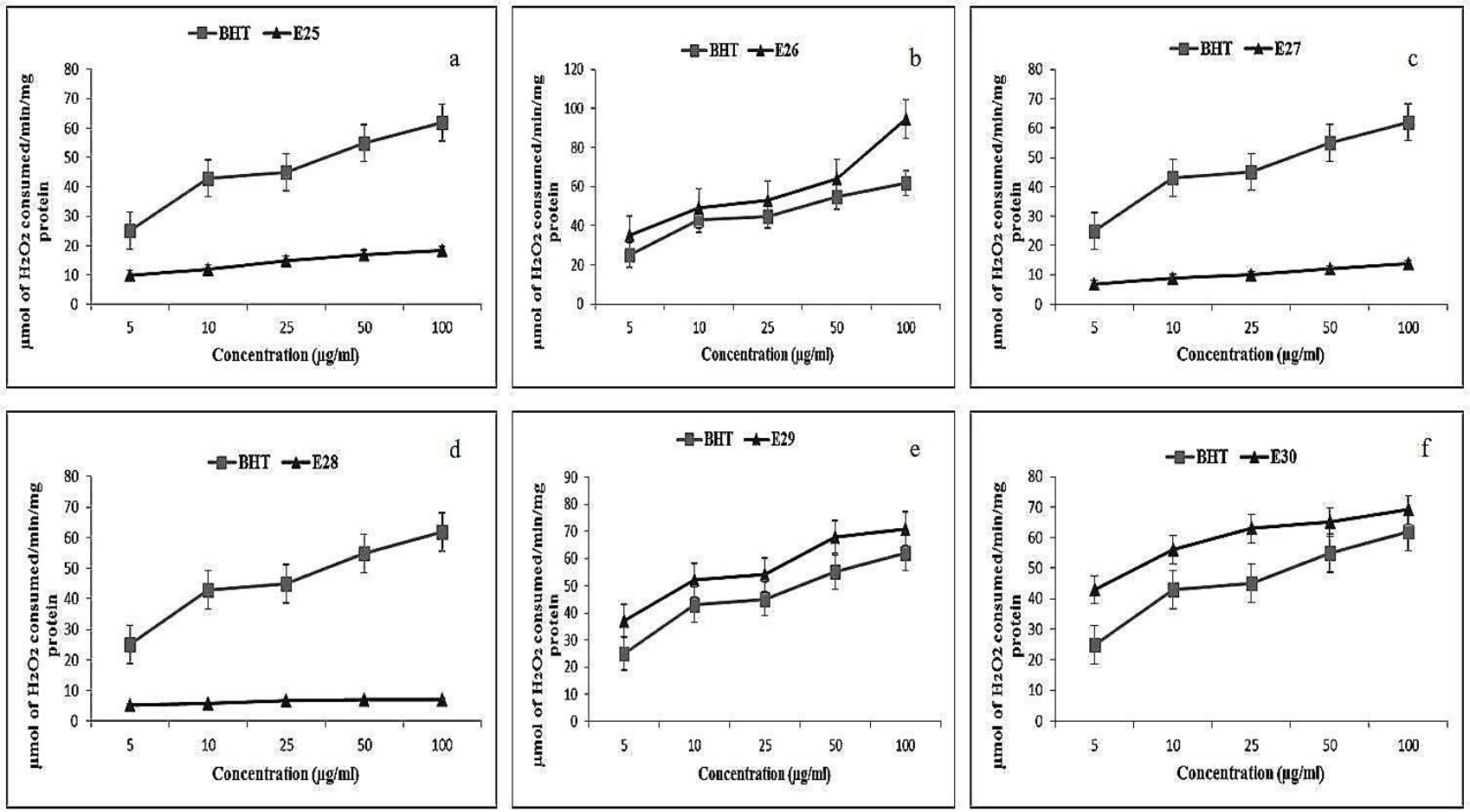


Fig.**7.5** Variation of Catalase (CAT) activity in E25-E30

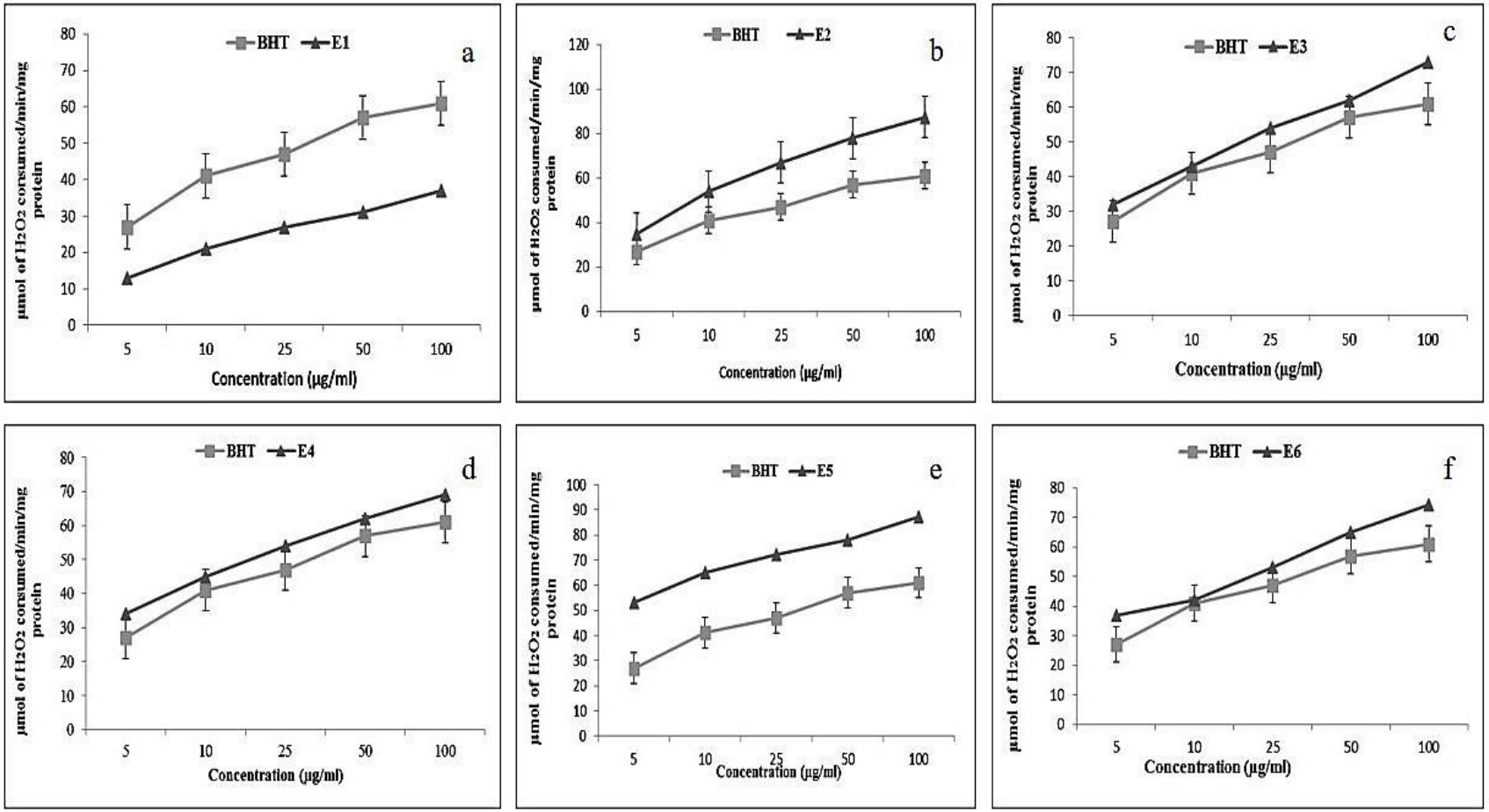


Fig.7.6 Variation of Ascorbate Peroxidase (APOX) activity in E1-E6

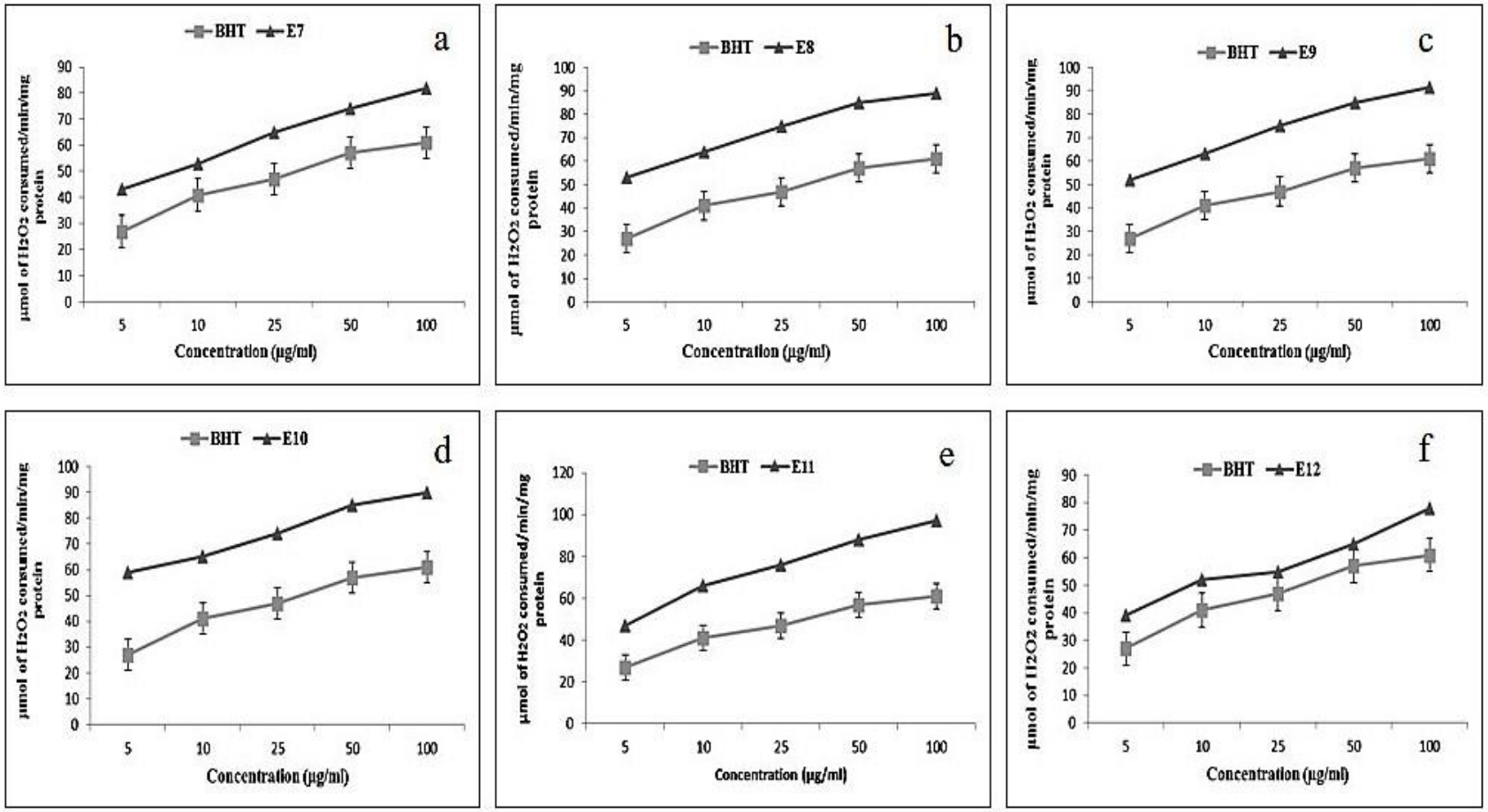


Fig.7.7 Variation of Ascorbate Peroxidase activity in E7-E12

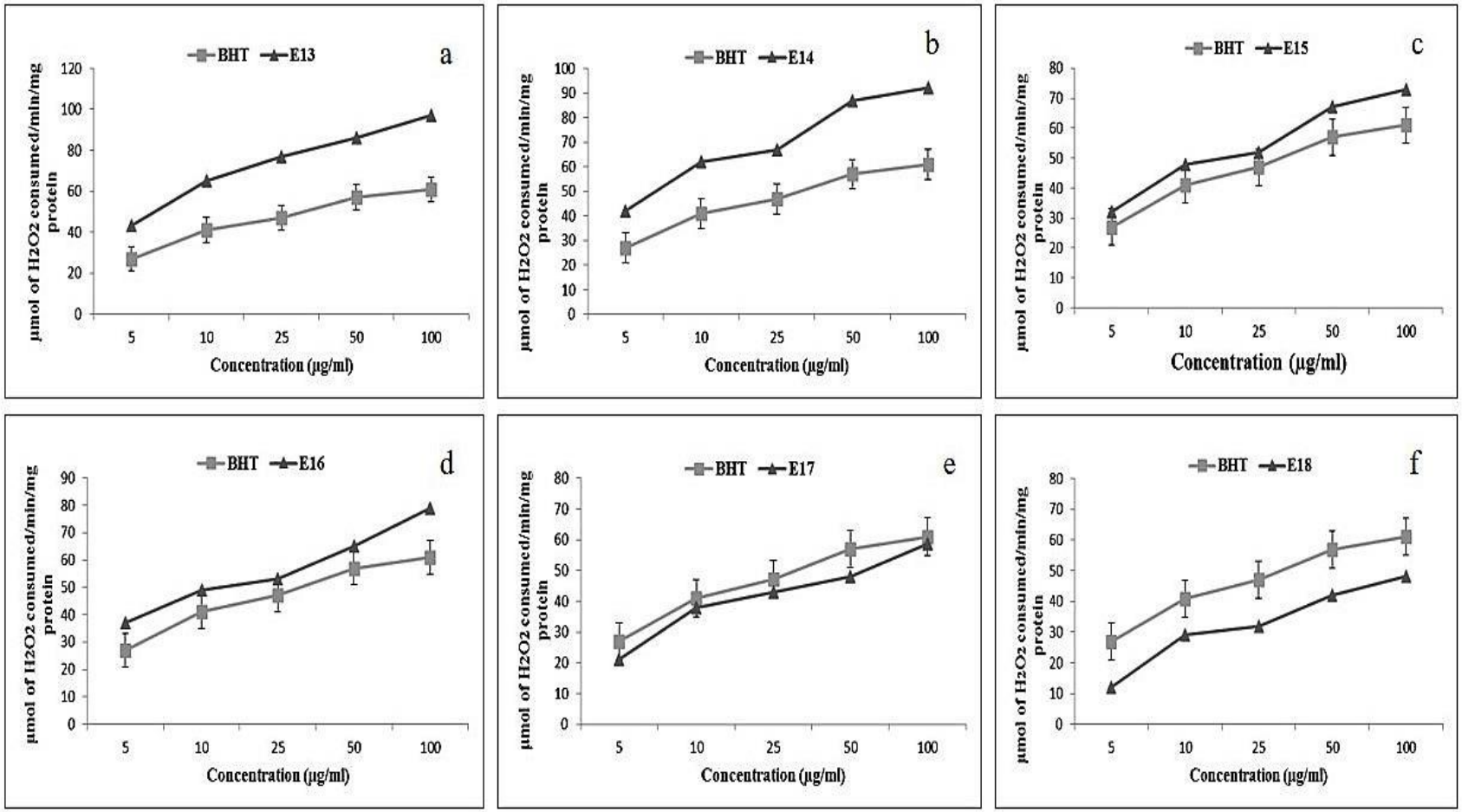


Fig.7.8 Variation of Ascorbate Peroxidase activity in E13-E18

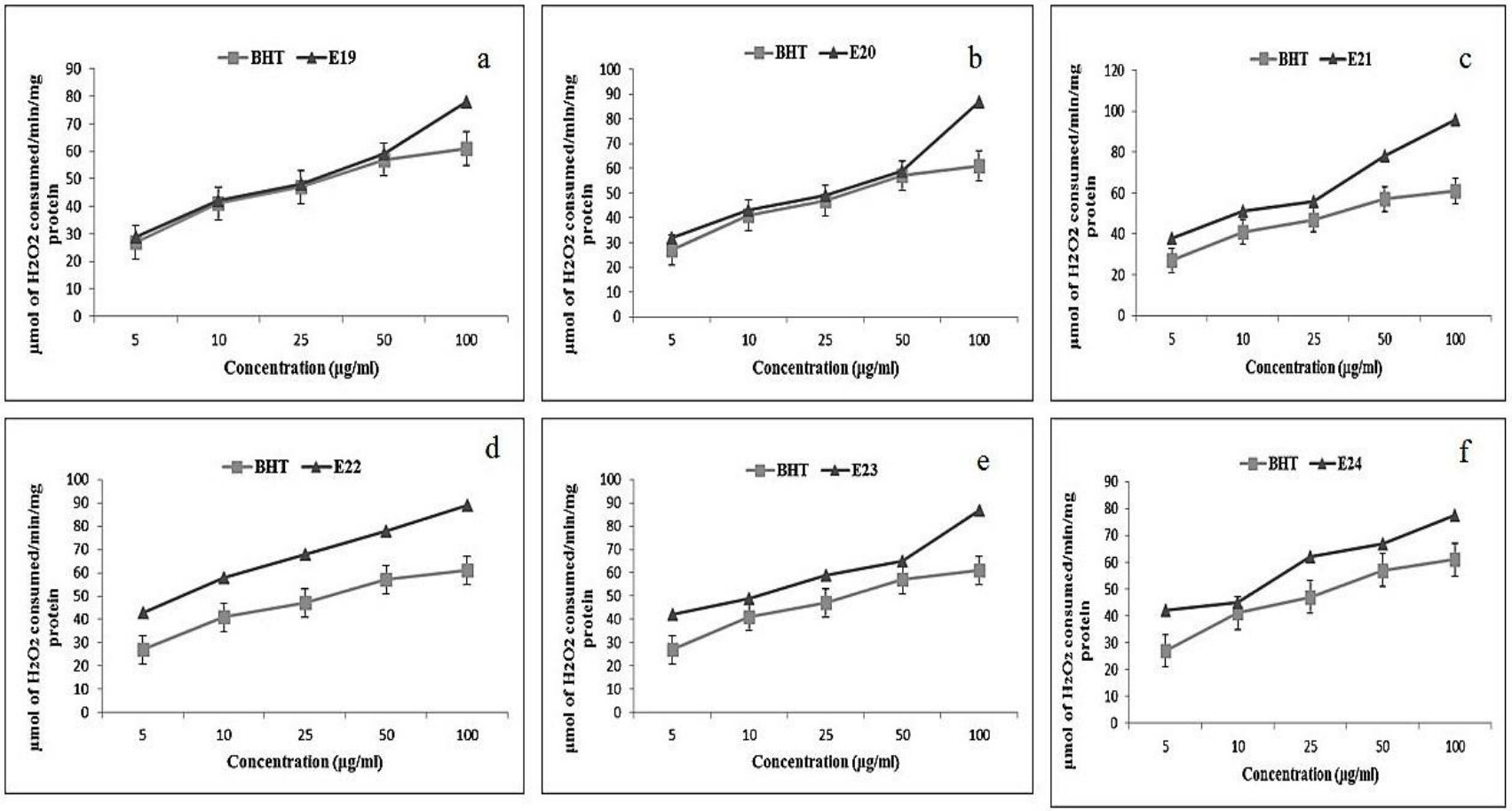


Fig.7.9 Variation of Ascorbate Peroxidase activity in E19-E24

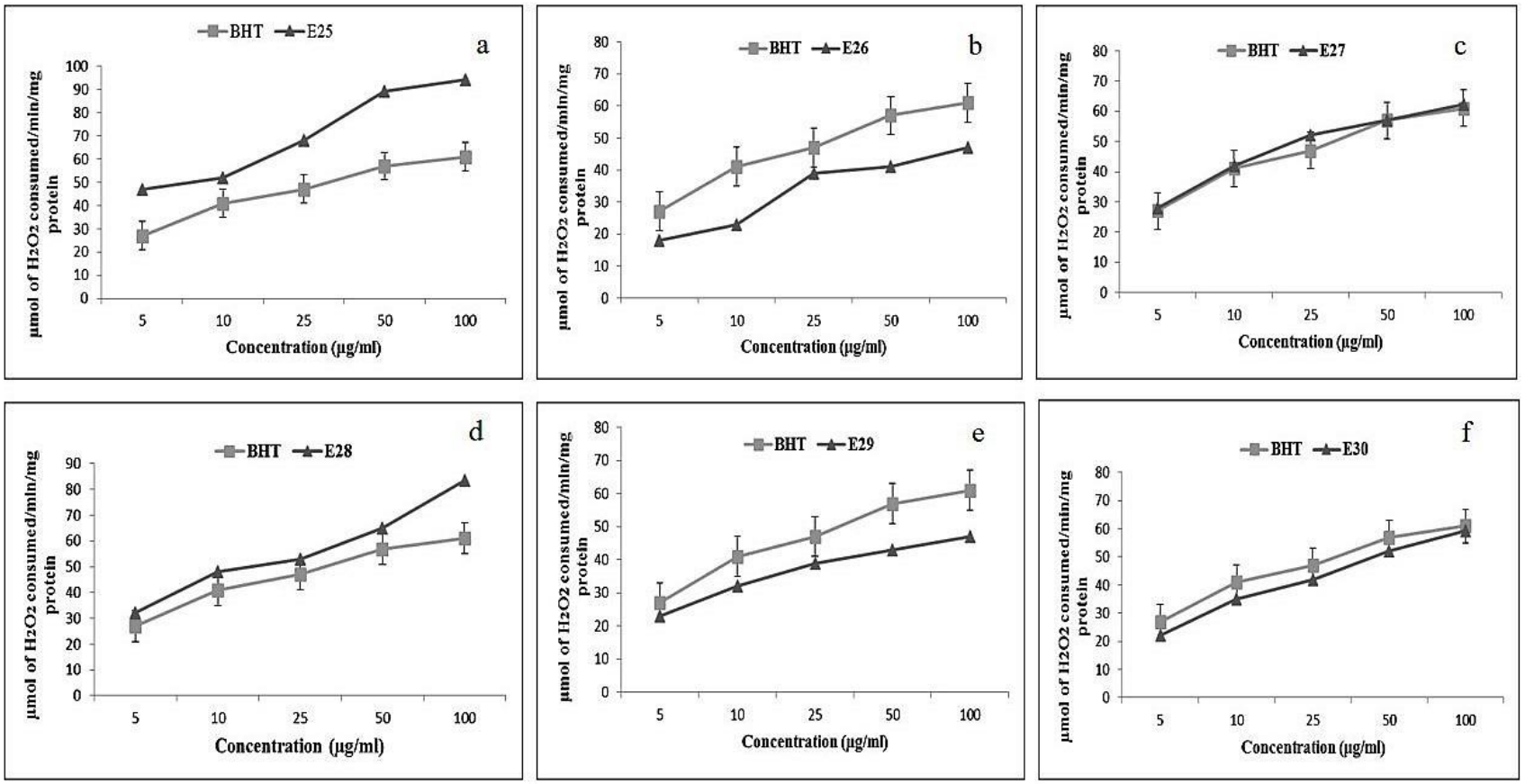


Fig.7.10 Variation of Ascorbate Peroxidase activity in E25-E30

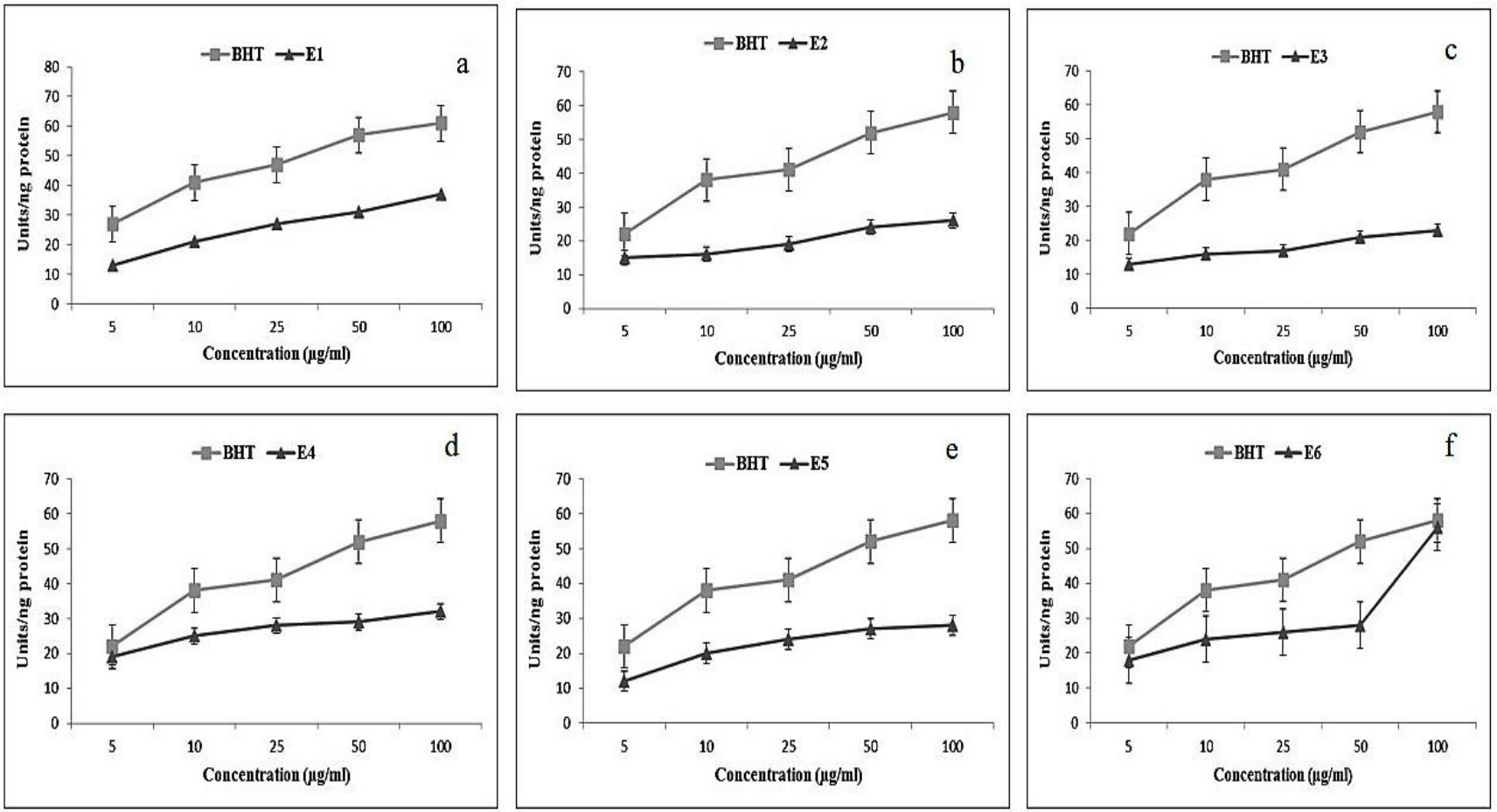


Fig. **7.11** Variation of Glutathione reductase (GR) activity in E1-E6

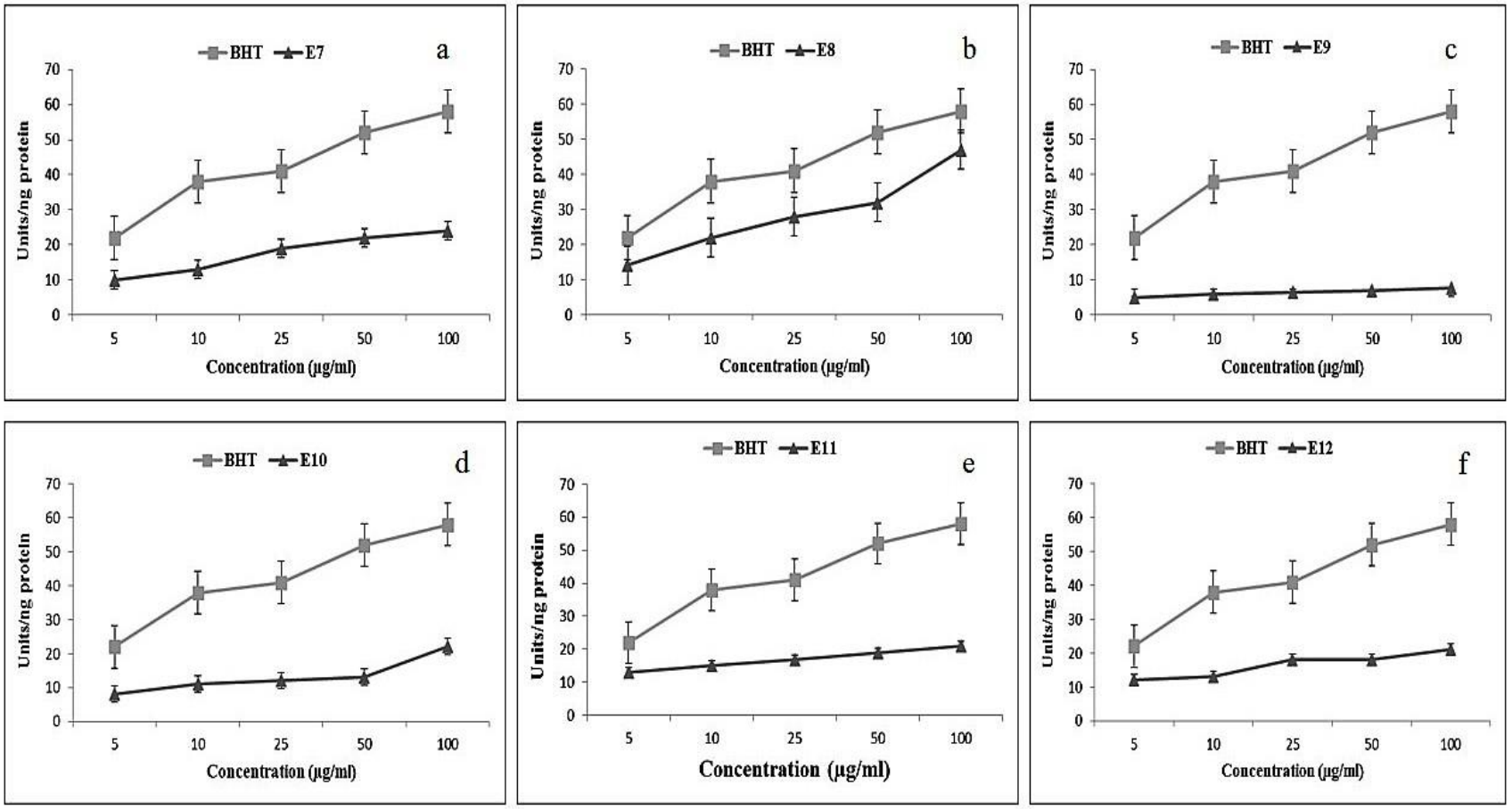


Fig. **7.12** Variation of Glutathione reductase (GR) activity in E7-E12

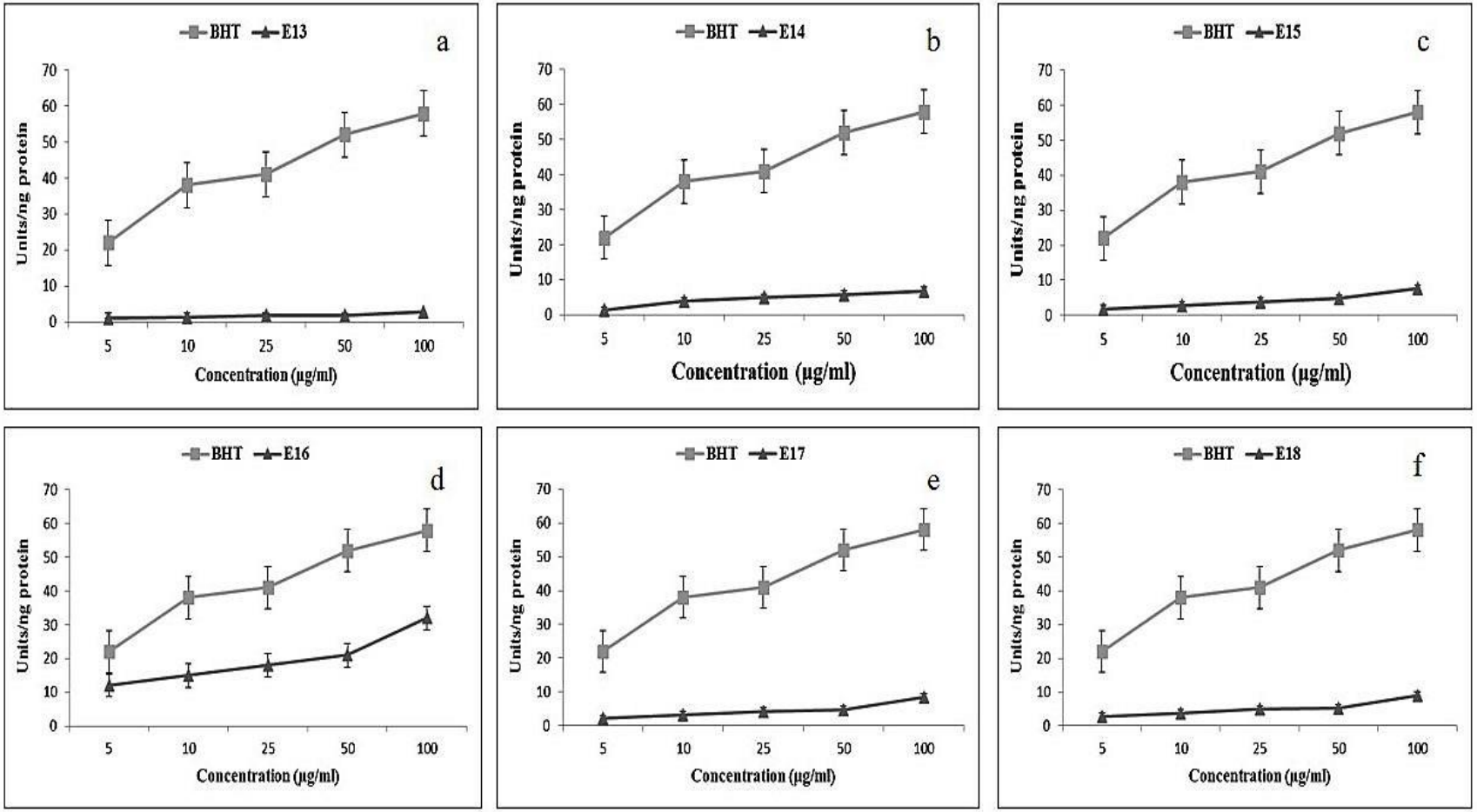


Fig. **7.13** Variation of Glutathione reductase (GR) activity in E13-E18

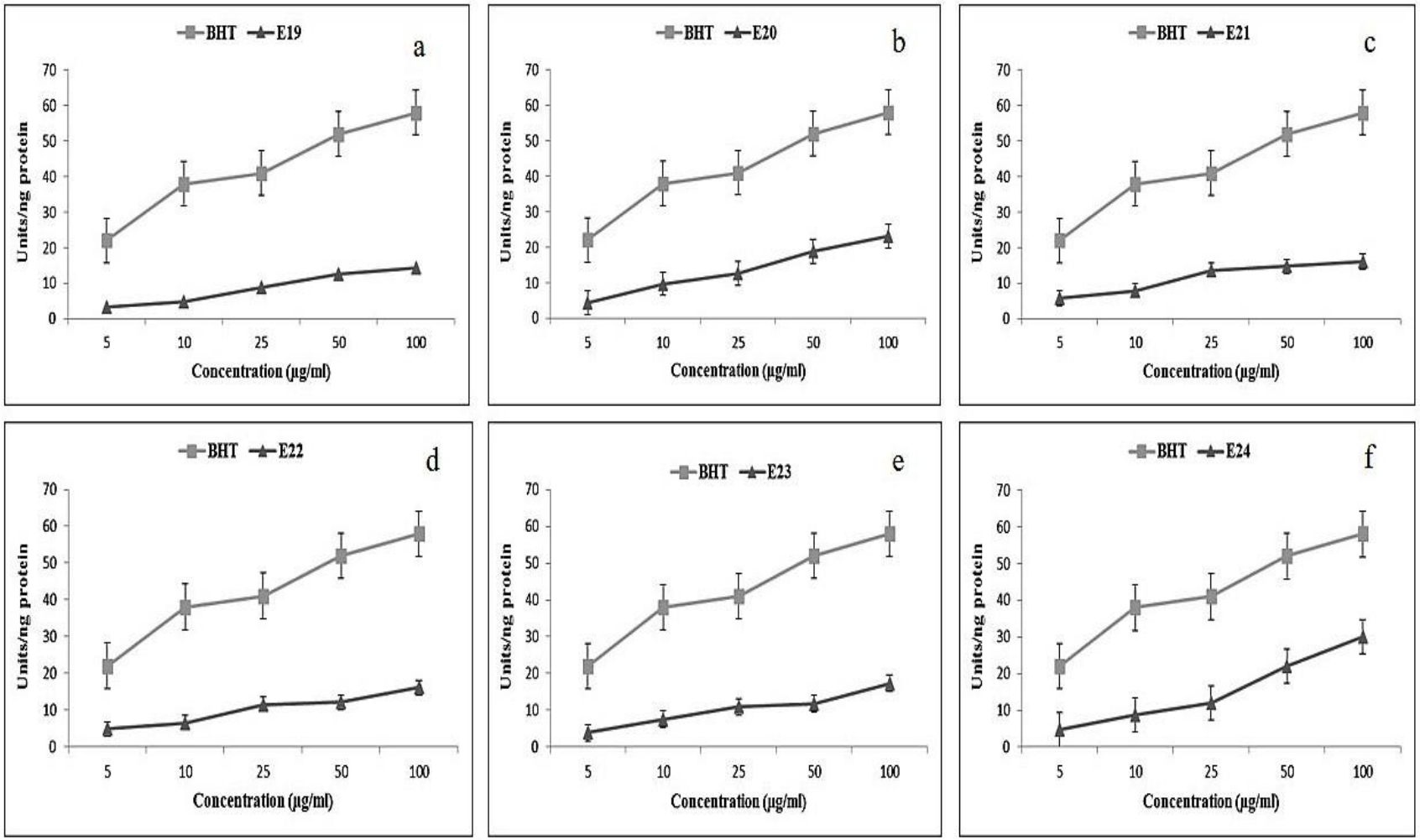


Fig. **7.14** Variation of Glutathione reductase (GR) activity in E19-E24

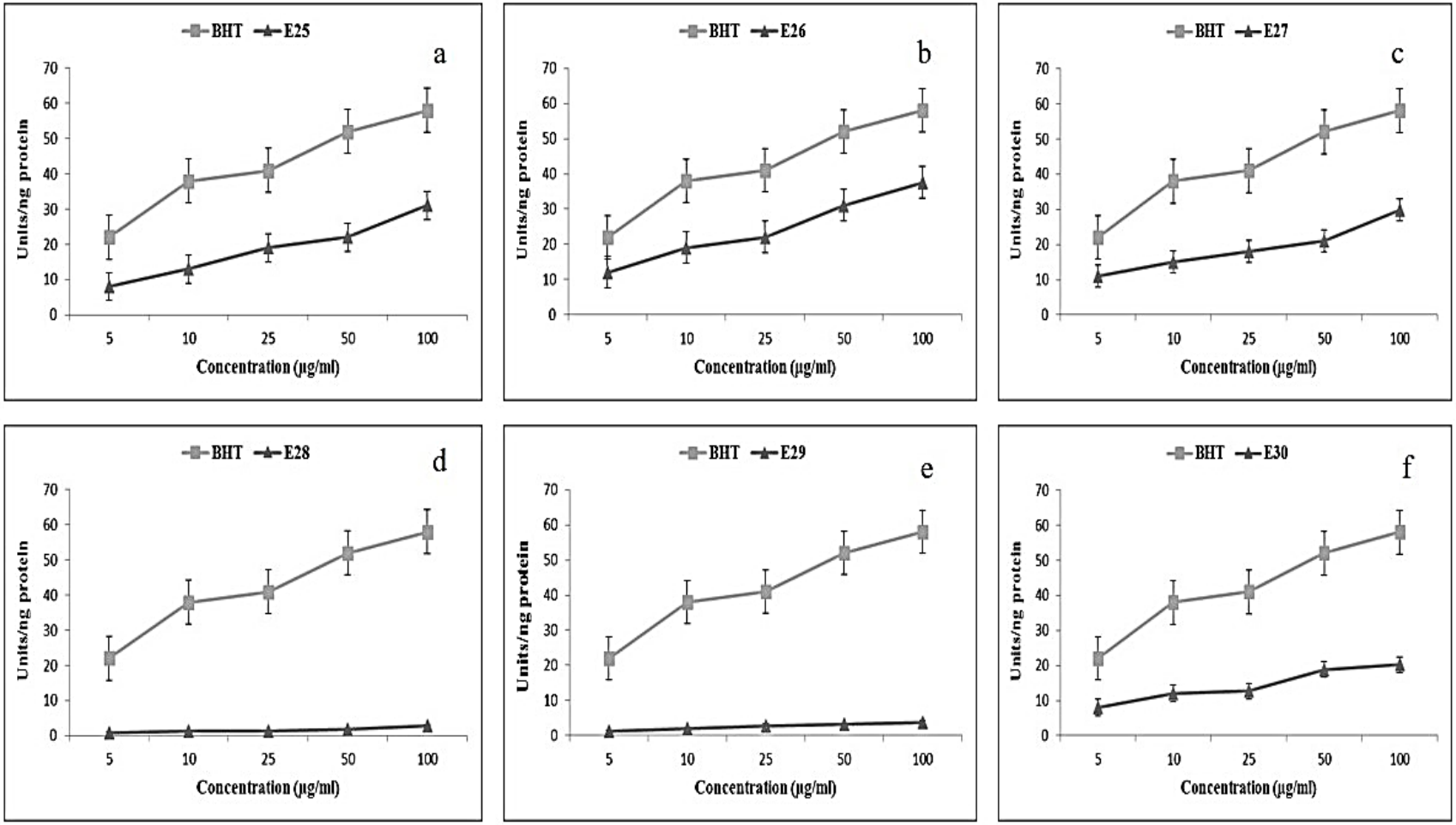


Fig. **7.15** Variation of Glutathione reductase (GR) activity in E25-E30

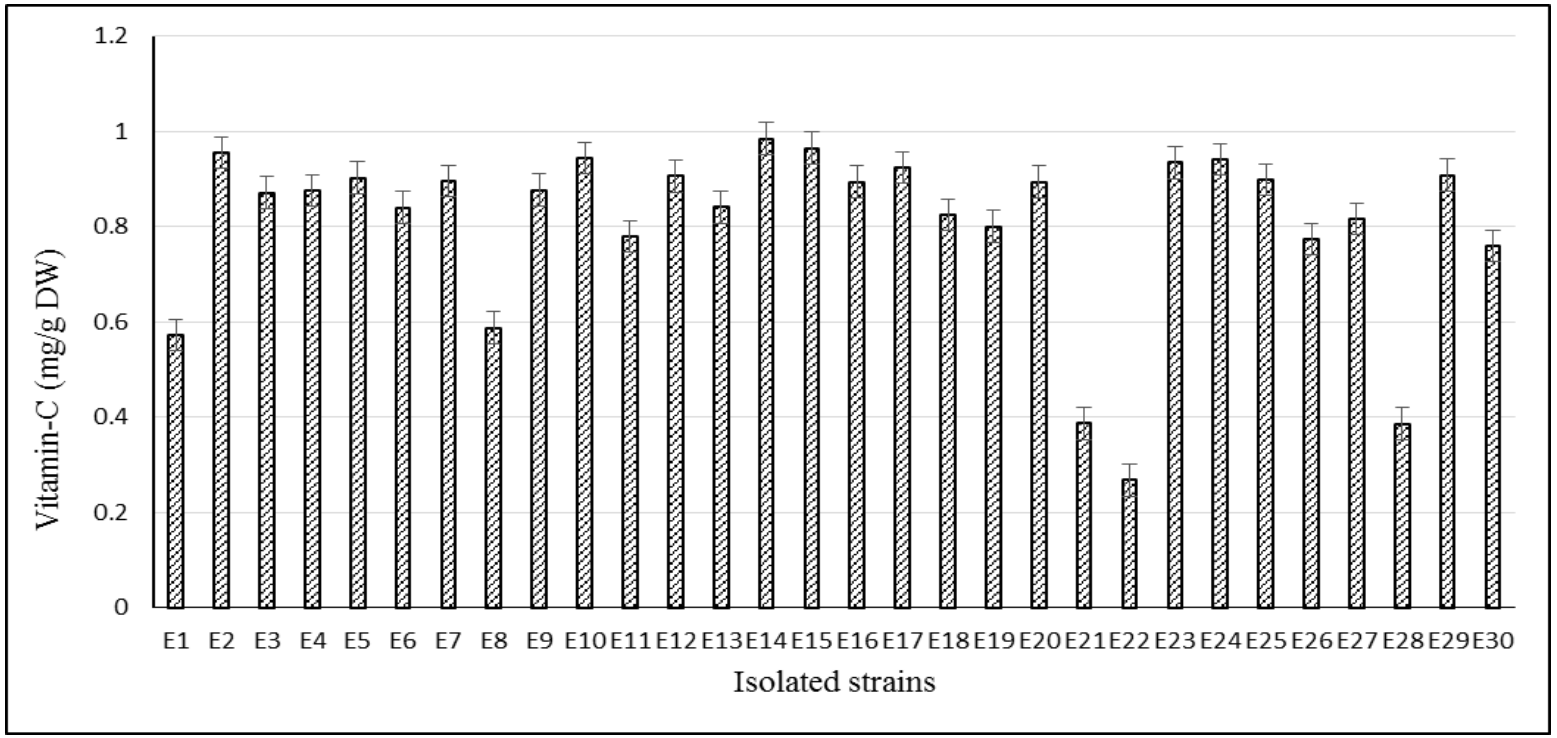


Fig. 7.16. Variation of Vitamin-C of E1-E30

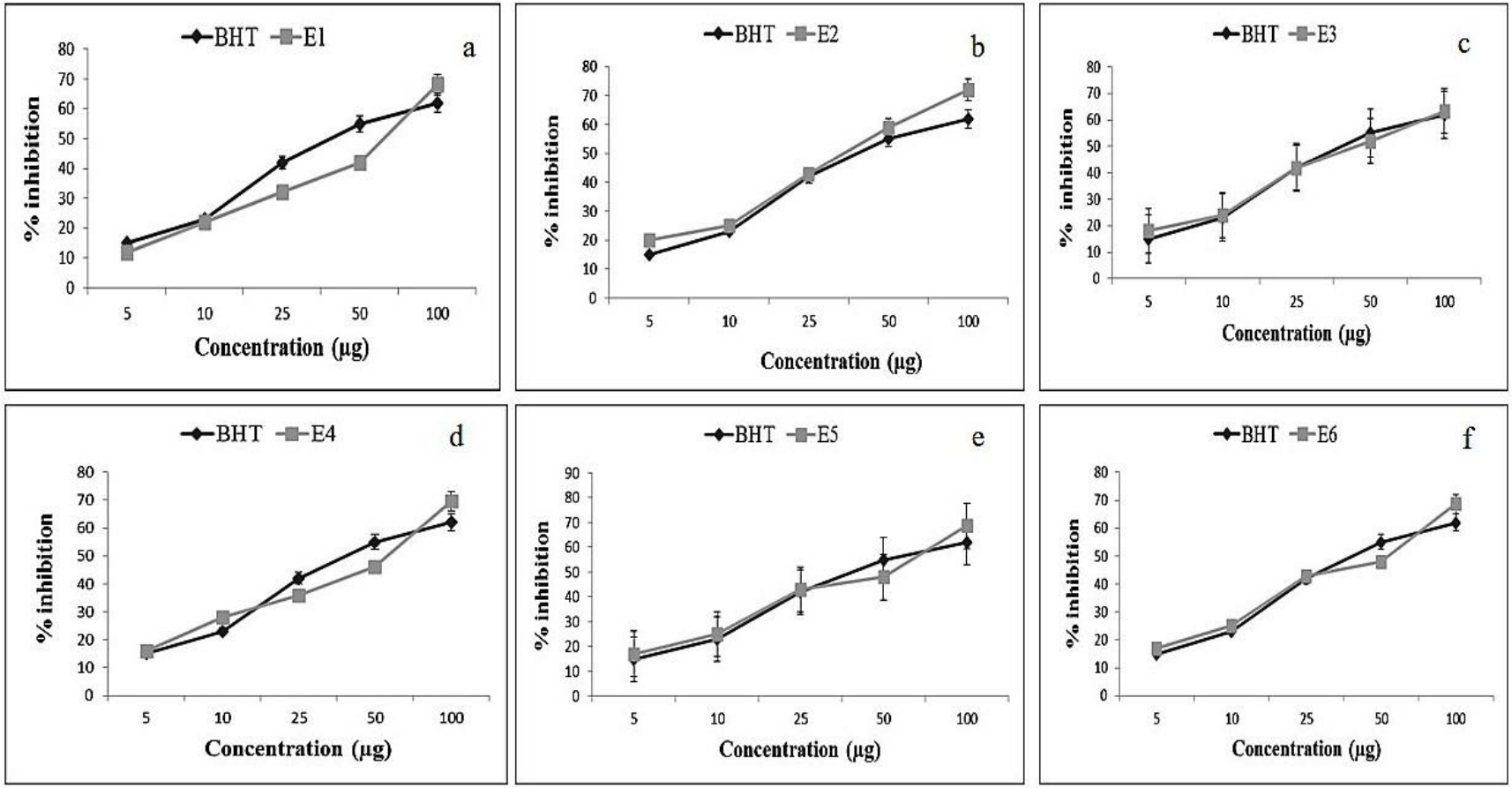


Fig.7.17 DPPH radical scavenging activity of E1-E6

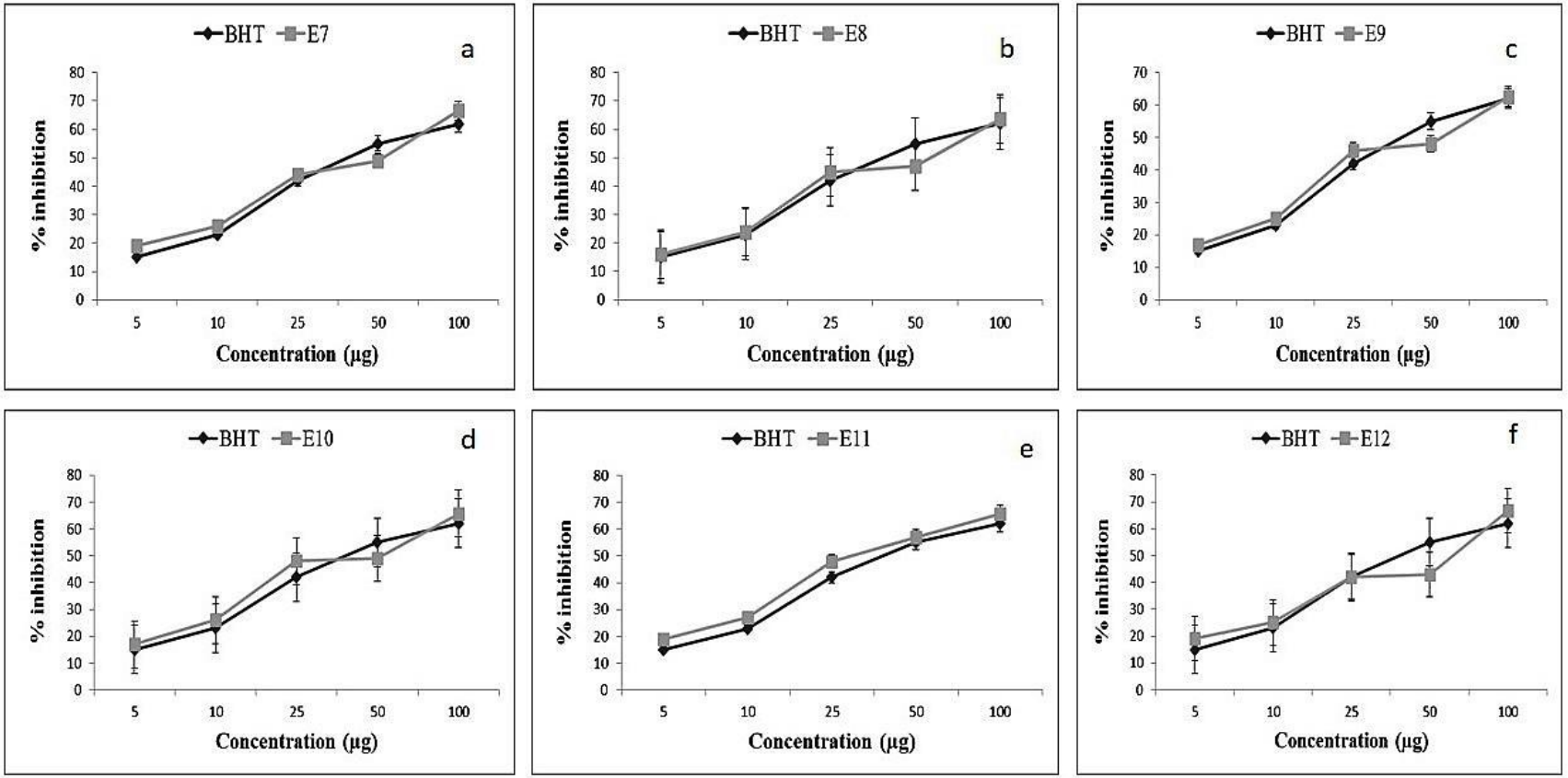


Fig.7.18 DPPH radical scavenging activity of E7-E12

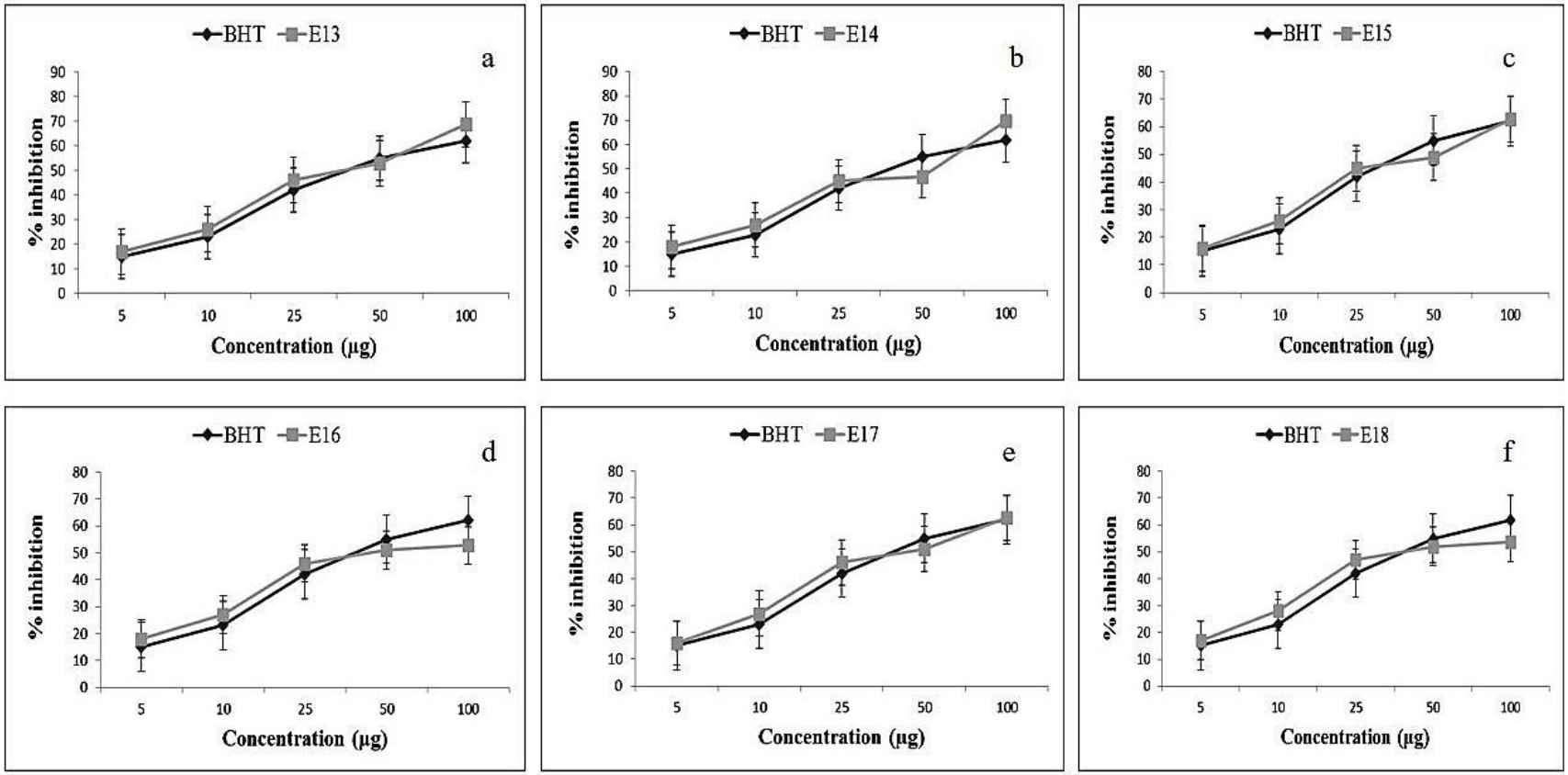


Fig.7.19 DPPH radical scavenging activity of E13-E28

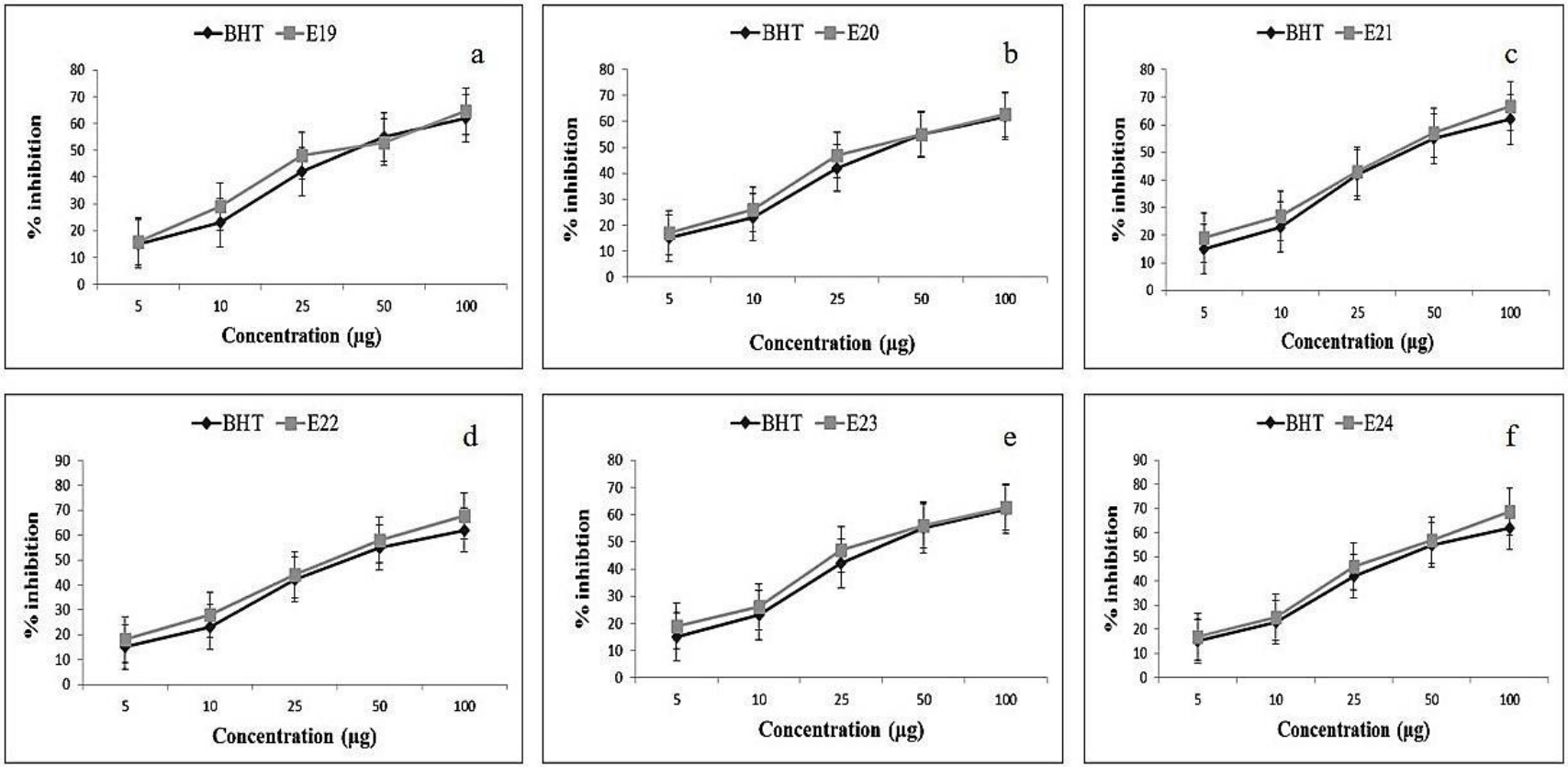


Fig.7.20 DPPH radical scavenging activity of E19-E24

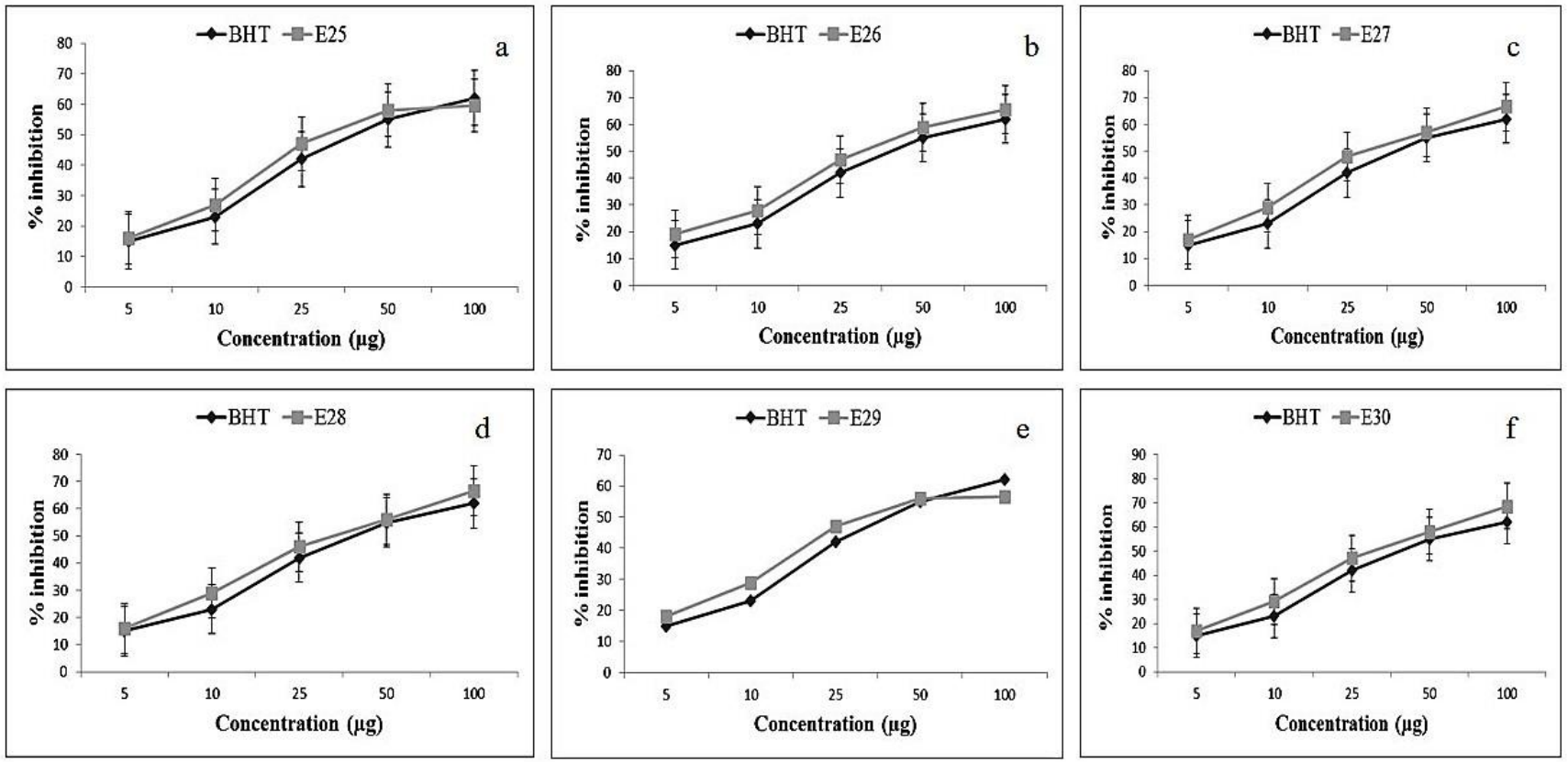


Fig.7.21 DPPH radical scavenging activity of E25-E30

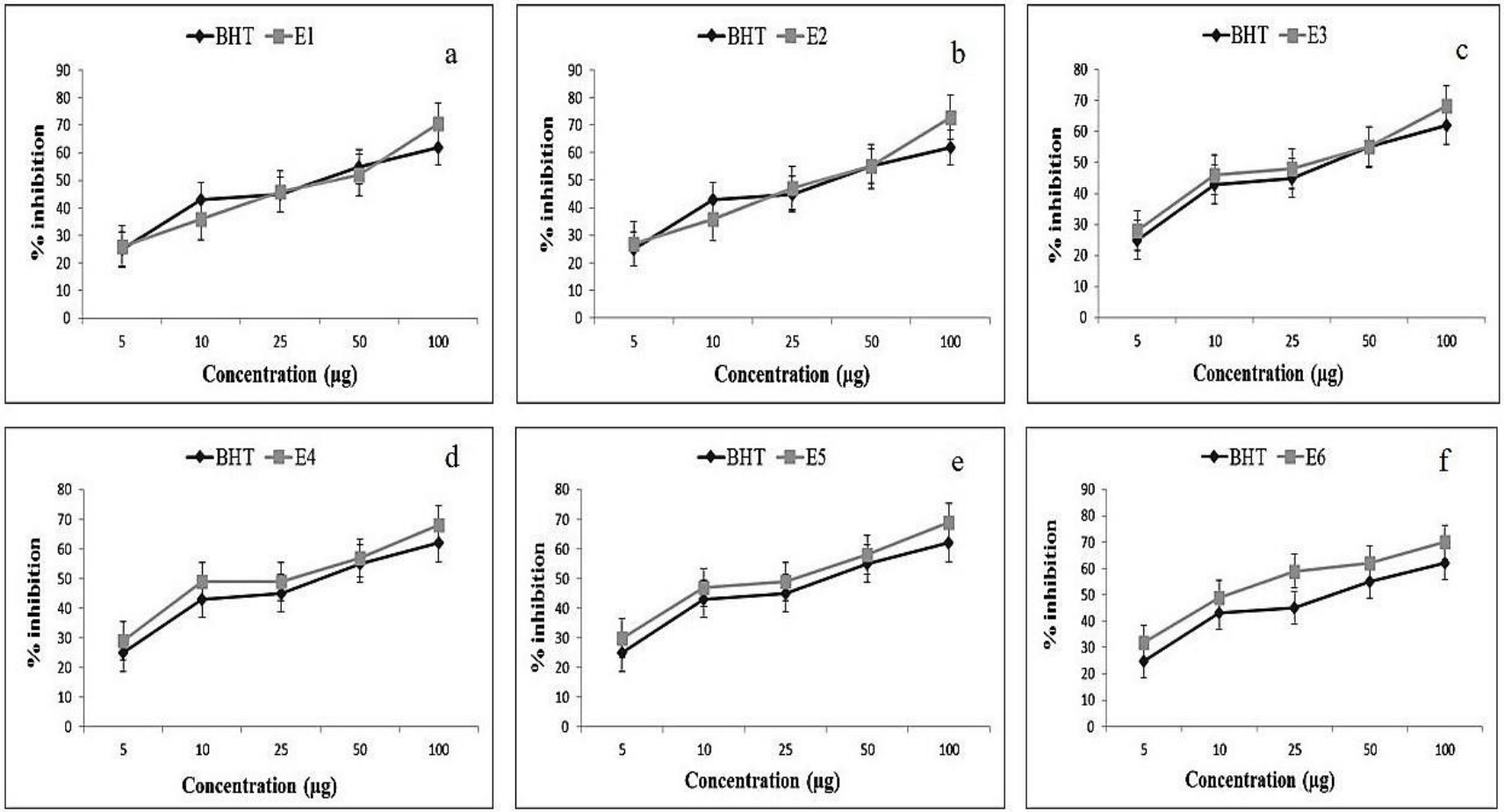


Fig.7.22 Hydrogen peroxide radical scavenging (H_2O_2) activity of E1-E6

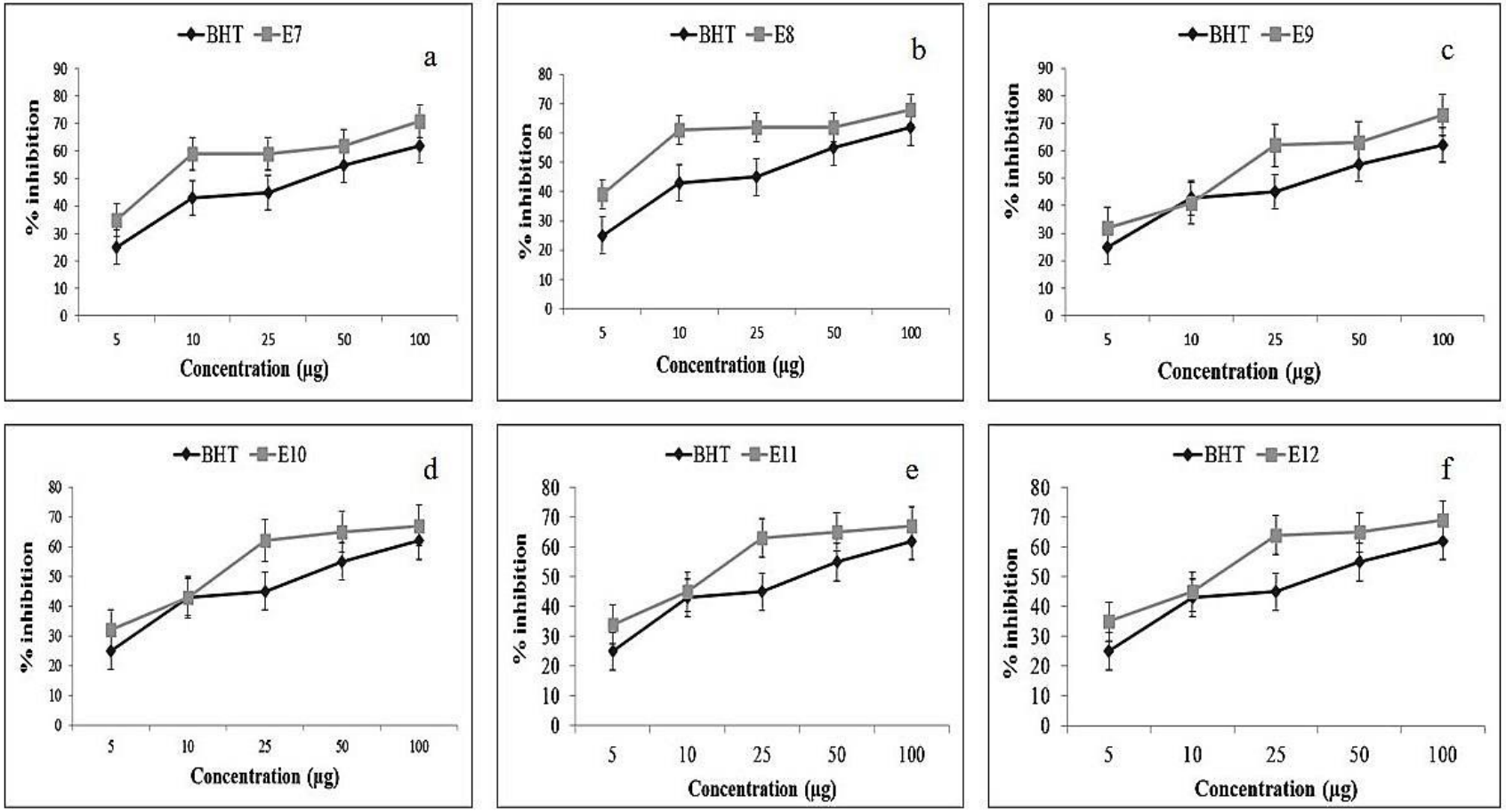


Fig.7.23 Hydrogen peroxide radical scavenging (H_2O_2) activity of E7-E12

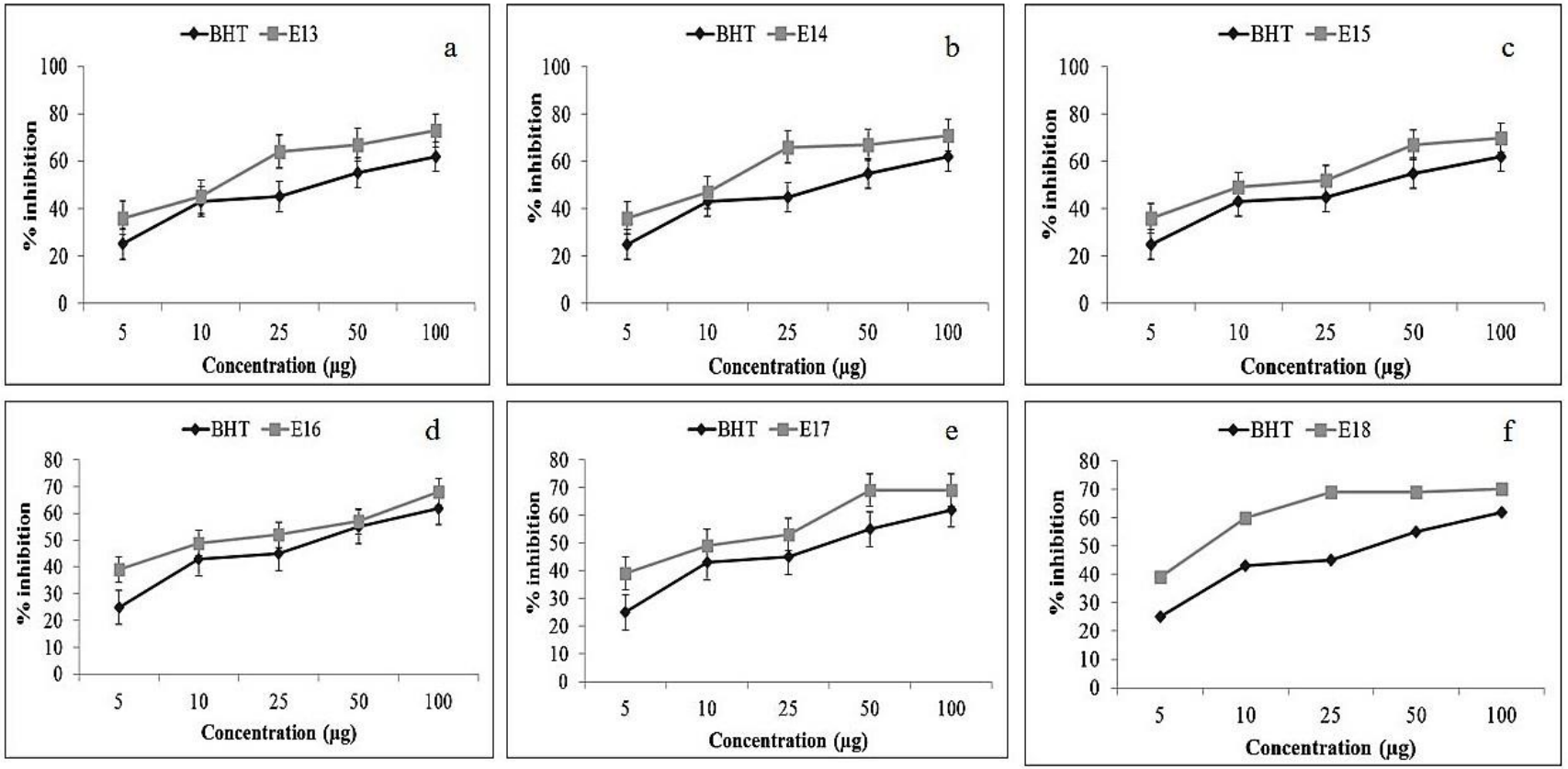


Fig.7.24 Hydrogen peroxide radical scavenging (H_2O_2) activity of E13-E18

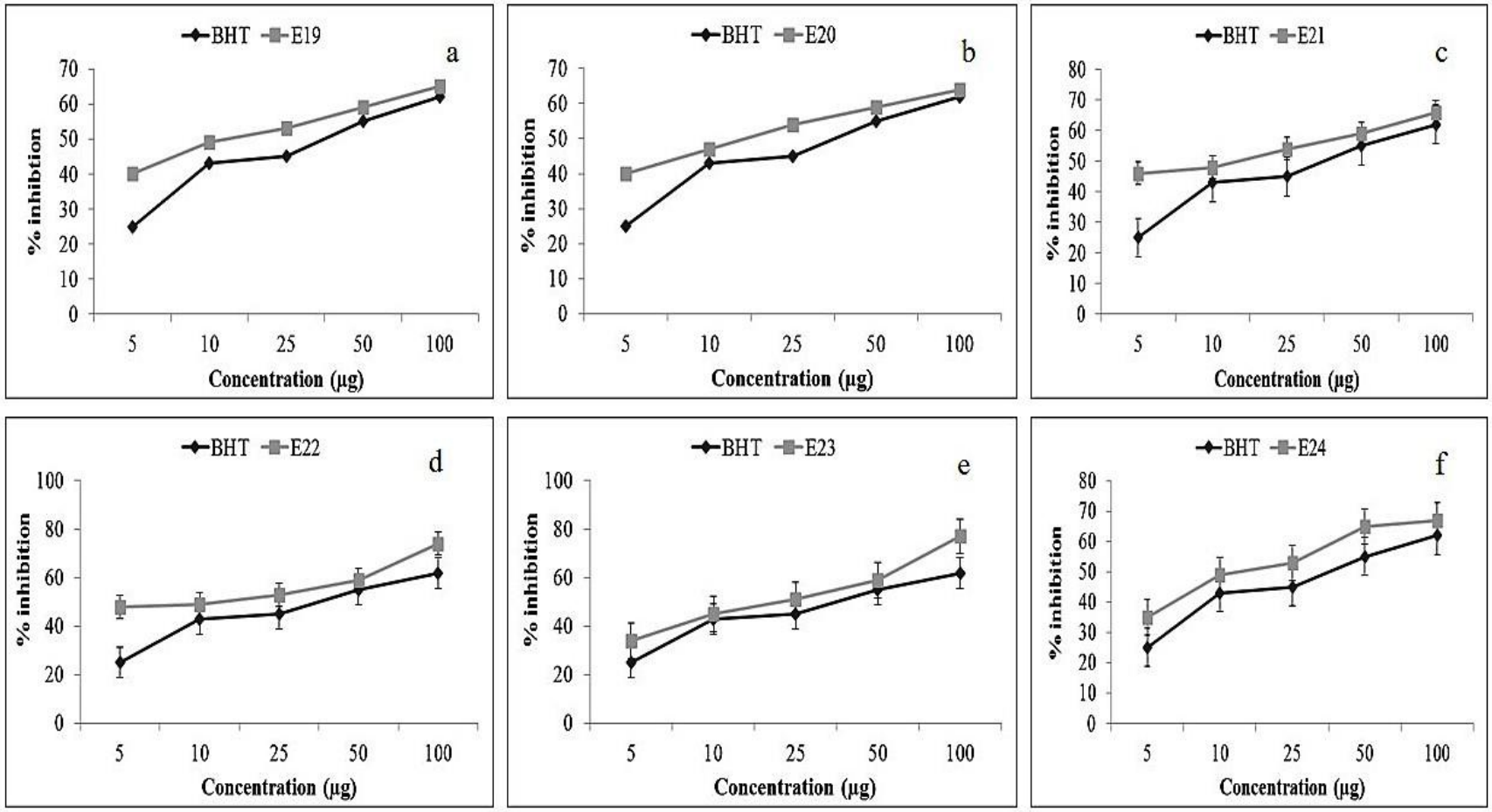


Fig.7.25 Hydrogen peroxide radical scavenging (H_2O_2) activity of E19-E24

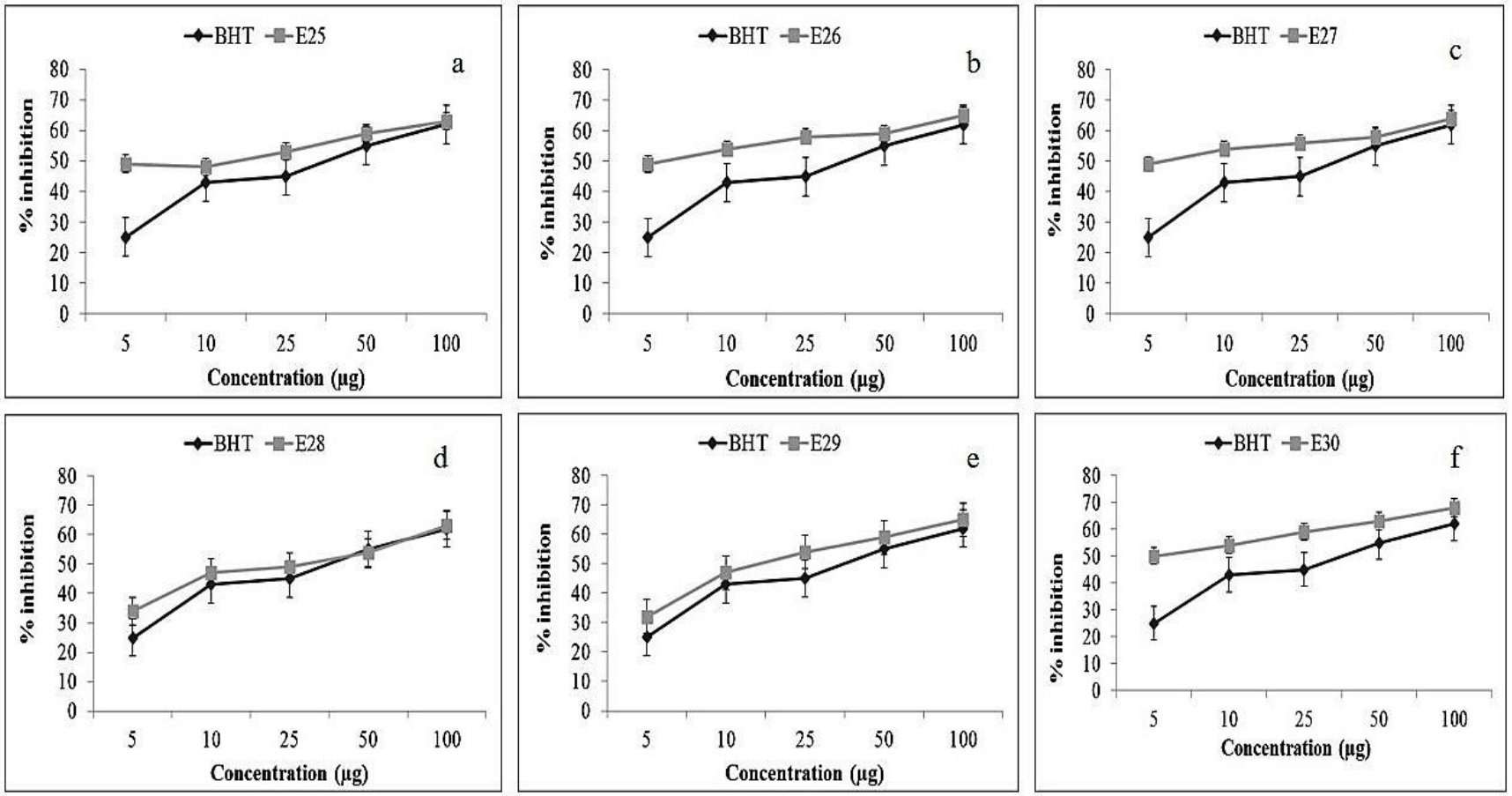


Fig.7.26 Hydrogen peroxide radical scavenging (H_2O_2) activity of E25-E30

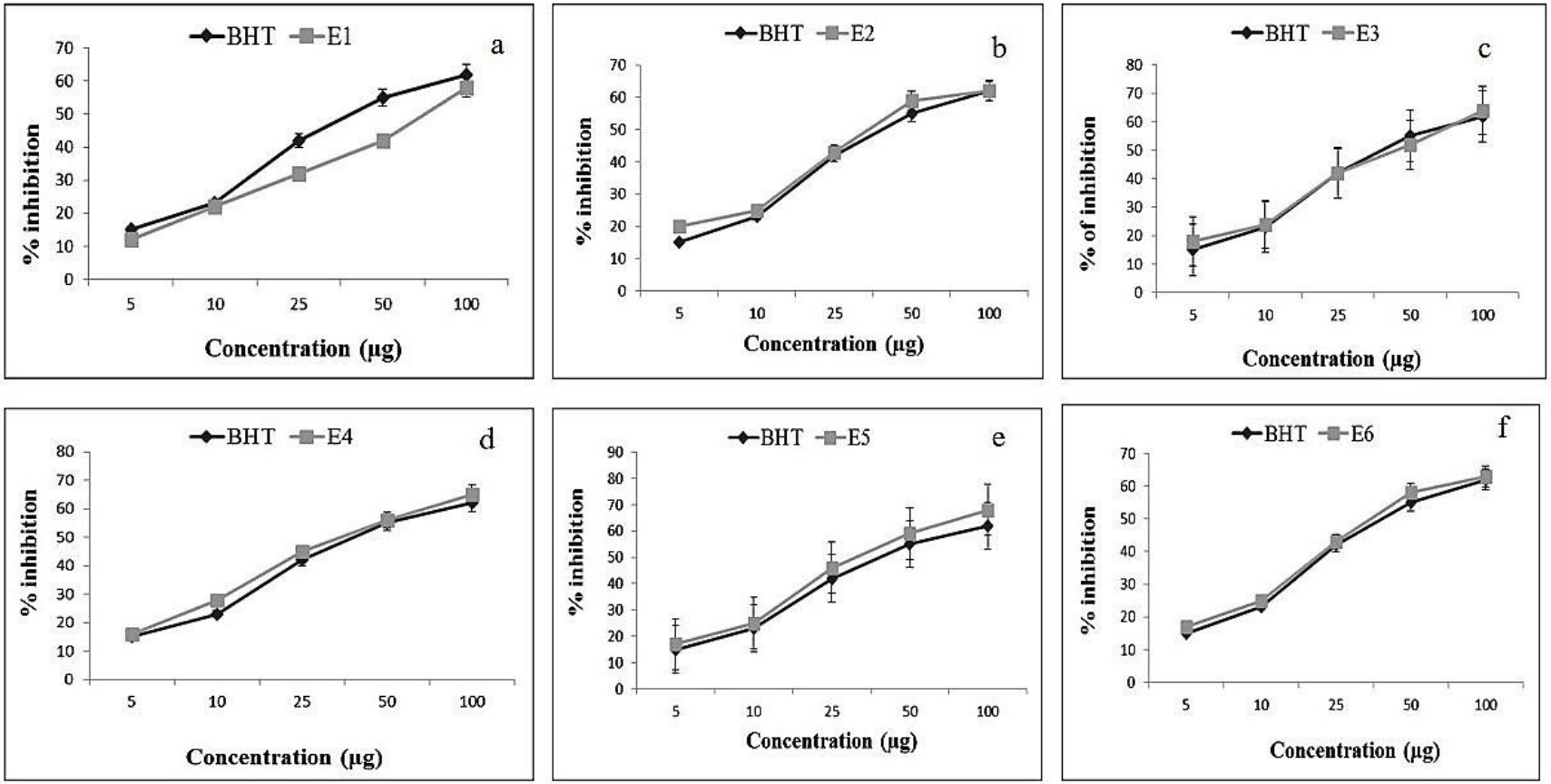


Fig.7.27 Total antioxidant activity of E1-E6

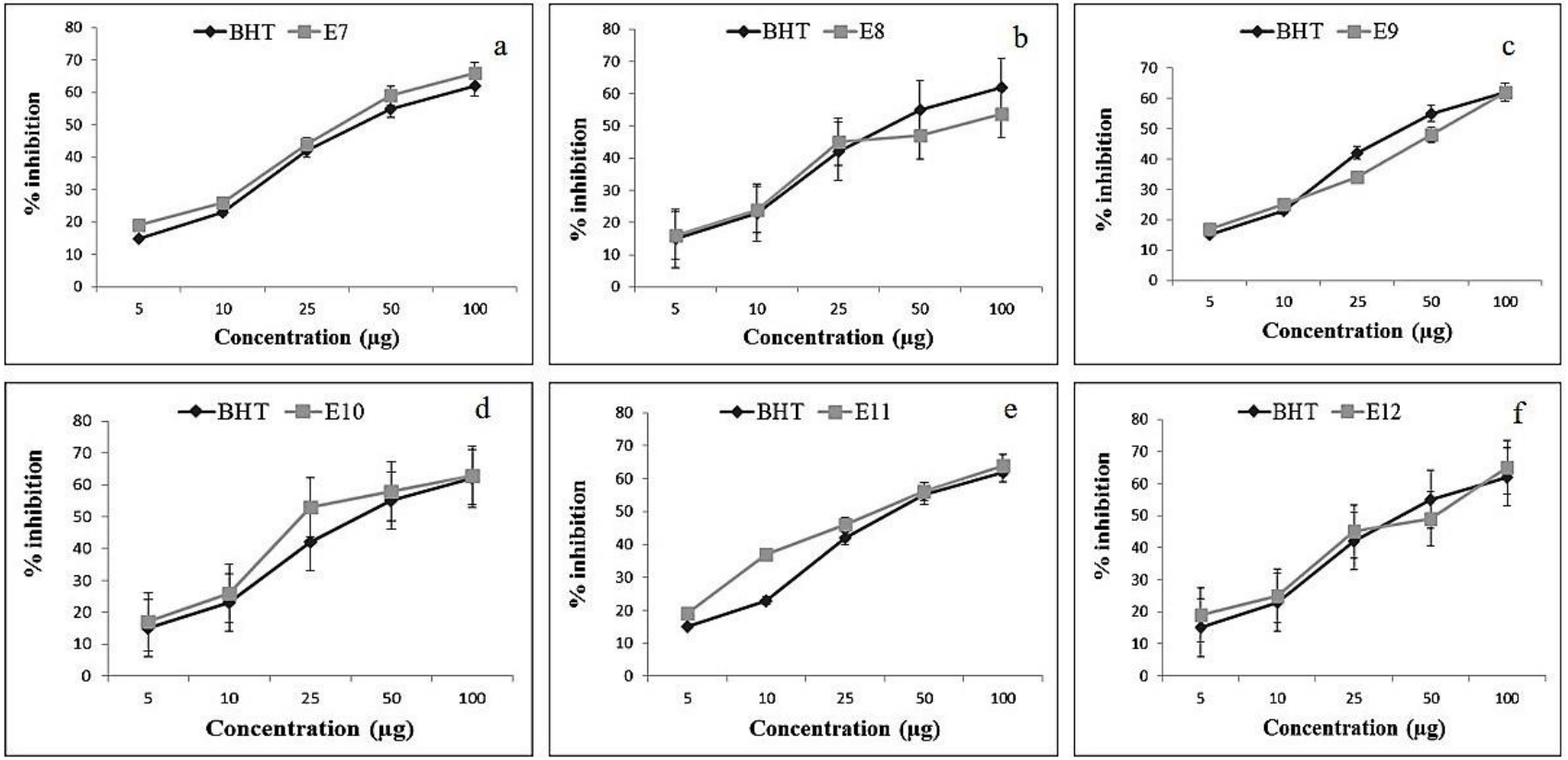


Fig.7.28 Total antioxidant activity of E7-E12

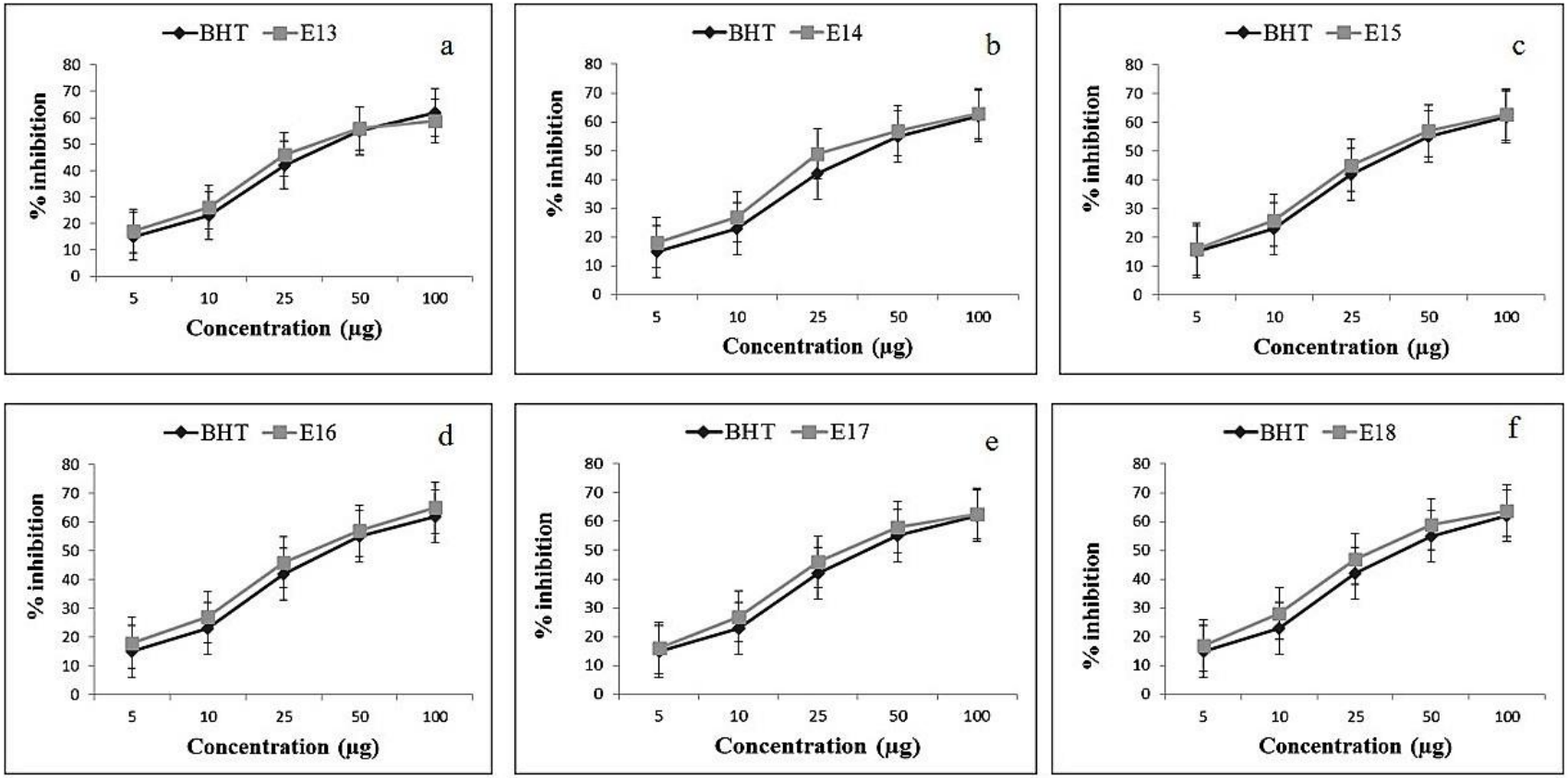


Fig.7.29 Total antioxidant activity of E13-E18

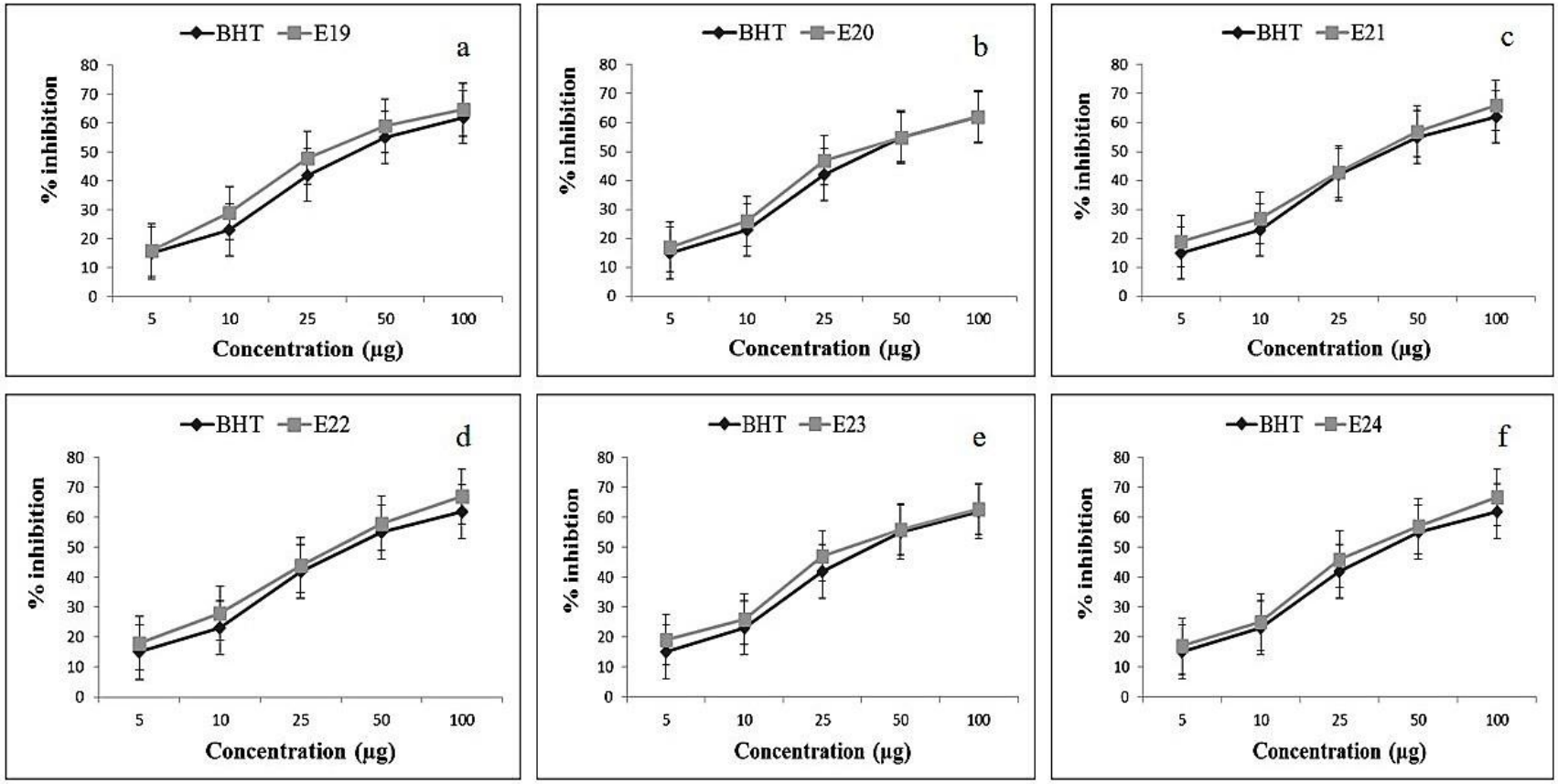


Fig.7.30 Total antioxidant activity of E19-E24

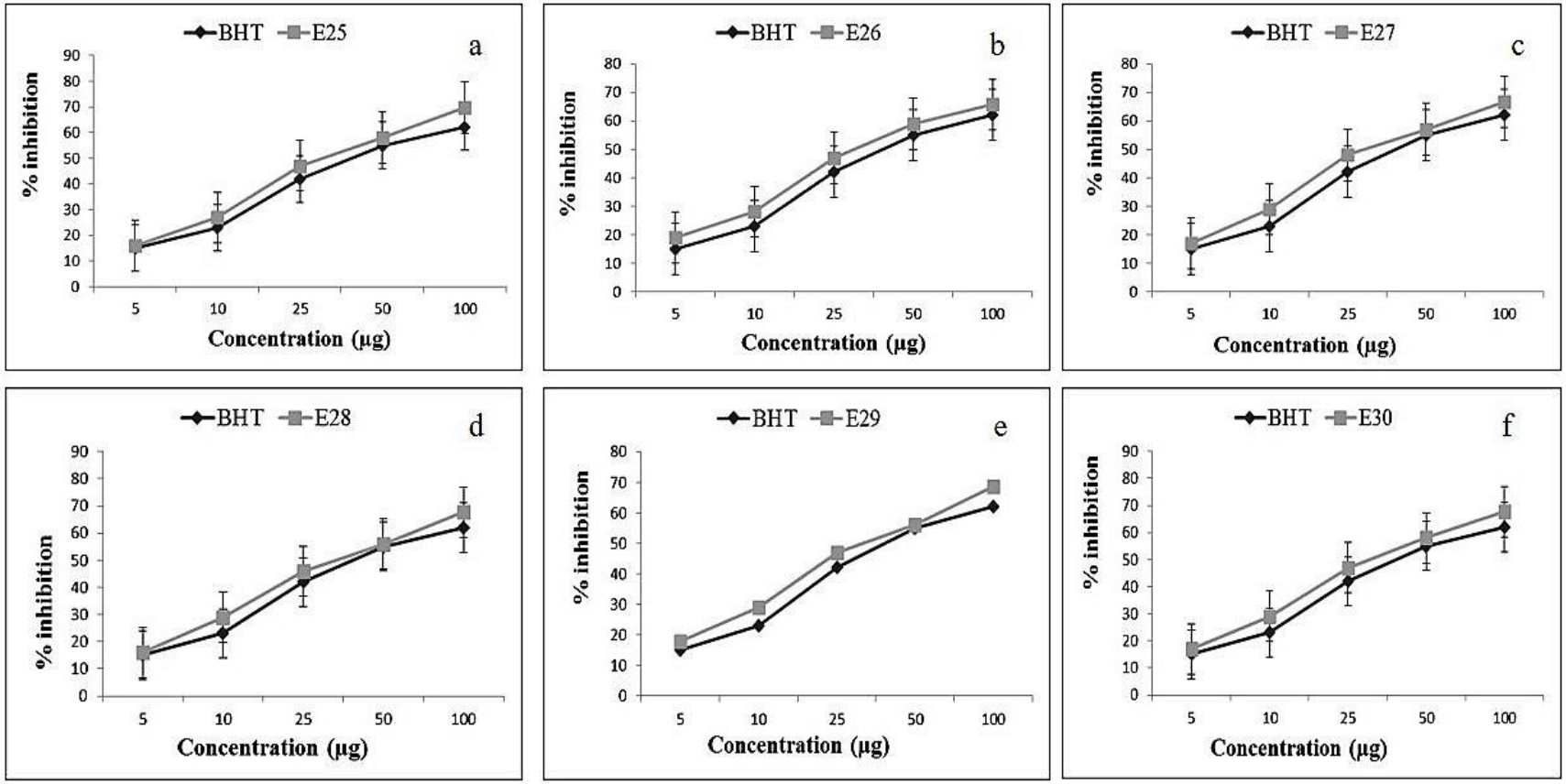


Fig.7.31 Total antioxidant activity of E25-E30

extreme environmental conditions (Mukund *et al.,* 2014). The isolated algae are rich in photosynthetic pigments. It is pertinent here to mention that photosynthetic pigments and phytochemicals present in the algal isolates plays an important role in protecting the chloroplasts against photodamage, by scavenging several active oxygen species (ROS) such as $^1O_2$, O_2^-, H_2O_2, hydroxyl radicals (HO^-) and peroxy radicals (Burton, 1989; Krinsky 1989; Munir *et al.,* 2013).

In a previous study, *Chlorella vulgaris* and *Acutodesmus obliquus* (*Scenedesmus obliquus*) were cultivated in normal water, Bold's medium and sewage water and found to be rich in lipid and antioxidants. Phenolic extract, lipid and antioxidant potential were found to maximum in sewage water as compare to normal water and Bold's medium (Shetty et al. 2015). In a recent study, the green alga *Acutodesmus obliquus* found to be rich in non-enzymatic antioxidants (ascorbate, glutathione) and metabolic profile (Piotrowska-Niczyporuk et al. 2018).

7.5 Conclusion

The results of the above study clearly indicated the presence of enzymatic and non – enzymatic antioxidant in algal species that could protect against oxidant and other free radical diseases. The isolated algal species from submerged polythene surface in sewage water are found to be the rich source of several bioactive compounds which have potential for pharmacological and biotechnology industries.

Chapter 8

PHYCOREMEDIATION OF DOMESTIC SEWAGE WATER USING SOME SELECTED ALGAL ISOLATES

8.1 Introduction

Algae have the biotransformation potential and can transform pollutants to recover nutrients and xenobiotic from sewage water and carbon dioxide from waste air (Mehta and Gaur, 2005). Physical adsorption is a process in bioremediation in which the metal ions are adsorbed over the cell surface very quickly just in a few seconds or minutes and chemisorptions is a process in which ions are transported slowly into the cytoplasm (Dwivedi, 2012). The microorganism has received the attention of the researchers due to its potential for application in phycoremediation. Some algae have been used for removal of heavy metal from contaminated sites. Use of algae for heavy metal remediation have

been documented - *Oscillatoria tenuis* (Ajavan *et al.,* 2011), *Oscillatoria quadripunctulata* (Rana *et al.,* 2013; Azizi *et al*., 2012), *Spirogyra hatillensis* (Dwivedi, 2012), *Spirogyra hyaline* (Kumar and Oommen, 2012), *Cladophora glomerata, Oedogonium rivulare* (Dwivedi, 2012), *Chlorella vulgaris*, *Spirulina maxima* (Chan *et al*., 2014) and *Chlorella vulgaris* (Aung *et al*., 2013; Edris et al., 2012). Azarpira *et al.,* (2014) studied cyanobacteria species in phycoremediation of municipal wastewater. Recent studies on remediation of municipal waste water by *Chlorella sorokiniana* have revealed that it can grow in waste water without pretreatment or additional nutrient sources. The species was efficient in removing nitrogen, phosphorus and organic carbon from waste water (Ramsundar *et al.,* 2017)

Most of the Indian cities are not sufficiently planned or systematically managed to contain large population and some small towns grow eccentrically into cities by arbitrary settlements. Water is one of the major product of nature used by human beings generating enormous waste water or sewage alongside.

The nutrient uptake potential of genus *Chlorella* received attention of researchers around the globe as they can be cultivated in nutrient rich wastewater without any dilution or sterilization - *Chlorella sorokiniana* (Ogbonna *et al.,* 2000), *Chlorella vulgaris* (Chan *et al.,* 2014; Aung *et al.,* 2012; Edris *et al.,* 2012, Feng and Zhang, 2011, Mohan *et al.,* 2009 and Megharj *et al.,* 1992), *Cholrella vulgaris* and *Cholrella protothecoides* (Marchao *et al.,* 2017).

Accordingly, incorporated in this chapter feasibility and assessment of raw domestic sewage water as growth media for the cultivation of selected species, *Oscillatoria subbrevis, Nostoc carneum* and *Chlorella ellipsoidea,* and biomass production thereof with an integrated approach of nutrient uptake from the sewage water.

8.2 Methodology

Details of the method of estimation, procedure for nutrient removal, growth curves and biochemical analysis were already described at **Chapter 3**. The abbreviations used for the phycoremediation different strains of microalgae are given in **Table 3.6**. One-way analysis of variance (ANOVA) was used to evaluate the differences among the treatments. The triplicate sets of data were evaluated in accordance with the experimental design (Completely Randomized Design) with ANOVA (Analysis of Variance). The

comparisons between the different means were made using post hoc least significant differences (LSD) calculated at P level of 0.05. Standard deviation values are depicted in the graphs as bars.

8.3 Results and discussion

8.3.1 Growth kinetics of the isolated strains in Domestic sewage water and mixture of media and Domestic sewage water

Results of growth study of *Oscillatoria subbrevis*, *Nostoc carneum* and *C. ellipsoidea* in different treatments are shown in **Fig. 8.1-8.3.** Chlorophyll accumulation of *Oscillatoria subbrevis* in different treatment was highest in BG11 (60%) + (40%) DSW and BG11 (80%) + (20%) DSW and lowest in BG11 (50%) + (50%) DSW on 20th day. Medium growth was observed in BG11 media grown *Oscillatoria subbrevis*. Same growth was observed in domestic sewage water grown *Oscillatoria subbrevis* (1b).

1g= 1h >1c > 1b > 1a >1e >1i >1d >1f

During lag phase, *Oscillatoria subbrevis* grown in BG11 (20%) + (80%) (1d) DSW have showed maximum growth, while BG11 (90%) + (10%) DSW (1i) have showed minimum carbohydrate production.

During log phase, maximum growth of *Oscillatoria subbrevis* was found to be in BG11 (40%) + (60%) DSW (1e) while BG11 (90%) + (10%) DSW have showed minimum growth.

During stationary period, maximum growth of *Oscillatoria subbrevis* was found to be in BG11 (40%) + (60%) DSW (1e while BG11 (90%) + (10%) DSW (1i) have showed minimum growth.

During death period, maximum growth of *Oscillatoria subbrevis* was found to be in BG11 (10%) + (90%) DSW (1c) while BG11 (90%) + (10%) DSW (1i) have showed the minimum growth.

Among the different treatments of domestic sewage water and culture media, chlorophyll accumulation was found to be maximum in 2b treatment i.e., *Nostoc carneum* grown in domestic sewage water (100%). Minimum growth was observed in BG11 (10%) + (90%) DSW (1i).

2b > 2a > 2d = 2e > 2c > 2g > 2i > 2h = 2f

During lag phase, *Nostoc carneum* grown in BG11 (60%) + (40%) DSW (2g treatment) have showed maximum growth while BG11 (10%) + (90%) DSW (2c) have showed minimum growth. During log phase, BG11 (40%) + (60%) DSW (2e) showed shown maximum growth while BG11 (90%) + (10%) DSW (2i) showed shown minimum growth in *Nostoc carneum*. During stationary period, BG11 (40%) + (60%) DSW (2e) have showed maximum growth while BG11 (90%) + (10%) DSW (2i) have showed minimum growth. During death period, BG11 (10%) + (90%) DSW (2c) showed showedmaximum growth while BG11 (1a) have showed the minimum growth.

Among the different treatments, chlorophyll accumulation was found to be highest in *Chlorella ellipsoidea* grown in only domestic sewage water (100%). The BG11 (20%) + (80%) DSW showed lowest growth, 3b > 3c > 3a > 3i > 3g = 3h > 3e > 3f > 3d

During lag phase, *Chlorella ellipsoidea* grown in BG11 (40%) + (60%) DSW (3e) have showed the maximum growth and BG11 (3a) minimum growth. During log phase maximum growth was observed in DSW grown (3b) (100%), and BG11 (3a) minimum growth. During stationary period, BG11 (50%) + (50%) DSW (3f) showed maximum growth while BG11 (3a) grown have showed the minimum growth. During death period, BG11 (60%) + (40%) DSW (3g) have showed the maximum growth while minimum growth was observed in BG11 (3a).

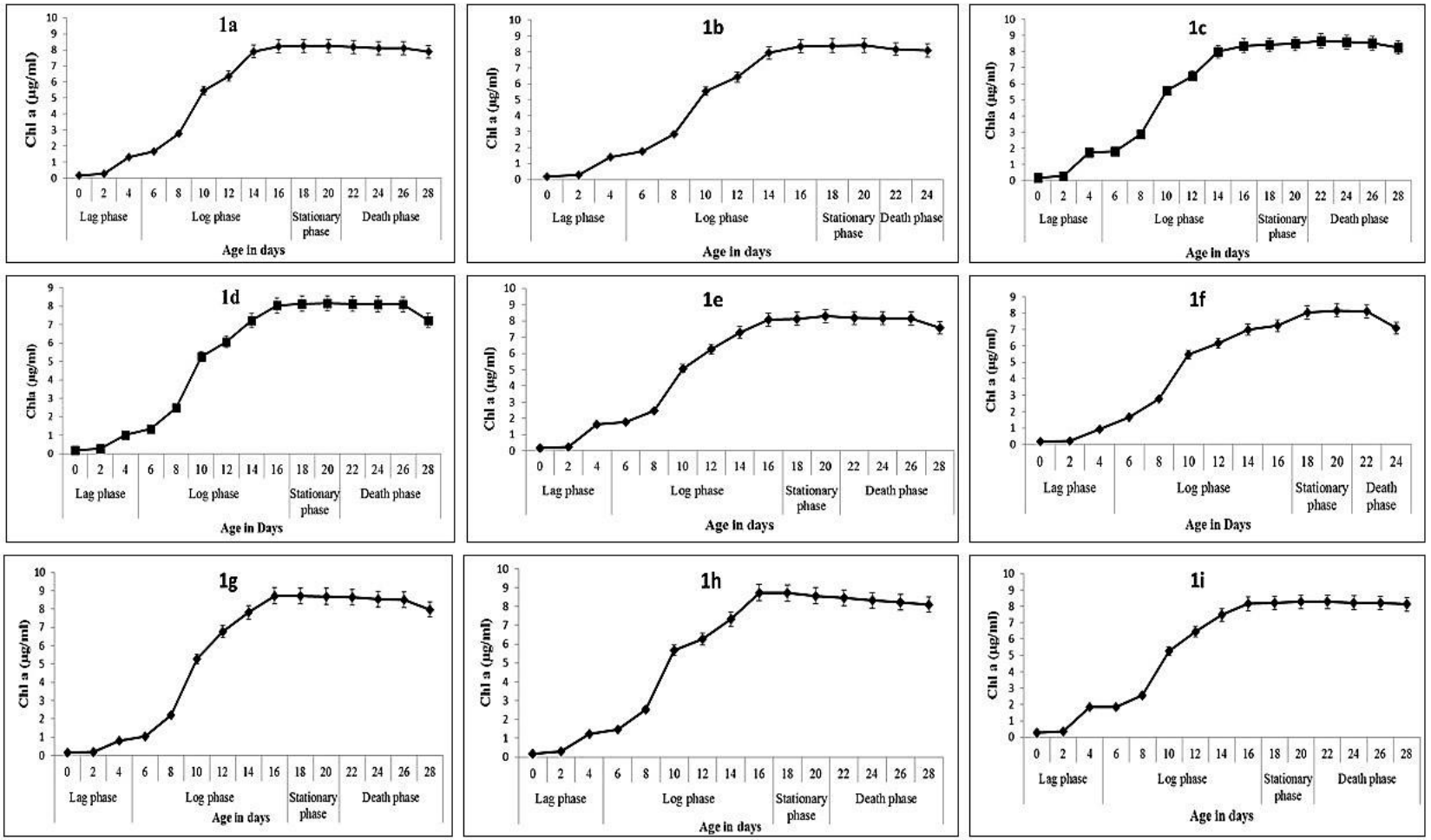


Fig. 8.1 Growth curves of *Oscillatoria subbrevis* for different treatment (1a-1i)

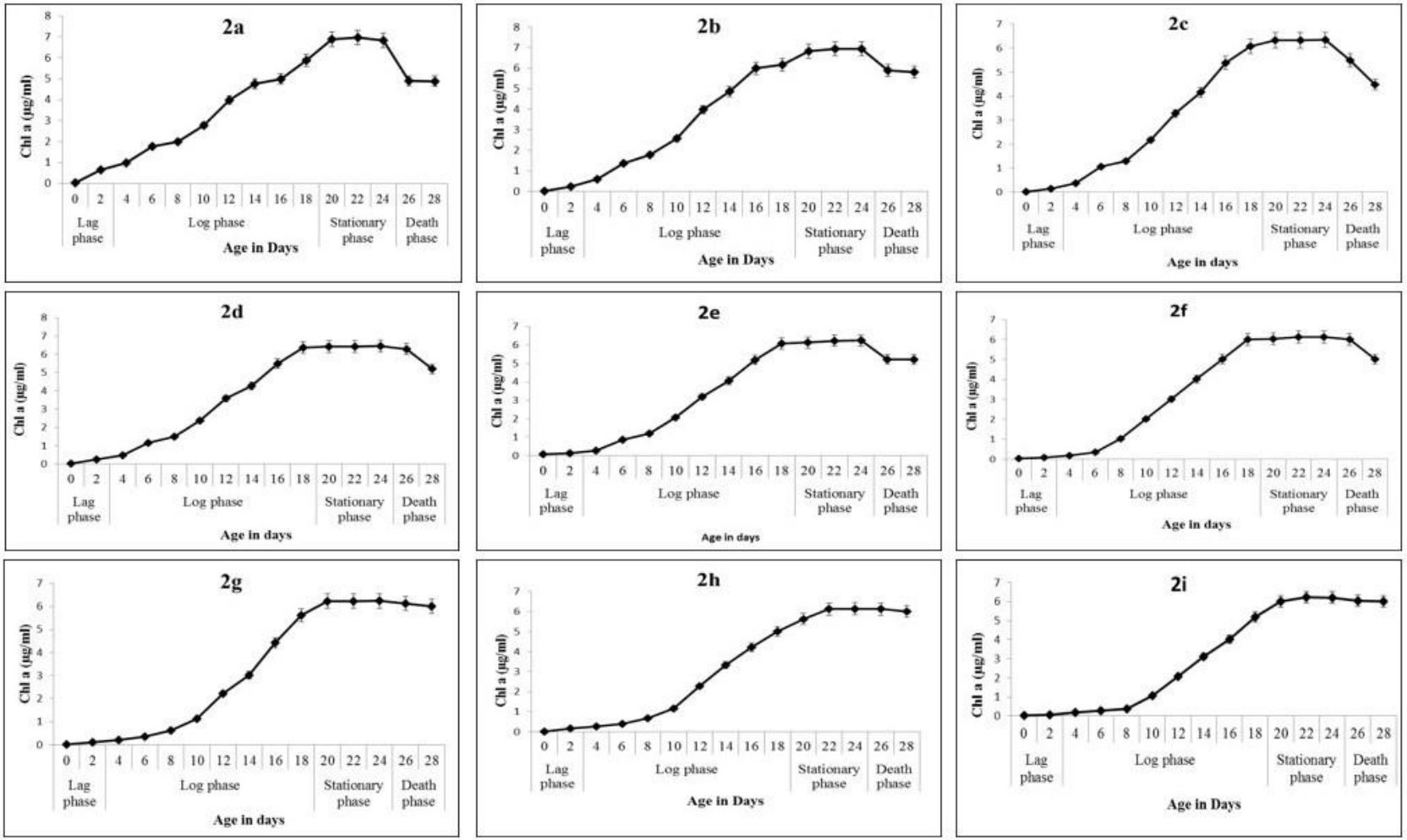


Fig. 8.2 Growth curves of *Nostoc carneum* for different treatment (2a-2i)

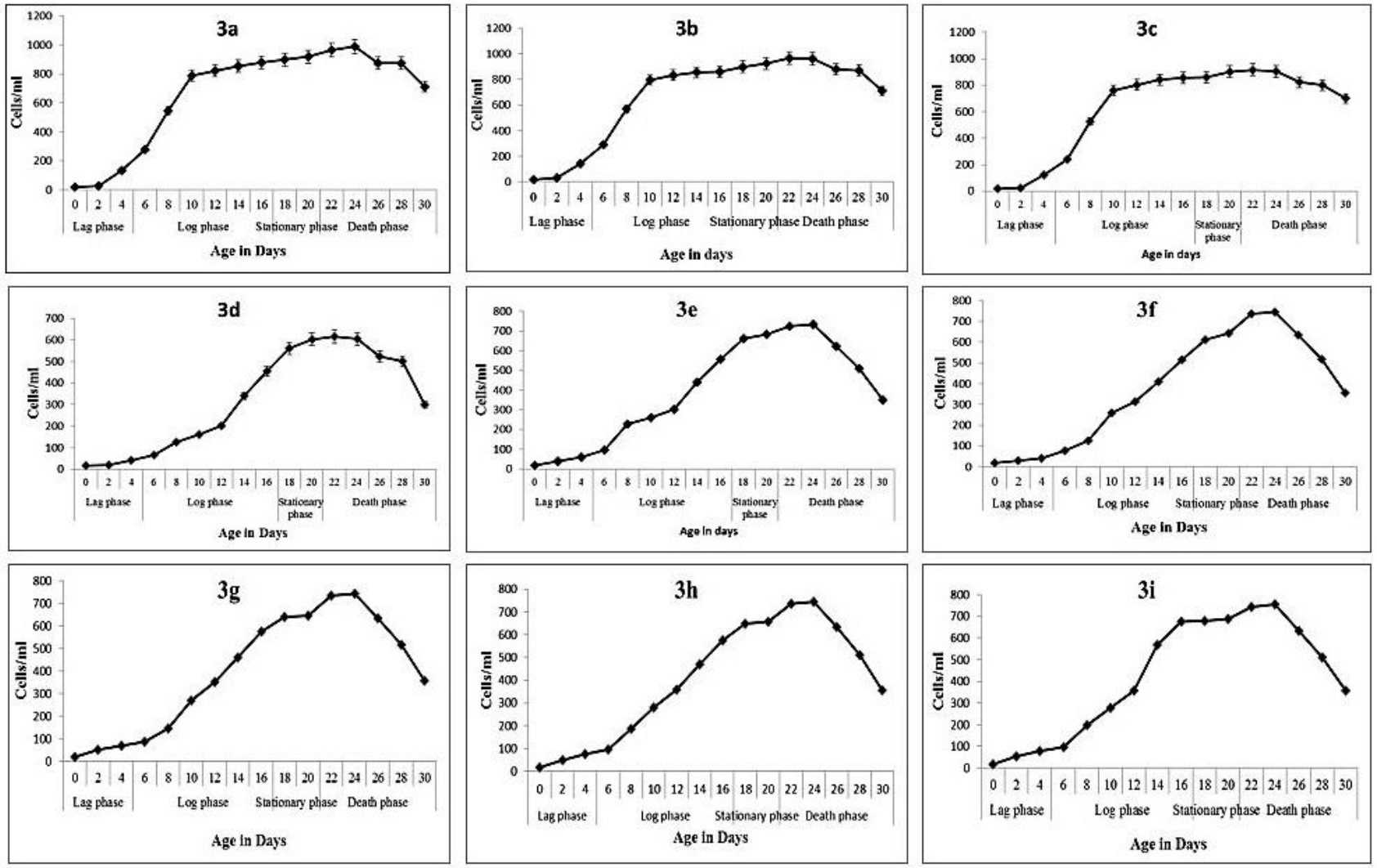


Fig. 8.3 Growth curves of *Chlorella ellipsoidea* for different treatment (3a-3i)

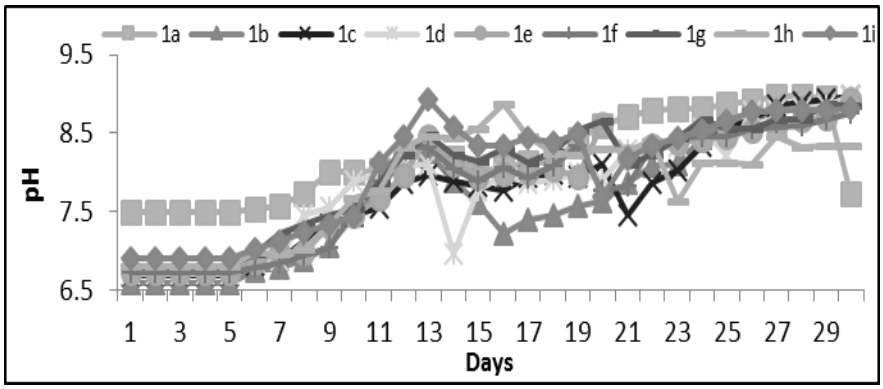


Fig.8.4 Variation of pH of media in 1a-1i treatment for *Oscillatoria subbrevis*

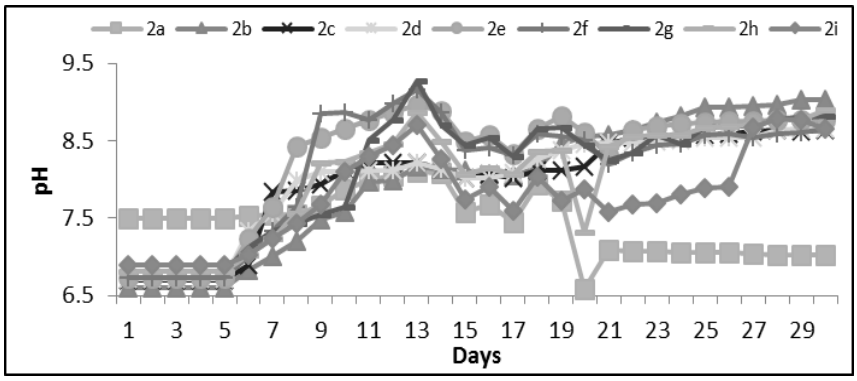


Fig.8.5 Variation of pH of media in 2a-2i treatment for *Nostoc carneum*

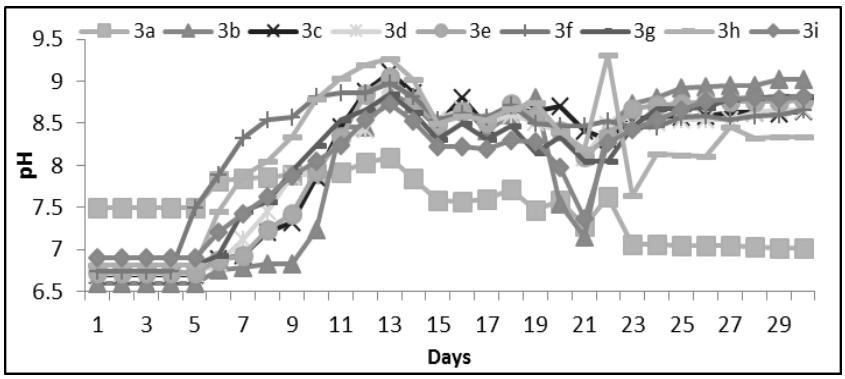


Fig.8.6 Variation of pH of media in 3a-3i for *Chlorella ellipsoidea*

Variation of pH in different treatments for three algae *Oscillatoria subbrevis*, *Nostoc carneum* and *Chlorella* sp over a period of 30 days are presented in **Fig. 8.4-8.6**. The pH of *Oscillatoria subbrevis* in BG11 was 7.5 in the initial day of the experiment and nearly 7 at the end of 30 days.

In case of domestic sewage water and (DSW) mixture of DSW and media, BG11, pH was found to be around 6.3 in the initial days which rose to around 9 at the end of 30 days. In case of *Nostoc carneum* and *Chlorella ellipsoidea*, similar results were obtained as that of *Oscillatoria subbrevis.* In a previous work, related to cultivation of microalgae in domestic sewage water a pH of around 9 has been recorded (Thomas *et al.,* 2016).

Phycoremediation of effluent of a soft drink manufacturing industry by *Chlorococcum* sp*, Chlorella conglomerata* and *Desmococcus* sp. with a special emphasis on nutrient removal has been studied (Sivasubramanian *et al.,* 2012). Nitrate and phosphate were removed very rapidly both under the laboratory and outdoor conditions. Phycoremediation efficiency of three micro algae, *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* were studied (Dominic *et al.,* 2009. Uptake of nutrients like phosphate, nitrate and nitrite was very high. The species, *Gloeocapsa gelatinosa* showed more efficiency than *Chlorella vulgaris* and *Synechocystis salina* in phycoremediation). In the present study, removal rate for nutrients was comparatively higher, *Oscillatoria subbrevis* being more efficient . The pH for all the treatments were monitored for a period of 30 days. The BG-11 media, served as control, showed lowest pH compared to domestic sewage water (DSW) treatments and BG11+DSW treatments.

8.3.3 Dry cell weight

Dry cell weight of three algae in different treatments are shown in **Fig.8.7-8.9**. Dry cell weight was found to be highest in domestic sewage water grown *Oscillatoria subbrevis* and *Nostoc carneum* (1b & 2b). In case of 3f *treatment Chlorella ellipsoidea*, have showed maximum dry cell weight (BG11 (50%) + (50%) DSW.

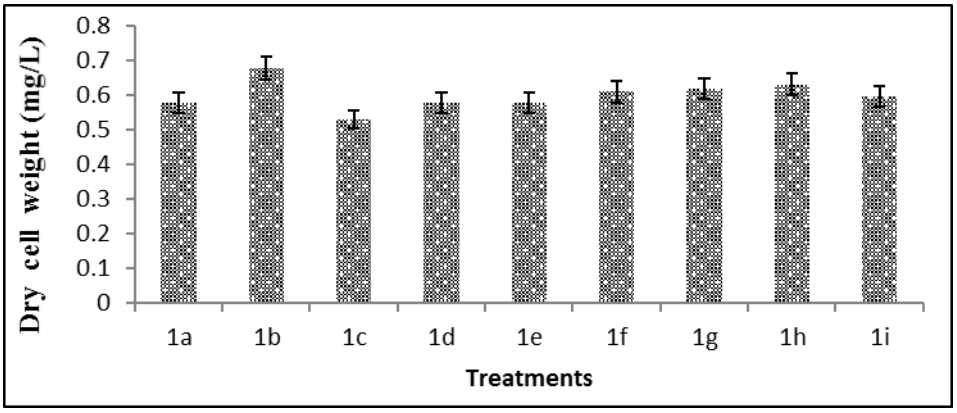


Fig.8.7 Dry cell weight of *Oscillatoria subbrevis*

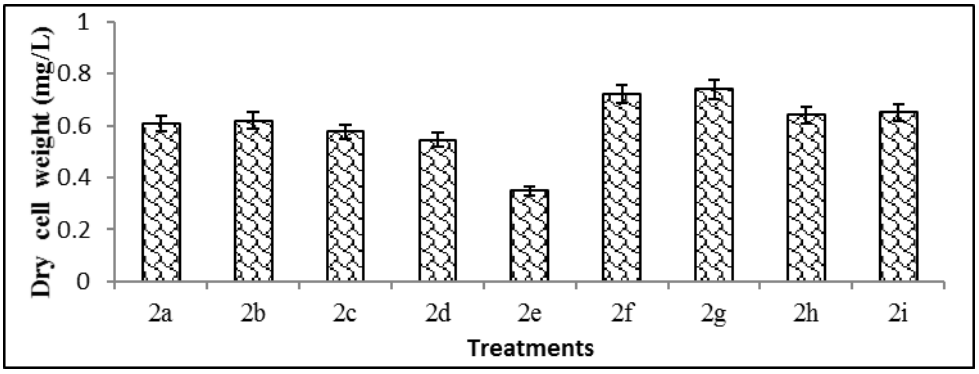


Fig.8.8 Dry cell weight of *Nostoc carneum*

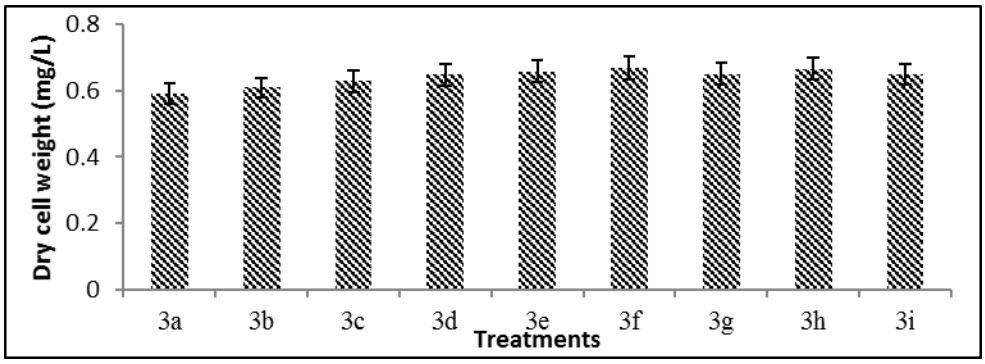


Fig.8.9 Dry cell weight of *Chlorella ellipsoidea*

8.3.4 Phaeopigment and physiological stress indices

Phaeopigment and physiological stress indices has been shown in Fig.8.10-8.12. Both revealed a trend of 1b >1i >1e >1c >1h >1d >1g >1f >1a for *Oscillatoria subbrevis*, 2f >2h >2b >2g >2c >2d >2i >2e >2a for *Nostoc carneum* and 3d >3i >3h >3c >3f >3e >3g >3b >3a for *Chlorella ellipsoidea* on 20th day.

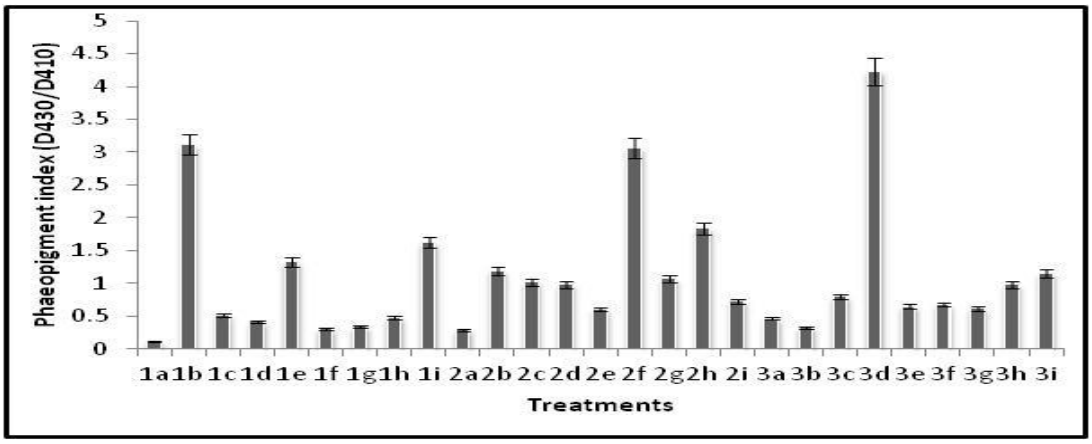


Fig.8.10 Phaeopigment index of 1a-1i, 2a-2i, 3a-3i treatments

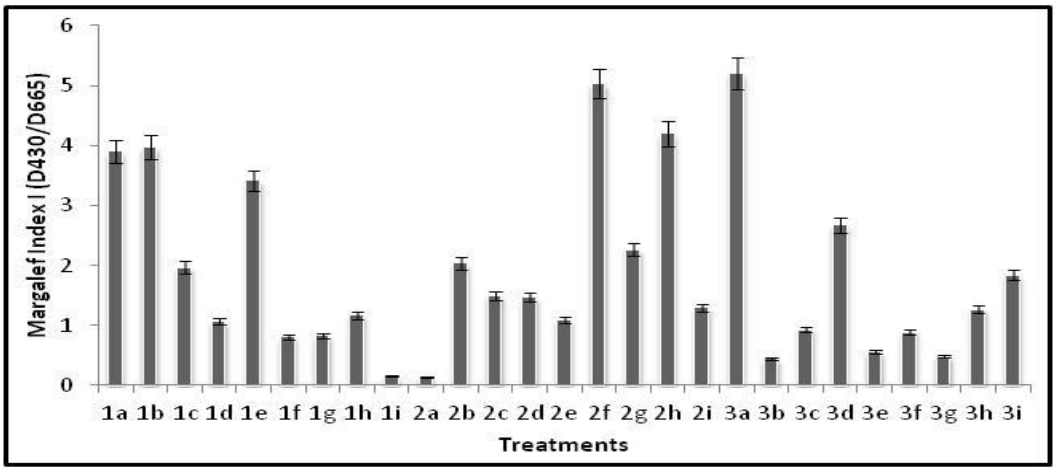


Fig.8.11 Margalef index I of 1a-1i, 2a-2i, 3a-3i treatments

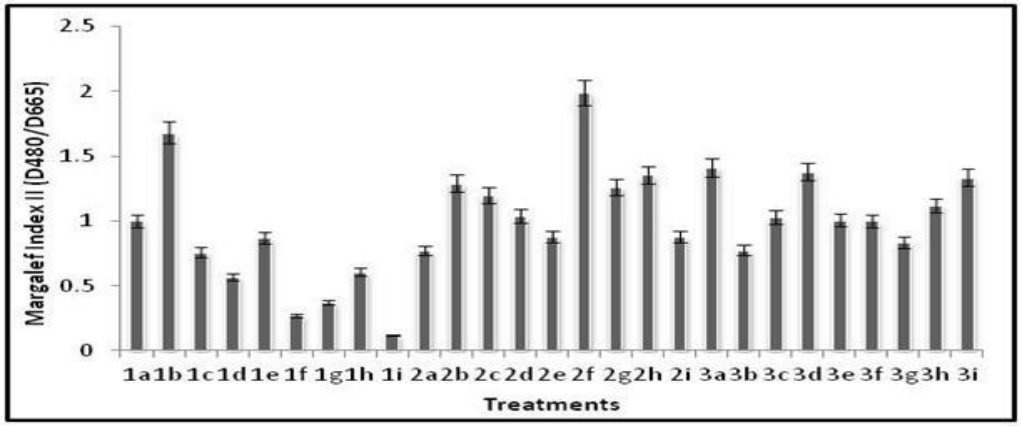


Fig.8.12 Margalef index I of 1a-1i, 2a-2i, 3a-3i treatments

Photosynthetic pigment composition is widely used for evaluating stress in different organisms such as algae (Marker *et al.,* 1980), bryophytes (Spitale, 2009), and vascular plants (Megateli *et al.,* 2009). The ratio of physiological stress index (D665/D665a) and other stress indices - phaeopigment index (D430/D410), Margalef index I (D430/D665) and Margalef index II (D480/D665) decreases in organisms in response to stresses such as heavy metal pollution (Megateli *et al.,* 2009; Spitale, 2009). In the present study, it revealed the stress indices were stable in domestic sewage water and media mixture as compared to 100% domestic sewage water and media treatments.

8.3.4. Variation of biochemical composition for different treatments during phycoremediation of domestic sewage water

Variation of carbohydrates and proteins for different treatments of three isolates has been presented in Fig.8.13-8.18. During lag phase, *Oscillatoria subbrevis* grown in BG11 (20%) + (80%) (1d) DSW have showed maximum carbohydrate, BG11 (90%) + (10%) DSW (1i) have showed minimum carbohydrate production. During log phase, BG11 (40%) + (60%) DSW (1e) have showed maximum carbohydrate while BG11 (90%) + (10%) DSW have showed the minimum carbohydrate. During stationary period, BG11 (40%) + (60%) DSW (1e) have showed maximum carbohydrate while BG11 (90%) + (10%) DSW (1i) have showed the minimum carbohydrate. During death period, BG11 (10%) + (90%) DSW (1c) have showed maximum carbohydrate while BG11 (90%) +

(10%) DSW (1i) have showed the minimum carbohydrate. During lag phase, *Nostoc carneum* grown in BG11 (60%) + (40%) DSW (2g) have showed the maximum growth while BG11 (10%) + (90%) DSW (2c) the showed minimum carbohydrate . During log phase, BG11 (40%) + (60%) DSW (2e) have showed the maximum carbohydrate while BG11 (90%) + (10%) DSW (2i) have showed the minimum carbohydrate. During stationary period, BG11 (40%) + (60%) DSW (2e) showed maximum carbohydrate while BG11 (90%) + (10%) DSW (2i) have recorded the minimum carbohydrate. During death period, BG11 (10%) + (90%) DSW (2c) have showed maximum carbohydrate while BG11 (1a) recorded minimum carbohydrate. During lag phase, *Chlorella ellipsoidea* grown in BG11 (40%) + (60%) DSW (3e) have showed maximum carbohydrate and BG11 (3a) minimum carbohydrate. During log phase, DSW grown (3b) have showed the maximum carbohydrate and BG11 (3a) minimum. During stationary period, BG11 (50%) + (50%) DSW (3f) have showed the maximum carbohydrate while BG11 (3a) grown exhibited minimum value. During death period, BG11 (60%) + (40%) DSW (3g) have showed the maximum carbohydrate while BG11 (3a) showed shown the minimum carbohydrate. The strain, *Oscillatoria subbrevis* showed maximum carbohydrate and protein content in BG-11+ domestic water treatment series. A recent study observed 41% carbohydrate and high lipid production (20%) when *Desmodesmus* spp. and *Scenedesmus obliquus* were grown in municipal wastewater (Hernández-Garcia *et al.*, 2019). In an investigation, the microalga, *Asterarcys quadricellulare* grown in waste water recorded maximum amount of lipid and carbohydrate production (Oliveira *et al.*, 2017).

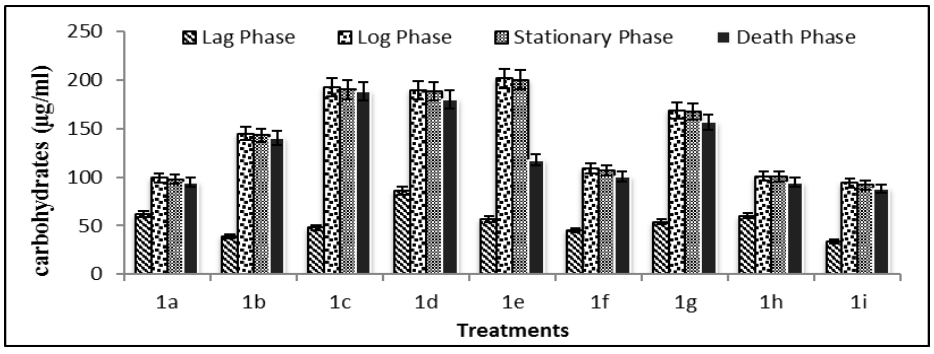


Fig.8.13 Variation of carbohydrate (µg/ml) *Oscillatoria subbrevis* in 1a-1i

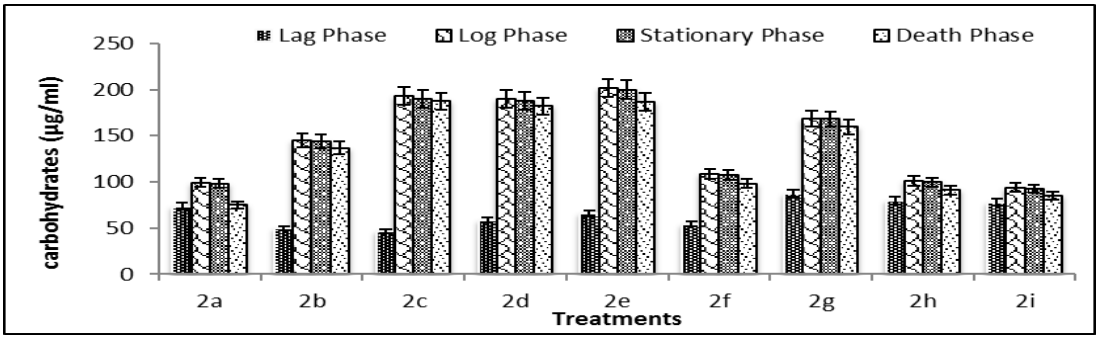


Fig.8.14 Variation of carbohydrate (µg/ml) *Nostoc carneum* in 2a-2i

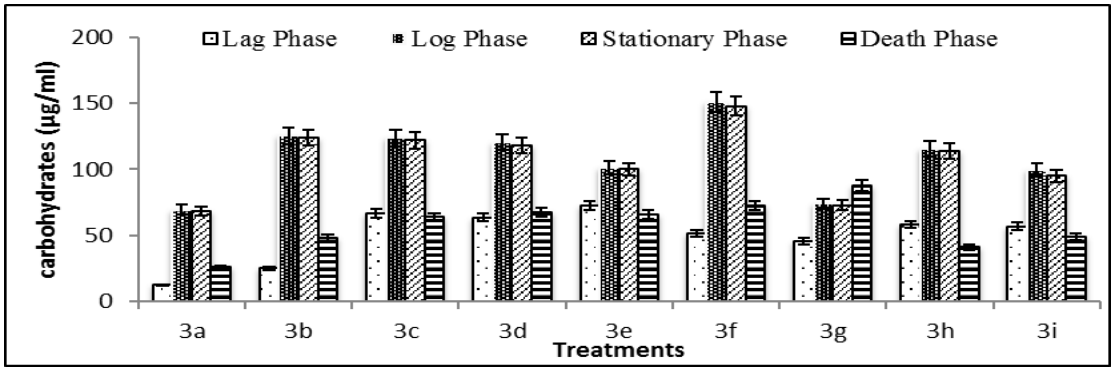


Fig.8.15 Variation of carbohydrate (µg/ml) *Chlorella ellipsoidea* in 3a-3i

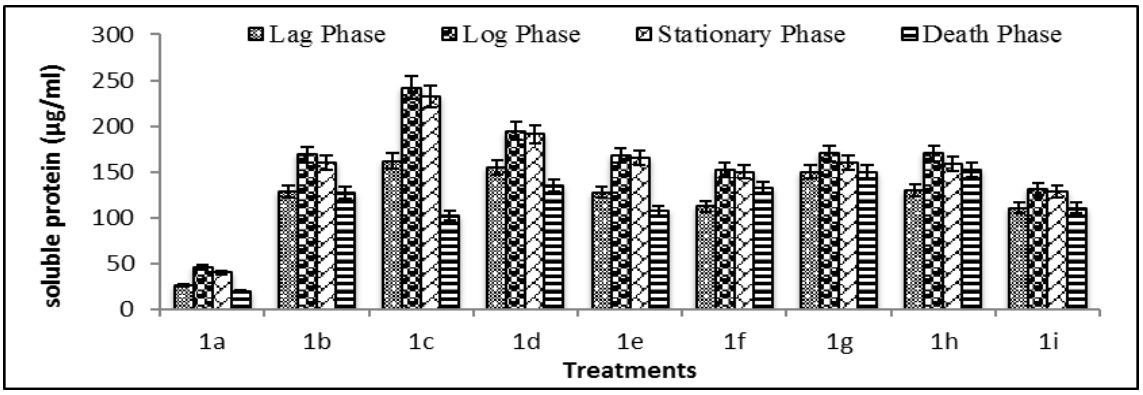


Fig.8.16 Variation of protein (µg/ml) *Oscillatoria subbrevis* in 1a-1i

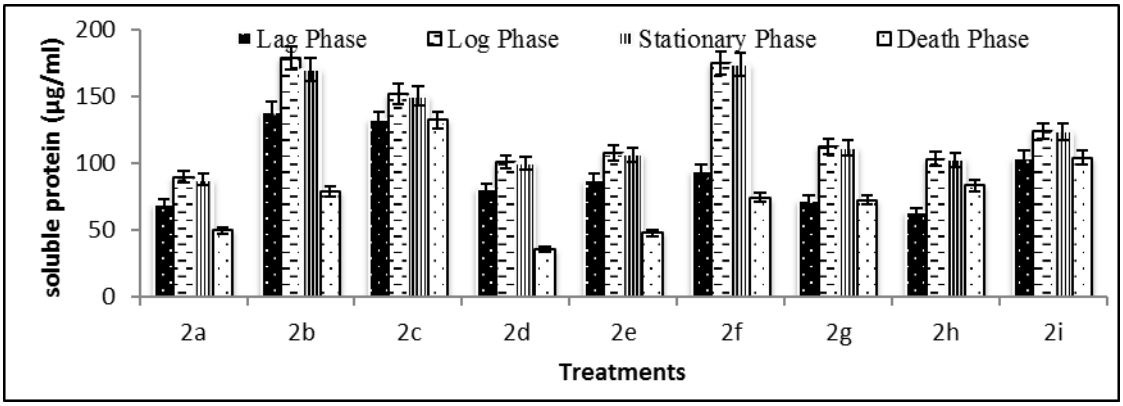


Fig.8.17 Variation of protein (µg/ml) content *Nostoc carneum* in 2a−2i

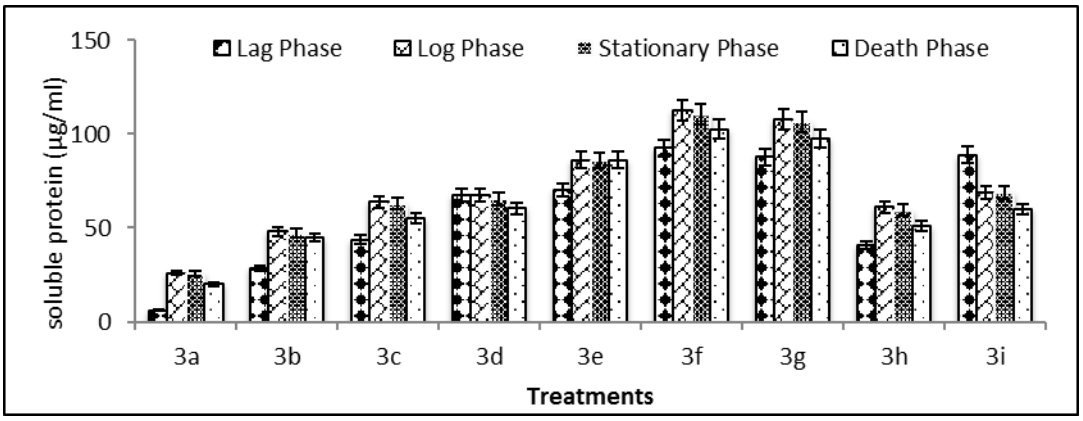


Fig.8.18 Variation of protein content (µg/ml) *Chlorella ellipsoidea* in 3a− 3i

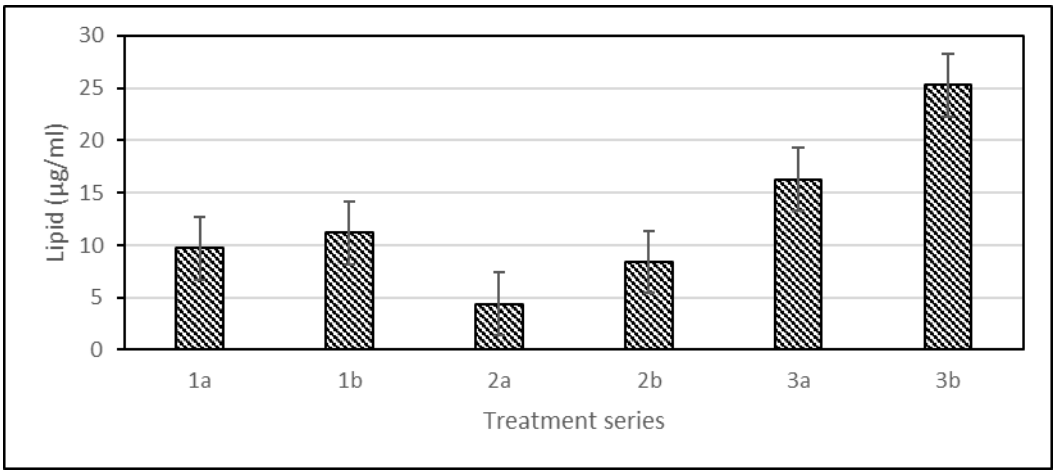


Fig.8.19. Variation of lipid content (µg/ml) in control BG11 (a) and domestic sewage water (b)

8.3.5. Enzyme production

Enzyme activity for different treatments of three algae are presented in 8.20-8.23. Laccase production was found to be highest in 1c (BG11 10% + 90% DSW) for *Oscillatoria subbrevis*, 2c (BG11 10% + 90% DSW) for *Nostoc carneum* and 3h (BG11 80% + 20% DSW) for *Chlorella ellipsoidea*. Peroxidase was found to be highest in (BG11 50% + 50% DSW) in 1f for *Oscillatoria subbrevis* and 2g BG11 (60%) + (40%) DSW for *Nostoc carneum* and (BG11 50% + 50% DSW) in 3f treatment for *Chlorella* sp. The catalase activity was found to be highest in 1b treatment (DSW) for *Oscillatoria subbrevis*, (2g) BG11 (60%) + (40%) DSW for *Nostoc carneum* and 3h BG11 (80%) + (20%) DSW for *Chlorella* sp. The GR was found to be highest in 1g treatment, BG11 (60%) + (40%) DSW for *Oscillatoria subbrevis* , 2f treatment BG11 (50%) + (50%) DSW for *Nostoc carneum* and 3d BG11 (20%) + (80%) DSW for *Chlorella ellipsoidea*.

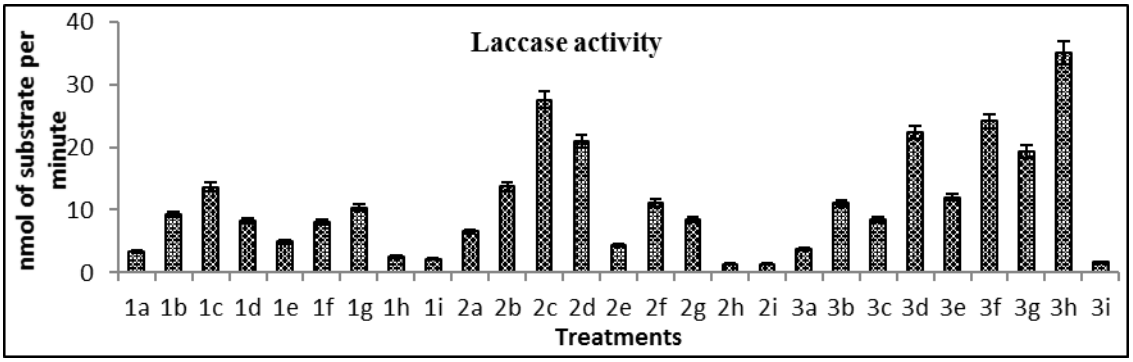


Fig.8.20 Variation of laccase in the treatments of 1a-1i, 2a-2i, 3a-3i

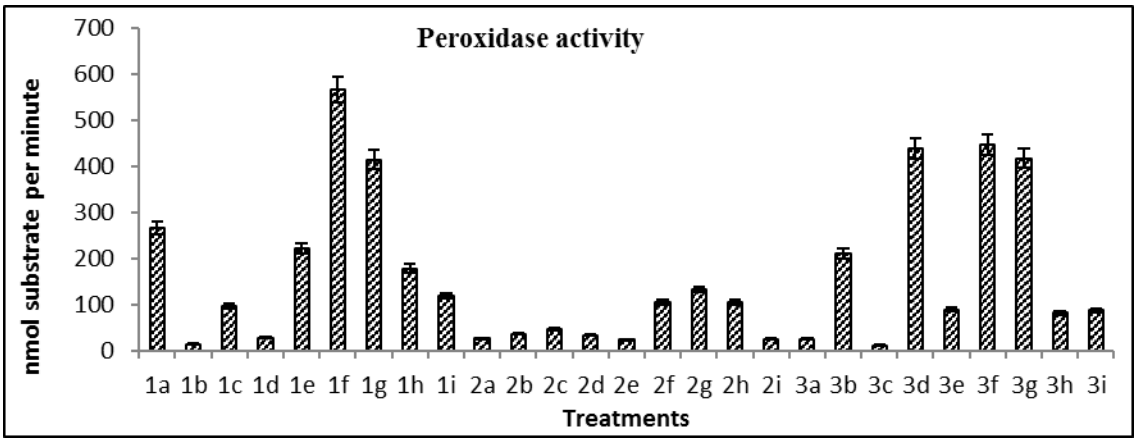


Fig.8. 21 Variation of peroxidase in the treatments of 1a-1i, 2a-2i, 3a-3i

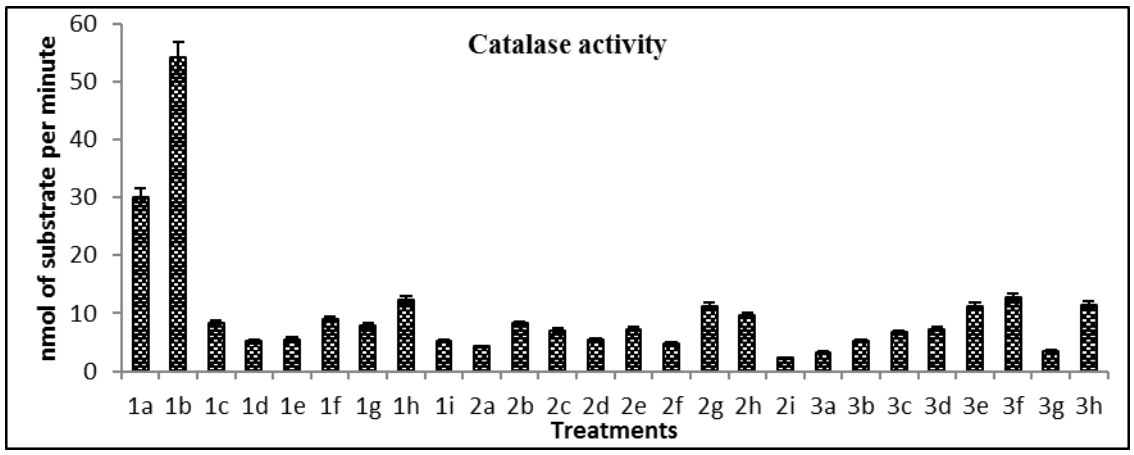


Fig.8.22 Variation of catalase in the treatments of 1a-1i, 2a-2i,3a-3i

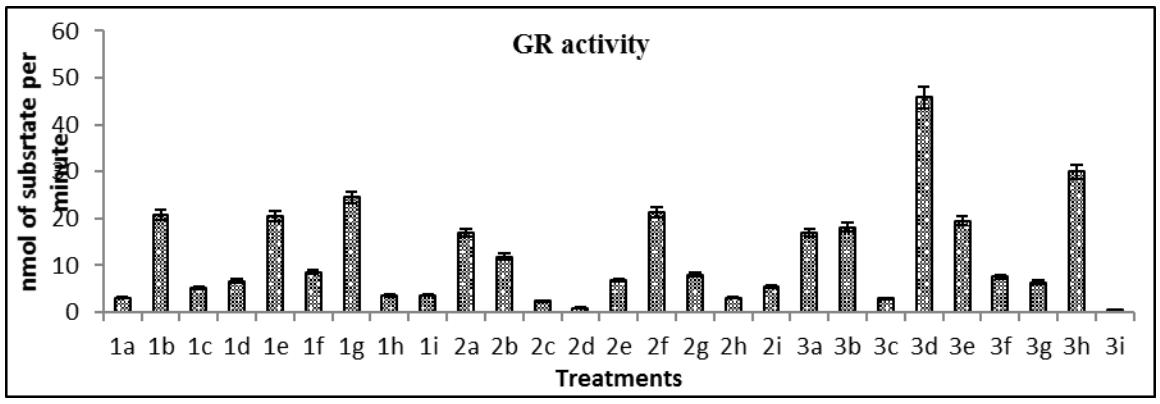


Fig.8.23 Variation of Glutathione reductase in the treatments of 1a-1i, 2a-2i,3a-3i

Active oxygen species (AOS) generated through photosynthesis causes oxidative stress which are defended by using antioxidative enzymes namely, SOD, CAT, POD, APX, GPX, GR, and low molecular weight antioxidants, ascorbic acid and glutathione (Asada, 1999; Noctor and Foyer, 1998).The differential expressions of the above enzymes suggested a strong response of antioxidative enzymes of *Oscillatoria subbrevis*, *Nostoc carneum* and *Chlorella* sp. under tested growth conditions. Both removal of combined-nitrogen and addition of algae to the growth medium enhanced the expressions of most of the antioxidative enzymes (SOD, POD, CAT, and APX) suggesting that the organism experienced oxidative stress as a consequence of AOS overproduction. The ability of a non-nitrogen fixing cyanobacterium *Oscillatoria subbrevis* to survive and decolourize

domestic sewage water, when grown in nitrogen-depleted medium supplemented with domestic sewage water is an adaptive.

8.3.6.Nutrient Removal

Nutrient Removal for different treatments of three treatments are presented in **Fig.8.24-8.27.** Nitrate removal was found to be maximum in *Oscillatoria subbrevis* 1b (91.13%), *Nostoc carneum* 2d (74.25%) and *Chlorella ellipsoidea* 3h (86.37%). Phosphate removal rate was found to be highest in *Oscillatoria subbrevis* 1b (97%), *Nostoc carneum* 2b (95%) and *Chlorella ellipsoidea* 3d (84%) (Fig.11). Ammonia removal rate was found to be highest in *Oscillatoria subbrevis* 1b (99.78%), *Nostoc carneum* 2g (97.82%) and *Chlorella ellipsoidea* 3f (36.93%) (Fig.12). The BOD removal rate was found to be maximum in *Oscillatoria subbrevis* 1e (99.57%), *Nostoc carneum* 2g (98%) and *Chlorella ellipsoidea* 3c (98.85%). The TDS removal rate was found to be maximum in *Oscillatoria subbrevis* 1h (99.16%), *Nostoc carneum* 2b (97.32%) and *Chlorella ellipsoidea* 3g (98.92%).

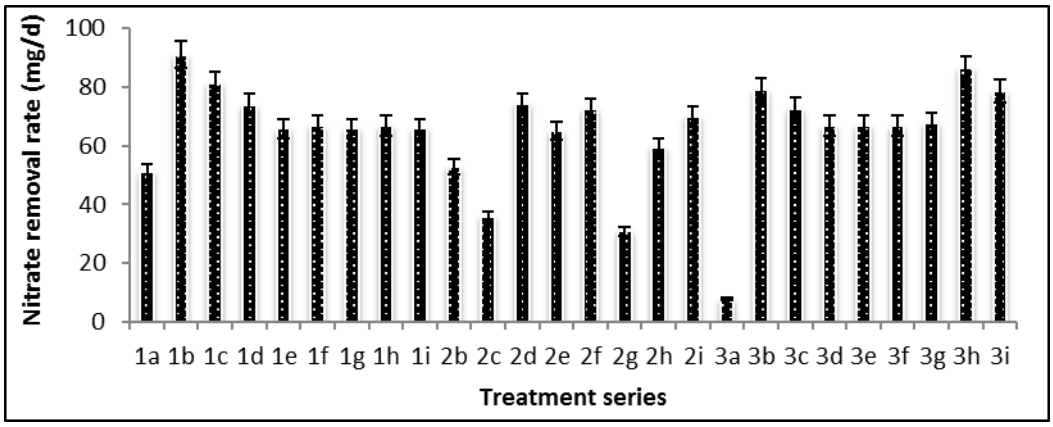


Fig.8.24 Removal rate of Nitrate NO_3-N; by different microalgae treatments on the 20th day of incubation.

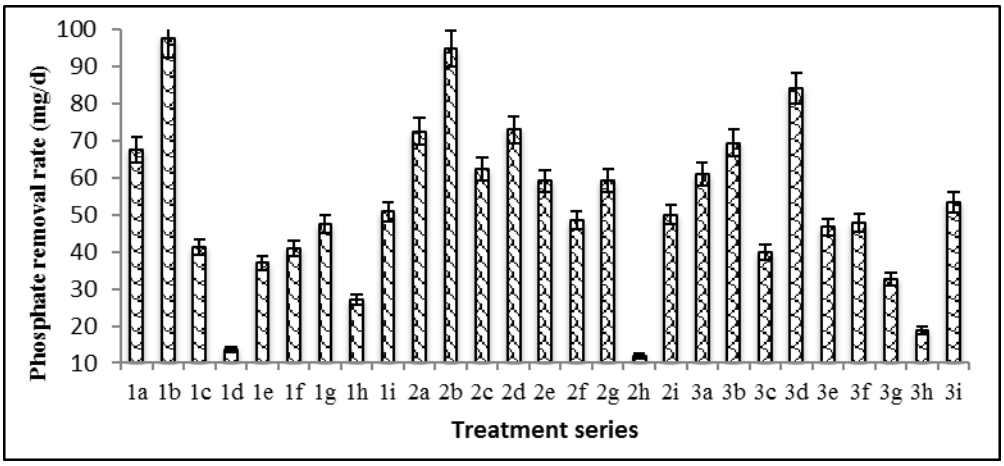


Fig.8.25 Removal rate of Phosphate PO_4-P; by different microalgae treatments on the 20th day of incubation.

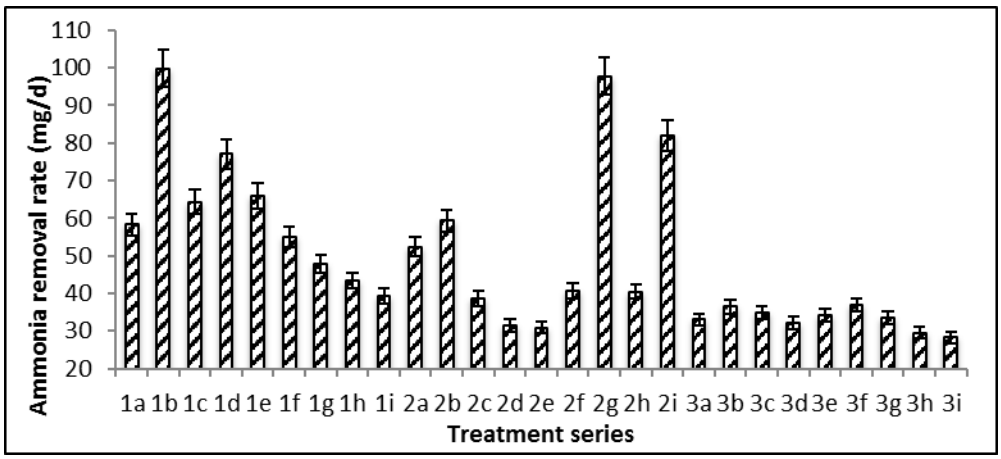


Fig.8.26 Removal rate of Ammonia by different microalgae treatments on the 20th day of incubation.

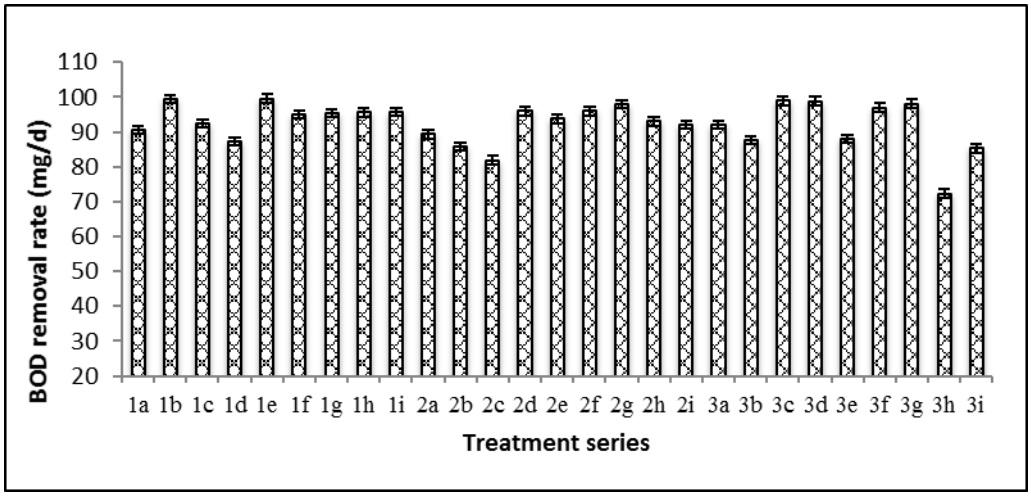


Fig.8.27 Removal rate of BOD by different microalgae treatments on the 20th day of incubation.

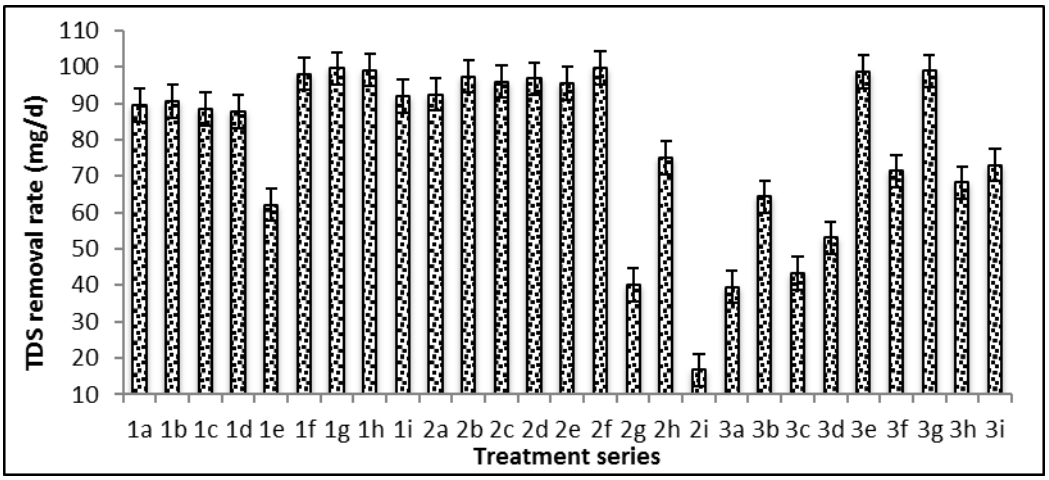


Fig.8.28 Removal rate of TDS by different microalgae treatments on the 20th day of incubation.

Thomas *et al.,* (2016) have studied microalgae cultivation in domestic sewage water for biofuel application. The microalgae, *Chlorococcum humicola*, *Chlorella* vulgaris and *Selenastrum* sp. were isolated and purified from domestic sewage water. The species *C. humicola* treatment of domestic sewage water resulted in nearly 60% of BOD and more than 80% removal of total N. The TDS and TS reduction by microalgae was approximately 65%.Total phosphorus was reduced to nil by all the microalgae. Reduction of all the parameters was in the range of 60-65% by the microalga *C. vulgaris*, except

total N which was nearly 85%. The treatment efficiency was less by *Selenastrum* sp. when compared to that of *C. humicola* and *C. vulgaris.* The results indicated that *Chlorella ellipsoidea* was able to remove the nutrients in a range of 80-95%. The nutrients, nitrate, phosphate, ammonia, BOD and TDS removal rate was highest by *Oscillatoria subbrevis*. The species *Oscillatoria subbrevis* have showed maximum nutrient removal while *Nostoc carneum* have showed the minimum rate, and *Chlorella ellipsoidea* occupied a middle rank in three nutrients nitrate, phosphate and ammonium removal rate. This can be cited that *Oscillatoria subbrevis* and *Chlorella ellipsoidea* nitrogen dependence from sources from external environment, as compared to nitrogen fixing *Nostoc carneum* used in our experiment (Renuka *et al.,* 2013).

Nitrate, phosphate and ammonia were measured during the growth curve of each treatments, it can be said that decreasing concentration of chlorophyll *a* towards the stationary period also affects the nutrient removal rate ($p < 0.05$).Reduction in nutrients concentration significantly decreased the biochemical parameters (carbohydrates, proteins) ($p < 0.05$). Similar studies has been done by Renuka *et al.,* 2013), phycoremediation of sewage water by microalgae.Their results showed all the parameters showed significant difference between the parameters and among the groups of parameters .

8.3.7. Chlorophyll *a*

Chlorophyll accumulation of different treatments of three algae are shown in Fig. 8.28-8.31. Among the different treatments, chlorophyll accumulation of *Oscillatoria subbrevis* was found to be maximum in BG11 (60%) + (40%) DSW and BG11 (80%) + (20%) DSW and minimum in BG11 (50%) + (50%) DSW on 20th day. Among the different treatments, chlorophyll accumulation of *Nosto*c *carneum* was found to be maximum in Domestic sewage water, 2b on 20th day. Among the different treatments, chlorophyll accumulation of *Chlorella ellipsoidea* was found to be maximum in domestic sewage water, 3b.

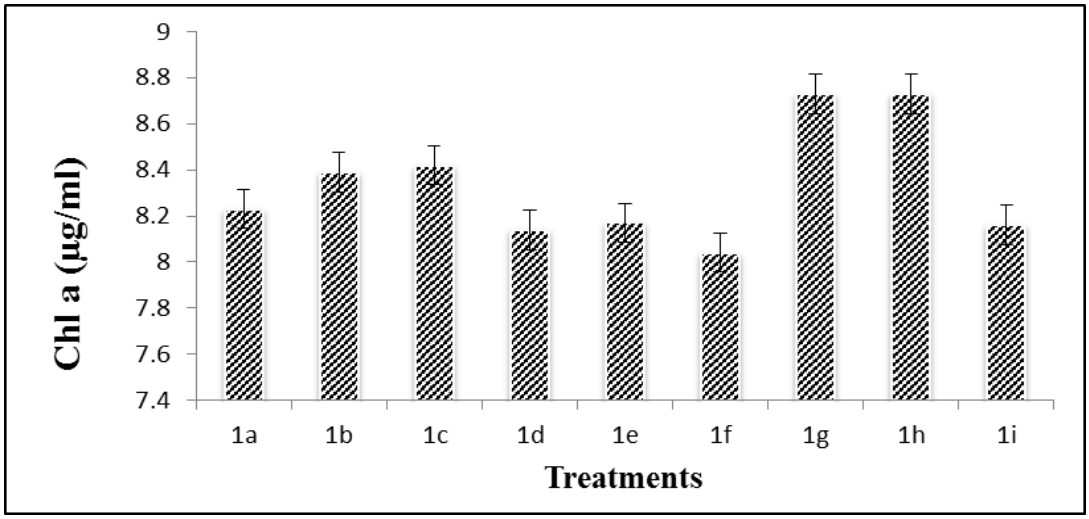


Fig.8.29 Chlorophyll *a* concentration of *Oscillatoria subbrevis* in 1a,1b,1c,1d,1e,1f,1g,1h,1i

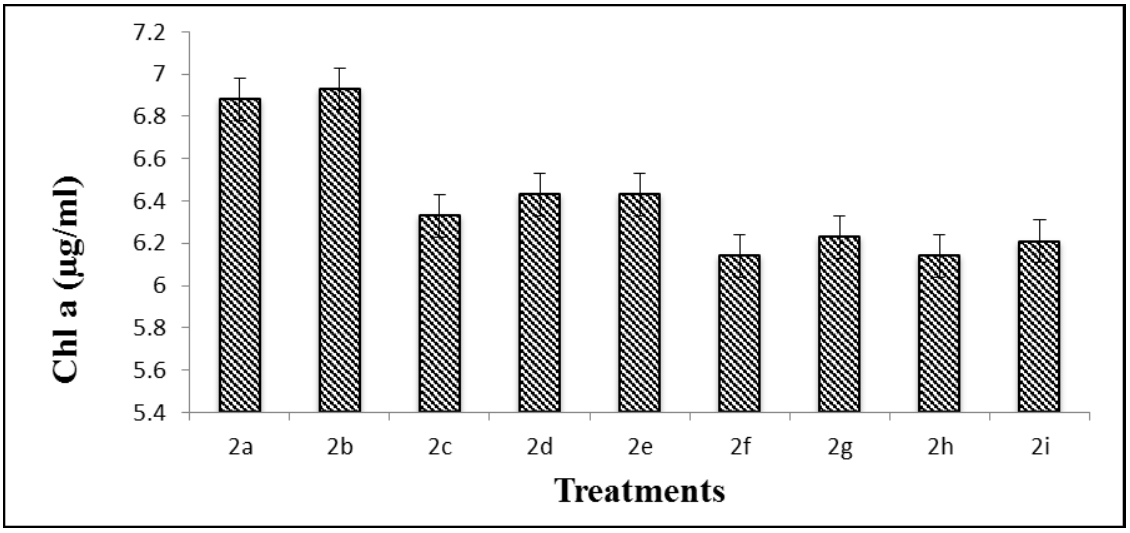


Fig.8.30 Chlorophyll *a* concentration of *Nostoc carneum* in 2a, 2b, 2c, 2d, 2e, 2f, 2g, 2h, 2i

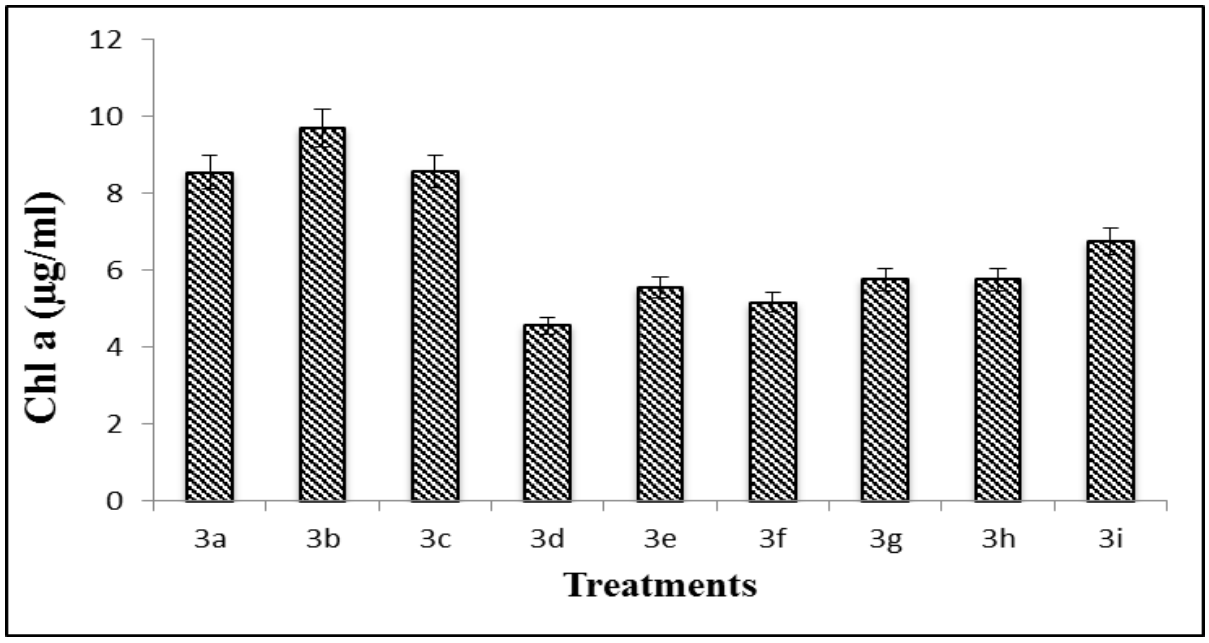


Fig.8.31 Chlorophyll *a* concentration of *Chlorella ellipsoidea* in 3a, 3b, 3c, 3d, 3e, 3f, 3g, 3h, 3i

8.4 Conclusion

The present study revealed that a filamentous microalga *Oscillatoria subbrevis* exhibited the potential to compete with native microalgae in sewage water and was promising in terms of growth and biomass production in domestic sewage water and domestic sewage water and BG11 mixture. *Oscillatoria subbrevis* was able to substantially sequester N and P, lower BOD levels and lower TDS below permissible limits. This illustrates its potential for its inclusion in the development of an environment friendly phytoremediation technology of sewage water. Such a technology can also be combined with simultaneous production of biomass for use as biofertilizers and source of pigments and bioactive compounds.

Chapter 9

BIODEGRADATION OF LOW DENSITY POLYETHYLENE (LDPE) BY SOME SELECTED CYANOBACTERIAL ISOLATES

9.1 Introduction

Polyethylene (PE) is a synthetic polymer consisting of long chain monomers of ethylene and 140 million tones of PE are produced worldwide (Shimao,2001). Due to low cost, durability PEhave myriad application as packaging purposes such as carrying food materials,packaging and transportation of textiles,manufacturing laboratory instruments and automobile components all over the world (Arutchelvi *et al.*,2008). The recalcitrant nature of polythene bags due to high molecular weightcomplex three dimensionalstructures and hydrophobic nature cause these polythene bags resistant to natural environment (Shah *et al.*, 2009, Nanda *et al.*, 2010). Polythene bags after use are usually thrown to landfills and or natural water bodies. Consisting of carbon and hydrogen polymers, polythene are remarkably resistant to

biological decay, it can be degraded to some extent by sunlight and oxygen resulting brittleness and loss of tensile strength without proportionate loss of mass while degradation by mechanical forces may merely lead to smaller pieces (Potts, 1984).Algae are known to colonise on submerged polythene in sewage water or domestic solid waste dumping site (Suseela and Toppo 2007;Sharma *et al.,* 2014). Colonisation of microbial communities on polythene surfaces depends largely on the environmental factors which may provide an ideal substratum for the colonisation (Pathak and Navneet, 2017). Direct degradation of LDPE by microorganisms utilising only the polymer as sole carbon source has been documented (Roy *et al.,* 2008). Biodegradation is the natural process in which the microorganisms such as bacteria, fungi or algae helps in the degradation process (ASTM, 1993).

The degradation rate of polythene depends on the crystallinity, surface treatment, additives, molecular weight, and surfactants present in the polyethylene. Both extracellular and intracellular types of enzymes play a crucial role in biological degradation of LDPE (Gu 2003).While polythene degradation by bacteria and fungi has been quite extensively studied, algae have received only meagre attention from researchers (Suseela and Toppo 2007; Kumar *et al.,* 2017). Previous research involving LDPE biodegradation potential of *Anabaena spiroides*, *Scenedesmus dimorphus* and *Navicula pupula* have demonstrated efficient biodegradation potential of algae (Kumar *et al.,* 2017).Selection of microorganisms for biodegradation of polythene plays an important role. The microorganisms with enhanced capacity to produce the oxidative and lignolytic enzymes are more efficient in the biodegradation of polythene (Nayak and Tiwari, 2011).Accordingly, the present chapter deals with LDPE biodegradation potential of five cyanobacterial species, *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum* and *Cylindrospermum muscicola* screened from submerged algal colonised polythene substrates in domestic sewage water. The mechanism of biodegradation has been expounded by spectroscopic, morphological, mechanical and thermal studies of control and treated LDPE.

9.2 Methodology

The detail methodology for evaluation of biodegradation of low density polythene (LDPE) were already mentioned in **Chapter 3.** The biodegaradation studies of LDPE using five dominant cyanobacterial strains and the obtained results are depicted in the tables, graphs and figures below.

9.3 Results and discussion

Biological treatment

The five cyanobacterial species, *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum*, *Cylindrospermum muscicola* were selected for the biodegradation of LDPE based on dominance over the occurrence of other algal population that were screened from submerged polythene surface in domestic sewage water of Silchar town. After 6week of treatment with five cyanobacterial species, the pre-weighed LDPE strips (1cm×1cm) from each treatment were observed under the optical microscope. The cyanobacteria were adhered on the LDPE surfaces (**Plate 9.1**).Each conical flask incubated with LDPE polythene strips are represented in **Plate 9.2-9.4**. The cyanobacterial adhesion after 6 week of treatment are presented in **Plate 9.5.**

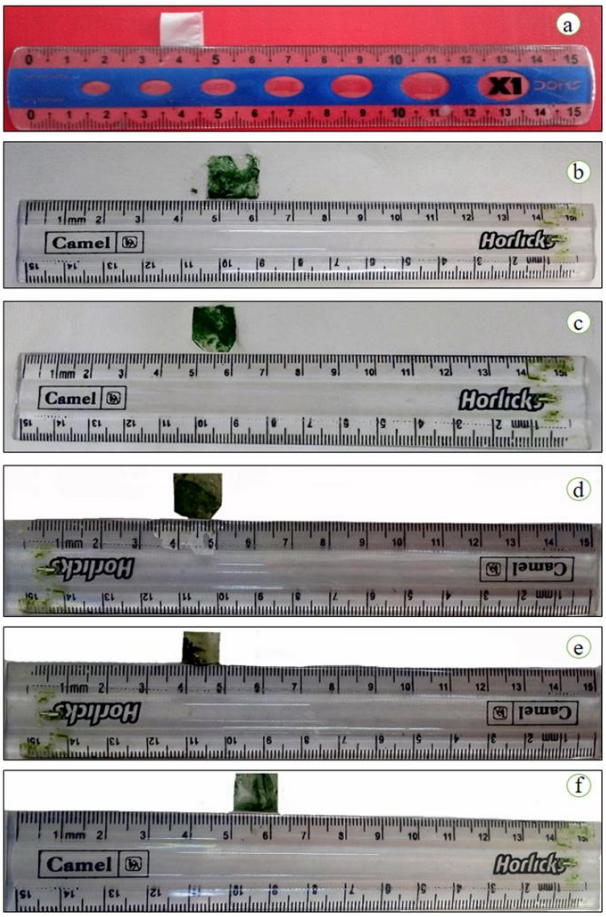


Plate 9.1. Algal colonization on PE surface (a) control; and *Phormidium lucidum* (b), *Oscillatoria subbrevis* (c), *Lyngbya diguetii* (d), *Nostoc carneum* (e), *Cylindrospermum muscicola* (f)

Plate 9.2. Biological treatment (6 weeks) of LDPE polythene films (a-control, b, c, d-*Phormidium lucidum*, e-control, f, g, h- *Oscillatoria subbrevis*)

Plate 9.3. Biological treatment (6 weeks) of LDPE polythene films (a-control, b, c, d-*Lyngbya diguetii*, e-control, f-h-*Nostoc carneum*)

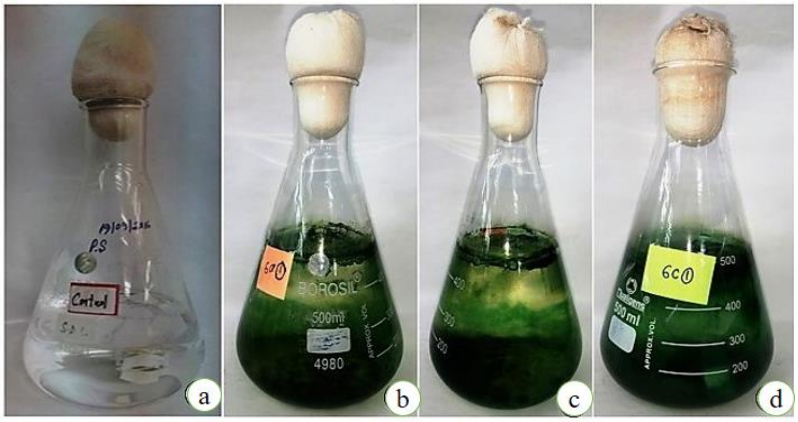


Plate 9.4 Biological treatment (6 weeks)of LDPE polythene films (a-control, b, c, d-*Cylindrospermum muscicola*)

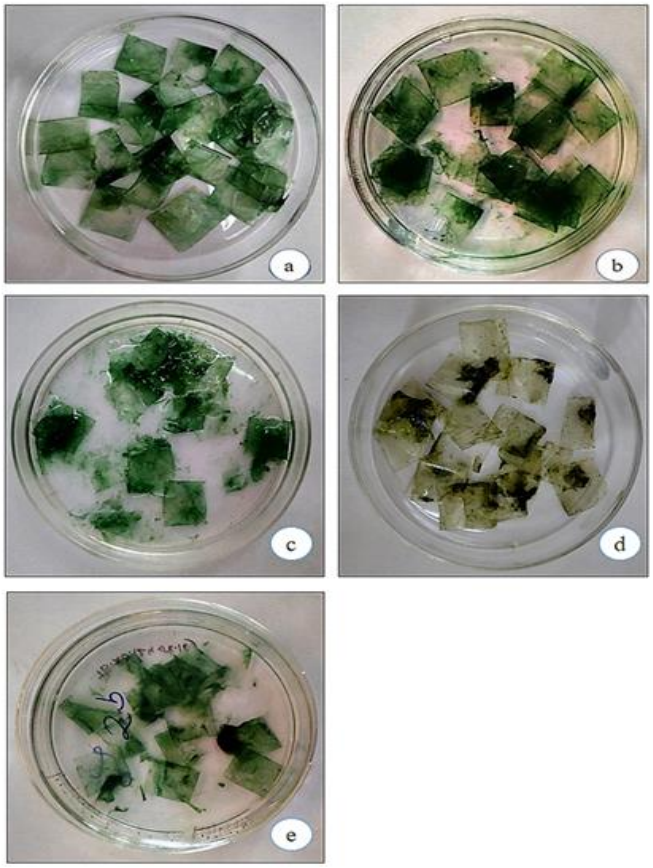


Fig.9.5 Cyanobacterial colonisation on PE surface in sixth week of the biological treatment.

Cyanobacteria growth study

The five cyanobacterial growth on PE surface was initiated from the first week of biological treatment itself.Growthof cyanobacterial species on the PE surfacewas studied with reference to biotic control and four distinguishable phases: lag phase, log phase, stationary phase and death phase were found to be observed (**Fig.9.1-9.5).** The fast growing cyanobacteria showed a longer lag period on PE surfaces.This can be attributed to the acclimatization of cyanobacterial species in the PE with the degraded carbon source from the PE (Arutchelvi *et al.,* 2008). The *Phormidium lucidum* showedhighest generation time (**G=182.21h)** on PE surfaces (**Table 9.1**).The cyanobacterial growth increased with days and after a period of 6 weeks the chl *a* production found to level off. The enhancement of carbohydrate and protein content of cyanobacteria on PE surface was also observed (**Fig. 9.6-9.7**). The rapid growth of cyanobacterial species on the PE surfaces may be expounded by metabolisable compounds from the PE (Koutny *et al.,* 2006a). The enhancement of photosynthetic pigment (chl*a*), protein and carbohydrate contents in cyanobacterial species growing on PE surface were also observed (Shang *et al.,* 2009; Koutny *et al.,* 2006a). It may be assumed that rapid growth of cyanobacteria on the PE may be due to release of some low molecular weight compounds to the liquid phase of the medium (Koutny *et al.,* 2006b). The enhanced production of cellular contents under abiotic stress is considered as defensive mechanism of the strain (Paliwal *et al.,* 2017). This therefore suggests

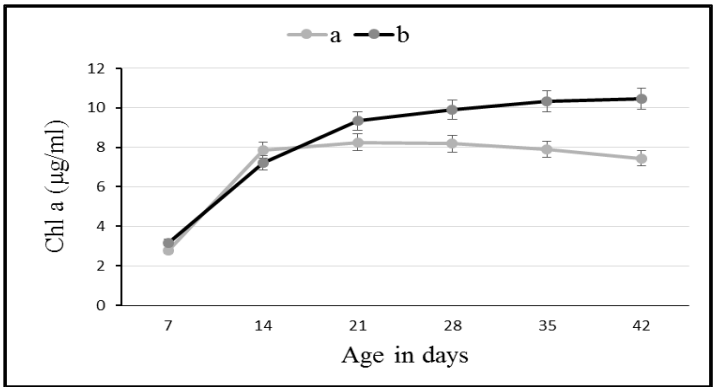


Fig. 9.1. Growth pattern of *Phormidium lucidum* on PE for a period of 6 weeks. Biotic control (a) and PE strips (b).

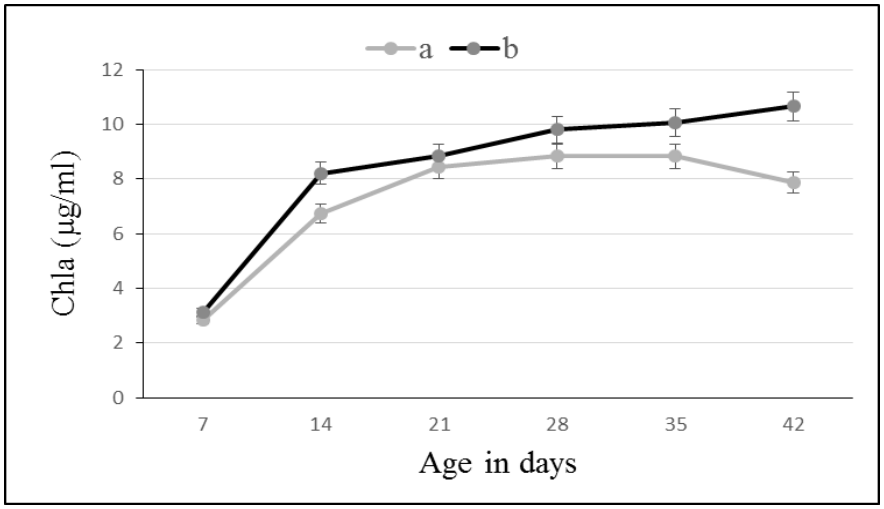


Fig. 9.2. Growth pattern of *Lyngbya diguetii* on PE for a period of 6 weeks. Biotic control (a) and PE strips (b).

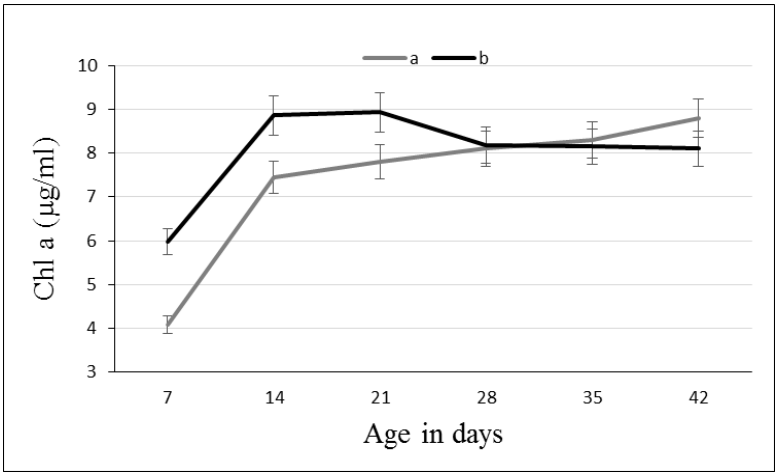


Fig. 9.3. Growth pattern of *Lyngbya diguetii* on PE for a period of 6 weeks. Biotic control (a) and PE strips (b).

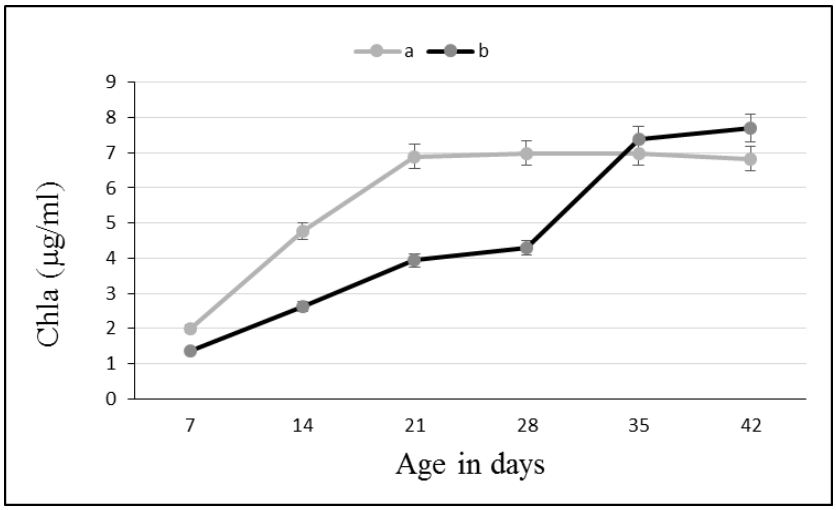


Fig. 9.4. Growth pattern of *Nostoc carneum* on PE for a period of 6 weeks. Biotic control (a) and PE strips (b).

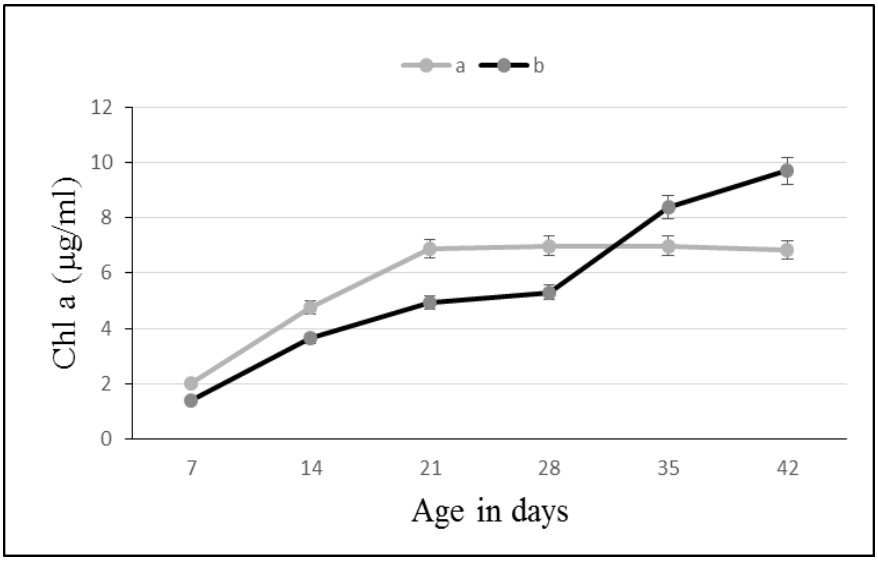


Fig. 9.5. Growth pattern of *Cylindrospermum muscicola* on PE for a period of 6 weeks. Biotic control (a) and PE strips (b).

Table 9. 1 Specific growth rate (K) and generation time (G) of the isolates

Serial no	Cyanobacterial isolates	K (μd^{-1})		G (h)	
		Biotic control	**LDPE surfaces**	**Biotic control**	**LDPE surfaces**
1	*Phormidium lucidum*	0.134 ±0.12	0.123 ± 0.12	**178.25 ±0.45**	**182.21 ± 0.23**
2	*Oscillatoria subbrevis*	**0.158 ±0.23**	**0.143 ± 0.23**	151.34 ±0.67	156.24 ± 0.37
3	*Lyngbya diguetii*	0.138 ±0.13	0.128 ± 0.13	173.67 ±0.54	178.32 ± 0.37
4	*Nostoc carneum*	0.152 ±0.14	0.142 ± 0.12	157.21 ±0.43	177.21 ± 0.23
5	*Cylindrospermum muscicola*	0.136 ±0.16	0.132 ± 0.16	153.89 ±0.23	156.45 ± 0.23

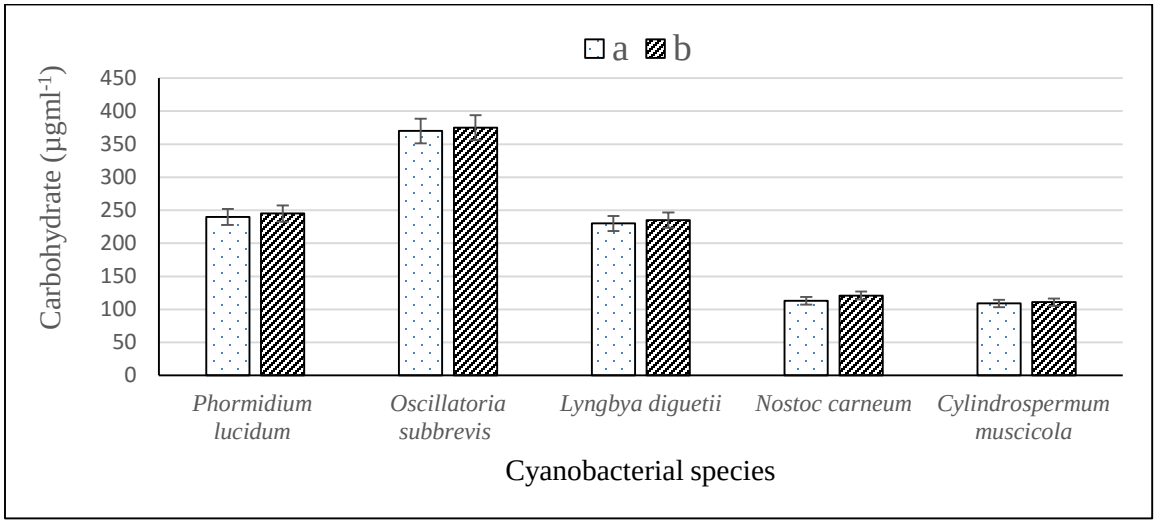


Fig.9.6 Carbohydrate composition of (a) biotic control and (b) on LDPE surfaces

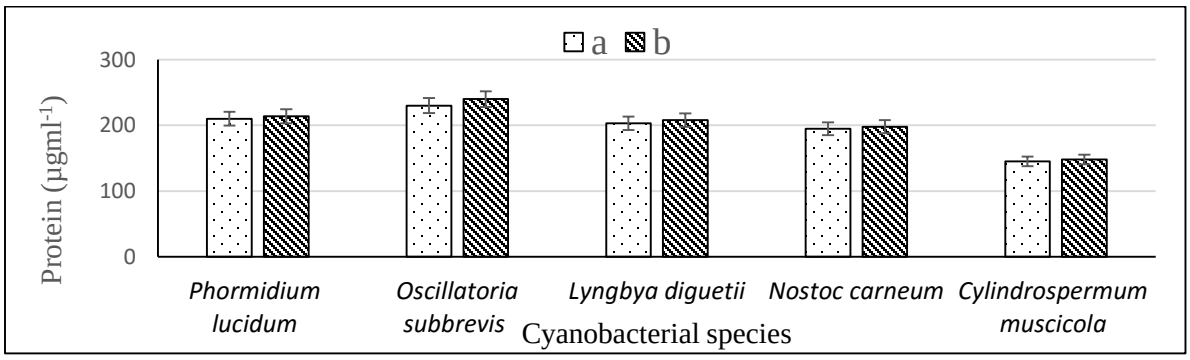


Fig.9.7 Protein composition of (a) biotic control and (b) on LDPE surfaces

that cyanobacterial species must have use LDPE as carbon source. For biotic control, the death phase were arrived earlier than PE grown cyanobacteria, it may be due to that for biotic control, carbon source may be depleted after a period of 5 weeks, but in polythene grown cyanobacteria they have shown a longer stationary phase as they used the polythene as carbon source (Gilan et al. 2004).

Optical microscopy

The attachment of cyanobacteria on the PE surfaces were observed under the optical microscope(**Plate 9.6-9.16).** Attachment of cyanobacteria on the polythene was regraded as the first criteria to initiate LDPE biodegradation (Arutcheli et al. 2008, Longo et al. 2011).Cyanobacterial adhesion on PE surface got initiated in the first week of the treatment. The cyanobacterial biomass on the surfaces were started increasing in the second week of the biological treatment.

For *Phormidium lucidum*, colonization was initiated in the first week, a small colony was observed.In the second week, a thin layer of algae was observed.*Phormidium lucidum*adherence showed full coverage on the polythene surfaces in fifth and sixth week of the treatment.For *Oscillatoria subbrevis*, rapid colonization was observed. In the third week of treatment, full colonization on the surfaces was occurred.The adherence pattern of *Lyngbya diguetii* was very similar to that of *Phormidium lucidum*. The adherence pattern of *Oscillatoria subbrevis* indicated that in the third week of treatment it reached much higher Chl*a* production than *Phormidium lucidum* and *Lyngbya diguetii*.

Plate 9.6. Optical micrography of PE under control condition (a, b, c)

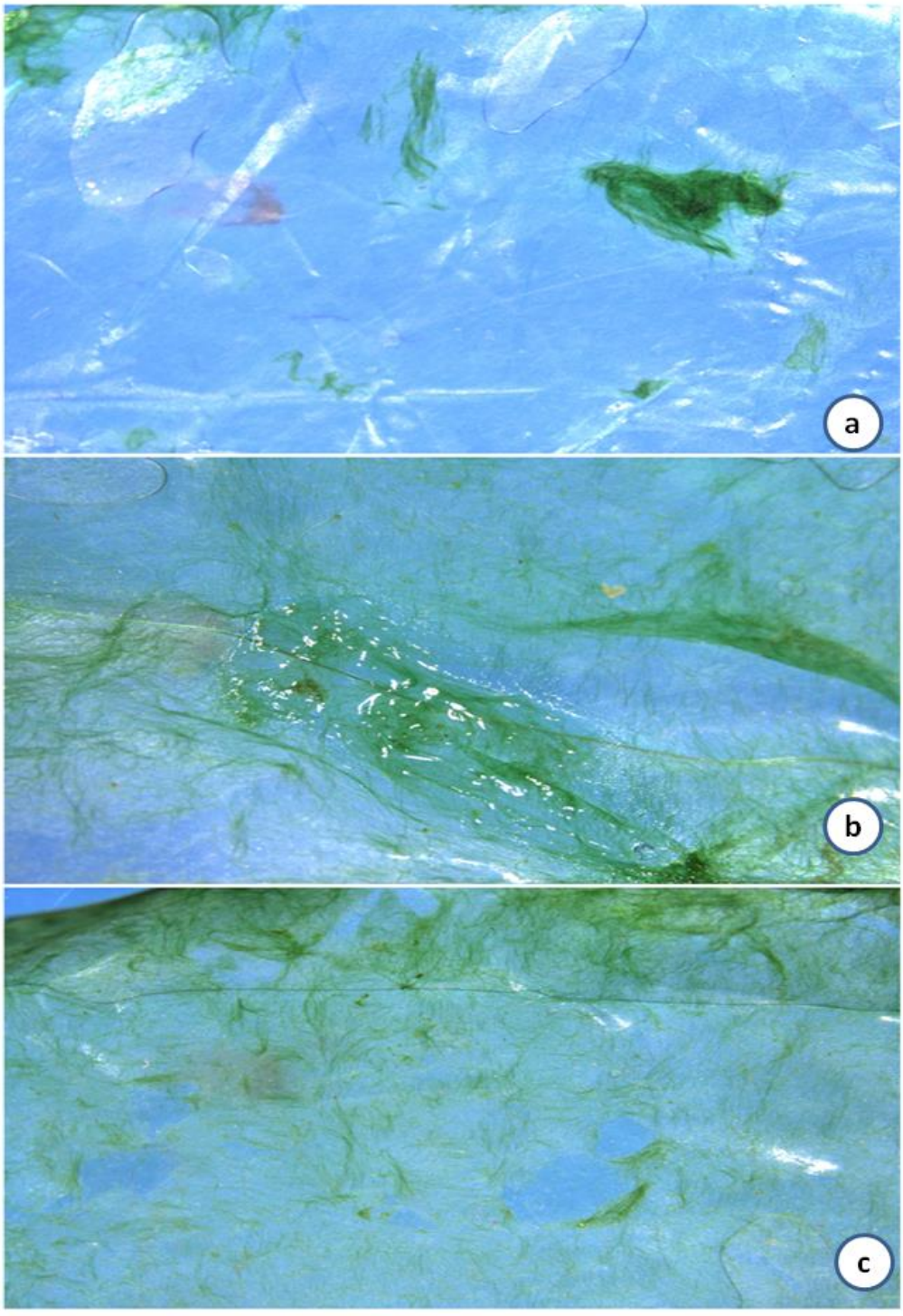


Plate 9.7. Optical micrography of PE treated by *Phormidium lucidum* of first week (a), second week (b) and third week (c) of treatment showing evidence of algae adherence

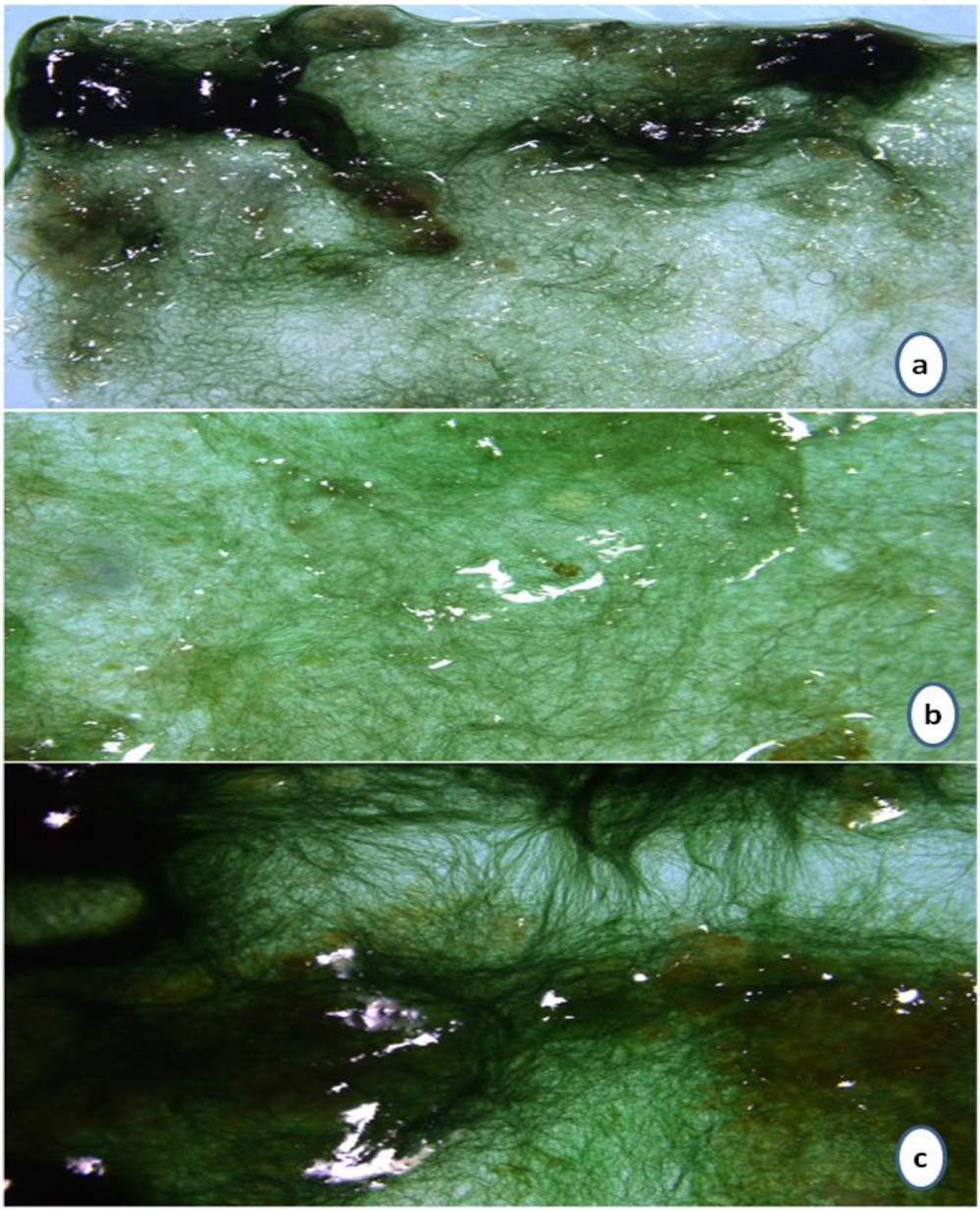


Plate 9.8. Optical micrography of PE treated by *Phormidium lucidum* of fourth week (a),fifth week (b) and six week (c) of treatment showing algal adherence

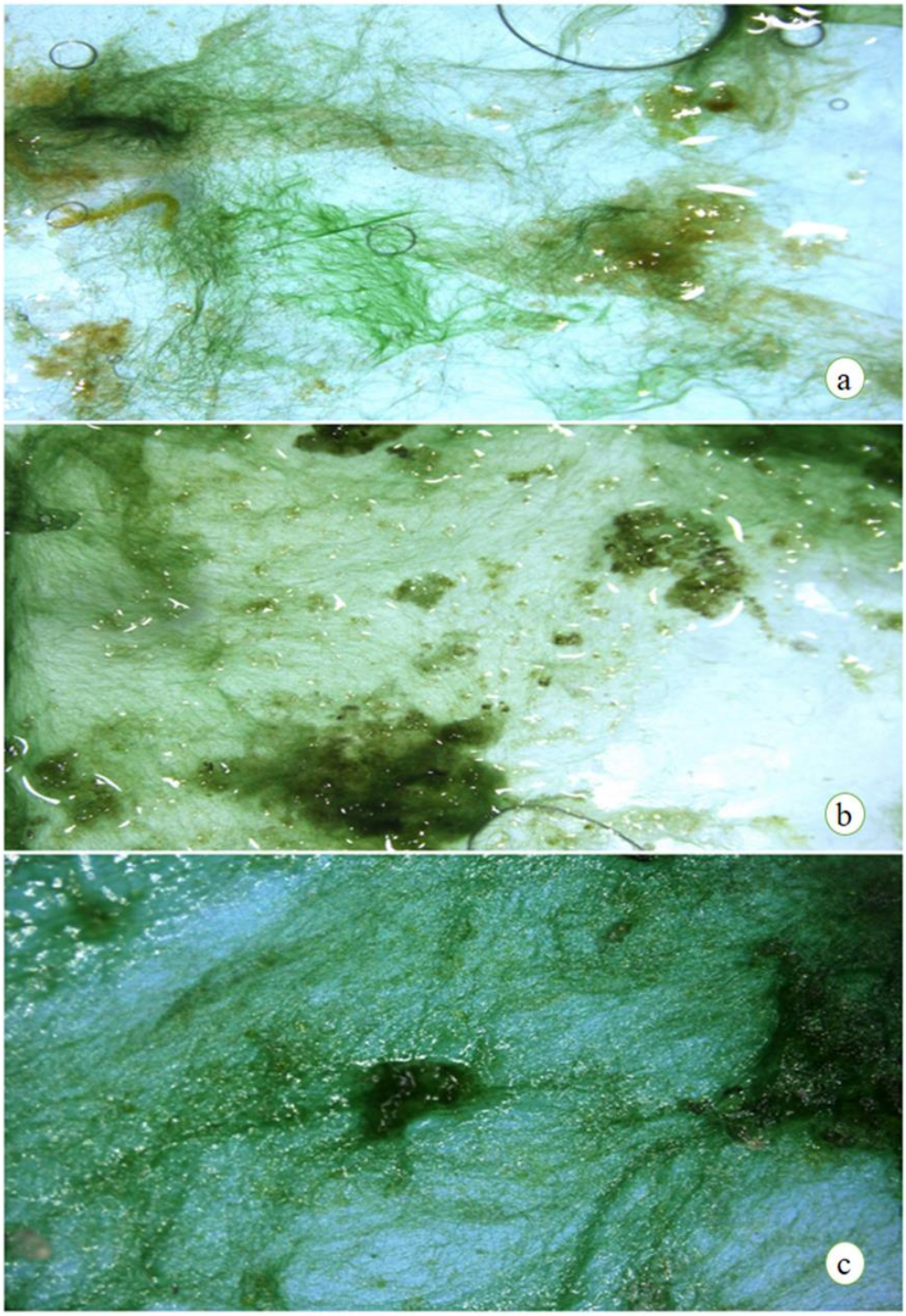


Plate 9.9. Optical micrography of PE treated by of *Oscillatoria subbrevis* first week (a),second week (b) and third week (c) of treatment showing algal adherence

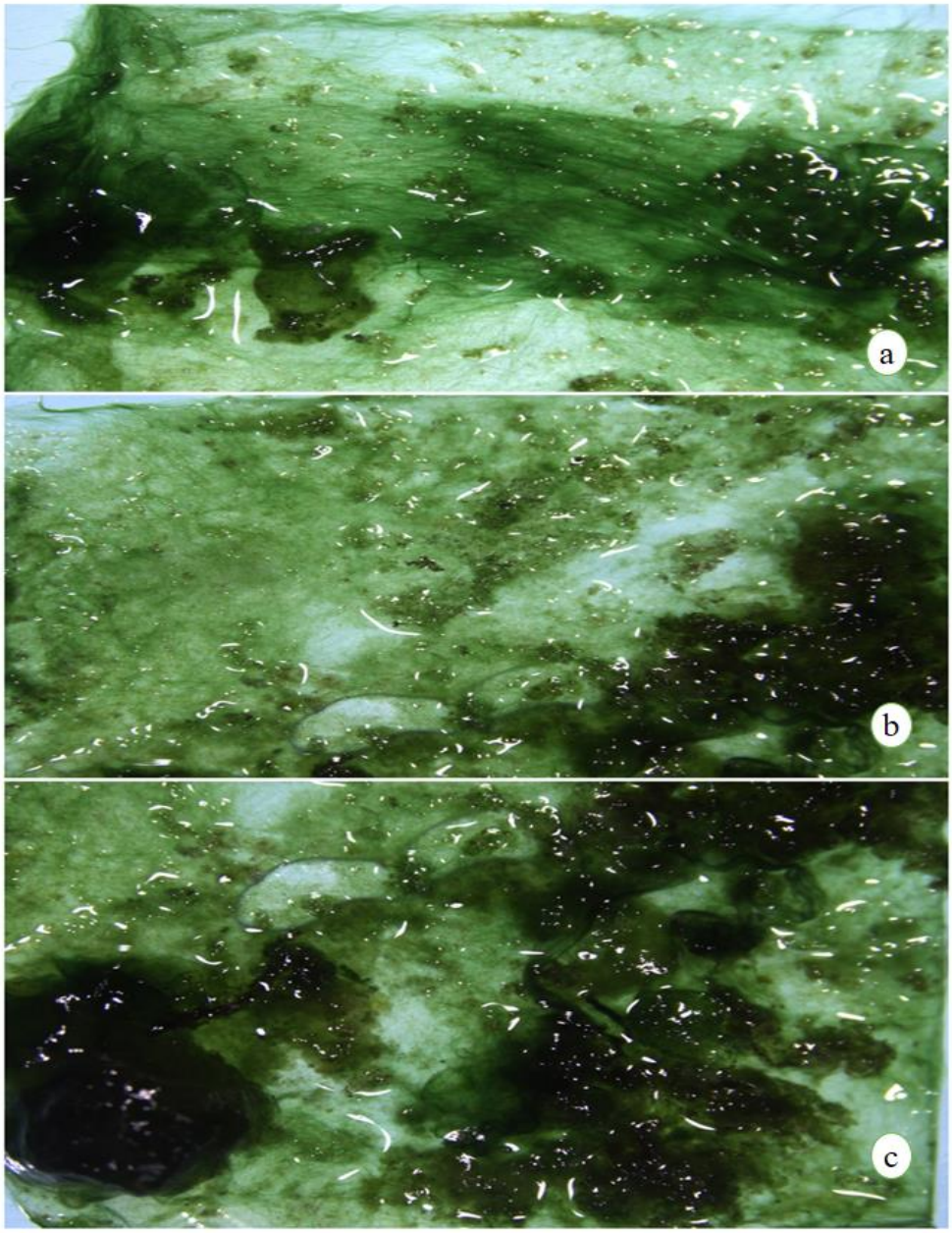


Plate 9.10. Optical micrography of PE treated by *Oscillatoria subbrevis* of fourth week (a),fifth week (b) and six week (c) of treatment showing algal adherence

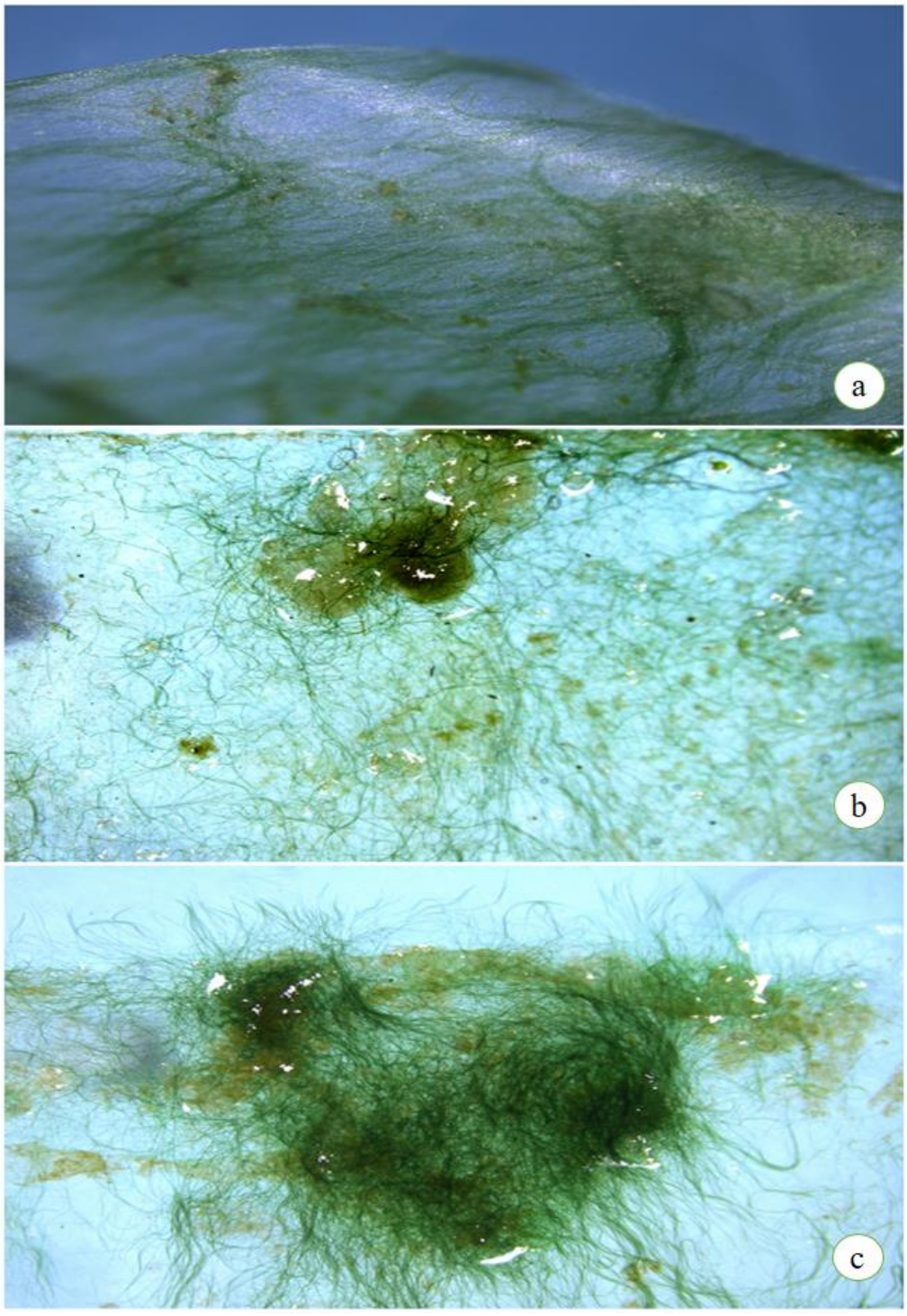


Plate 9.11. Optical micrography of PE treated by *Lyngbya diguetii* of fourth week (a), fifth week (b) and six week (c) of treatment showing algal adherence

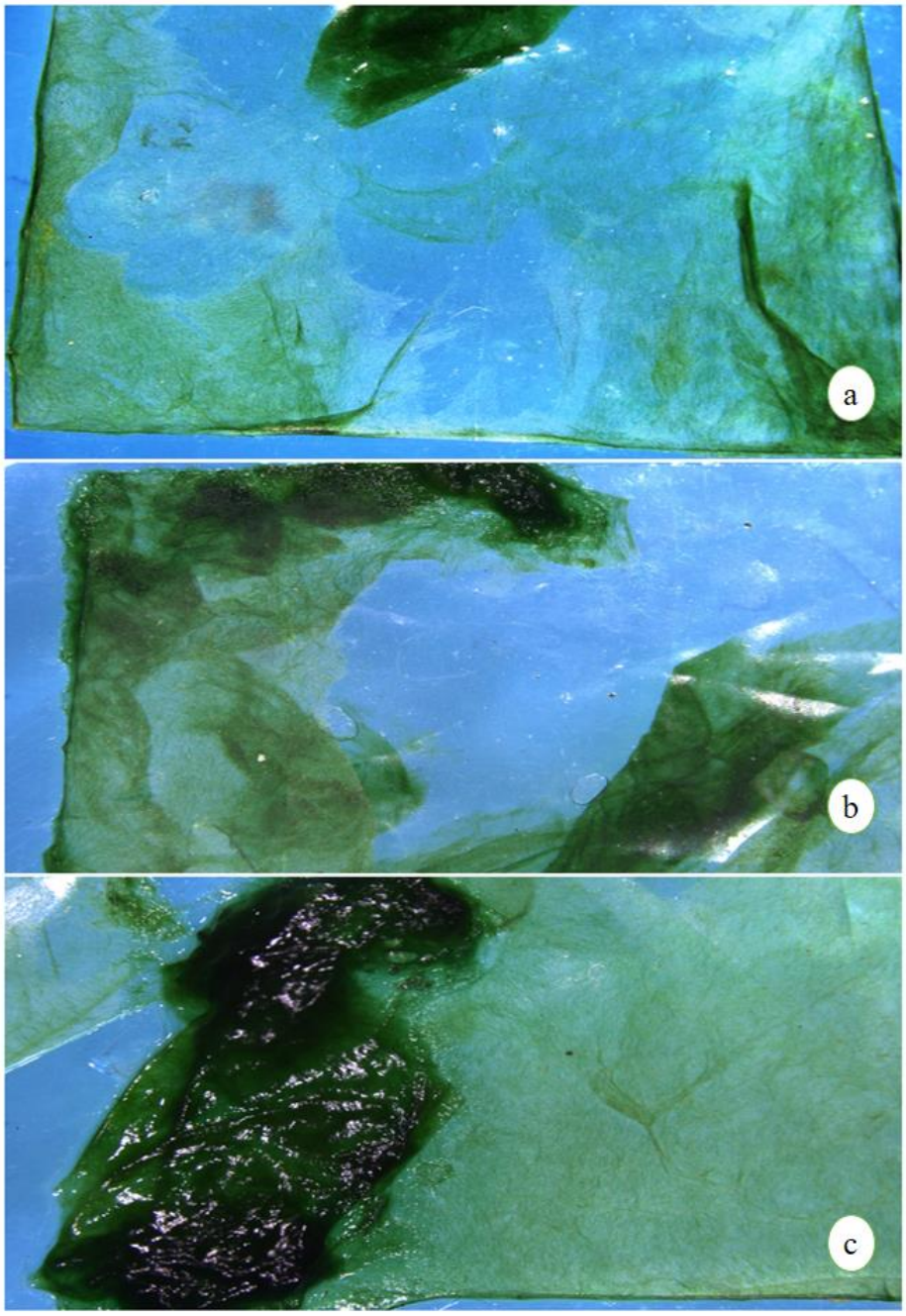


Plate 9.12. Optical micrography of PE treated by *Lyngbya diguetii* of fourth week (a),fifth week (b) and six week (c) of treatment showing algal adherence

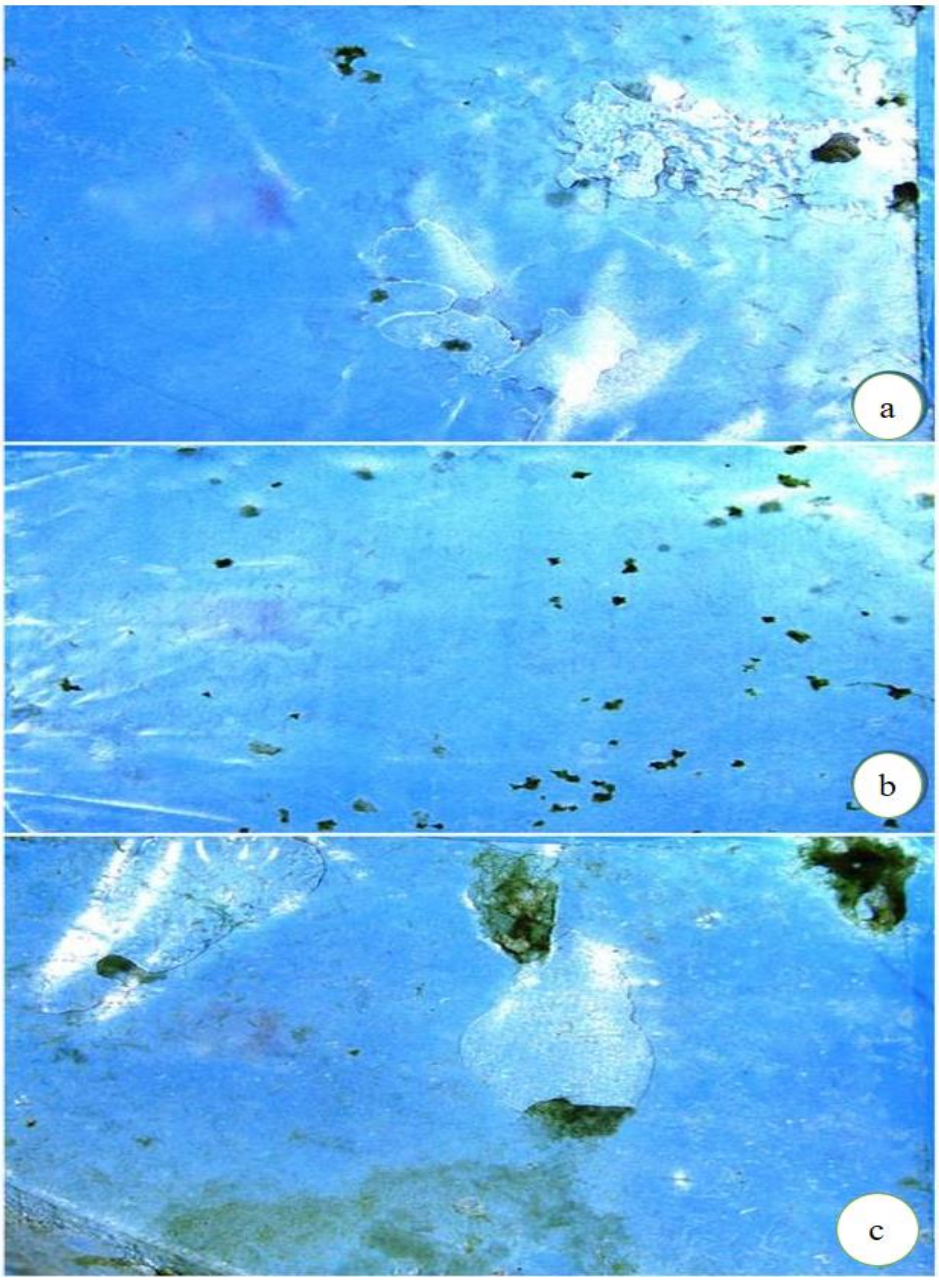


Plate 9.13. Optical micrography of PE treated by *Nostoc carneum* of first week (a), second week (b) and third week (c) of treatment showing algal adherence

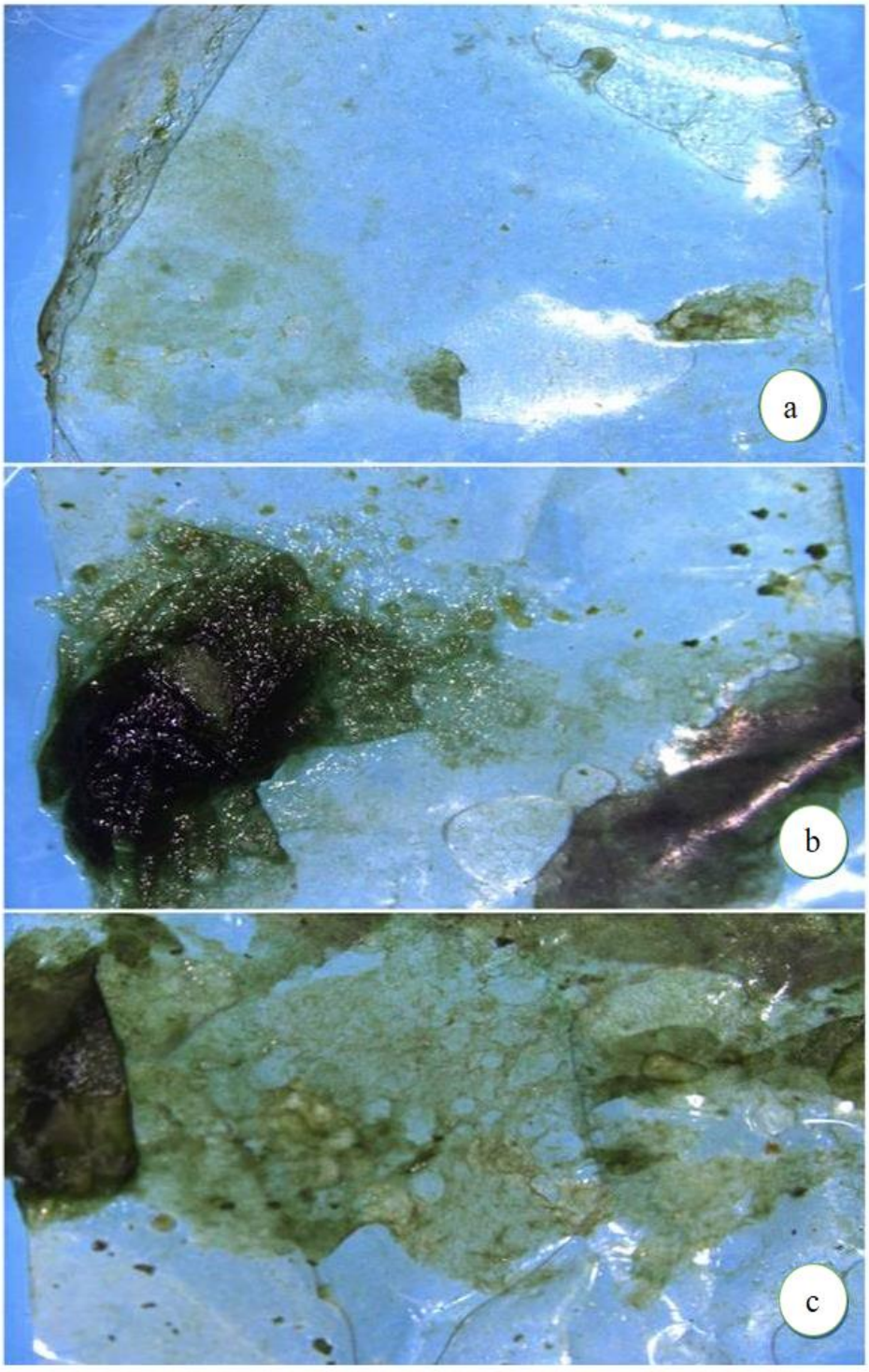


Plate 9.14. Optical micrography of PE treated by *Nostoc carneum* of first week (a), second week (b) and third week (c) of treatment showing algal adherence

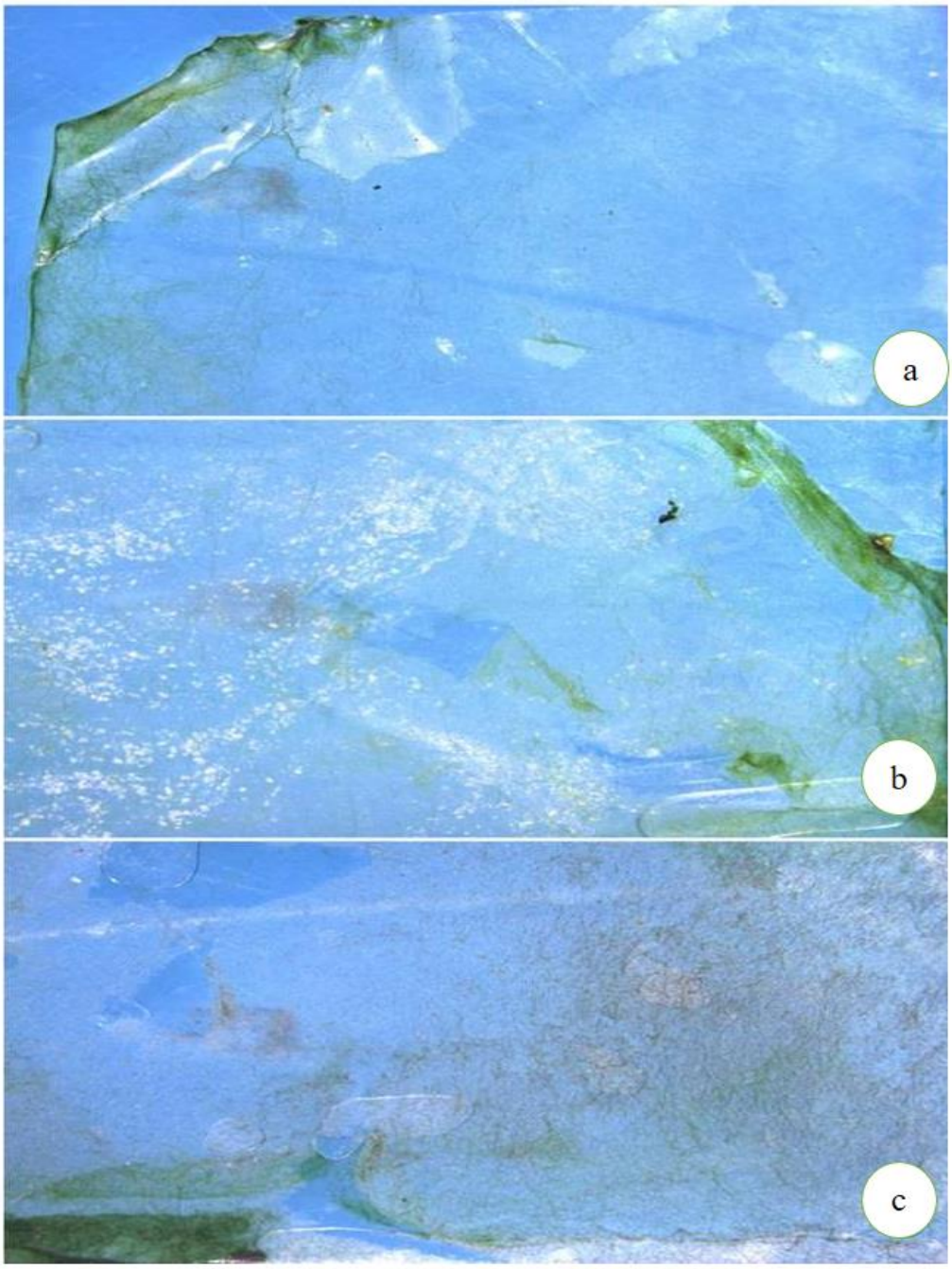


Plate 9.15. Optical micrography of PE treated by *Cylindrospermum muscicola* of first week (a), second week (b) and third week (c) of treatment showing algal adherence

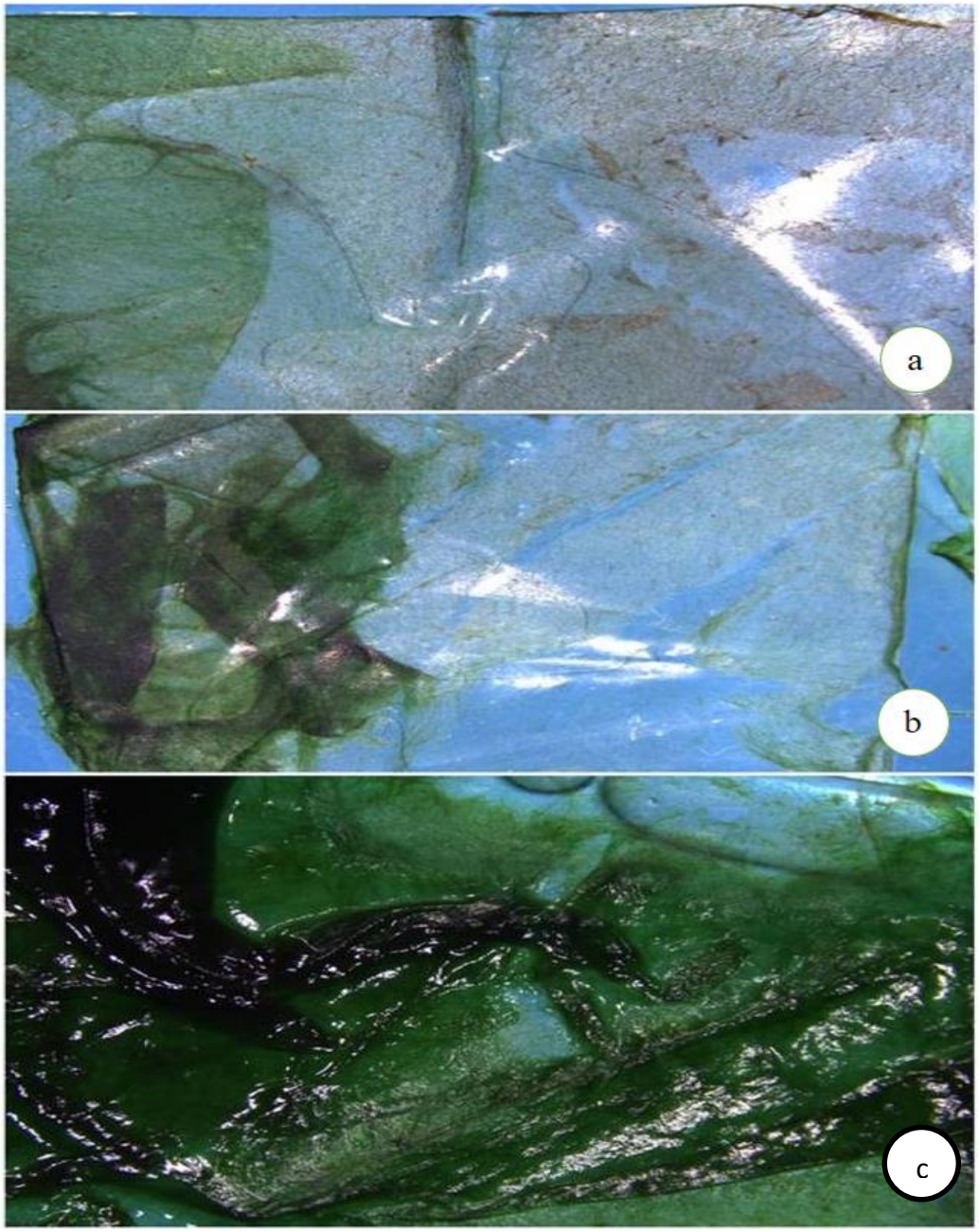


Plate 9.16. Optical micrography of PE treated by *Cylindrospermum muscicola* of first week (a),second week (b) and third week (c) of treatment showed evidence of algae adherence

The adherence pattern of *Nostoc carneum* and *Cylindrospermum muscicola* on LDPE surfaces were slower than other three cyanobacteria. Distinct signs of bioerosion were observed on the LDPE by the *Nostoc carneum*. It was observed that *Nostoc carneum* able to form a dense filamentous thallus on the polythene surface. The adherence of the cyanobacterium was most progressive during the fifth week. No marked changes were noted after this period. Some black dots observed initially were surrounded by new olive green colonies of the cyanobacterium species in the third week.

The adherence pattern of *Cylindrospermum muscicola* on LDPE surfaces was significantly slower than other cyanobacteria. Colonization was initiated in the first week of the treatment itself, but in the fourth week of treatment colonization was visible to naked eyes. It may be due to that gelatinous nature of cyanobacteria did not permit to grow on the LDPE surfaces.

The hydrophobic nature of the LDPE may resist the adherence and establishment of new colonies on the LDPE surfaces. After a week of incubation, the cyanobacterial species found to grow well on the surfaces, it may be due to that initial degradation leading to the insertion of hydrophilic groups to the polythene strips (Vasile 1993). Previous studies suggesting that algal colonization on polythene surfaces may be due to that algae can used polythene as sole carbon source (Kumar et al. 2017).

Scanning electron microscopy (SEM)

Surface erosion, formation of holes and cavities on the surfaces of LDPE were observed after 6weeks of treatment**(Plate 9.17-9.22).**The LDPE surface suffered considerable surface damage, through an increase in surface roughness and an inhomogeneous morphology, with multiple holes and all of which indicated substantial surface degradation. The breakdown of complex polymeric form into its monomeric form was evidenced by cyanobacterial treatment (Sanin et al. 2003). The grooves and cracks confirmed the fragility of the polythene may be breakdown by the cyanobacterial species. The grooves on the LDPE surfaces breakdown the polyethylene branching which upset to form new branching (Manzur et al. 2004). Previous studies using Anabaena spiroides and Navicula pupula colonization on polythene surface showed rather identical surface features (Kumar et al. 2017). The corrosion of the surfaces were not uniform indicating the amorphous region of the polythene was more susceptible to

cyanobacterial adhesion and LDPE degradation. It is generally believed that near surface accessible region of the polythene was mainly attack by the cyanobacteria (Albertsson et al. 1994).

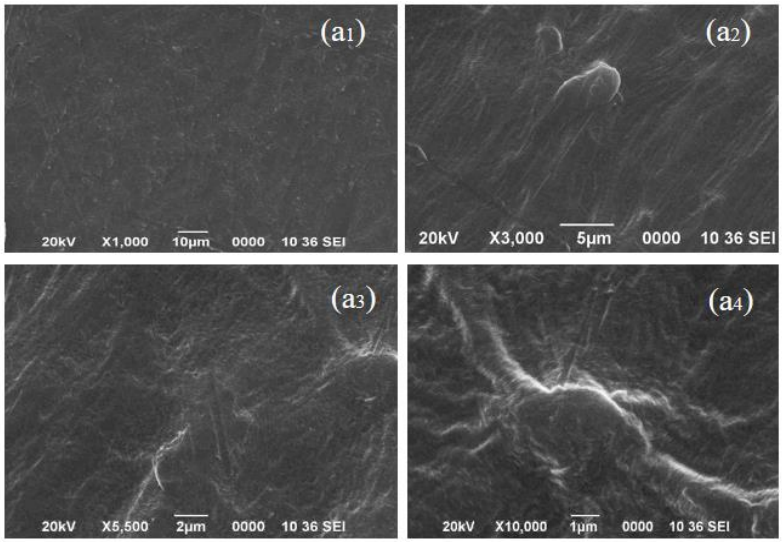


Plate 9.17 Scanning electron micrographs of the surface of control LDPE polythene strip (a_1; 1000x); (a_2; 3000x); (a_3; 5500x); (a_4; 10000x) (washed with 2% SDS)

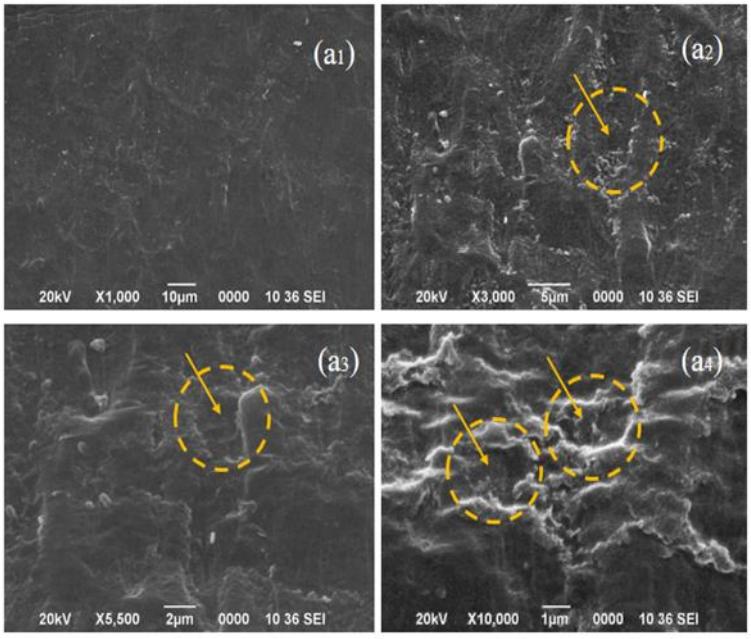


Plate 9.18 Scanning electron micrographs of the surface of LDPE polythene strip after incubation with *Phormidium lucidum* (a_1; 1000x); (a_2; 3000x); (a_3; 5500x); (a_4; 10000x) (washed with 2% SDS)

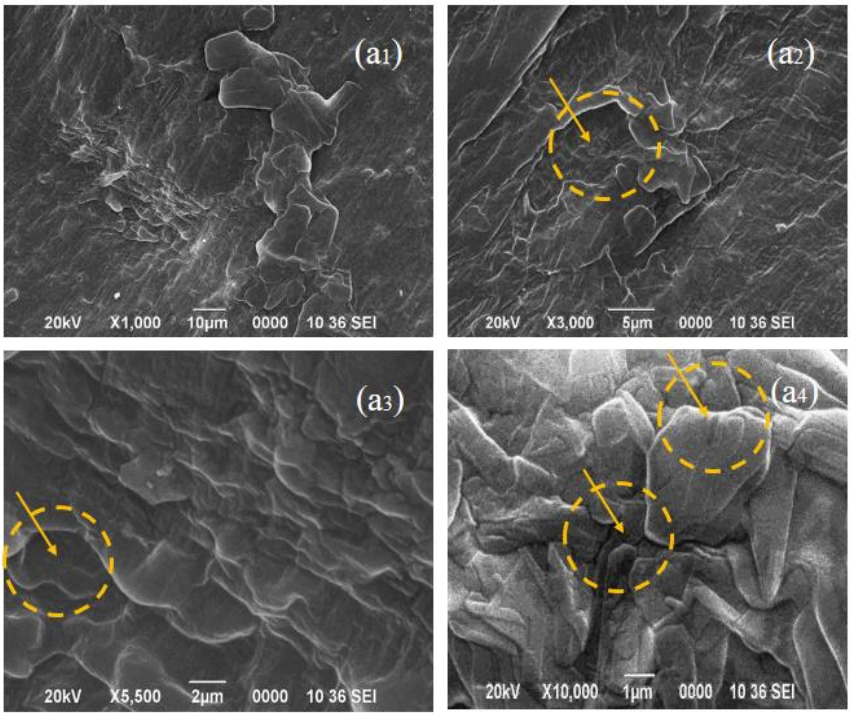


Plate 9.19 Scanning electron micrographs of the surface of LDPE polythene strip after incubation with *Oscillatoria subbrevis* (a_1; 1000x); (a_2; 3000x); (a_3; 5500x); (a_4; 10000x) (washed with 2% SDS)

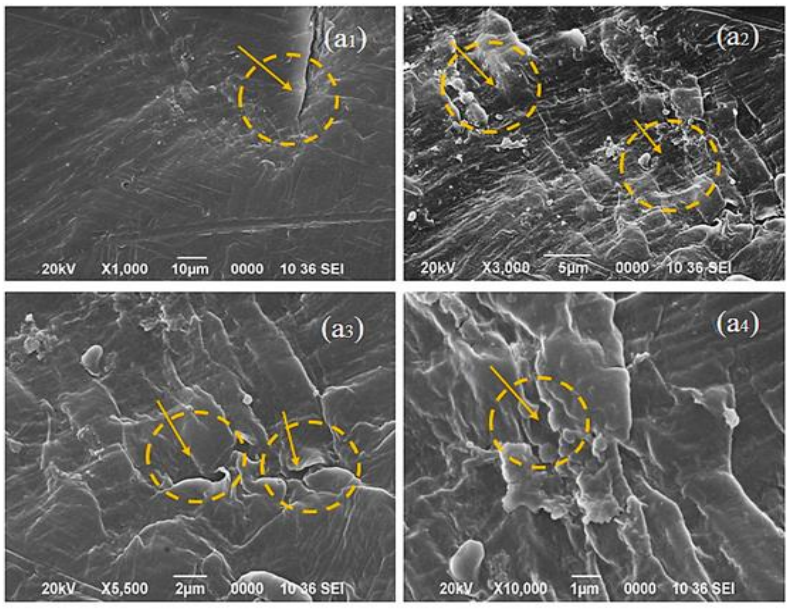


Plate 9.20 Scanning electron micrographs of the surface of LDPE polythene strip after incubation with *Lyngbya diguetii* (a_1; 1000x); (a_2; 3000x); (a_3; 5500x); (a_4; 10000x) (washed with 2% SDS)

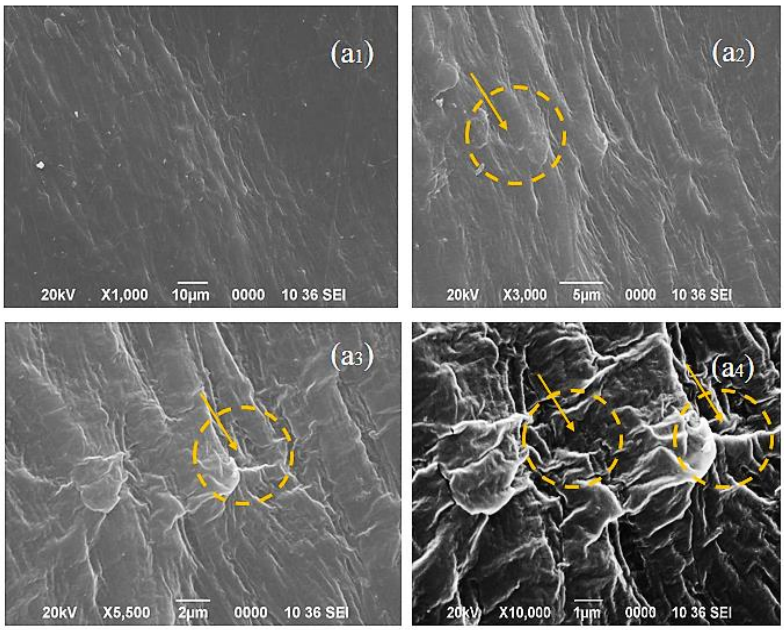


Plate 9.21 Scanning electron micrographs of the surface of LDPE polythene strip after incubation with *Nostoc carneum* (a_1; 1000x); (a_2; 3000x); (a_3; 5500x); (a_4; 10000x) (washed with 2% SDS)

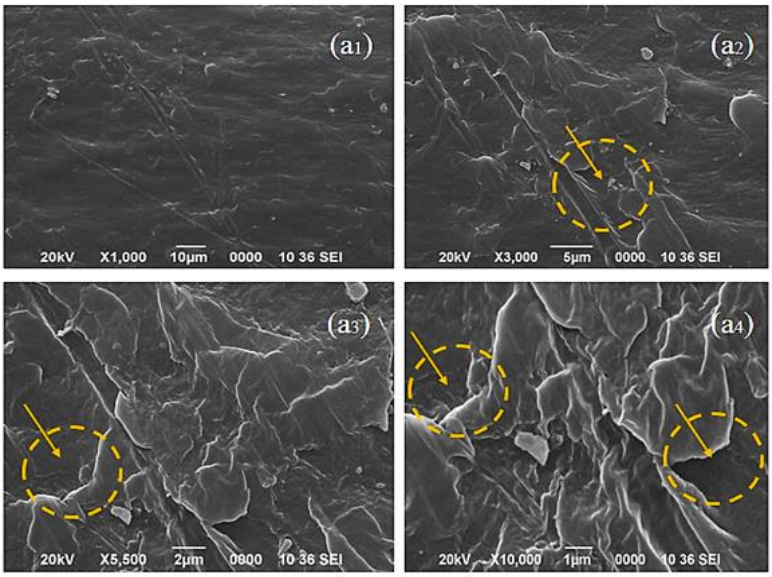


Plate 9.22 Scanning electron micrographs of the surface of LDPE polythene strip after incubation with *Cylindrospermum muscicola* (a_1; 1000x); (a_2; 3000x); (a_3; 5500x); (a_4; 10000x) (washed with 2% SDS)

Fourier transform infra-red (FT-IR) spectroscopy

FT-IR spectroscopy (**Table 9.2**) is frequently used to in analyse the biodegradation of polythene strips. Vibrational changes in cyanobacterial treated polythene were noted (**Fig.9.6-9.7**). Significant changes in polyethylene chain were prominent for fivecyanobacteria treated polythene. In *Phormidium lucidum* treated LDPE strips, the peak observed at 2919cm^{-1} attributed to free OH combination. The peak at 3422cm^{-1} assigned to υCO mode indicating the occurrence of carboxylic acid in *Phormidium lucidum* treated LDPE strips. In *Oscillatoria subbrevis* treated sample, some peaks (2364cm^{-1}) reduced more than those in the control. Some peaks 2326 and 2850cm^{-1} became sharper in *Oscillatoria subbrevis* treated sample than those in control LDPE. A new peak at 1633cm^{-1} corresponding to C=O in *Oscillatoria subbrevis* adduced support to depolymerization activity of the cyanobacterial isolates. The peak at 1629cm^{-1} for υCO mode originated from the presence of carboxylic acid group. For *Oscillatoria subbrevis* treated polythene, peaks at 667 and 468cm^{-1} confirmed the presence of nitrogen containing bio-ligands. The υCO peak at 1633cm^{-1} is concordant with the presence of carboxylic acid group.A peak at 1369cm^{-1} in the control polythene strip usually observed for 1000C short branching is found missing in the treated polythene strips presumably owing to cyanobacterial degradation (Blitz and McFaddin 1994). The peak at 3448cm^{-1} for υCO modeoriginated from the presence of alcohol group for *Lyngbya diguetii* treated polythene. The band at 3449cm^{-1} for υCO indicated the presence of aldehyde group. The υSH peak at 2630cm^{-1} is concordant with the presence of thiol group in *Lyngbya diguetii* treated polythene. The band at 1627cm^{-1} for υCO indicated the presence of ester group. The peak at 1459cm^{-1} and 1363cm^{-1} has been attributed to the C-H stretching. The vibrational absorptions of *Nostoc carneum* treated LDPE polythene strip at 3432cm^{-1}assigned to υOH mode originated from alcohol. The band at 1629cm^{-1}for υCO indicated the presence of carboxylic acid group. Peaks at 733and 716cm^{-1} confirms the presence of aromatic group. Occurrence of alkanes are attested by C-H bands at 2922 and 2860cm^{-1}.The peak at 2355cm^{-1}has been attributed to the C=N stretching. Significant changes in the ester, keto, vinyl and internal double bond index validated the LDPE biodegradation. It is also important to note that the stretching vibrational feature at 2355cm^{-1} is characteristic of the pyridine group augmenting their involvement in the biodegradation process.The peak at 3447cm^{-1} has been attributed to the O-H stretching in *Cylindrospermum muscicola* treated LDPE. The FT-IR spectrum of *Cylindrospermum*

muscicola treated polythene showed peak at 3422cm^{-1} assigned to υOH mode indicating the occurrence of alcohol. The band at 1643cm^{-1} for υCO indicated the presence of ester.

The formation of acid, ester and double bonds in the treated polythene were indicated the process of biodegradation (Balasubramanian et al. 2010). The FT-IR signatures for treated samples of ester, keto,vinyl and internal double bond indices were relativelyindicative and the describe the presents LDPE biodegradation (Albertsson et al. 1987; Gardette 2006). The KCB, ECB, VB, and IDB indices were all found to be higher in the treated samples than those of control. It is presumed that higher value of bond indices in treated samples were ascribed the enzymatic activity of the cyanobacterial isolates (Albertsson et al. 1994) (**Fig. 9.8**).

The keto and ester carbonyl indices in the treated LDPE samples have been stated as major degraded products of polyethylene in the presence of enzyme oxidoreductase(Karlsson and Albertsson 1998). The Norrish type II photochemical reaction has been found to be prompted the formation of double bonds in the treated LDPE (Albertsson *et al.,* 1987; Chiellini *et al.*, 2003; Chiellini *et al.,* 2007).The crystallinity of polythene strips were found to decrease up to 2%, 62%, 58%, 4%, and 7% after incubation with *Phormidium lucidum,Oscillatoria subbrevis,Lyngbya diguetii*, *Nostoc carneum*, and*Cylindrospermum muscicola*respectively. The crystalline order of the polyethylene may have disrupted by the free radical driven chain scission (Restrepo-Flórez et al. 2014). The cyanobacteria, *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum*, and *Cylindrospermum muscicola*, in the present case, are believed to access the amorphous regions of the polymermost (Roy *et al.,* 2008; Khabbaz *et al.*, 1999; Patani and Sorrentino 2013). The introduction of additional groups such as 'carbonyl' that can be utilised by the cyanobacteria in the polyethylene chain may have increase the surface hydrophilicity (Albertsson 1980; Ibiene*et al.,* 2013). It is pertinent to mention herein that 2% SDS buffer solution has been used for washing the LDPE before recording the FT-IR spectra to remove any extraneous substances such as microbial metabolites or culture media from the strips (Gu, 2017).

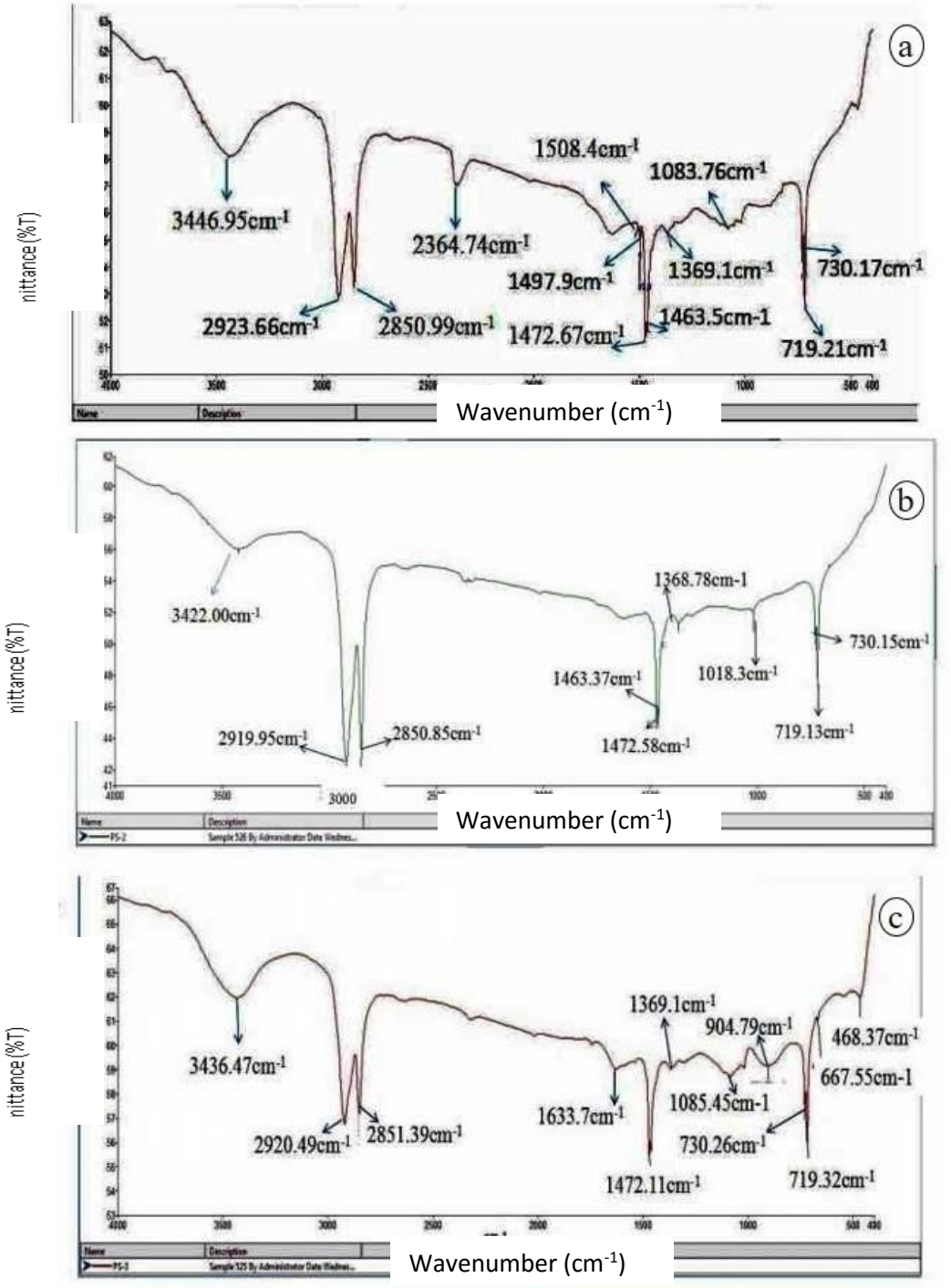


Fig 9.6. FT-IR spectra of LDPE polythene strip: (a) control; (b) *Phormidium lucidum* treated; (c) *Oscillatoria subbrevis* treated.

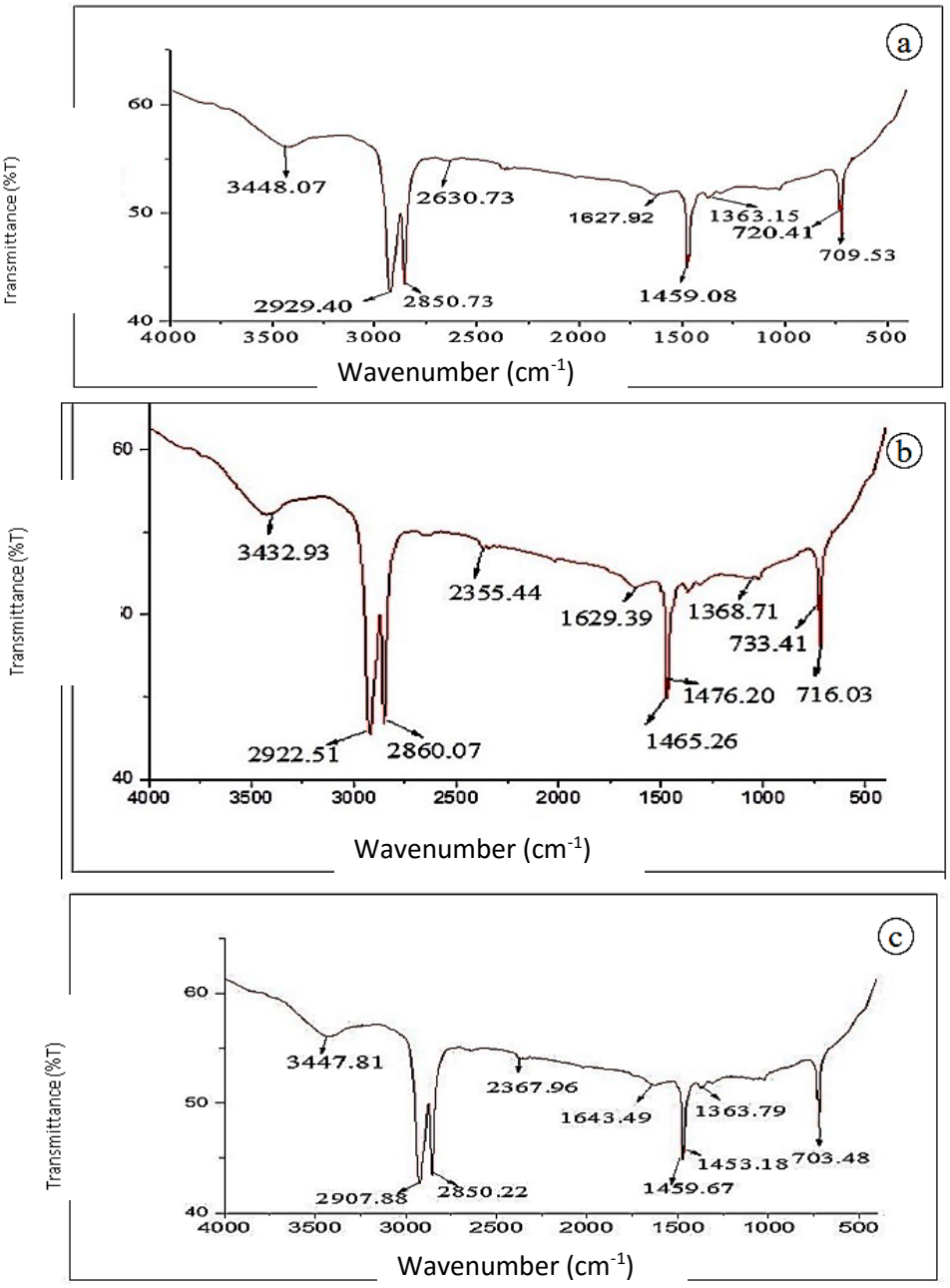


Fig 9.7. FT-IR spectra of LDPE polythene strip: (a) *Lyngbya diguetii* treated; (b) *Nostoc carneum* treated; (c) *Cylindrospermum muscicola* treated.

Table 9.2 FTIR spectra and assigned peaks of LDPE polythene strip

control LDPE strip	*Phormidium lucidum* treated strip	*Oscillatoria subbrevis* treated strip	*Lyngbya diguetii* treated strip	*Nostoc carneum* treated strip	*Cylindrospermum muscicola* treated strip
3446 (-upper stretching >C=O)	3422 (upper stretching >C=O)	3436 (-upper stretching >C=O)	3448 (O-H alcohol)	3432 (alcohol/phenol OH Stretching)	3447 (O-H alcohol)
2850 (-CH_3 Stretching)	2850 (-CH_3 Stretching)	2851 (-CH_3 Stretching)	3449 (H-C=O,C-H aldehydes)	2922 (-C-H-alkane)	2907 (Free OH combination)
2923 (-CH_3 Stretching)	2919 (Free OH combination)	2920 (-CH_3 Stretching)	2630 (S-H thiol)	2860 (-C-H-alkane)	2850 (-C-H-alkane)
2364 (combination of gamma CH^+)	1463 (-CH_3 scissoring)	1633 (C=O)	1627 (-C=O acid, esters, ethers)	1629 (C=C Stretch)	2367 (combination of gamma CH^+)
1508 (-CH_3 scissoring)	1472 (-CH_3 scissoring)	1472 (-CH_3 scissoring)	1459 (-C-H alkanes)	1476 (C-H scissoring)	1643 (-C=O acid, esters, ethers)
1497 (-CH_3 scissoring)	1358 (-CH_3 scissoring)	1308 (Characteristic of $–CH_2$ and $–CH_3$ groups)	1363 (-C-H alkanes)	1465 (C-H scissoring)	1459 (-CH3 scissoring)
1403 (-CH_3 scissoring)	-		-	1368 (C-H methyl rock)	1453 (-CH3 scissoring)
1472 (-CH_3 scissoring)	730 (-C=C Conjugation)	904 (-$N(CH_3)_2$)	-		1363 (CH3 scissoring)
1369 (-CH_3 scissoring)	719 (-C=C Conjugation)	730 (-C=C Conjugation)	-	733(=C-H methyl rock)	
-	-	719 (-C=C Conjugation)	-	-	
730 (-C=C Conjugation)	-	667 (N-containing bioligands)	720 (C=C alkene)	-	
719 (-C=C Conjugation)	-	468 (-N-containing bioligands)	709 (C=C alkene)	716(=C-H methyl rock)	703 (C=C alkene)

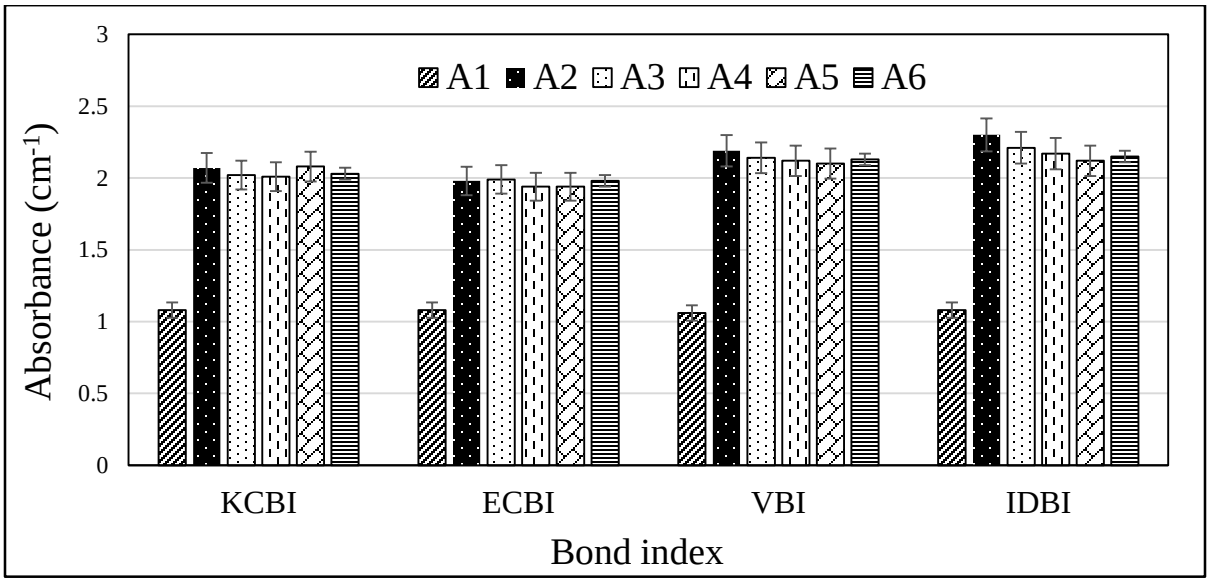


Fig. 9.8. Fourier transform infrared of high-density polyethylene exposed to five cyanobacteria for 42 days(KCBI – Keto carbonyl bond index; ECBI – Ester carbonyl bond index;VBI – Vinyl bond index; IDBI – Internal double bond index)A1-control, A2-*Phormidium lucidum*, A3- *Oscillatoria subbrevis*, A4- *Lyngbya diguetii*, A5- *Nostoc carneum*, and A6-*Cylindrospermum muscicola.*

Carbon, hydrogen, nitrogen (CHN) analysis

The control polythene strips after 6 weeks of incubation revealed the carbon content to be 84%. *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum* and *Cylindrospermum muscicola* utilised about 3, 3, 4, 4 and 3% carbon from the LDPE polythene strips (**Table 9.3**).

Table 9.3 CHN-elemental analysis of PE strips

	Elemental composition (%)		
Biological treatment of PE	**C**	**H**	**N**
Control PE	84.08	14.50	0.12
Phormidium lucidum treated	81.31	14.64	0.09
Oscillatoria subbrevis treated	81.33	14.47	ND
Lyngbya diguetii treated	80.33	16.67	0.11
Nostoc carneum treated	80.21	12.32	0.09
Cylindrospermum muscicola treated	81.23	11.21	0.10

*ND – Not detected

In a previous study, *Achromobacter denitrificans* have shown 2% of carbon being utilised from polythene substrata (Devi *et al.*, 2015).The growth of cyanobacteria may be limited by low nitrogen availability (0.12%).The cyanobacterial growth on LDPE may have been facilitated by the presence of nitrogen in the LDPE. The colonisation of cyanobacteria on LDPE may be attributed to the low carbon availability in the medium and confirms its ability to use the polythene as a carbon and energy source (Awasthi *et al.*, 2017a).The graph co-relating algal colonization in terms of chl *a* with the extent of carbon utilization has been shown in Fig.9.24. The algal colonization was found to be maximum in *Oscillatoria subbrevis* and minimum in *Nostoc carneum.*

TGA-DSC analysis

Melting points for the treated LDPE polythene strips were found to be slightly lower than the control (**Fig.9.9-9.10**). Both control and cyanobacteria treated LDPE polythene strips were heated to 0-200ºC at the heating rate of 10ºC/min.For the control polythene strip, a characteristic peak observed at 125.22°C with a heat enthalphy -35.49mJ has been considered as melting temperature. For *Phormidium lucidum*, the melting temperature was 121.56°C, heat enthalphy -42.17mJ and that for *Oscillatoria subbrevis* it was 121.37°C, heat enthalphy -74.41mJ. For *Lyngbya diguetii*, the melting temperature was 123.20°C, heat enthalphy -71.41mJ (Fig.7) and for *Nostoc carneum*, it was 123.69°C, heat enthalphy -45.49mJ. For *Cylindrospermum muscicola*, the melting temperature was 122.53°C, heat enthalphy -41.49mJ (Table 9.4).The results from the present study

demonstrated that heat release was relatively much higher in *Oscillatoria subbrevis*. The melting and crystallization temperature of LDPE treated by *Phormidium lucidum*and *Nostoc carneum* are found to be higher than that treated by *Oscillatoria subbrevis*, *Lyngbya diguetii* and *Cylindrospermum muscicola*. Similar reduction in melting temperatures by about 1.2°C wrt the control has been noted in *Aspergillus* sp. treated polythene (Raaman *et al.*, 2012). The higher melting and crystallization temperature can be correlated with high molecular weight substances in *Phormidium lucidum* and *Nostoc carneum* (Farukkawa *et al.*, 2006).

The TGA traces (**Fig. 9.11-9.12**) of cyanobacteria treated polythene strips showed that initial decomposition temperature of the control polythene strip (only culture medium)occurred at 300^0C indicating high thermal stability. A steady weight loss has been registered thereafter showing 40% weight loss at 600^0C. For *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum* and *Cylindrospermum muscicola*treated species, weight loss of the polythene strip were observed at 100^0C only, the start temperature. A sharp decline in percentage weight loss were noticed thereafter showing a 20% weight loss at 600^0C. The weight loss were most prominent for the *Phormidium lucidum*, *Oscillatoria subbrevis* species. The thermal stability results, thus, clearly demonstrate the degradation of the algae treated polythene strips. Similar decrease in the onset temperatures and corresponding weight loss were also observed in fungi treated polythene films, though, in the presence of pro-oxidant (Corti *et al.*, 2010).The lamellar thickness (Lc~110nm) of control polythene strip following cyanobacterial exposure got reduced to 97.78nm, 54.45nm, 57nm, 98.12nm and 97.67nm for *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum* and *Cylindrospermum muscicola* treatments, respectively. Considering that no pro-oxidant were used in the present experiments, the results are very significant.

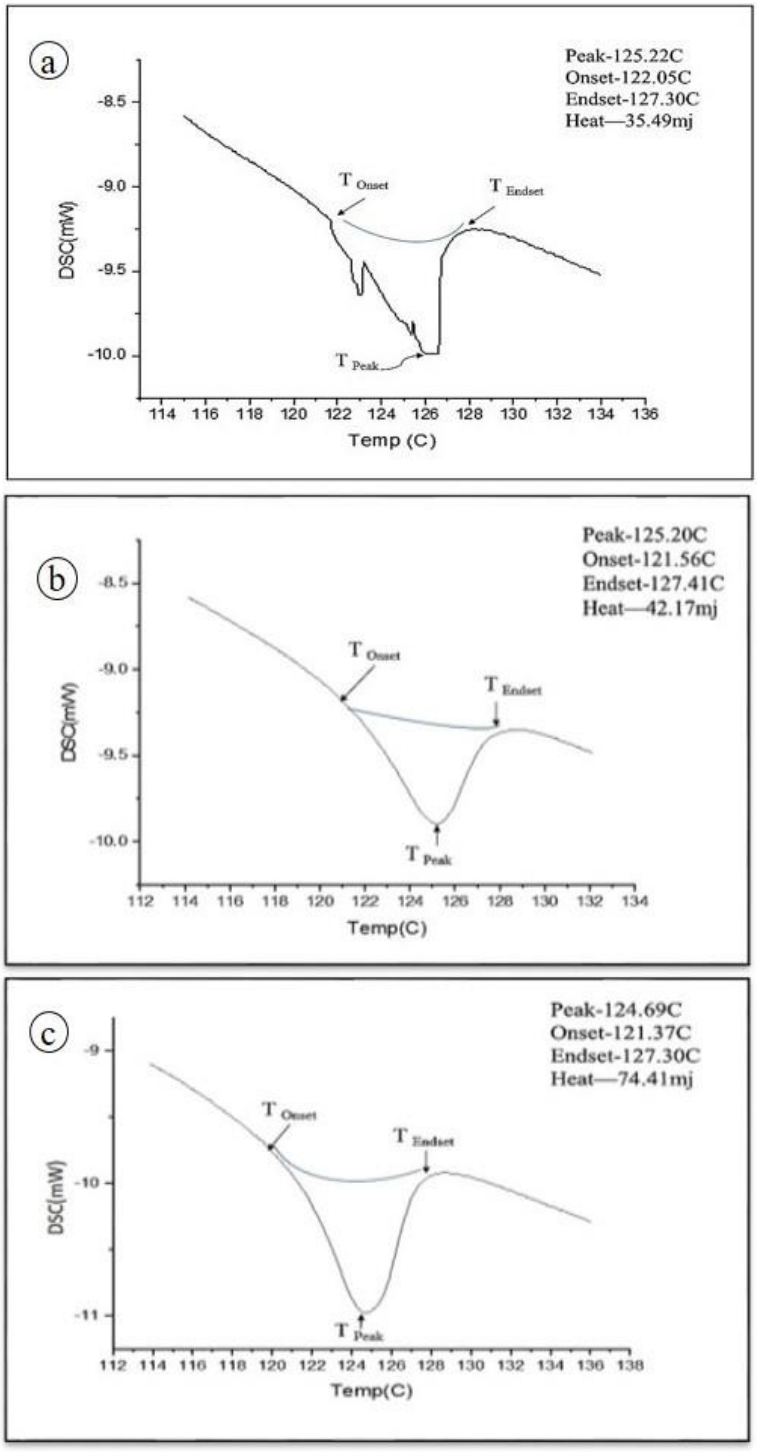


Fig 9.9. Melting points of polythene strip: (a) control; (b) *Phormidium lucidum* treated; (c) *Oscillatoria subbrevis* treated

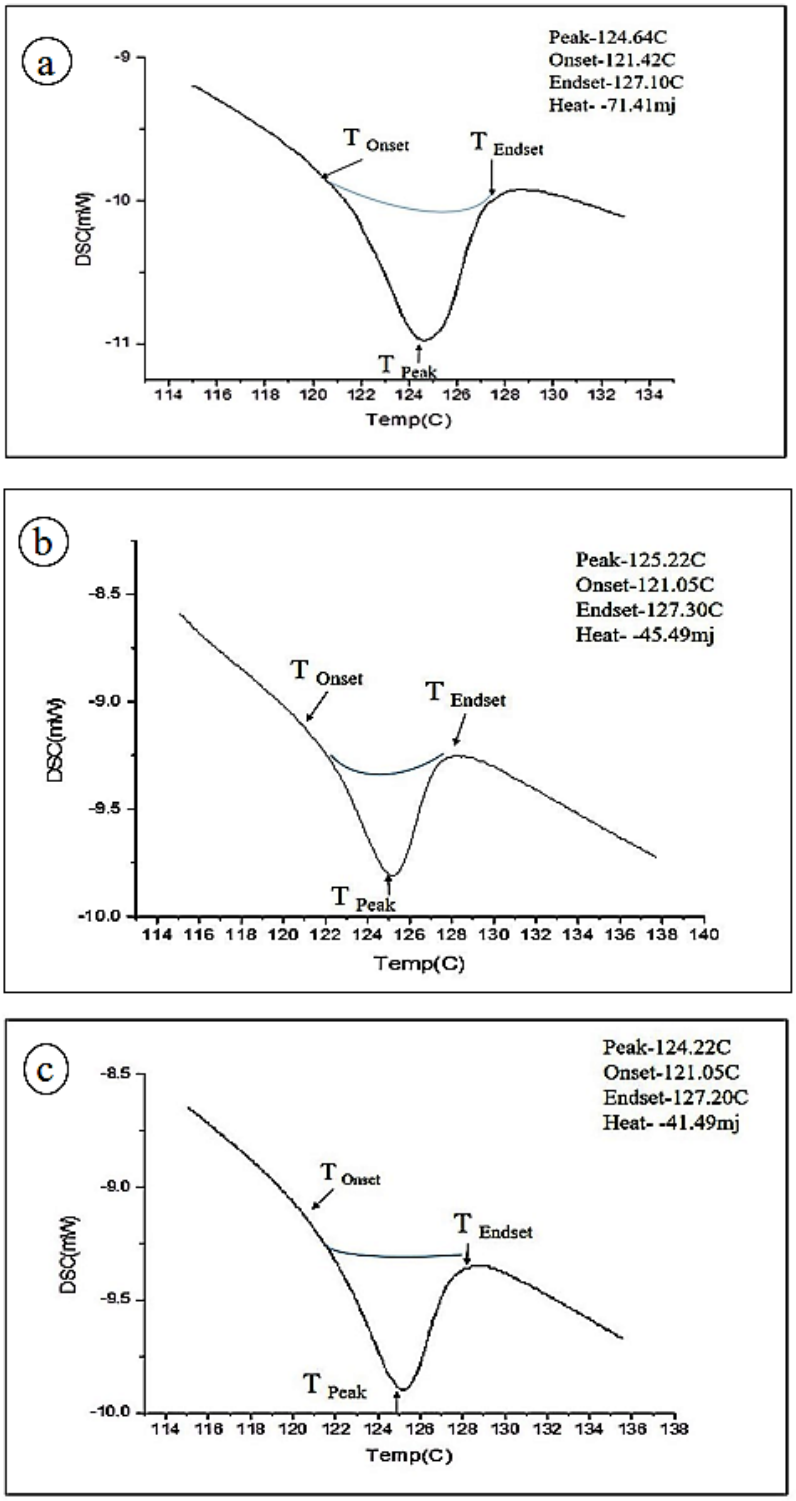


Fig 9.10. Melting points of polythene strip: (a)*Lyngbya diguetii* treated; (b) *Nostoc carneum* treated; (c) *Cylindrospermum muscicola* treated

Table 9.3 DSC data of polythene strip

Sample	**Peak (ºC)**	**Onset(ºC)**	**Endset(ºC)**	**Heat (mJ)**
Control-PE	125.22	122.05	127.30	-35.49
Phormidium lucidum treated	125.20	121.56	127.41	-42.17
Oscillatoria subbrevis treated	124.69	121.37	127.30	**-74.41**
Lyngbya diguetii treated	124.64	121.42	127.10	-71.41
Nostoc carneum treated	125.22	121.05	127.30	-45.49
Cylindrospermum muscicola treated	124.22	121.05	127.20	-41.49

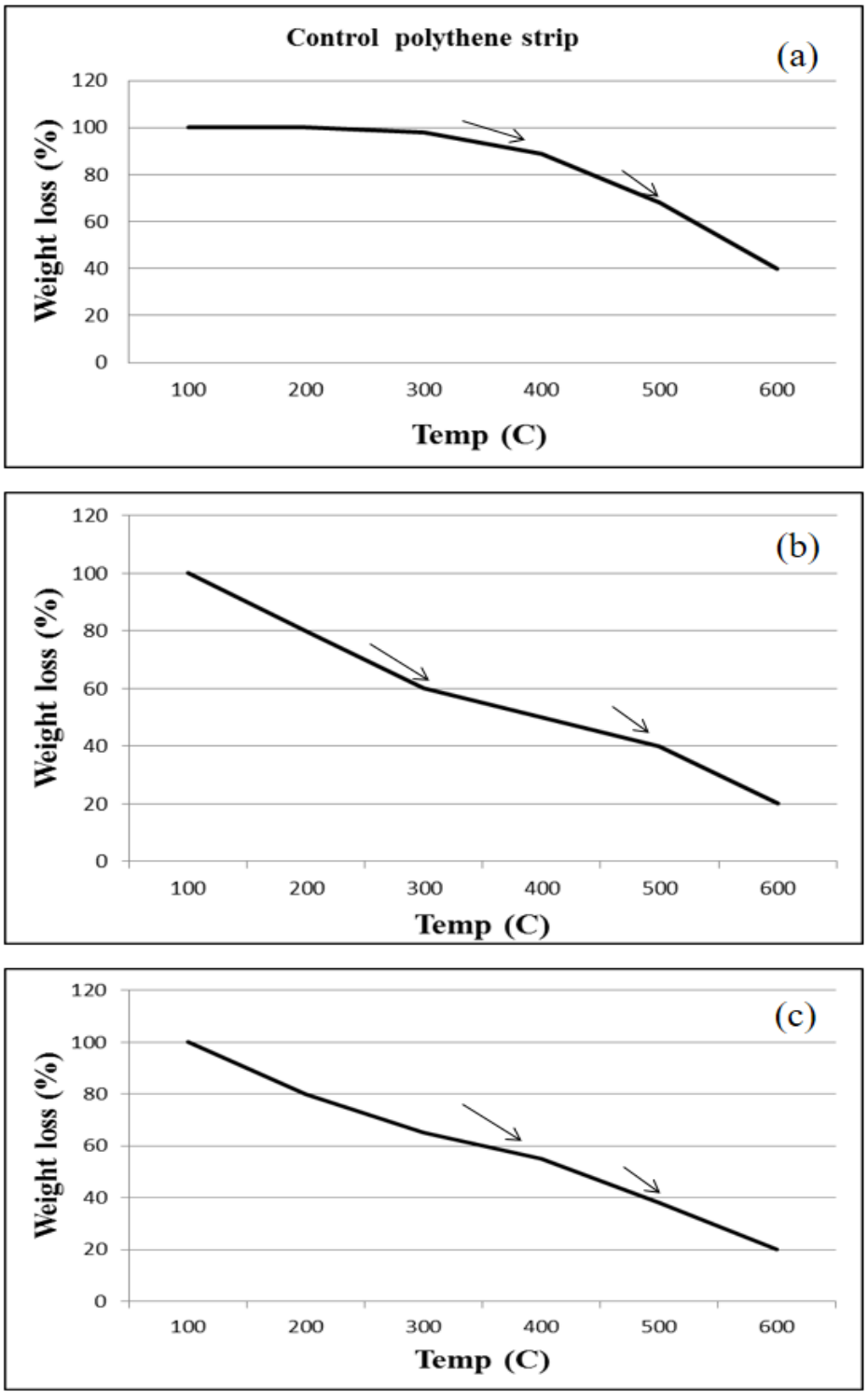


Fig. 9.11.TGA profile - variation inthethermal behavior of LDPE polythene strips due to cyanobacterial exposure (a-control, b-*Phormidium lucidum*, c-*Oscillatoria subbrevis*)

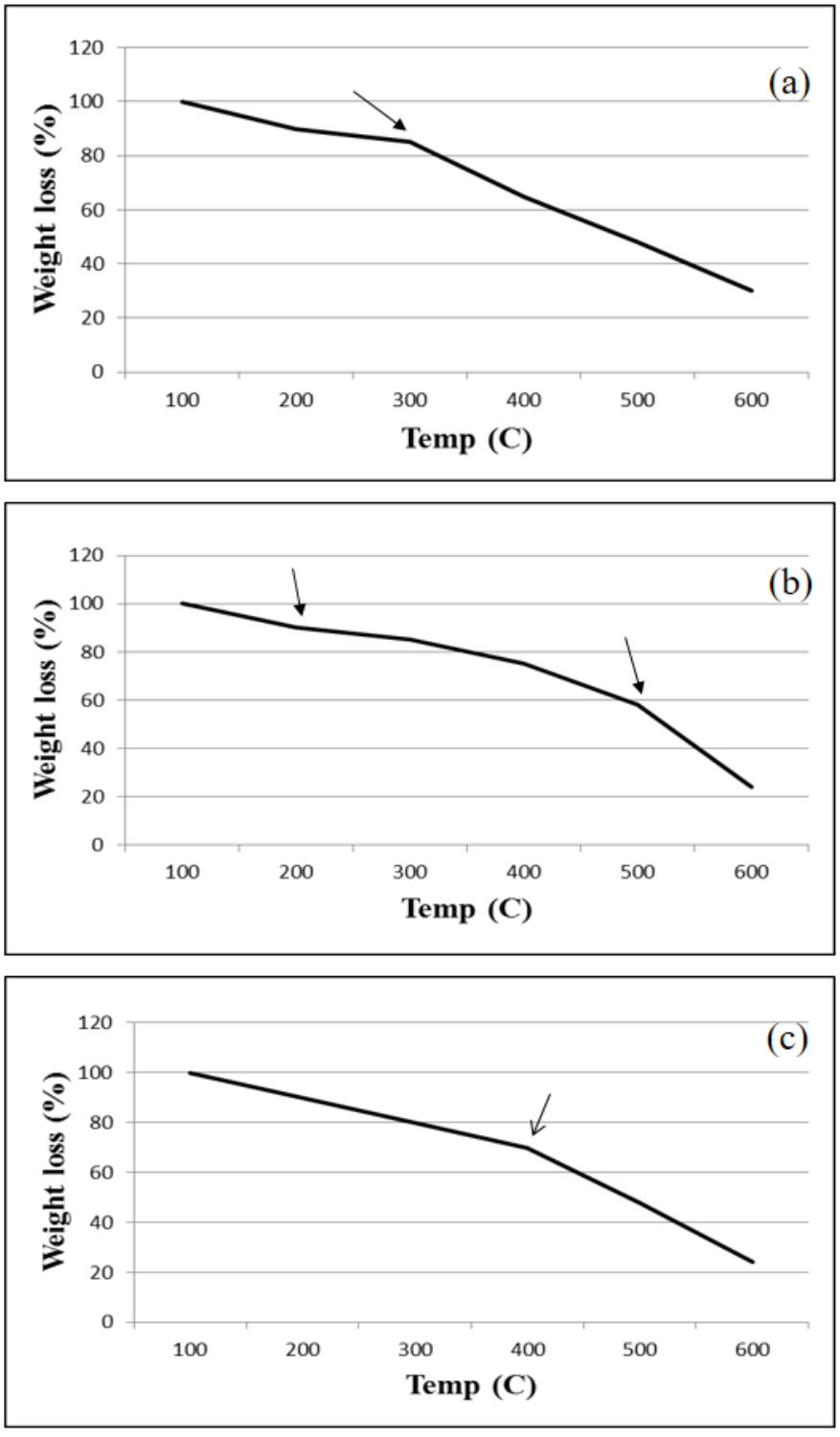


Fig. 9.12. TGA profile - variation in thethermal behavior of LDPE polythene strips due to cyanobacterial exposure (a- *Lyngbya diguetii*, b- *Nostoc carneum*, c- *Cylindrospermum muscicola*)

Nuclear magnetic resonance spectroscopy (NMR)

Micro structural changes in the polythene were detected when the biodegraded strips were characterized by ^{13}C NMR spectroscopy (**Fig.9.14-9.15**).^{13C} NMR chemical shift (ppm) and assignment (1-*Phormidium lucidum* treated; 2- *Oscillatoria subbrevis* treated; 3-*Lyngbya diguetii* treated; 4-*Nostoc carneum* treated; 5-*Cylindrospermum muscicola* treated) are shown in **Table 9.4**.The absorption peak centered at 20ppm in the control LDPE strip is believed to have originated from common plastic additives like phosphoric acid esters. The peaks at 35.1ppm and 35.7ppm, conspicuously absent in the control,are due to esters and formation of some new -CH_2 group indicating the formation of ethyl propanoate. These chemical shifts are compatible with substituted or unsubstituted -CH and -CH_2 groups (**Table 9.4**), showing that these compounds are derivatives of oxidized polythene fragments (Kouty *et al.,* 2006a). Multiplet character of the absorption peak at 34ppm in the cyanobacteria treated polythene can be assigned tocarbonyl group of the acid moiety. The enhanced intensity of absorption peak at 34.7ppm in the treated LDPE suggested short chain branching (Brandolini and Hills 2000).The degraded polythene provides an organic soluble fraction implying a carbon uptake process linked to the metabolic pathway of the cyanobacterial species (Miyazaki *et al.,* 2012; Balasubramanian *et al.,* 2014). Formation of carboxylic acid and other byproduct of polythene degradation reduces the molar mass of the polythene and facilitate carbon assimilation (Arnaud *et al.,* 1994).

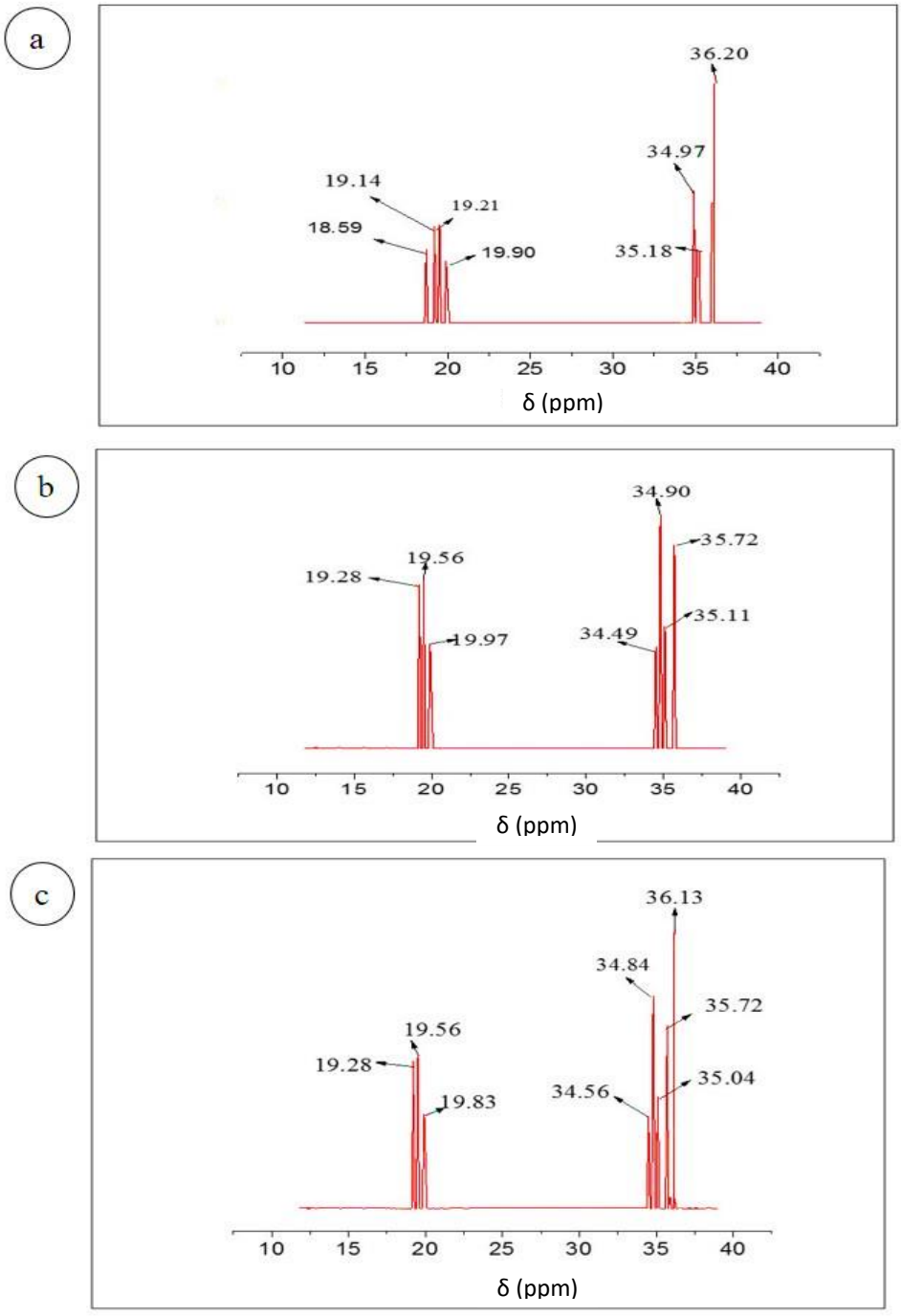


Fig.9.14. ^{13}C NMR of LDPE polythene strip: (a) control; (b) *Phormidium lucidum* treated; (c) *Oscillatoria subbrevis* treated.

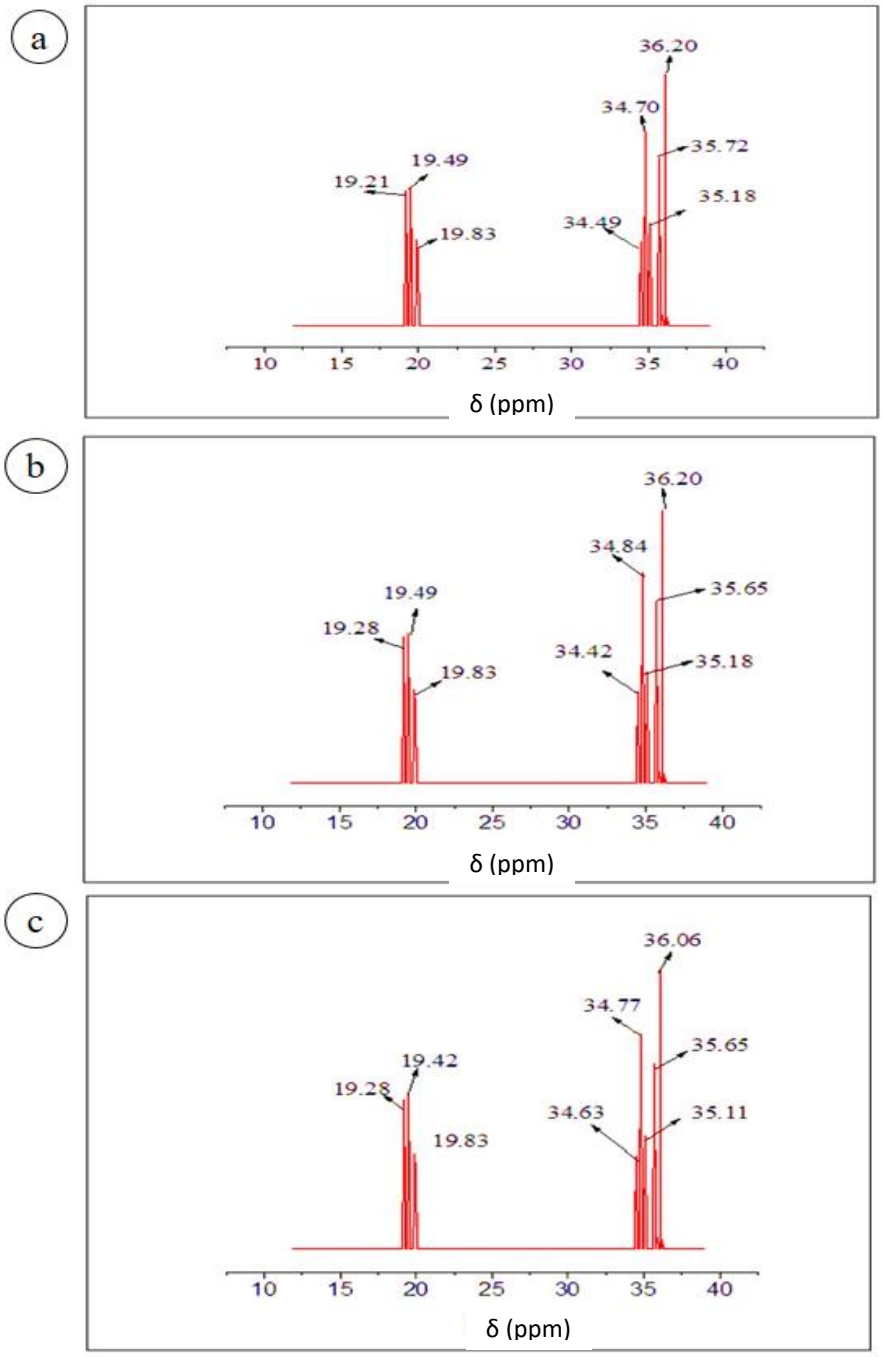
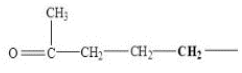
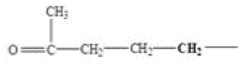
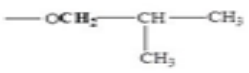
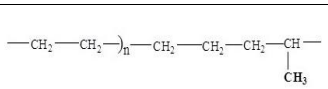
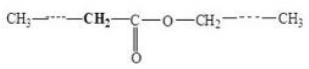
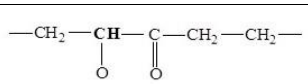
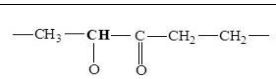
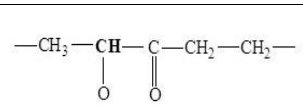
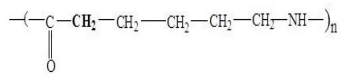


Fig.9.15. ^{13}C NMR of LDPE polythene strip: (a) *Lyngbya diguetii* treated; (b) *Nostoc carneum* treated; (c) *Cylindrospermum muscicola* treated.

Table 9.4^{13}C NMR chemical shift (ppm) and assignment (1-*Phormidium lucidum* treated; 2- *Oscillatoria subbrevis* treated, 3- *Lyngbya diguetii* treated, 4- *Nostoc carneum* treated, 5- *Cylindrospermum muscicola* treated)

	Chemical shift (ppm)					
Structural unit	Control (LDPE)	1	2	3	4	5
	18.59	-	-	-	-	-
	19.14	19.28	19.28	-	19.28	19.28
	19.21	19.56	19.56	19.21	19.49	19.42
	19.90	19.97	19.83	19.83	19.83	19.83
	34.97	34.49	34.56	34.49	-	34.63
	35.18	34.90	34.84	35.18	-	34.77
	-	35.11	35.04	-	-	35.11
	-	35.72	35.72	35.65	-	35.65
	36.20	-	-	36.20	36.20	36.06

Tensile testing/Mechanical properties

The changes in the tensile strength and elongation at break due to cleavage of the polymer chain by the treatment with cyanobacterial species were monitored. The cyanobacteria treated polythene strips expectedly registered a reduction in tensile strength to~7 from 11.8Mpa. The elongation break of Oscillatoria subbrevis treated polythene strip were observed as highest as compared to other cyanobacteria (**Table 9. 5).** In a previous study, involving biodegradation of LDPE was recorded about a reduction of 42.5% tensile strength of LDPE polythene, 31.5% reduction inelongation and 28.8% reduction in modulus (Devi *et al.,* 2015).

Table 9. 5. Mechanical properties of treated PE after one month of incubation with cyanobacteria

PE material	**Tensile Strength (Mpa)**	**% Elongation at break**	**Modulus (MPa)**
Control	11.8±0.12	299±0.002	128.3±0.002
Phormidium lucidum treated	6.7±0.002	254±0.002	101±0.002
Oscillatoria subbrevis treated	6.7±0.003	243±0.001	103±0.001
Lyngbya cinerescens treated	6.6±0.003	245±0.001	101±0.02
Nostoc carneum treated	6.7±0.001	250±0.001	105±0.03
Cylindrospermum musicola treated	6.8±0.002	252±0.10	102±0.002

Weight changes of polythene

Weight loss of polythene is an important and well recognized indicator of biodegradation. In the present study, the weight loss associated with the cyanobacterial biodegradation of LDPE found to be about 30% after 6 weeks (**Fig. 9.16-9.21**). In the abiotic control (without inoculation), no significant weight loss was observed thus ruling out any effect of the nutrient chemicals of the culture medium on the polythene substratum. In a previous study involving polythene degradation by algae, *Anabaena spiroides*, *Scenedesmus dimorphus* and *Navicula pupula* recorded weight loss upto 8% only (Kumar *et al.,* 2017). Percent reduction of weight loss in cyanobacteria treated strips in the present study *vis-a-vis* the dual control used in the experiment unequivocally represent significant biodegradation by the tested cyanobacteria(Awasthi et al. 2017b). The highest reported weight loss so far by any microorganism appears to be ~60% after 3 months (Rajandas *et al.,* 2012).

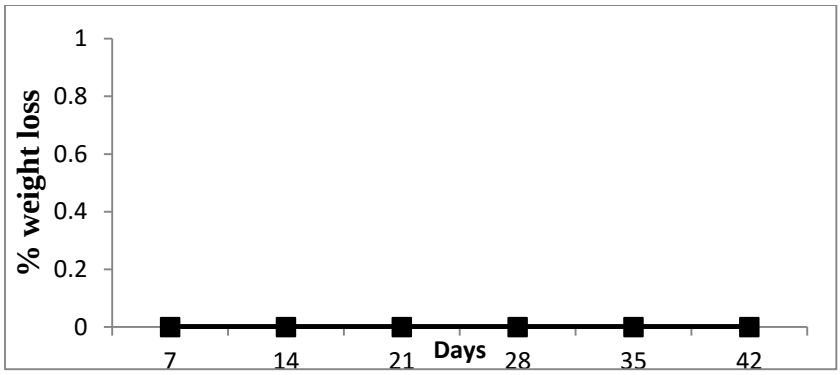


Fig. 9.16. Percentage of weight loss of control LDPE

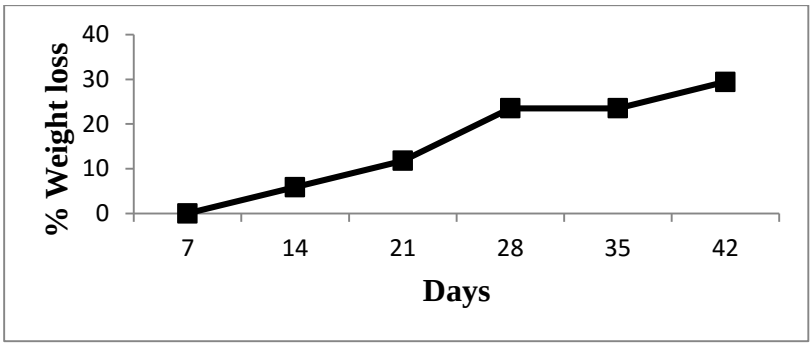


Fig. 9. 17. Percentage of weight loss of LDPE treated by *Phormidium lucidum*

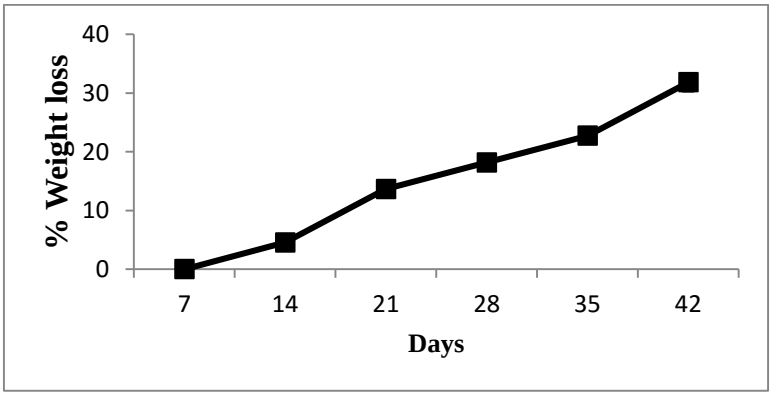


Fig. 9.18. Percentage of weight loss of LDPE treated by *Oscillatoria subbrevis*

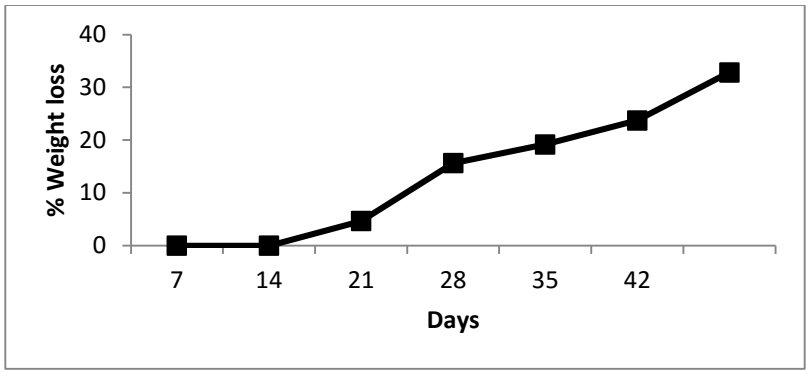


Fig. 9.19. Percentage of weight loss of LDPE treated by *Lyngbya diguetii*

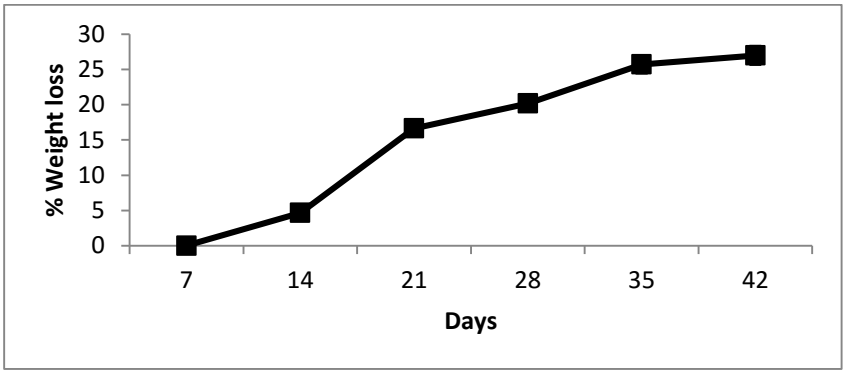


Fig. 9.20. Percentage of weight loss of LDPE treated by *Nostoc carneum*

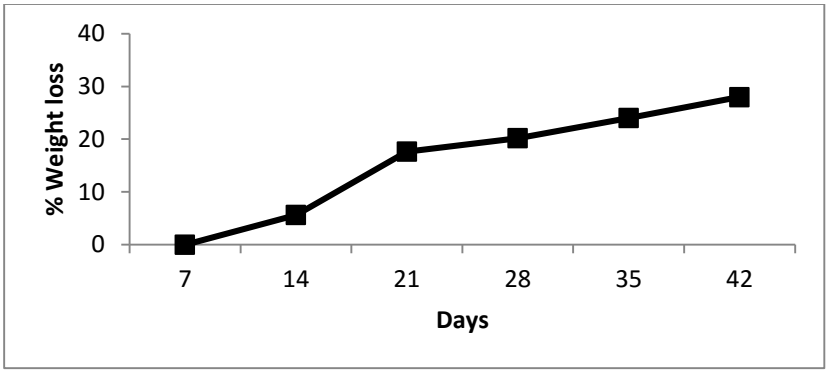


Fig. 9.21. Percentage of weight loss of LDPE treated by *Cylindrospermum muscicola*

Enzymatic activity

Activity of laccase (0.009IU/ml) was higher as compared to manganese peroxidase (0.0075IU/ml) after 6 weeks treatment for the cyanobacterial species. High molecular weight of the polymer limits the enzymatic reactions leading to biodegradation of LDPE. Two key mechanism are believe to be operative in LDPE degradation, one is the reduction of molecular weight and second oxidation of molecules (Yoon *et al.,* 2012). The break-down of large polyethylene molecules is in fact believed to have been initiated by enzymatic action accompanied by molecular weight reduction and enhancement of keto-carbonyl index (Santo *et al.,* 2012). Microorganisms are known to produce necessary oxidative and degradative enzymes and assimilate the polymeric carbon into their biomass (Hadad et al., 2005; Tribedi and Sil 2013). Cyanobacterial enzymes present in the liquid phase of the media interact with the macromolecules available at the surface of the polythene strips triggering biodegradation (Chinaglia *et al.,* 2017).

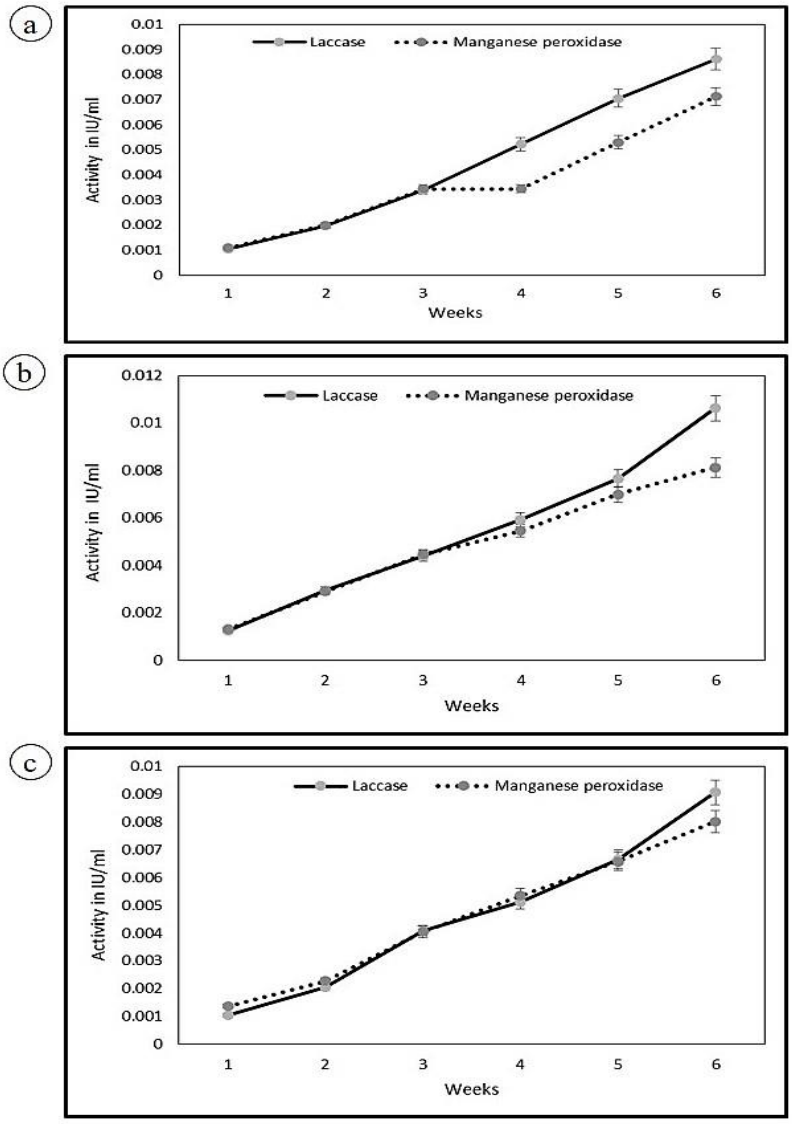


Fig. 9.22. Enzymatic activity of *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii* incubation with LDPE

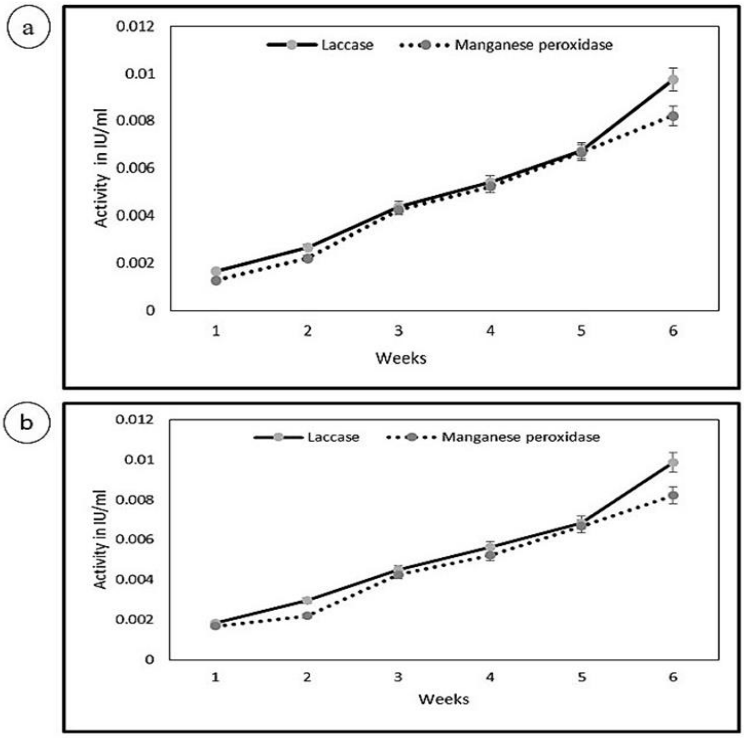


Fig. 9.23. Enzymatic activity of *Nostoc carneum*, *Cylindrospermum muscicola* incubation with LDPE

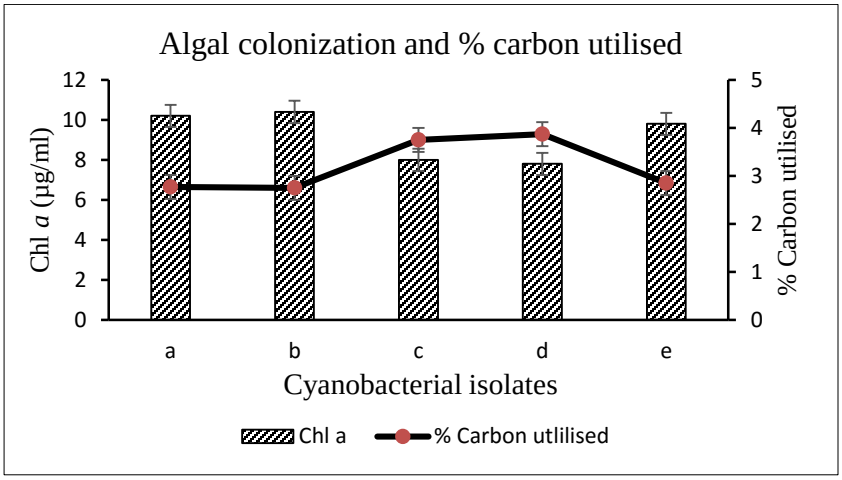


Fig. 9.24 Algal colonization and carbon utilization (%) after 42 days)

Conclusion

Spectroscopic, enzymatic, thermal, mechanical and morphological studies in relation to dual experimental control clearly demonstrated efficient LDPE biodegradation by the cyanobacterial species. The fast growing, readily available, easily isolable and non-toxic cyanobacteria are capable of effectively colonising on the LDPE polythene utilising carbon without any pro-oxidant additives or pretreatment. The results are of significance for development of biodegradation protocol for polythene using freshwater nontoxic cyanobacteria which are more efficient besides being convenient, easy to handle and less hazardous as compared to other bacteria or fungi. Under natural conditions, these cyanobacterial species have the potential to degrade polythene even more efficiently furnishing a tangible alternative solution to polythene waste management.

Chapter 10

General Discussion

Polyethylene are well known for packaging films, as well as in making commercial polyethylene bags. They degrade very slowly in comparison to pro-oxidant containing polyethylene. The degradation of polyethylene depends on the presence of impurities, carbonyl and hydroperoxides groups introduced during manufacturing processes. Indiscriminately dumping of polythenes into the sewage water and landfills are known to emit dangerous methane and carbon dioxide gases during their decomposing stages as well as highly toxic leachates (Simmons, 2005). It effectively blocks sewerage pipe lines, litters agricultural lands, canals, rivers and oceans. They are not biodegradable or take incredibly long time to break down into powdery plastic dusts which contaminate the soil and the water adversely affecting all life forms (Stevens, 2001). Algae are known to colonise on polyethylene surfaces submerged in waste water and domestic solid waste dumping site. Study of growth of algal species on such polythene substrata are important in the context of biodegradation of polythene. The physico-chemical properties of natural water bodies

are important for algal growth. In pursuance of the objectives of the present research, a total of 122 algal species belonging to 41 genera under 4 classes were found to be distributed on the polyethylene surfaces in Silchar town during the present study. Cyanophyceae were found to be the dominant class with 57 species spread over 13 genus. Chlorophyceae represented by 15 genus with 25 species. Bacillariophyceae were also found to colonise on polyethylene surfaces represented by 2 genus and each with 1 species. The species *Oscillatoria* was the largest genus with 29 species found to form mat like colonisation. Highest algal diversity was observed in Link road 1st (Site 1) during premonsoon season with maximum Shannon-Wiener diversity index (H=2.81 ± 0.15), minimum Simpson's dominance index (D=0.035 ± 0.14) and maximum Pielou's evenness index (J=0.98 ± 0.01). The algal distribution in Club road during post monsoon was observed to be least diverse with minimum Shannon Wiener diversity index (H=0.98 ± 0.14), maximum Simpson's dominance index (D=0.70 ± 0.03) and minimum Pielou's evenness index (J=0.26 ± 0.33).

Pearson's correlation coefficients calculated between various physico-chemical properties of water and total Cyanobacterial species present on submerged polythene bags in domestic sewage water drains have been presented in Table 4.29. Water pH has positive correlation (**r=0.942**, p<0.01**) with total Cyanobacterial species. BOD has positive correlation with total Cyanobacterial species (r = **0.311****, p <0.01) in the domestic sewage water. DO has positive correlation (r=0.656**, p<0.01) with total Cyanobacterial species. Total alkalinity has positive correlation (r=**0.923****, p<0.01) with total Cyanobacterial species. Sulphate, nitrate and calcium has a positive correlation (r=**0.585**, 0.446**, 0. 440**)** with total Cyanobacterial species.

The soil pH has negative correlation with total Chlorophyceae species (r= **-0.992****, p<0.01). BOD has negative correlation with total Chlorophyceae species (r=**-0.226***, p<0.05). Total alkalinityhas negative correlation with total Chlorophyceae species (r=-0.995**, p<0.05). Suspended solid (SS) has negative correlation with total Chlorophyceae species (r=**-0.974****, p<0.05).Nitrate has negative correlation with total Chlorophyceae species (r=**-0.475****, p<0.05). Free CO_2 has negative correlation with total Chlorophyceae

species (r=**-0.670****, $p<0.05$). All the physico chemical parameters were found to be negatively correlated with total Bacillariophyceae species.

Cyanobacterial population reached maximum number during pre-monsoon and decreased thereafter. *Oscillatoria limosa*, *O. princeps*, *O. subbrevis*, *O. tenuis*, *O. willei*, *Nostoc carneum*, *N. linckia*, *Phormidium lucidum*, *Cylindrospermum muscicola* and *Lyngbya diguetii*were found to be the most dominantspecies during pre-monsoon period on submerged polythene surface in sewage water. The physico-chemical properties of sewage water were found to influence the cyanobacterial colonisation on the polythene surface. The free CO_2 and pH value of sewage water were found to favor the cyanobacterial growth on the polythene surface. During monsoon period, *Oscillatoria limosa* and *O. princeps* were found to mat-like colonisation on the polythene surface. Sometimes, *Lyngbya diguetii* were found to colonise on the polythene surface during post-monsoon. In-between, pre-monsoon and monsoon, when the dissolved oxygen were found to be low, some *Oscillatoria* and *Phormidium* were found to dominate on the polythene surface.The value of pH, free CO_2, phosphate, nitrate and chloride concentration of sewage water were found to be responsible for the colonisation of blue-green algae on the polythene surface (Sarojini 1996; Tarar and Bodhke, 2002). The higher value of nitrate in sewage water are attributed to the colonisation of blue-green algae on the polythene surface (Jarousha, 2002).

Algae shows various forms that can acclimatize to different habitats through varying growth rates with variable genomes, different genes under different environmental conditions (Manoylov, 2014). Most of the cyanobacteria are covered by thick gelatinous sheath and some of the thallus morphology are also inconspicuous due to very fine thread like trichome with insignificant cross walls (Banerjee and Pal, 2017). Morphological characterization of cyanobacteria on the basis of trichomes, presence or absence of sheath, heterocyst present or absent, terminal or intercalary heterocyst, diffluent sheath forming free or floccose or soft mucilaginous thallus are necessary keys that leads to species identification. Morphological characterization play an important role in next level of taxonomy such as chemotaxonomy and phylogenetic studies of the cyanobacteria. In the present study, morphological characters of the isolates with cultural developmental history were found to be very helpful to avoid wrong identification of the isolates. A total of 33

algal isolates were taxonomically assigned to 13 genera and were identified as *Anabaena*, *Anabaenopsis*, *Aphanothece*, *Calothrix*, *Chlorella*, *Cylindrospermum*, *Fischerella*, *Hapalosiphon*, *Lyngbya*, *Nostoc*,*Oscillatoria*, *Phormidium*, and *Westiellopsis*. Maximum number of isolates were belonged to the genus *Anabaena* (6), followed by *Nostoc* (5), *Calothrix* (5), *Oscillatoria* (4), *Cylindrospermum* (2), *Westiellopsis* (2), and *Lyngbya* (2). The cultural and growth studies of isolates were performed in the present study. The maximum growth rate was revealed has by *Oscillatoria subbrevis* (E2) ($0.158\mu d^{-1}$) followed by *Anabaena oscillatoriales* (E30) ($0.157\mu d^{-1}$). The generation time was maximum in *Anabaena anomala* (E23) (277.86h) and minimum in *Calothrix* sp. (E20) (109.69h).

The biochemical constituents of algal isolates from polythene surface submerged in domestic sewage water showed isolates contain high cellular constituents of chla, carotenoids, protein, carbohydrate, vitamin C, lipid, phycobiliproteins, total phenolic content, polyphenol content and total flavonoid content. Significant differences were observed in biochemical constituent species wise. The characteristic morphological and physiological attributes of the species might be ascribed to typical physico-chemical properties of domestic sewage water. It has been reported that the cellular composition of cyanobacteria depend on the nature of strains, physiological state of the isolates and the nutrient conditions of environment from where they have collected (Vargas *et al.,* 1998; Subhashini *et al.,* 2003; Rosales *et al.,* 2005; Smith and Schindler, 2009). Phycobiliproteins content of the algal isolates were found to be direct relation with the environmental condition of species where from they were isolated. Grossman *et al.,* (1993) opined that environmental condition of species might alter the composition and abundance of phycobiliproteins. In the present study, the physic-chemical parameters were at variance in all the five sites. This, we believe, might have caused a variation in the total phycobiliproteins in the species studied.

The presence of enzymatic and non-enzymatic antioxidants in algal isolates clearly demonstrated its role against oxidant and other free radicals. The occurrence of enzymes viz., catalase, and peroxidaseand glutathione reductase in the algae are key factors to its adaptation to extreme environmental conditions (Mukund *et al.,* 2014). The algal isolates

showed the inhibition percent to DPPH radical scavenging activity, hydroxyl radical scavenging activity and total antioxidant activity. The algal isolates are believed to have developed defense against photo-oxidative damage by various antioxidative mechanisms to detoxify and remove highly reactive oxygen species (ROS) by producing several oxidative and radical stressors such as phenolic compounds and carotenoids (Tsao and Deng, 2004). As the algae were screened from submerged polythene surface in sewage water, it is anticipated that it might have gradually developed a system of either accumulating or releasing intra- or extracellular compounds to cope with the stress (Grossman *et al*., 1993; Ward and Singh, 2005, Paliwal *et al*., 2017). In the present study, the algal isolates were found to be rich in carotenoid content. The algal isolates were also revealed the percent inhibition of antioxidant activity. It is assumed that the presence of those carotenoids in algal isolates may be responsible for the antioxidant activity (Matsukuwa *et al.,* 2000). Algalisolates are found to be rich in various other natural compounds and pigments such as chlorophyll, phycocyanins, phenolic compounds, carotenoids, vitamins. Algal antioxidants have important role in regulating various diseases such as cardiovascular diseases, anti- inflammatory and immune protective, enhancing eye health, and increasing muscle strength. They are also effective in providing defence and antioxidant mechanism system via enzymatic and non-enzymatic antioxidants. The enzymatic and non-enzymatic antioxidants contain several mettalo-isoenzymes that can neutralise the harmful effects of ROS (Ahmad *et al.*, 2000; Sharma *et al.*, 2017).

In the present study, a total of 31 species of algae were isolated based on collection from polythene surfaces in domestic sewage water and solid waste dumping site. Five species of cyanobacteria based on dominance over occurrence for biodegradation of polyethylene. The cyanobacterial species, *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum* and *Cylindrospermum muscicola* were selected for polyethylene degradation. The products of degradation were tested by SEM, FTIR, NMR, tensile strength, and CHN analysis. In the present study, polyethylene degradation were observed in minimal carbon BG11 media. It was observed after 42days of experiment, the cyanobacterial species were grown better on $1cm^2$ polyethylene surface than BG11 liquid media. From the present study, it was evident that the cyanobacteria isolated from polythene surface in sewage water and solid waste dumping site are capable of utilizing

polyethylene as nutritional sources. In a recent study, capabilities of cyanobacteria in polyethylene degradation has been demonstrated (Kumar *et al.,* 2017).Studies also revealed that gram-negative bacteria could easily adapt to the environment rich in polyethylene and similar polymers.Since, cyanobacteria are gram-negative, they are found to be colonise on the polythene surface and utilizing the polyethylene as carbon source (Dey *et al.,* 2012).The structure of cell wall of Gram-negative is simple, they comprise of either homopolysaccharides or heteropolysaccharides. These molecules impart mechanical stability and are pivotal to adhesion and cohesion on the polymeric surface, and evasion from harsh dynamic environmental conditions. They consolidate the biofilm structure (Garrett *et al.,* 2008). The cyanobacterial colonisation on the polyethylene surface were observed to be maximum at the end of log phase. Extracellular polysaccharide of cyanobacteria helps in intercellular adhesion of cyanobacteria to the polyethylene surface ((Dunne, 2002). In the present study, EPS of cyanobacterial were measured and found to be 19.99, 22.43, 16.17, 21.44, 34.46µg/ml, respectively, *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum* and *Cylindrospermum muscicola*. Large surface area and organic content of polythene combined with cyanobacterial cell wall secretions render polythene surface more hydrophobic enabling fast colonisation (Sivan, 2011; Kumar *et al.,* 2017).

The rate of PE biodegradation was determined by weight loss in minimal carbon BG11 media. The present study revealed about 30% degradation of polyethylene for the tested species i.e., *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum* and *Cylindrospermum muscicola.* Previous studies reported 8% of weight reduction for PE by few cyanobacterial species over a period of 120 days (Kumar *et al.,* 2017). However, the present study suggested 27-30% of degradation for PE by the cyanobacterial isolates. The cyanobacterial isolates were found to grow better on polythene than BG11 media. The production of cellular constituents were found to be maximum on the polyethylene grown cyanobacteria. The pigment, protein and carbohydrate contents were found to be higher on polythene grown cyanobacterium than the biotic control (Shang *et al.,* 2009; Koutny *et al.,* 2006a). The enhanced production of cellular contents under minimal carbon source is considered as defensive mechanism of the cyanobacterial isolates (Paliwal *et al.,* 2017).

The FT-IR spectra of treated PE strip demonstrated several peaks in the range of 500-3446 cm^{-1} in comparison with the control PE. The FTIR spectra of treated PE also showedminor structural variation (peaks between 730-1500 and 2364-3446cm^{-1}) in comparison with the control.Present study confirmed that PE treated with cyanobacterial species underwent major structural changes which are a direct indication of biodegradation (Corti *et al.*, 2010; Esmaeili *et al.*, 2013) by the cyanobacterial species.

There were considerable differences in the percentage of elongation of treated PE after 42days by cyanobacterial isolates in comparison with the control. The tensile strength (TS) and elongation at break (EAB) for the control PE were found to be 11.8±0.12% MPa and 299±0.002%, respectively. The TS and EAB of treated PE by cyanobacterial species were found to be 6.7 ± 0.43 % and 243 ± 0.43 % after 42days.The reduction in tensile strength for tested PE in comparison with the control PE samples indicates structural changes of PE by the cyanobacterial species. All the findings related to tensile strength analysis of the degraded polymers are in accordance with the previous reports by Lee *et al.*, (1991); Orhan and Büyükgüngör (2000); Jakubowicz *et al.*, (2011).

After 42days of incubation in BG11 carbon minimal media, erosion, formation of pits and cavities were apparent on the surface of PE. However, in the present study, control PE revealed no erosion.The attachment of cyanobacterial species on the polyethylene surface is regarded as one of main criteria for biodegradation mechanism which were revealed by optical microscopy (Das and Kumar, 2015). The presence of cracks and cavities on the PE surface are considered as break-down the complex polyethylene form into its monomeric forms (Manzur *et al.*, 2004). The deformities on the polyethylene surface was interpreted in terms of enzymatic activities by the cyanobacterial species (Bhatia *et al.*, 2014).

Laccase and manganese peroxidase activities were measured for polyethylene degradation by the cyanobacterial species. Activity of laccase (0.009IU/ml) was found to higher as compared to manganese peroxidase (0.0075IU/ml) after 42days treatment. In biodegradation of polyethylene by cyanobacterial species, there are two key mechanism are believe to be operative, one is the reduction of molecular weight and second oxidation of molecules (Yoon *et al.*, 2012). The break-down of large polyethylene molecules is in fact believed to have been initiated by enzymatic action accompanied by molecular weight

reduction and enhancement of keto-carbonyl index (Santo *et al.,* 2012). Microorganisms are known to produce necessary oxidative and degradative enzymes and assimilate the polymeric carbon into their biomass (Hadad *et al.,* 2005; Tribedi and Sil, 2013). Cyanobacterial enzymes present in the liquid phase of the media interact with the macromolecules available at the surface of the polythene strips triggering biodegradation (Chinaglia *et al.,* 2018). Carbon analysis after 42 days of incubation revealed the percentage of carbon in the control PE to be 84%.The extent of carbon ultilised by the cyanobacterial species from the treated PE was around 4% for the cyanobacterial species. The adhesion mechanism of the cyanobacteria to polyethylene surface may be attributed to the low carbon availability in the medium and confirms its ability to use the polyethylene as a carbon and energy source (Awasthi *et al.,* 2017a).The NMR spectra of PE control have revealed some absorption peak centered at 20ppm in is believed to have originated from common plastic additives like phosphoric acid esters. The absorption peaks at 35.1ppm and 35.7ppm, conspicuously absent in the control, are due to esters and formation of some new -CH_2 group indicating the formation of ethyl propanoate. Multiplet character of the signal at 34ppm in the treated polyethylene can be assigned to carbonyl group of the acid moiety. The enhanced intensity of peak at 34.7ppm in the treated polythene suggested short chain branching (Brandolini and Hills 2000).The degraded polythene provides an organic soluble fraction implying a carbon uptake process linked to the metabolic pathway of the cyanobacteria (Miyazaki *et al.,* 2012; Balasubramanian *et al.,* 2014). Formation of carboxylic acid and other byproduct of polyethylene degradation reduces the molar mass of the polythene and facilitate carbon assimilation (Arnaud *et al.,* 1994).

Based on the research undertaken and analysis of the results, we furnish herein some generalized conclusions:

- A total of 122 algal species were found to colonize on the polythene surface in sewage water and domestic solid waste dumping site. The colonization pattern were found to anticipated that algal communities may use the polythene as a carbon source, and the submerged polythene in sewage water and domestic solid waste dumping site certainly serves as substratum for colonization.
- Factors like phosphate, nitrate, ammonia, temperature of the sewage water play an important role in colonization of algae on the polythene surface. As for the algal colonization on the polythene surface in domestic solid waste the organic carbon, total nitrogen, available phosphorus and potassium of soil were found to have significantrole.
- A total of 31 species of algae were isolated as pure cultures.
- The algal species isolated from submerged polythene surface in domestic sewage water are demonstrated to be a rich source of carbohydrate, proteins, lipids, vitamin C, phycobiliproteins, total phenolic, total flavonoids, carotenoid, and antioxidants.
- Some of the isolated algal species cultivated in untreated municipal sewage water without any additional nutrients afforded remarkable growth and biomass production. The cyanobacterial species, *Oscillatoria subbrevis* and *Nostoc carneum* were found to be capable of sequestering the nutrients from the sewage water. The lipid rich green alga, *Chlorella ellipsoidea* was able to significantly sequester nitrate and phosphate, increase the DO level, and lower the TDS well below the permissible limit. This also demonstrate that the algal species are capable of efficiently remediate sewage water, mitigate carbon dioxide as it grow proficiently in polluted water.
- The green alga, *C. ellipsoidea* was found to produce higher percentage of lipid in sewage water relative to the control medium may be exploited for its feasibility in biofuel generation.
- The antioxidants produced by the algal species in sewage water is anticipated to be of significance in pharmaceutical, food and cosmetic applications.

- Employing sewage water to harvest algae for production of value added chemicals could thus serve as an integrated approach for manifold applications.
- Spectroscopic, enzymatic, thermal, mechanical and morphological studies in relation to dual experimental control clearly demonstrated efficient polyethylene biodegradation by the cyanobacterial species.
- The cyanobacterial species, *Phormidium lucidum, Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum* and *Cylindrospermum muscicola* were found to be efficient in biodegradation of LDPE polyethylene.
- The cyanobacterial species are capable of effectively colonising on the LDPE polythene surface utilising carbon without any pro-oxidant additives or pretreatment.
- The results of FT-IR and NMR spectroscopy corroborated the presence of alcohol and carboxylic acids as the degradation end products of polyethylene.
- The faster growth of the cyanobacterial species on the polyethylene surface is associated with greater weight loss and thinning of PE.
- The amorphous regions of the polyethylene are more easily degraded and that small crystals are likely to be consumed by the cyanobacterial species, but the rate of consumption of the smaller crystals were not investigated.
- It might be of interestto test the polyethylene under natural conditions.These cyanobacterial species have the potential to degrade polythene even more efficiently furnishing a tangible alternative solution to polythene waste management.
- The future investigations will likely to throw more mechanistic insights to the problem of polyethylene biodegradation. Isolation and identification of the enzymes able to oxidize and break polyethylene chains is a primary goal to elucidate the mechanisms of degradation of polyethylene. In the present research, laccase and manganese peroxide enzyme activity were monitored.
- Another important area of future research is the identification of biodegradation pathway involved inside cyanobacterial species.

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APPENDIX I LIST OF PUBLICATIONS

1. Sarmah, P.; Rout, J. (2017). Colonisation of *Oscillatoria* on submerged polythenes in domestic sewage water of Silchar town, Assam (India), ***J. Algal Biomass Utln.***, 8: 135-144

2. Sarmah, P.; Rout, J. (2018a). Biochemical profile of five species of cyanobacteria isolated from polythene surface in domestic sewage water of Silchar town, Assam (India), ***Curr. Trends Biotechnol. Pharm***., 12:7-15

3. Sarmah, P.; Rout, J. (2018b). Phytochemical screening and antioxidant activity of a cyanobacterium, *Oscillatoria limosa* isolated from polythene surface in domestic sewage water, ***J. Algal Biomass Utln***., 9: 48-54

4. Sarmah, P.; Rout, J. (2018c). Algal colonisation on polythene carry bags in a domestic solid waste dumping site of Silchar town in Assam, ***Phykos,*** 48(1): 67-77

5. Sarmah, P.; Rout, J. (2018d). Efficient biodegradation of low density polyethylene by cyanobacteria isolated from submerged polythene surface in domestic sewage water, ***Environ. Sci. Pollut. Res.*** https://doi.org/10.1007/s11356-018-3079-7

Paper presented in Seminar/Conference

1. Presented an oral presentation on "Distribution pattern of cyanobacterial community in domestic sewage water of Silchar town, Assam" in International conference on Harnessing natural resources for sustainable development: global trends, 29-31 January, 2014, Organized by Cotton College.

2. Presented a poster on "Filamentous cyanobacteria from domestic sewage water as a source of phycobiliproteins pigments" in National seminar on Advances in biotechnology research: current trends and future prospects, 25-26 March, 2014, Organized by Assam University.

3. Presented a poster on "Cyanobacterial diversity in soil of domestic solid waste dumping ground in Silchar town, Assam, India" in National seminar on Charting and managing biodiversity: perceptions and priorities: 8th and 9th March, 2016, Organized by Assam University.

4. Presented an oral presentation on " Biochemical screening and antioxidant activity of four cyanobacterial species isolated from domestic sewage water of Silchar town, Assam (India)" in National Seminar on Recent advances and scope in herbal technology: challenges and prospects, 9th-10th September, 2016, Organized by Assam University.

5. Presented a poster on " A study on non-heterocystous cyanobacterial species (*Oscillatoria*) from domestic sewage water of Silchar town" in National seminar on Ethnobiology and traditional knowledge in biodiversity conservation- approaches and dimensions, 2nd & 3rd February, 2017, Organized by Assam University.

6. Presented a poster on "Biodegradation of polythene by a cyanobacterium, *Lyngbya diguetii*" in National conference on Emerging materials, 20-22 March, 2018 Organized by Assam University.

Conference attended

1. International conference on Global ecosystems, biodiversity and Environmental sustainability in the 21st century, 15-17 February, 2012, Organized by Assam University.

2. Workshop on Statistical tools in scientific research, 7th-12th April 2012, Organized by Assam University

3. Grassroot innovators' meet, 14th May, 2013, Organized by Assam University

4. Workshop on Basic statistical using software, 14-18th August, 2014, Organized by Assam University

5. ISI-NIT Silchar spring school on computational biology: tools and techniques, 20-24 March, 2015, Organized by NIT Silchar

6. National workshop at Ecological issues and concern: quantitative applications, 23-24 March, 2015, Organized by Assam University

7. National workshop on Innovating India, 25-26 November, 2013, Organized by NIT College, CSIR-NISTADS, New Delhi, IIM Kashipur

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Biochemical profile of five species of cyanobacteria isolated from polythene surface in domestic sewage water of Silchar town, Assam (India)

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Abstract

Disposal of polythene into waste water poses a serious problem as they get accumulated in the environment. Submerged polythene in waste water offers an ideal substratum for algae to colonize. The present paper highlights the biochemical composition of five cyanobacteria isolated from submerged polythene surface in domestic sewage water, Silchar town, Assam, (India). The carbohydrate, protein, lipid, vitamin C and pigments (Chl*a*, carotenoids, phycobiliproteins) contents of five cyanobacterial species, *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum*, and *Cylindrospermum muscicola* isolated from submerged polythene surface were analysed. Maximum amount of total protein, carbohydrate and lipid content were found in *Oscillatoria subbrevis* and minimum in *Cylindrospermum muscicola*. Vitamin C was found to be highest in *Oscillatoria subbrevis* and *Nostoc carneum* and minimum in *Cylindrospermum muscicola*. The total phycobiliproteins was maximum in *Oscillatoria subbrevis* and minimum in *Cylindrospermum muscicola*.One-way analysis of variance (ANOVA) showed significant differences among the biochemical parameters of cyanobacteria isolated from polythene surface.

Keywords: biochemical; cyanobacteria; domestic sewage water; polythene bags; Assam

Introduction

Cyanobacteria are known to occupy a broad range of habitats across all latitudes and are believed to be the earliest inhabitants of earth. They are not only widespread in freshwater, marine and terrestrial ecosystems but also occur in extreme habitats such as hot springs, hypersaline localities, freezing environments and arid deserts (1). Besides such natural habitats, algae including cyanobacteria are capable of growing on artificial substrates as well. Made from non-renewable fossil fuel, introduced around 1970s(2), plastic carry bags are indiscriminately dumped into landfills worldwide and emit dangerous methane and carbon dioxide gases during their decomposing stages as well as highly toxic leachates (3). It effectively blocks sewerage pipe lines, litters agricultural lands, canals, rivers and oceans. They are not biodegradable or take incredibly long time to break down into powdery plastic dusts which contaminate the soil and the water adversely affecting all life forms (4). Algae are known to colonise on polythene surfaces submerged in waste water(5-6).Study of growth of algal species on such polythene substrata are important in the context of biodegradation of polythene (7). Biodegradation of polyethylene by algae constitute an attractive environment friendly and cost effective viable option(8).Algae and cyanobacteria are rich source of several bioactive compounds such as

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proteins, polyunsaturated fatty acids (PUFAs), sterols,enzymes,vitamins and pigments(9).Their vast potential in varied applications such as food,feed, fuel, fertilizer, medicine,industry and in combating pollution(10-16) have been explored. Given the huge diversity of algae and the phytochemicals they produce, exploring their biochemical contents has remained a favourite pastime of researchers.Accordingly, the present study addresses the biochemical screening of five cyanobacteria isolated from submerged polythene surface in the domestic sewage water of Silchar town in the state of Assam, India.

Material and Method

Study area : The study was carried out in the urban area of Silchar town of Cachar district located in the state of Assam, India (Fig. 1) during the 2014. The study area lies between latitude $24^0$49Ê North and longitude $92^0$48$^{\hat{E}}$ East and altitude of 114.69 meters above sea level on the banks of river Barak. The domestic sewage drains carries waste from household and medium scale industries. A view of the study site showing algae colonized on polythene bags is presented in Fig. 2

Physico-chemical properties of sewage water : The water samples from domestic sewage drains were collected, transferred into pre-cleaned plastic bottles and stored for further analysis. The pH was measured using a digital pH meter. Biological oxygen demand (BOD) and dissolved oxygen (DO) measured by titrimetric method (17). Chemical oxygen demand (COD) was measured by open reflux method. Alkalinity, free CO_2 and magnesium and calcium were measured by titrimetric method (18). Total dissolved solid (TDS) and suspended solid (SS) were measured by gravimetric method. Chloride was measured by argentometric method (19). Sulphate was estimated by turbidimetric method (17). Nitrate was measured by brucine method (20). Soluble reactive phosphate was estimated by molybdate blue method(21). Ammonia was determined by phenol-hypochlorite method (22).

Isolation of cyanobacteria : A total of 20 dumped waste polythene bags colonised by algae were collected from domestic sewage water drains of Silchar town, Assam and brought into laboratory.

Fig. 1. Map of the study area showing the location of study sites

Fig.2. Close view of algae colonizing on submerged polythene bags (2A-2B)

The polythene bags were cut into 1cm^2 size pieces with a sterilized blade. The algae samples from polythene surfaces were scrubbed with a sterilized brush and observed under microscope. The method used for isolation and purification of cyanobacteria was according to Rippka *et al*. (23). The algal samples were homogenized in sterile water with glass beads, centrifuged at 3000 rpm for 10 minutes with repeated washing.The pellets were suspended in sterilized BG-11 medium and placed onto the agar petri plates by pour plate method. The plates were incubated for 15 days under continuous illumination (2000lux) at 24±1^{0}C. The pure colonies developed in the agar plates were picked upandsub-cultured in 500ml Erlenmeyer flasks. The cultures were observed under microscope and the isolated cyanobacterial species were identified using standard keys (24-25). Photomicrographs of five cyanobacterial species were presented in Fig.3.The BG-11 medium without combined nitrogen source was used for the isolation and maintenance of *Nostoc*and *Cylindrospermum.*

Biochemical analysis : The total carbohydrate was determined according to anthrone method (26). Total protein was estimated by modified method of Herbert*et al*.(27).The chl*a* and carotenoids were estimated by the standard methods of Strickland and Parsons (28) and Parson (29), respectively. Phycobiliproteins estimation has been carried out as per Bennet and Bogorad (30). Lipid content was estimated by the standard method of Bligh and Dyer (31). Vitamin C content was evaluated using the method of Roe and Keuther (32). Growth rate was measured in terms of chl *a* as biomass component(33). Growth kinetics in terms of specific growth rate (K) and generation time(G) were evaluated (34).

Statistical analysis : The statistical analyses were performed using the software Statistical Package for Social Sciences (SPSS Version 21.0). One-way analysis of variance (ANOVA) was used to

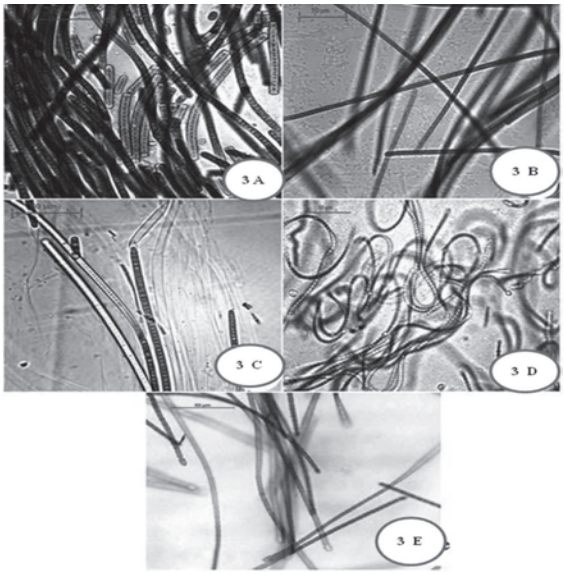

Fig. 3. Photomicrographs of five cyanobacteria isolates
3 A- Phormidium lucidum, 3 B- Oscillatoria subbrevis,
3 C- Lyngbya diguetii, 3D- Nostoc carneum,
3 E- Cylindrospermum muscicola

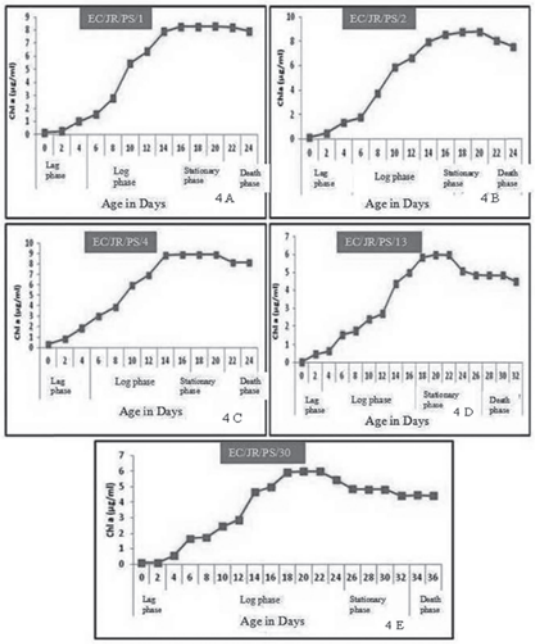

Fig. 4. Growth curve of isolated cyanobacteria (4 A-Phormidium lucidum, 4B- Oscillatoria subbrevis , 3 4C- Lyngbya diguetii, 4D- Nostoc carneum, 4 E-Cylindrospermum muscicola)

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evaluate the differences among the biochemical parameters.The triplicate sets of data were evaluated inaccordance with the experimental design (Completely Randomized Design) with ANOVA (Analysis of Variance). The comparisons between the different means were made usingpost hoc least significant differences (LSD) calculated at p level of 0.05 (5%), and representedas CD (Critical Differences) values in Table 3 and 4 with standard deviations.

Results

The physico chemical propertites of domestic sewage water are presented in Table 1. The colour of domestic sewage water was black to yellowish grey. The temperature ranged from 28 to 34°C. The site 2 recorded maximum water temperature while site 4 recorded the minimum. The pH values of the different sites were quite at variance with each other. The domestic sewage water of site 2 was slightly acidic while that for site 4 was found to be alkaline.The value of BOD varied from 383 to 600 mg/L with site 3 registering maximum value. The COD values were in the range of 1511 to 2189 mg/L with maximum being at site 5. The DO concentrations varied from 1.3 to 2.4mg/Lwith maximum being at site 4 and minimum at site 1. Alkanity of domestic sewage water varied from 9 to 11mg/L. Free CO_2 was in the range 38-42mg/L with maximum at site 2. Nitrate ranged from 12 to 65 mg/L with maximum being at site 4. Magnesium ranged from 25 to 178mg/L, maximum being at site 4 and minimum at site 2. The TDS of sewage water were within the range of 500 to 3210mg/L, it was maximum being at site 2 .The SS of sewage water was in the range of 50 to 200mg/L and being it was maximum at site 2 and minimum at site 3. The chloride concentration were in the range of 35 to 78mg/L, being maximum at site 2 .The calcium content of sewage water was found in the range 54 - 69mg/L, , being maximum at site 4. The sulphate of domestic sewage varied from a minimum of 50mg/L at site 3 to maximum of 897mg/L at site 4.The minimum ammonia value was found to be 28mg/L at site 1 andmaximum at34mg/L at site 4.The phosphate of sewage water varied from a minimum of 58mg/L at site 5 to a maximum of 72mg/L at site 4.

The growth curves of five cyanobacterial species were presented in Fig.4. The maximum growth rate (Table 2)has been shown by *Oscillatoria subbrevis*(0.158μd^{-1})followed by *Nostoc carneum* (0.152μd^{-1}). The growth rate was lowest in *Phormidium lucidum* (0.134μd^{-1}).The generation time was maximum in *Phormidium lucidum* (178.25h) and minimum in *Oscillatoria subbrevis* (151.34h).

The biochemical analysis of five species of cyanobacteria (Table 3) revealed the carbohydrate to be in the range109-370 μgml^{-1}**.**The maximum carbohydrate present in *Oscillatoria subbrevis* (370 μgml^{-1}**)** and minimum in *Cylindrospermum muscicola* (109 μgml^{-1}**)**.The carbohydrate present in *Phormidium lucidum*, *Lyngbya diguetii*, *Nostoc carneum* were 240 μgml^{-1}**,** 230 μgml^{-1} and 113 μgml^{-1}, respectively**.** The protein range was145-230 μgml^{-1}.The maximum protein content was found in *Oscillatoria subbrevis* (230 μgml^{-1}**)** and minimum in *Cylindrospermum muscicola* (145 μgml^{-1}**)**. The protein present in *Phormidium lucidum*, *Lyngbya diguetii*, *Nostoc carneum* were 210 μgml^{-1}, 203 μgml^{-1} and 195 μgml^{-1}. The range of vitaminC was 0.3-0.9 μgml^{-1}. The maximum vitamin C content was observed in *Oscillatoria subbrevis*and *Nostoc carneum*(4.2μgml^{-1}) , minimum in *Cylindrospermum muscicola* (1.2 μgml^{-1}). The vitaminC present in *Phormidium lucidum*, *Lyngbya diguetii* were 0.5 μgml^{-1} and 0.8 μgml^{-1},respectively.The range of lipid content in cyanobacterial isolates was 4.2-11.2μgml^{-1}. The maximum lipidcontent was noted in *Oscillatoria subbrevis* (11.2 μgml^{-1}) and minimum in *Cylindrospermum muscicola* (4.2 μgml^{-1}**)**. The lipid content present in *Phormidium lucidum*, *Lyngbya diguetii*, *Nostoc carneum* were 8.7 μgml^{-1},7.3 μgml^{-1} and 5.1 μgml^{-1} ,respectively.

Phycocyanin (PC) content (Table 4) was maximum in *Lyngbya diguetii* (17.5 μgml^{-1}) and minimum in *Nostoc carneum* (12.1 μgml^{-1}). Phycoerythrin (PE) content was maximum in *Oscillatoria subbrevis* (48.7 μgml^{-1}**)** and minimum

in *Cylindrospermum muscicola* (15.34 μgml^{-1}). Allophycocyanin (APC) was maximum in *Lyngbya diguetii* (25 μgml^{-1}**)** and minimum in *Cylindrospermum muscicola* (15.34 μgml^{-1}**)**. Total phycobiliproteins content was maximum in *Oscillatoria subbrevis* (81.4 μgml^{-1}) and minimum in *Cylindrospermum muscicola* (45.98 μgml^{-1}**)**.

One way ANOVA revealed significant differences among the biochemical parameters, chl*a* ($p = 0.02$), carotenoids ($p = 0.01$), protein ($p = 0.02$), carbohydrates ($p = 0.04$), vitaminC ($p = 0.02$), lipids ($p = 0.04$). Significant variationin phycobiliproteins concentrations, PE ($p = 0.02$), PC ($p = 0.01$) and APC ($p = 0.04$) were observed.It is noteworthy that total phycobili proteins in *Oscillatoria subbrevis* contains almost double the amount of *Cylindrospermum muscicola* **.**

Discussion

The biochemical constituents of cyanobacteria isolated from polythene surface submerged in domestic sewage water showed that *Oscillatoria subbrevis*, *Phormidium lucidum*, *Lyngbya diguetii*, *Nostoc carneum* and *Cylindrospermum muscicola* contain high cellular constituents of chl*a*, carotenoids, protein, carbohydrate, vitamin C, lipid and phycobili proteins. Significant differences were observed in biochemical constituents species wise. In the present study, *Oscillatoria subbrevis* was found to thrive in an alkaline condition (pH = 8.1, site 4) while *Phormidium lucidum* was collected from an acidic (pH = 5.8, site 2) sewage water. The species *Lyngbya diguetii isolated* was found to grow under slightly acidic sewage water (pH = 6.3, site 3) and *Nostoc carneum* and *Cylindrospermum muscicola* was collected from slightly alkaline condition (pH = 7.3, site 1) and moderately acidic condition (pH = 6.4**,** site 5)**,** respectively.The characteristic morphological and physiological attributes of the species might be ascribed to typical physico-chemical properties of domestic sewage water. It has been reported that the cellular composition of cyanobacteria depend on the nature of strains, physiological state of the isolates and the nutrient conditions of environment from where they have collected (35-38).

The dissolved oxygen of domestic sewage water varied from 1.3-2.4mg/L. The growth of algae was found to be directly proportional to the available nutrients and oxygen level of water which in turnmight alter the oxygen level of sewage water.The cyanobacteria in the present study has been found to adapt well to the oxygen depleted condition of the sewage water and it was presumed that in the absence of additional nitrogen source in domestic sewage water, the flow of carbon fixed in photosynthesis is switched from the path of protein synthesis to that leading to higher production of biochemical constituents of microalgae (39).The extent of dissolved solid and suspended solid of domestic sewage water was quite different in all the five sites. Therefore, the sunlight penetration on submerged polythenes is anticipated to be different.Grossman *et al*.(40) opined that environmental condition of species might alter the composition and abundance of phycobiliproteins. In the present study, the physico chemical parameters were at variance in all the five sites. This, we believe, might have caused a variation in the total phycobiliproteins in the species studied.

In a previous study, the algal species *Phormidium angustissimum*, *Lyngbya holdenii*, *Anabaena doliolum*, *Calothrix marchica* and *Fischerella muscicola* isolated from lime sludge waste of a paper mill in the district showed higher accumulation of chl *a*, phycocyanin, carbohydrates and protein (41). Lime sludge waste is rich in organic carbon and thus contributes to the nutrition of cyanobacterial growth. Lipids content was recorded highest in *Oscillatoria subbrevis* and lowest in *Cylindrospermum muscicola*.In the present study, carbohydrate and protein are found to be highest in *Oscillatoria subbrevis* and lowest in *Cylindrospermum muscicola*, respectively. This is in conformity with the observation made by Zhu *et al.* (42) wherein it has been shown that proteins are present as large fraction of biomass in growing algae.

Table 1: Physico-chemical properties of domestic sewage drain water

Water Parameters	Site 1 (Link road)	Site 2 (Sonai road)	Site 3 (NH road)	Site 4 (Premtola)	Site 5 (Ambikapatty)
Colour and odour	*Black, present*	*Black, present*	*Black, present*	Yellowish grey, present	Yellowish grey, *present*
Temperature	32°C	34°C	29°C	28°C	35°C
pH	7.3±0.23	5.8±0.10	6.3±0.13	8.1±0.23	6.4±0.21
BOD (mg/l)	586.3±0.45	483±0.14	600±0.18	383±1.2	509±2.3
COD (mg/l)	1511±0.67	1520±0.56	1520±0.18	1764±0.24	2189±0.78
DO (mg/l)	1.3±0.12	2.3±0.15	2.2±0.23	2.4±0.21	2.2±0.12
Alkalinity(mg/l)	9±0.34	9.8±0.12	10±1.4	11±0.12	9.8±0.23
Free CO_2 (mg/l)	38±0.13	42±0.13	36.98±0.13	39±0.21	36±1.2
TDS (mg/l)	500±1.2	3210±1.4	500±0.14	1546±2.4	578±2.5
Suspended solids (mg/l)	51±0.56	200±0.13	50±0.35	53±0.13	58±0.23
Chlorides(mg/l)	62±0.21	78±0.34	60±0.06	73±0.21	35±0.12
SO_4^{-2} (mg/l)	880±1.3	876±1.4	50±1.6	897±3.2	783±0.23
Nitrate (mg/l)	43±0.13	44±0.12	12±1.5	65±0.13	46±1.2
Mg (mg/l)	32±0.24	25±0.67	30±1.1	178±1.3	176±2.1
Ammonia (mg/l)	28±0.12	32±0.34	30±1.2	34±0.12	32±0.23
Appearance	Not clear	Not clear	Not clear	Not clear	Not clear

Table 2: Specific growth rate (K) and generation time (G) of the isolates

Sl. No	Cyanobacterial isolates	K (μd^{-1})	G (h)
1	*Phormidium lucidum*	0.134	**178.25**
2	*Oscillatoria subbrevis*	**0.158**	151.34
3	*Lyngbya diguetii*	0.138	173.67
4	*Nostoc carneum*	0.152	157.21
5	*Cylindrospermum muscicola*	0.136	153.89

Vitamin C, a wide spectrum antioxidant not synthesized in the body is obtained from dietary sources (43). In the present study, vitamin C contents was found to be highest in *Oscillatoria subbrevis*, *Nostoc carneum* and lowest in *Cylindrospermum muscicola*. Algae with brighter thalli were reported to be rich in vitamin C (44). In the present study, *Oscillatoria subbrevis* and *Cylindrospermum muscicola* were found to colonize with brighter blue-green and olive green colour thalli on polythene bags. *Oscillatoria subbrevis* formed bright blue-green loop like thallus floating over the liquid medium and *Cylindrospermum muscicola* formed olive green finger like projection on the petri plate surface in laboratory culture.

Conclusion

Five species of cyanobacteria isolated from submerged polythene surface in domestic sewage water are demonstrated to be a rich source of carbohydrate, proteins, lipids, vitamin C and phycobiliproteins.The results are anticipated to be of relevance to biodegradation of polythenes, aquaculture, pharmaceutical

Table 3: Biochemical composition of five cyanobacterial isolates from submerged polythene bags in domestic sewage water

Biochemical parameters	Cyanobacterial isolates					
	Phormidium lucidum	*Oscillatoria subbrevis*	*Lyngbya diguetii*	*Nostoc carneum*	*Cylindros permum muscicola*	CD (0.05%)
Chl*a* (µgml^{-1}	8.47±0.12	7.32±0.23	6.92±0.34	3.02±0.21	4.23±0.42	0.02
Carotenoid (µgml^{-1})	2.10±0.02	2.89±0.01	1.89±0.03	0.89±0.01	1.02±0.02	0.01
Protein(µgml^{-1})	210±o.54	230±0.51	203±0.32	195±0.43	145±0.34	0.02
Carbohydrate (µgml^{-1})	240±0.73	370±1.02	230±0.45	113±0.56	109±0.67	0.04
Vitamin C (µgml^{-1})	0.5±0.01	0.9±0.03	0.8±0.01	0.9±0.01	0.3±0.01	0.02
Lipid (µgml^{-1})	8.7±0.02	11.2±0.21	7.3±0.03	5.1±0.12	4.2±0.12	0.04

Table 4: Phycobiliproteins of five cyanobacterial isolates from submerged polythene bags in domestic sewage water

Phycobiliproteins	Cyanobacterial isolates					
	Phormidium lucidum	*Oscillatoria subbrevis*	*Lyngbya diguetii*	*Nostoc carneum*	*Cylindros permum muscicola*	CD (0.05%)
PE(µgml^{-1})	14.2±0.12	13.1±0.21	17.5±0.12	12.1±0.21	15.3±0.34	0.02
PC(µgml^{-1})	42.4±0.02	48.7±0.23	36±0.23	45±0.04	15.34±0.15	0.01
APC(µgml^{-1})	18±0.01	19.6±0.12	25±0.05	21±0.24	15.34±0.02	0.04
Total Phycobiliproteins (µgml^{-1})	74.6±0.15	81.4±0.56	78.5±0.4	78.1±0.49	45.98±0.51	0.05

applications and biofuel.Due to rich biochemical contents these cyanobacteria may have the potential for use in the food industry as high value nutritional products.

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Colonisation of *Oscillatoria* on submerged polythenes in domestic sewage water of Silchar town, Assam (India)

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Abstract

Disposal of polythene to waste water is a environmental concern. Submerged polythene plays an ideal substratum for colonising of algae. The present paper hightlights the colonisation of *Oscillatoria* on submerged polythene in domestic sewage water, Silchar town, Assam (India). A total of 20 species of *Oscillatoria* were found to be distributed on submerged polythene. *Oscillatoria princeps* , *O. subbrevis* , *O. limosa* , *O. amoena* ,*O. vizagapatensis* , *O. okeni* , *O. limosa and O. laete-virens* are the most common species encountered on the submerged polythene bags. The quality of domestic sewage water were investigated and correlation was made with total *Oscillatoria* species. Water temperature, pH, BOD, nitrate, calcium, sulphate and free CO_2 showed positive correlation with total *Oscillatoria* species.

Key words*:* domestic sewage water, *Oscillatoria*, polythene bags , Silchar, water quality
Running title: *Oscillatoria* on polythene bags

Introduction

Plastics usage across the world has increased enormously in recent times. A substantial current production involves items of packaging which are rapidly disposed. While plastics bring many societal benefits offering future technological advances, the concerns about usage and their disposal are diverse are fraught with many challenges and opportunities. The accumulation of plastic waste in landfills and in natural habitats leads to ingestion, leaching of chemicals to the environment (Richard *et al.*, 2009). Domestic sewage water mostly coming from bathrooms, kitchen and laundry sources and small scale industries consists of nitrate and phosphates in addition to moderately small concentrations of suspended and dissolved organic and inorganic solids. The polythene carry bags after use are generally thrown into landfills or drains which eventually head to other minor water bodies. Algae are known to colonise on such polythenes submerged in waste water (Suseela and Toppo, 2007 and Sharma *et al.*, 2014). Studies of growth of algal species on such polythene substrata are important in the context of sustainable plastic bag waste management. Biodegradation of polyethylenes by algae constitute an attractive environment friendly and cost effective option (Kumar *et al.*, 2017, Michaud *et al.*, 2007). Accordingly, the present work has been carried to study the algal colonisation pattern on low density polyethylene (LDPE) surface with emphasis on a filamentous non-heterocystous blue-green *Oscillatoria* species in the domestic sewage water sites of Silchar town in the state of Assam. Correlation study has been performed to ascertain the influence of sewage water parameters on *Oscillatoria* diversity.

Material and methods

Study area

The study was carried out in the urban area of Silchar town of Cachar district located in the state of Assam, India (Fig. 1). The study area lies between latitude $24^0 49'$ North and longitude $92^0 48'$ East and altitude of 114.69 meters above sea level on the banks of river Barak. The domestic sewage drains carries waste from household and medium scale industries. The overview of study sites of algae colonized on polythene bags have been shown in plate 1.

A total of 45 samples were collected from five different study sites during July-Dec., 2013. The water samples were collected in the morning and transferred into pre-cleaned polythene bottles and stored for further analysis. The pH and dissolved oxygen (DO) were measured in the field immediately after sampling and other parameters were determined in the laboratory following standard analytical procedure as recommended by APHA (2005). Algal colonised polythene bags were collected from domestic sewage water drains of Silchar town, observed under

microscope and identified using standard keys (Prescott, 1951; Desikachary, 1959) and preserved in 4.5% formalin for further study. The algal samples were finally counted following Lackey's drop method (Trivedy and Goel, 1986).

The surface colonisation of algae on polythene bags collected from fields were viewed at 80x magnification using an optical reflection microscope StereoZoom Leica S8 APO equipped with a polarized and coupled to a computer.

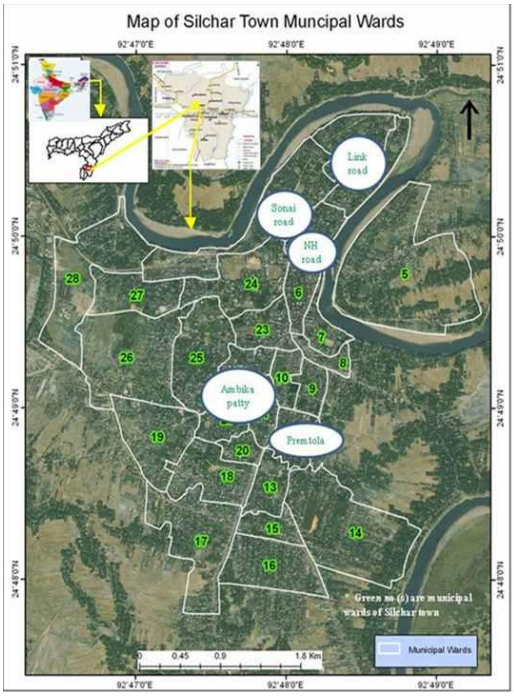

Fig 1. Map of the study area showing the locations of study sites.

Plate 1 The close view of algae colonizing on submerged polythene bags (a – d)

Results

The physico chemical propertites of domestic sewage water are shown in Table 1. The colour of domestic sewage water was black to yellowish grey. The temperature of domestic sewage water ranged between 28-34 °C. The site 2 recorded maximum water temperature while site 4 recorded minimum water temperature. The pH values of the different sites were quite at variance with each other. The domestic sewage water of site 2 was slightly acidic while that for site 4 was found to be alkaline. Biological oxygen demand (BOD) ranged between 383-600 mg/L. Maximum BOD has been observed for site 3 while site 4 recorded the minimum. Chemical oxygen demand (COD) ranged between 1511-2189 mg/L. Site 5 recorded highest COD, while site 1 recorded lowest COD. Dissolved oxygen ranged between 1.3-2.4 mg/L. Site 1 recorded lowest DO while site 4 showed highest DO. Alkanity of domestic sewage water ranged between 9-11 mg/L, site 1 recorded minimum value and site 4 recorded maximum value. Free CO_2 ranged between 38-42 mg/L. Site 1 recorded minimum value, site 2 recorded maximum value. Nitrate ranged between 12-65 mg/L. Magnesium ranged between 25-178 mg/L with site 4 recording highest value and site 3 lowest. The value of total dissolved solid (TDS) ranged between 500-3210 mg/L. Site 2 recorded highest value, site 1 recorded lowest value. The value of suspended solid (SS) ranged between 500-3210 mg/L. Site 2 recorded highest value, site 1 recorded lowest value. Chlorides ranged between 35-73 mg/L. Site 5 recorded highest value, site 4 recorded lowest value. Calcium ranged between 54-69 mg/L. Site 4 recorded highest value, site 1 recorded lowest value. Sulphate ranged between 50-897 mg/L. Site 4 recorded highest value, site 3 recorded lowest value. Ammonia ranged between 28-34 mg/L. Site 4 recorded highest value, site 1 recorded lowest value. The value of phosphate ranged between 58-72 mg/L. Site 5 recorded highest value while site 4 recorded lowest value.

Table 1: Physico-chemical properties of domestic sewage drain water

Water Parameters	Site1(Link Road)	Site2(Sonai Road)	Site3(National Highway road)(NH road)	Site4(Premtola)	Site5(Ambikapatty)
Colour and odour	Black, present	Black, present	Black, present	Yellowish grey,present	Yellowish grey,present
Temperature	32°C	34°C	29°C	28°C	35°C
pH	7.3±0.23	5.8±0.10	6.3±0.13	8.1±0.23	6.4±0.21
BOD (mg/l)	586.3±0.45	483±0.14	600±0.18	383±1.2	509±2.3
COD (mg/l)	1511±0.67	1520±0.56	1520±0.18	1764±0.24	2189±0.78
DO (mg/l)	1.3±0.12	2.3±0.15	2.2±0.23	2.4±0.21	2.2±0.12
Alkanity(mg/l)	9±0.34	9.8±0.12	10±1.4	11±0.12	9.8±0.23
Free CO_2 (mg/l)	38±0.13	42±0.13	36.98±0.13	39±0.21	36±1.2
TDS (mg/l)	500±1.2	3210±1.4	500±0.14	1546±2.4	578±2.5
Suspended solids (mg/l)	51±0.56	200±0.13	50±0.35	53±0.13	58±0.23
Chlorides(mg/l)	62±0.21	78±0.34	60±0.06	73±0.21	35±0.12
Ca (mg/l	54±0.13	65±0.23	60±0.13	69±0.23	63±0.13
SO_4^{-2} (mg/l)	880±1.3	876±1.4	50±1.6	897±3.2	783±0.23
Nitrate (mg/l)	43±0.13	44±0.12	12±1.5	65±0.13	46±1.2
Mg (mg/l)	32±0.24	25±0.67	30±1.1	178±1.3	176±2.1
Ammonia (mg/l)	28±0.12	32±0.34	30±1.2	34±0.12	32±0.23
Phosphate(mg/l)	68±0.23	70±0.12	70±0.12	72±0.24	58±0.23
Appearance	Not clear	Not clear	Not clear	Not clear	Not clear

On the submerged polythene surfaces, *Oscillatoria,* a member of a filamentous non-heterocystous blue green alga was found to be a dominant species. A total of twenty species of *Oscillatoria* were found to colonize on the surfaces of polythene bags. Plate 2 describes the clear colonization pattern of *Oscillatoria* as observed under the microscope**.** Attachment of *Oscillatoria princeps* was clearly seen on the polythene surfaces with a thallus spread over (Plate 2a) . The *Oscillatoria subbrevis* filamentous net like forms with a mixed consortia with other algae is also prominent on the polythene surfaces (Plate2b). A massive growth of *Oscillatoria limosa* along with *Lyngbya* species is observed and an initiation of the polythene degradation is under progress (Plate 2c). Photomicrographs of twenty species of *Oscillatoria* have been presented in Plate 3 & Plate 4. During our field observation, it has been noted that the *Oscillatoria* mat comes up to the surface of the sewage water from the polythene surfaces. It has been observed that growth of *Oscillatoria* species and formation of mats was more prominent during bright sunshine. A giant form of *Oscillatoria* belonging to *O.peronata* was found to contribute to the waste water system as well as the polythene surface colonization. The *Oscillatoria princeps* present in all the study sites formed dark blue green mats on the polythene surface. During bright sunshine period, a brownish mats has also been obserevd. The *Oscillatoria tenuis* when present was found to occur as a solitary member on the polythene surface. The *Oscillatoria geitleriana* has been found in combination with *Oscillatoria earlei.* Among the other common species encountered on submerged polythene bags were *Oscillatoria subbrevis*, *O.princeps*, *O. limosa, O. amoena*, *O. vizagapatensis*, *O. okeni, O.limosa and O. laetevirens*. The characteristic features alongwith the thallus morphology of twenty *Oscillatoria* species have been described below.

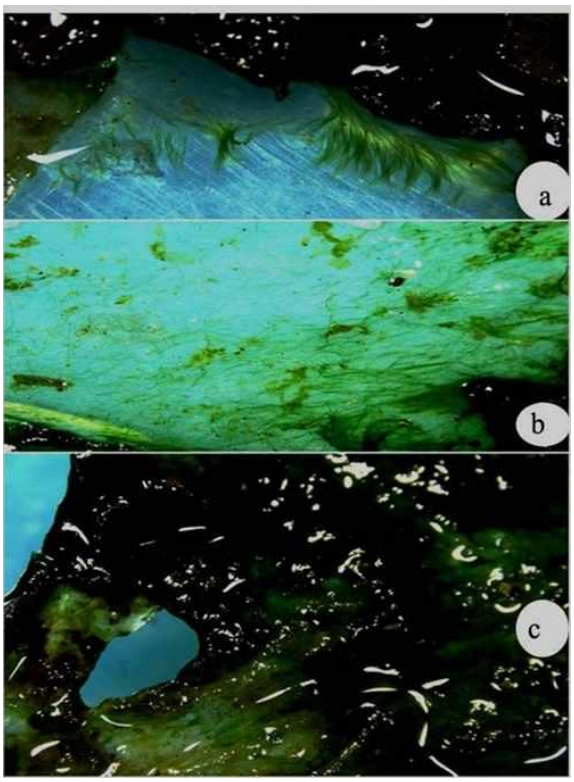

Plate 2. Micrography (OM) of some selected polythene bags showing colonization of algae found in domestic sewage

Plate 3 Photomicrographs of some of the *Oscillatoria* species during study period

1-*Oscillatoria curviceps* Ag.(after Gomont),2- *O.limosa* Ag.(after Gomont),3- *O.princeps* Vaucher (orig.),4-*O.subbrevis* Schmidle (orig.), 5- *O.amoena* Gom.(after Skuja) ,6–*O.chalybea* Martens (after Gomont),7- *O.peronata* Skuja (after Skuja),8 *O.tenuis*Ag.(afterGomont),,9-*O.willei* Gardner em.Drouet (after Gardner),10- *O.rubescens* DC (after Gomont),11- *O.vizagapatensis* Rao (after Rao,C.B)

Plate .4 Photomicrographs of some of the *Oscillatoria* species during study period

12- *Oscillatoria geitleriana* (Frémy) Elenkin (after Frémy),13- *O. formosa* Bory (after Frémy),14- *O. splendida* Grev. (after Gomont),15- *O. okeni Ag.(after Gomont)*,16-*O. limnetica* Lemm.(Orig.),17- *O. earlei* Gardner (after Gardner),18- *O. salina* biswas f.major f.n. (orig.).,19- *O. laetevirens* v.*minimus* Biswas (after Biswas),20- *O. acuminata* Gom.(after Frémy)

1. ***Oscillatoria subbrevis* Schmidle (orig.), Desikachary, 1959, Plate 40, Fig.1**
   Thallus yellow grey to green- yellowish; trichome single, straight, slightly tapering at the end; cells 8 µm x 2 µm; apical cell rounded, without calyptra.

Locality : Link road,Sonai road and Premtola
Collection No. and Date :EC/JR/PS/CY2 and 12/07/2013

2. ***Oscillatoria limosa* Ag.(after Gomont), Desikachary, 1959, Plate 38, Fig.1**
Thallus expanded bright blue-green to brown; trichome, straight or rarely curved; cells 12 µm x 2 µm, cross wall frequently granulated; apical cell flat or obtuse rounded with thickened wall.
Locality : Link Road,Sonai Road,and National highway Road
Collection No. and Date: EC/JR/PS/CY11 and 23/08/2013
3. ***Oscillatoria princeps* Vaucher (after Frémy), Desikachary, 1959, Plate 37, Fig.13,14**
Thallus expanded, attached, forming mats blue green, brownish; trichome mostly straight, slightly attenuated at the apices, occasionally with thin sheaths; cells 10µm x 2 µm; apical cell flatly rounded, slightly capitate, truncate, fragmented part containing two horns like structure
Locality : Link Road,Sonai Road,and National highway Road and Premtola
Collection No. and Date: EC/JR/PS/CY13 and 24/08/2013
4. ***Oscillatoria okeni* Ag.(after Gomont), Desikachary, 1959, Plate 38, Fig.17**
Thallus dark blue green, trichome 5.5-9 µm in diameter straight,distinctly constricted at joints, apical cell somewhat pointed, not capitate, Calyptra none, cell 4µm×2µm in length, apical cell somewhat quadrate up to 8 µm in length, cell content finely granular.
Locality : Link Road,Sonai Road,and National highway Road and Premtola
Collection No. and Date: EC/JR/PS/CY17 and 13/09/2013
5. ***Oscillatoria acuminata* Gom.(after Frémy), Desikachary, 1959, Plate 40 , Fig.13**
Thallus blue-green; trichome more or less straight, not constricted at cross-walls, 4-5µ broad, at the ends briefly tapering, sharply pointed, bent; cells longer than broad, 6-8µ long, sometimes granulated at the cross-walls.
Locality : Link Road,Sonai Road,and National highway Road
Collection No. and Date: EC/JR/PS/CY19 and 23/09/2013

6. ***Oscillatoria willei* Gardner em.Drouet (after Gomont), Desikachary, 1959, Plate 38, Fig.4, 5**
Trichomes pale blue green, bent at the ends or screw like, cells 7µm×3µm in length constricted at the cross walls, ends not attenuated, not capitate at the cross walls, end cells rounded without a thickened membrane.
Locality : Link Road and Sonai Road
Collection No. and Date: EC/JR/PS/CY18 and 14/10/2013

7. ***Oscillatoria splendida* Grev. (after Gomont), Desikachary, 1959, Plate 38, Fig.10**
Trichomes solitary and scattered,rarely aggregated in small, flake-like masses; straight or curved ,tapering for a long distance to a fine hair at the apex.Apical cell conical and capitate.Cells 2.2-2.8µ in diameter,7.2-9µ long,not constricted at the cross walls; cell contents finely granular or homogeneous,pale blue-green.
Locality : National highway Road and Ambikapatty
Collection No. and Date: EC/JR/PS/CY20 and 11/11/2013
8. ***Oscillatoria tenuis* Ag.(after Gomont), Desikachary, 1959, Plate 42, Fig.15**
Thallus forming flat mats, blue green; trichome straight or slightly curved; cells 6 µm x 3 µm; apical cell rounded with slightly thickened wall.
Locality : Link Road,Sonai Road,and National highway Road
Collection No. and Date: EC/JR/PS/CY22 and 12/11/2013
9. ***Oscillatoria limnetica* Lemm.(Orig.), Desikachary, 1959, Plate 37, Fig.3**
Thallus blue green, trichomes straight or slightly bent, constricted at the cross walls, pale blue green, 1-2 µm broad, filaments not attenuated, not capitate, cells 1.5 µm broad, 3 µm long, usually 2 1/2 -6 times as long as broad,
Locality : National highway Road and Premtola
Collection No. and Date: EC/JR/PS/CY24 and 13/11/2013
10. ***Oscillatoria peronata* f.attenuata Skuja (after Skuja), Desikachary, 1959, Plate 41, Fig.9**
Trichome, pale blue- green, erect or flexuous, apices briefly attenuated or curved; cells 10 µm x 3 µm, distinctly constricted and granulated at the cross wall; apical cell hemispherical without calyptra.

Locality : Premtola
Collection No. and Date: EC/JR/PS/CY7 and 22/11/2013

11. ***Oscillatoria geitleriana*** **(Frémy) Elenkin (after Frémy), Desikachary, 1959, Plate 40, Fig.9**
Trichome single, long flexible, blue-green, 4.1 µ broad not constricted at the cross-walls, ends erect, not attenuated, sub capitates, cells 1½ times longer than broad, 4-5 µ long septa not granulated; end cells with a convex distinctly thick membrane
Locality : Sonai Road
Collection No. and Date: EC/JR/PS/CY9 and 23/11/2013
12. ***Oscillatoria chalybea*** **Marrens (after Gomont), Desikachary, 1959, Plate 38, Fig.3**
Thallus dark blue green, trichome nearly straight, constricted at cross walls, attenuated at the apex end bent, living trichomes show forward and rotatory movement, 3 µm broad cells, 1/2-1/3 times as long as broad or nearly quadrate (5 µm broad), 2 µm long, septa not granulated, end cells obtuse not capitates, without calyptra, gas vacuoles present.
Locality : Link Road,Sonai Road
Collection No. and Date: EC/JR/PS/CY11 and 25/11/2013
13. ***Oscillatoria laetevirens*** **v.minimus Biswas (after Biswas), Desikachary, 1959, Plate 39, Fig.2,3**
Thallus thin,membranous,green;trichome yellowish green,straight,fragile,slightly constricted at the cross-walls,3µ broad,apices attenuated undulate or bent,cells nearly as long as broad,2 µm long,sometimes granualated at the cross-walls;end-cells not capitate,more or less obtuse or conical,without calyptra.
Locality : Link Road
Collection No. and Date: : EC/JR/PS/CY32 and 26/11/2013
14. ***Oscillatoria salina*** **biswas f.major f.n. (orig.)., Desikachary, 1959, Plate 37, Fig.16,17**
Plant mass were forming a deep blue-green thin membrane extending over muddy soil and after separation floating on the water surface. Filaments are straight with single, trichome 5-3µm broad, cells shorter than broad, 2µ long, transverse septa indistinct cell content homogenous blue-green.
Locality : Sonai Road
Collection No. and Date: EC/JR/PS/CY25 and 30/11/2013
15. ***Oscillatoria rubescens*** **f.forma (orig.), Desikachary, 1959, Plate 37, Fig.9,18**
Trichome are nearly straight, at the ends gradually attenuated, 8µm broad, not constricted at the cross-walls, cells ½-1/3 as long as broad, 2 µm long, often granulated at the septa, with gas-vacuoles; end cell capitate, with convex calyptra
Locality : Sonai Road
Collection No. and Date: EC/JR/PS/CY22 and 02/12/2013
16. ***Oscillatoria vizagapatensis*** **Rao (after Rao,C.B.), Desikachary, 1959, Plate 39, Fig.16,18**
Thallus blue green; trichome straight or slightly bent, uniformly broad except at the extreme apex; cells 9µm x 2 µm; apical cell broadly rounded, forming a cap with slightly thickened outer wall.
Locality : Ambikapatty and Sonai road
Collection No. and Date: EC/JR/PS/CY26 and 04/12/2013
17. ***Oscillatoria earlei*** **Gardner (after Gardner), Desikachary, 1959, Plate 38, Fig.15**
Trichomes short,straight,bent at the ends,tip attenuated 3 µm broad,not constricted at the cross walls;cells quadrate,6 µm long;ends prominently pointed.
Locality : Link road
Collection No. and Date: EC/JR/PS/CY28 and 09/12/2013
18. ***Oscillatoria curviceps*** **Ag.(after Gomont), Desikachary, 1959, Plate 38, Fig.2**
Thallus bright blue green or blackish mats; trichome, straight, long, hooked or loosely spirally coiled at the end; cells 10 µm x 3 µm; apical cell rounded with thickened wall.
Locality : Sonai road
Collection No. and Date: EC/JR/PS/CY30 and 10/12/2013
19. ***Oscillatoria formosa*** **Bory (after Frémy), Desikachary, 1959, Plate 40, Fig.15**
Thallus dark blue green, trichomes 4µm in diameters, straight to flexuous, usually slightly constricted at the cross walls, apex of the trichome slightly tapering and bent end cells blunt conical, nearly obtuse, not capitate without calyptra, cells nearly quadrate up to 1/2 is long as broad, 2µm long septa some time granulated, cell content bright blue green.
Locality : Ambikapatty and National Highway road

Collection No. and Date: EC/JR/PS/CY33 and 12/12/2013

20. *Oscillatoria amoena* Gom.(after Skuja), Desikachary, 1959, Plate 41, Fig.1-4

Thallus green mass or scattered among other algae. Trichome long, straight, distinctly attenuated and slightly bend, single, 6 µm dia. Terminal cell conical, elongated with thick wall and distinct calyptra, septa clear, granulated.

Locality : Ambikapatty and National Highway road

Collection No. and Date: EC/JR/PS/CY27 and 23/12/2013

Pearson's correlation coefficients calculated between various physico-chemical properties of water and total *Oscillatoria* present on submerged polythene bags in domestic sewage water drains have been presented in Table 2**.** Water temperature has positive correlation ($r=0.583^{**}$, $p< 0.01$) with total *Oscillatoria* species. BOD has positive correlation with total *Oscillatoria* species ($r =0.942^{**}$,$p <0.01$) in the domestic sewage water. pH has positive correlation ($r=0.311^{**}$, $p< 0.01$) with total *Oscillatoria* species. COD has negative correlation with total *Oscillatoria* species ($r= -0.281^{*}$,$p<0.05$). DO has positive correlation with total *Oscillatoria* species ($r=0.656^{**}$, $p< 0.01$). Total alkanity (TLK) has positive correlation with total *Oscillatoria* species ($r=0.923^{**}$, $p< 0.01$). Suspended solid (SS) has positive correlation with total *Oscillatoria* species ($r=0.960^{**}$, $p< 0.01$).Sulphate,nitrate,calcium and free CO_2 has positive correlation with total *Oscillatoria* species ($r=0.585^{**}$,0.446^{**},0.440^{**} ,0.629^{**},$p<0.01$). Total dissolved solid showed no significant correlation on the distribution of total *Oscillatoria* species. This possibly could be the reason for their maximum colonisation on polythene surface during winter period.

Discussion:

A total of twenty species of *Oscillatoria* were found to colonize on the surfaces of polythene bags. *Oscillatoria subbrevis*, *O.princeps*, *O. limosa, O. amoena*, *O. vizagapatensis*, *O. okeni, O.limosa and O. laetevirens* are the common species found on submerged polythene bags. Site 4 recorded highest nitrate and phosphate concentration.Nitrate and phosphate concentration present in domestic sewage water affected the colonisation pattern and distribution of total *Oscillatoria* species. water temperature also affected the distribution of total *Oscillatoria* species . This findings are consistent with the observation made by Robarts and Zohary (1987), who explained that cyanobacteria blooms are likely to occur during the summer in temperate water. The ranged of pH and concentration of free CO_2 of domestic sewage water influenced the distribution pattern of total *Oscillatoria* species. The growth of cyanobacterial population were influenced by high pH and low free CO_2 (Shapiro,1990).

Due to heavy discharge by household and small scale industries to domestic sewage water drains, nitrate,sulphate and calcium were found to be present in high amount. Nitrate,sulphate and calcium showed positive correlation with total *Oscillatoria* species. Higher concentration of ammonium and phosphate were ascertained to be favourable for bloom of cyanobacterial populations (Chellappa *et al*., 2000; Gabriela and Alessandra, 2004 ; Vidya *et al.,* 2014). Nitrogen and phosphorus are both responsible for abundance of phytoplankton polulation (Gabriela and Alessandra, 2004).Nitrogen and phosphate concentration were found to be directly proportional to phytoplankton abundance in water (Coelho *et al*., 2003).

In the present investigation, dissolved oxygen ranged between 1.3-2.4mg/l revealing domestic sewage water is highly polluted. Low dissolved oxygen primarily results from excessive algae growth caused by phosphorus. The DO has positive correlation with total *Oscillatoria* species. Calcium concentration of domestic sewage water ranged between 54-69 mg/l. The abundance of cyanobacterial population is known to be influenced by calcium concentration (Sarojini, Y., 1996). Lower DO concentration and other physiochemical parameters showed that domestic sewage water are highly polluted. The genus *Oscillatoria* has been earlier noted to be tolerant to pollutants in water (Rai and Kumar, 1976). Free CO_2 value ranged between 36-42 mg/l in our present study and showed positive correlation with total *Oscillatoria* species. Lower free CO_2 value influenced the growth of cyanobacterial population. It has been observed earlier that green algae cannot thrive in low free CO_2 concentration (King, 1970). Effect of anthropogenic stressors, effluent influenced the growth of phytoplankton diversity. In our present investigation, high nutrients conditions influence the abundance of *Oscillatoria* population. The domestic sewage water drains of Silchar town carries domestic waste, municipal waste and small scale industries waste. Water qualities of the domestic sewage water were analysed and showed high amount of organic and inorganic substances present. Singh *et al.,* (2013) reported that physiochemical parameters greatly affected the cyanobacterial population in river Gomati. The domestic sewage, industrial waste, municipal waste water, human excreta, agricultural runoff and burning of corpse polluted

the Gomati river in Uttar Pradesh. A total number of 35 genera of algae, Cyanophyceae (11 genera), Chlorophyceae (12 genera), Euglenophyceae (1 genera) and bacillariophyceae (11 genera) were recorded in their observation. Jaiswal *et al*., (2017) reported the occurrence of organic pollutants in different water habitats and correlated the physico-chemical parameters with palmer algal genus index. They found that *Oscillatoria* alongwith frequently with other algal genera constituted around 49% of the total algal cover. This matches with the present findings of *Oscillatoria* occuring as largest genus on submerged polythene bags in domestic sewage water. A study on algal colonization on polythenes and their degradation revealed fifteen species including *Oscillatoria* in and around water bodies of Lucknow city in Uttar Pradesh (Suseela and Toppo, 2007). Sharma *et al*., (2014) detected ten algal species *Phormidium tenue, Oscillatoria tenuis, Navicula cuspidata, Monoraphidium contortum, Microcystis aeruginosa, Closterium costatum, Chlorella vulgaris* on polythenes on waste water of Kota city,Rajasthan. The species *Oscillatoria* thus may be useful in some monitoring programme (Cairns and Dickson,1971; James and Evison,1979) and also in the bidegaradation of polythene (Kumar *et al.,* 2017 , Sharma *et al*.,2014).

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Table 2: Bivariate correlation analysis of the physico-chemical and biological parameters using Pearson correlation coefficients

Correlations														
	Temp	BOD	pH	COD	DO	TLK	SS	Chlorides	sulphate	Nitrate	Calcium	TDS	FreeCO2	TOS
temp	1													
BOD	.145	1												
pH	-.099	**.280***	1											
COD	.226	**.519****	**-.327****	1										
DO	.072	.214	**.767****	**-.258***	1									
TLK	-.084	**.282***	**.997****	**-.322****	**.809****	1								
SS	-.060	-.060	**.614****	**-.543****	**.519****	**.610****	1							
Chlorides	-.034	.067	**.460****	**-.369****	**.334****	**.453****	**.351****	1						
sulphate	.106	**.468****	.113	**.536****	.090	.109	-.108	**-.476****	1					
Nitrate	-.146	-.125	**.496****	**-.510****	**.386****	**.494****	.232	**.774****	**-.553****	1				
Calcium	-.100	-.158	**.651****	**-.677****	**.483****	**.639****	**.925****	**.517****	**-.296***	**.499****	1			
TDS	-.177	**-.687****	-.041	**-.879****	-.020	-.042	**.369****	**.243***	**-.582****	**.288***	**.454****	1		
FreeCO2	-.070	**.381****	**.980****	**-.293***	**.775****	**.976****	**.617****	**.444****	.181	**.459****	**.640****	-.083	1	
TOS	**.583****	**.942****	**.311****	**-.281***	**.656****	**.923****	**.960****	-.084	**.585****	**.446****	**.440****	.131	**.629****	1

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Phytochemical screening and antioxidant activity of a cyanobacterium, *Oscillatoria limosa* isolated from polythene surface in domestic sewage water

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Running title: Phytochemicals of *Oscillatoria limosa*

Abstract

Carry bags made of polyethylene are widely used commodity in consumer products and packaging. These packaging materials are dumped into landfills and water bodies leading to major contamination of the environment. Phytochemical screening and antioxidant activity of *Oscillatoria limosa* collected from polythene surface in domestic sewage water of Silchar town, Assam (India) is the subject matter of the present work. The carbohydrate, protein, lipid, vitamin C, pigments (chlorophyll a, chlorophyll c, carotenoids, and phycobiliproteins), enzymatic antioxidants, non-enzymatic antioxidants, different radical scavenging, total phenolics, and flavonoids content of *O. limosa*were analysed. The carbohydrate, protein, total phenolics, and flavonoids were found to be 240µgml^{-1}, 378µgml^{-1}, 16.33mgGAE/gDW, and 4.4mgQE/gDW, respectively. The inhibition levels were found to be 63±0.21, 69±0.31, 68±0.21 percent at concentration of 100µg/ml, respectively, for DPPH radical scavenging activity, hydroxyl radical scavenging activity and total antioxidant activity.

Keywords: biochemical; cyanobacterium; sewage water; polythene; phytochemical

Introduction

Widely distributed in fresh, brackish and marine aquatic environments and in moist soil surfaces, cyanobacteria are very unique owing to their capacity to perform photosynthesis, fix nitrogen and grow in almost all types of extreme habitats including wastewater and highly polluted environments (Cuellar-Bermudez *et al*., 2017; Singh and Thakur, 2015; Dubey *et al*., 2011; Akoijam *et al*., 2015). Submerged polythene surfaces, ubiquitous in urban waste water is one such artificial substrate that is known to harbour algae including cyanobacteria (Suseela and Toppo, 2007; Sharma *et al.*, 2014; Kumar *et al.*, 2017; Sarmah and Rout, 2017). Given their ability to acclimatize to extreme environmental conditions, they are considered a rich source of secondary metabolites with potential biotechnological and pharmacological applications (Tan, 2007; Paliwal *et al.*, 2017; Sarmah and Rout, 2018). Such metabolites exhibit diverse biological activities, including antioxidant (Natrah *et al*., 2007), antimicrobial (Mugilan and Sivakami, 2016), anti-inflammatory(Deng and Chow, 2010), anticoagulant (Kim and Wijesekara, 2011), anti-mutagenic (Lahitová *et al*., 1994), antiproliferative (Sergey *et al*., 2013) and anti-cancer activities (Samarakoon *et al*., 2013). Growth of algae on such polythene substrata and their biochemical characterization are also important in the context of biodegradation of polythene (Rai and Rajashekhar 2015; Kumar *et al.*, 2017; Sarmah and Rout, 2018).Biochemical characterization ofrange of natural bioactive metabolites is the key pre-requisite for biotechnological applications. Algae generally considered as potent source of antioxidants due to higher contents of ascorbic acid, glutathione reductase, phenols and flavonoids (Wu *et al.*, 2010). Algal derived antioxidants, such as carotenoids, vitamin E (α-tocopherol), phycobiliproteins, polyphenols have drawn immense interest in health and pharmaceutical industry (Munir *et al.,*2013). The present study therefore addresses the phytochemical screening and antioxidant activity of a cyanobacterium, *Oscillatoria limosa* collected from submerged polythene surface in the domestic sewage water of Silchar town in the state of Assam, India.

Material and methods

Isolation of Oscillatoria limosa

The cyanobacterium was collected from submerged polythene surface in domestic sewage water of Silchar town (Assam, India). The study area lies between latitude 24º49´ North and longitude 92º48´East and altitude of 114.69

meters above sea level on the banks of river Barak. The species was isolated and purified (Rippka *et al.*, 1979). The culture was microscopically examined and identified as *Oscillatoria limosa* (Prescott, 1952; Desikachary,1959) (Fig.1). The BG11 agar petri plates containing the cyanobacterium were incubated for 15 days under continuous illumination (2000lux) at 24±1ºC. The pure colonies of the cyanobacterium were developed in agar petri plates. The pure colonies were diluted in sterilized distilled water and subcultured to 100mL of culture media in 250 mL conical flasks.

Fig. 1. Photomicrograph of *Oscillatoria limosa*

Physico-chemical properties of sewage water

Domestic sewage water was collected in clean polythene bottles, filtered to remove suspended particles, stored at 4°C andwas analysed (APHA, 2005).

Harvesting

The growth phases of the *O. limosa*were determined interms of their chlorophyll-*a* content (Kobayasi, 1961). The cultures of the cyanobacterium were harvested at exponential growth phase. The cyanobacterial biomass was separated from the BG-11 media by centrifugation at 3500r/min for 10 min.,collected by filtration and transferred to pre-weighed filter paper and oven-dried at 60ºC for 2 hours. The dried biomass was stored in vials at 4°C for further analysis.

Enzymatic and non-enzymatic antioxidants

Standard protocol were followed for catalase activity (Aebi, 1984), peroxidase activity (Kar and Mishra, 1976) andglutathione peroxidase activity (Rotruk*et al.*, 1973). Ascorbic acid content was estimated as per Roe and Keuther (1943). Glutathione reductase was assayed following the method of Scaedle and Bassham (1977).

DPPH free radical scavenging activity, hydroxyl radical scavenging activity, total antioxidant activity

DPPH free radical scavenging activity was measured according to Sanchez-Moreno *et al.*, 1995.
Hydroxyl radical scavenging activity was measured by the method outlined by Ruch*et al.*, 1989and that of total antioxidant activity was assessed by the method of Prieto *et al.*, 1999.

Phytochemical screening

The total phenolic content of the methanol extract was estimated by the Folin-Ciocalteu method (Singleton and Rossi, 1965).Total flavonoid content of the culture was determined by aluminium chloride method (Jia*et al.*,

1999).Chlorophyll *a*, c and carotenoids were estimated by standard methods(Strickland and Parsons, 1968and Parson, 1984). Vitamin C content was estimated using the method of Roe and Keuther (1943).Total carbohydrate was determined by anthrone method (Spiro, 1966). Total protein was estimated by modified method of Herbert *et al.*, (1971). Phycobiliproteins estimation has been made as per Bennet and Bogorad (1973). Lipid content was estimated by the standard method of Bligh and Dyer (1959).

Results

The physico chemical propertites of domestic sewage water is important to know the natural habitat conditions of *O. limosa*.The temperature of sewage water was 34±0.56ºC. Biological oxygen demand, chemical oxygen demand and dissolved oxygen of water was 600±0.18, 1520±0.18, and 2.1mg/L, respectively. The total alkalinity was 10±1.4mg/L, free CO_2 was 36.98±0.13mg/L and total dissolved solid of sewage water was found to be 500 ± 0.18mg/L. The suspended solid was 50±0.54mg/L. The chloride content and calcium concentration was found to be 60±0.67mg/L, 60±0.13mg/L, respectively. The sulphate, nitrate, magnesium and ammonia of sewage water was present at a concentration of 50 ± 1.6mg/L, 12 ±1.5mg/L, 30± 0.23mg/L and 30±0.45mg/L, respectively.The phosphate concentration found to be 70±0.45mg/L.

The growth rate of *O.limosa* (Fig.2) was found to be 0.158μd^{-1} with generation time 178.25h. The cyanobacterium showed a 28 days life cycle characterized by shorter duration log period with highest pigment production.
The catalase, peroxidase and glutathione reductase activity (Fig. 3) of methanol extract from *O.limosa* were found to be 45.11±0.41%, 87±0.34% and28±0.43% at for 100μg/ml, respectively.
DPPH radical scavenging activity, hydroxyl radical scavenging activity, total antioxidant activity of *O.limosa* (Fig 4) of the extracts were in the range of 5-100 μg/ml concentration. BHT was used as standards at the concentration 5-100 μg/ml concentration. The levels were found to be 63±0.21, 69±0.31, 68±0.21 percent at concentration of 100 μg/ml, respectively, for DPPH radical scavenging activity, hydroxyl radical scavenging activity and total antioxidant activity.
The total phenolic content (Table 1) in methanolic and acetonic extracts were found to be 16.33 and 14mg GAE/g DW, respectively. The total flavonoid content in methanol and acetone extracts were found to be 4.4 and 3.8mg QE/g DW, respectively. The vitamin-C content in the methanol and acetone extracts were found to be 1.2 and 0.9mg/g DW, respectively, in *O.limosa*. The chlorophyll *a* pigment in methanol and acetone extracts were found to be 7.44 and 6.7mg/g DW, respectively. The chlorophyll *c* content in methanol and acetone extracts were found to be 3.44 and 3.32mg/g DW, respectively. The carbohydrate, protein and lipid content in *O.limosa* were found to be 240, 378 and 14.3μgml^{-1}, respectively. The phycocyanin (60.7mg/g DW) and PE content (21.5mg/g DW) of the species were relatively high. Allophycocyanin content was found to be 20.35mg/g DW. Total phycobiliproteins was found to be of 102.55mg/g DW.

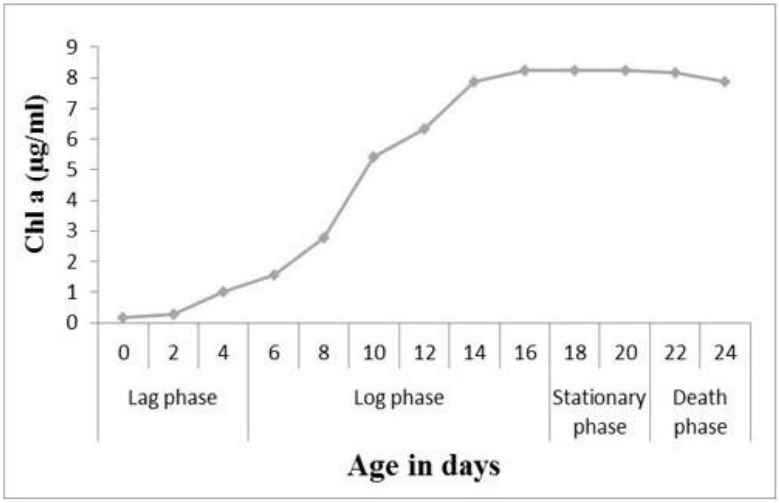

Fig.2. Growth of *Oscillatoria limosa*

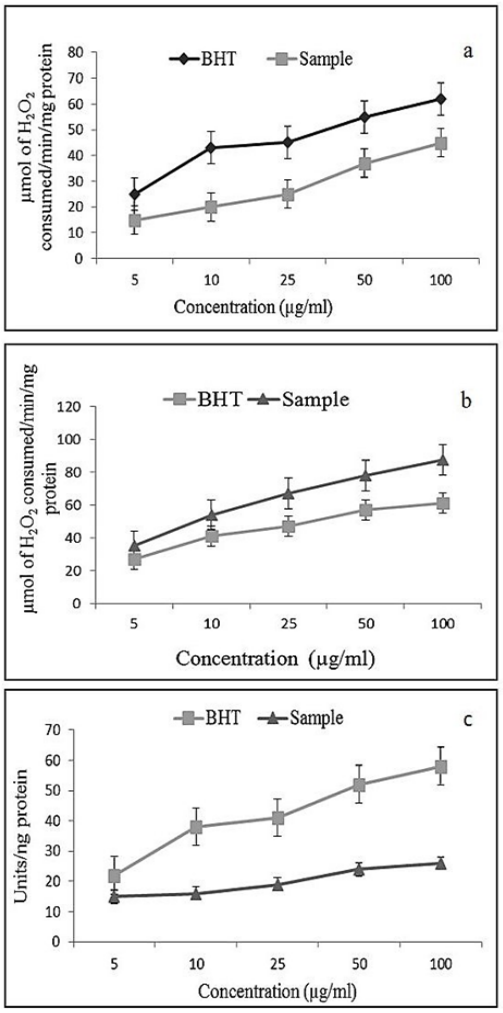

Fig.3. (a) Catalase; (b) Peroxidase; and (c) Glutathione reductase activity of *Oscillatoria limosa*

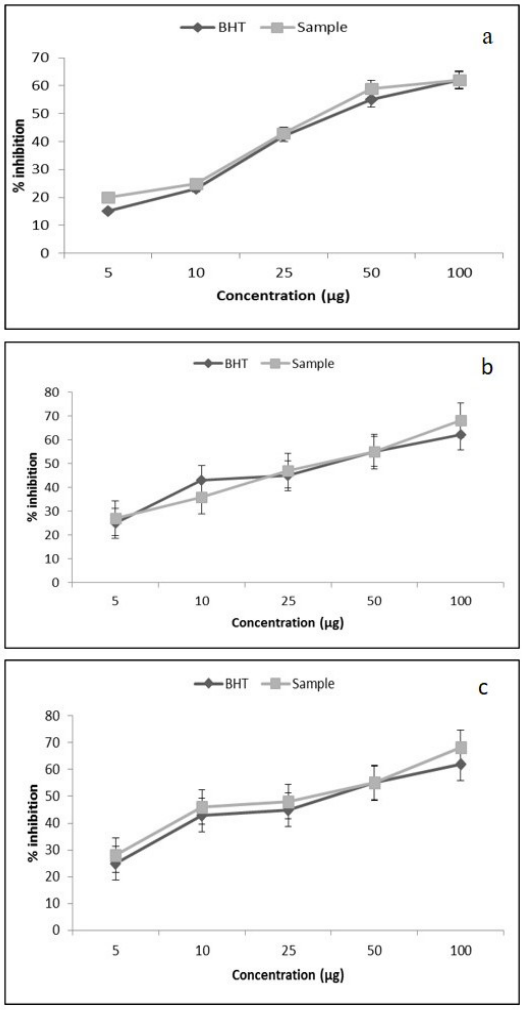

Fig. 4. (a) DPPH free radical scavenging activity; (b) Hydroxyl radical scavenging Activity; and (c) Total antioxidant activity of *Oscillatoria limosa*

Phytochemical											
Total phenolics (mg GAE/g DW)		**Total flavonoids (mg QE/g DW)**		**Vitamin C (mg/g DW)**		**Chlorophyll-a (mg/g DW)**		**Chlorophyll-c (mg/g DW)**		**Carotenoid (mg/g DW)**	
Methanol	Acetone	Methanol	Acetone	Methanol	Acetone	Methanol	Acetone	Methanol	Acetone	Methanol	Acetone
16.33 ± 0.12	14± 0.13	4.4± 0.16	3.8± 0.12	1.2± 0.11	0.9± 0.12	7.44± 0.18	6.7± 0.12	0.56 ± 0.02	0.43± 0.03	3.44± 0.01	3.32± 0.03

Discussion

The analysis of phytochemical constituents such as pigments, phycobiliproteins (PBP), protein, carbohydrate, vitamin C, lipid, total phenolics, and total flavonoids of the *O. limosa* revealed the relative concentration to be rather similar to those recently reported for *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum*, and *Cylindrospermum muscicola* isolated from the domestic sewage water of Silchar (Sarmah and Rout, 2018).

The presence of enzymatic and non-enzymatic antioxidants in *O.limosa* clearly demonstrated its role against oxidant and other free radicals. The occurrence of enzymes viz., catalase, peroxidase and glutathione reductase in the cyanobacterium are key factors to its adaptation to extreme environmental conditions (Mukund *et al*., 2014).The carotenoid content of methanol and acetone extracts were 3.44mg/g DW and 3.32mg/g DW, respectively. It is pertinent to mention herein that carotenoid play an important role in protecting the chloroplasts against photodamage, by scavenging several active oxygen species (ROS) such as $^1O_2$, O_2^-, H_2O_2, hydroxyl radicals (HO^-) and peroxy radicals (Burton, 1989; Krinsky, 1989; Munir *et al*., 2013).

The phenolic contents of methanolic and acetonic extracts of the cyanobacterium were 16.33 and 14mgGAE/g DW, respectively. Phenolic entity can donate a hydrogen atom or an electron in order to form stable radical intermediates (Jimenez-Escrig *et al*., 2001). The inhibition level in the methanolic extract of the *O.limosa*were found to be 68.11±0.21, 62.11±0.11, 69±0.31 percent at concentration of 100µg/ml, respectively, for DPPH radical scavenging activity, hydroxyl radical scavenging activity and total antioxidant activity. The cyanobacterium is believed to have developed defense against photo-oxidative damage by various antioxidative mechanisms to detoxify and remove highly reactive oxygen species (ROS) by producing several oxidative and radical stressors such as phenolic compounds and carotenoids (Tsao and Deng, 2004). As the cyanobacterium was collected from submerged polythene surface in sewage water, it is anticipated that it might have gradually developed a system of either accumulating or releasing intra- or extracellular compounds to cope with the stress (Grossman *et al*., 1993; Ward and Singh, 2005, Sarmah and Rout 2017; Paliwal *et al*., 2017).

The total phycobiliproteins (PBPs) of *O. limosa* was found to be of 102.55mg/g DWin in the methanolic extract. The PBPs are believed to be a strong antioxidant which have antiviral, antitumor, anti-inflammatory and antifungal activities (Rai and Rajashekhar, 2015). The natural habitat of the *O. limosa* is rich in inorganic nutrients such as nitrate, phosphate, calcium etc. and presumed to play an important role in growth and metabolic processes by transferring energy to cells, nucleic acid biosynthesis, phospholipid biosynthesis and membrane development (Reitan *et al.*, 1994;Khozin-Goldberg and Cohen, 2006; Sang *et al*., 2012). The species is found to be the natural source of several bioactive compounds which have potential for pharmaceutical applications. The luxuriant growth of the species in polluted habitat is relevant in the context of pollution abetment and monitoring (Cairns and Dickson, 1971; James and Evison, 1979) including biodegradation of polythene (Sharma *et al*., 2014; Kumar *et al*., 2017).

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Algal colonization on polythene carry bags in a domestic solid waste dumping site of Silchar town in Assam

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Abstract

Algal colonization on polythene carry bags in soild domestic waste disposal sites in Silchar town in Assam were investigated during June 2012-May, 2013. A total of 36 algae were found colonising on the surface of polythene carry bags in solid domestic sewage dumping sites. The most common algal species encountered were the *Oscillatoria*, *Phormidium*, *Lyngbya*, *Nostoc, Spirulina*, *Hydrocoleum*, *Chlorella*, *Pithophora, Stigeoclonium tenue*, *Anomoeoneis* and *Nitzchia*. Physio-chemical properties of soil from domestic solid waste dumping sites have been studied. Correlation study between soil parameters and total algal species were made. The cyanobacterial species showed positive correlation with organic carbon, total nitrogen, available phosphorus and potassium. The members of chlorophyceae showed positive correlation with total nitrogen.

Keywords: algae, colonisation, polythene bags, Silchar, solid domestic sewage

Introduction

Algae are the group of organisms which occupy a variety of terrestrial habitats, including soils, rocks and caves and also in living animals and plants (Hoffmann, 1989). Besides aqueous habitats, algae are found in non-aqueous habitats too (Zenova *et al*., 1995). Role of algae in soil formation, providing stability to mature soils (Metting, 1981) and in fixation of energy and matter to ecosystems is well recognised (Kuzyakhmetov, 1998). Algae contribute nitrogen to soil through the process of biological nitrogen fixation (Goyal, 1997). Previous studies have dealt with algae on submerged polythene sheets of municipal sewage water (Suseela and Toppo, 2007; Sharma *et al*., 2014). These polythenes gets degraded to some extent during rain and dry season and broken into small pieces by bacterial and algal attachment and released into the environment. The Silchar town, the headquarters of Cachar district is located in the state of Assam in India. It is the second largest city of the state in terms of population and municipal area in the state. Due to lack of inadequate regulations and monitoring of polythene carry bags usage, used polythene bags which are generally thrown to landfills or water bodies constitute the principal source of the town municipal solid waste. During the rainy season,the polythene bags were found colonized by algae. Study of growth of algae and their colonisation on such polythene substrata are important in the context of sustainable waste management.

The present study thus aims to assess the algal colonization on polythene carry bags of solid domestic sites of Silchar town. The Shannon diversity index, Simpson dominance index and evenness index were used to estimate the algal diversity. Correlation study has been performed to ascertain the influence of soil parameters on algal diversity.

Material and methods

The study was carried out at Silchar town area of Cachar district in the state of Assam. The study area lies between latitude $24^0$49´ North and longitude $92^0$48´ East and altitude of 114.69 meters above sea level on the banks of river Barak. A total of 10 different study sites in different locations were identified for the study (Fig.1). The colonisation of algae on polythene bags in solid domestic waste dumping site were shown in plate 1. The algae and soil samples were collected from each of these sites during the rainy season, June 2012-May 2013. The soils were sampled in triplicate from the different study sites. The soil pH was measured using Chemline digital pH meter. The conductivity of soil samples were measured using a Systronics direct reading conductivity meter, Type CM-82. Soil bulk density was estimated by soil corer method (Brady and Weil, 2004). The moisture content of the soil was determined by oven drying method (Soil Survey Standard Test Method,Gupta, 1999). Soil organic matter was analyzed by Walkey and Black's rapid titration method (Jackson, 1958). Nitrogen was analyzed by alkaline

permanganate method (Subbiah and Asija, 1956). Available Phosphorous was determined by Olsen's method (Olsen *et al.,* 1954) and Potassium by flame photometry (Black, 1965).

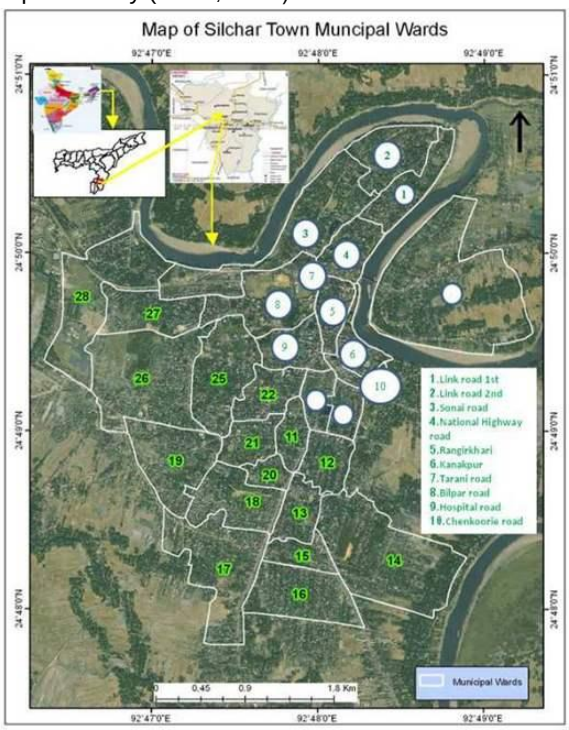

Fig 1. Map of the study area showing the location of study sites

The algal samples collected from different sites were observed under microscope and identified using standard keys (Prescott, 1951; Desikachary, 1959; Sarode and Kamat, 1984) and preserved in 4.5% formalin for further study. The algal samples were finally counted following Lackey's drop method (Trivedy and Goel, 1986).Correlation study was performed using SPSS-21.

Results

During the rainy season (June 2012- May 2013) the polythene bags were found colonized by algae in the form of dark green and dark brown patches. Sometimes light green colonization were also seen on the polythene surface. The physico-chemical parameters of soil for the 10 study sites are shown in Table 1. The soil pH ranged between 5.6 -7.4, slightly acidic to slightly alkaline. In the present study, highest pH was recorded in site 10 and lowest pH was recorded in site 4 and 7. Electrical conductivity(EC) ranged between 97-160µs/cm. In the present study, highest EC was recorded in site 2 and lowest in site 1.Water holding capacity of soil ranged in between 31-60%. The water holding capacity was highest in site 4 and lowest in site1. Moisture content of soil ranged between 34-54.8%, maximum being site 6, minimum in site 8. The content of organic carbon ranged from 0.87-2.6%, highest value being recorded in site 2 and lowest in site 4. Available NPK ranged from 0.07-0.77%, 2.8-6.6mg/kg and 141-191ppm, respectively.

Plate 2 shows the colonization pattern of algae as observed under the microscope**.** Attachment of *Oscillatoria tenuis* was clearly seen on the polythene surfaces with a thick mat on polythene surface (Plate 2a). *Oscillatoria brevis,* has been found to grow as a net like form in a mixed consortia of other algae (*Lyngbya* and *Navicula*) (Plate2b). A total of 36 species belonging to 25 genera had been enumerated. Highest number of species belonged to cyanophyceae (16) followed by chlorophyceae (10) and bacillariophyceae (10). The distribution of species for the genus are *Oscillatoria* (4), *Calothrix* (3), *Anabaena* (2), *Nostoc* (2), *Phormidium* (2), *Cosmarium* (2), and *Navicula* (2). Blue green algae were represented by 10 genera, *Anabaena* (2), *Aphanothece* (1), *Arthospira* (1), *Calothrix* (3), *Lyngbya* (1), *Nostoc* (2), *Oscillatoria* (4), *Phormidium* (2), *Spirulina* (1) and *Hydrocoleum* (1). The photomicrographs of 36 species of algae colonised on polythene bags have been presented in Plate 3, Plate 4, and Plate 5. The abundance, density and frequency of algae for the 10 study sites of Silchar town are shown in Table 2-6. At site 2 maximum value of Shannon index and evenness, and minimum Simpson index value indicated a higher algal

diversity. The site 2 is located in market area and also near to the small scale industries and residential area. Due to such reasons, the soil surface mostly remained moist causing high cyanobacterial diversity. At site 5, minimum value of Shannon index and evenness and maximum Simpson index were recorded. Karl Pearson's correlation coefficients calculated between various physico-chemical attributes of soil and density of algae present on polythene bags in domestic solid sewage dumping sites have been presented in Table 5. Total cyanobacterial species has positive correlation with organic carbon($r = 0.557$, $p < 0.05$), with total nitrogen ($r = 0.745$, $p < 0.01$), with available phosphorus ($r = 0.761$, $p < 0.01$) and with potassium ($r = 0.532$, $p < 0.05$). Total chlorophyceae species and bacillariophyceae species has positive correlation ($r = 0.507$, $p < 0.05$, $r = 0.474$ $p < 0.05$) with pH.

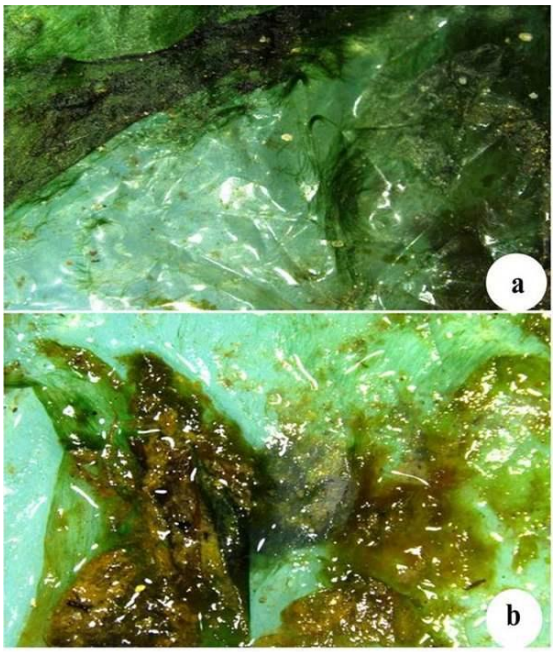

Plate 2. Micrography of some selected polythene bags showing colonization of algae found in domestic sewage solid dumping site (a,b)

Plate 3. Photomicrographs of algae species observed during study period

1- *Phormidium lucidum*, 2- *Phormidium* sp, 3- *Spirulina major*,4-*Arthospira plantensis*, 5-*Lynghya cinerescens*, 6-*Anabaena sp*,7- *Oscillatoria vizagapatensis*, 8-*O.tenuis*, 9-*Anabaena spiroides*,10-*Oscillatoria brevis*

Plate 4. Photomicrographs of some of algae species during study period

1-*Chlorella* sp, 2- *Scenedesmus quadricauda*,3-*Spirogyra sp*, 4- *Closterium* sp1, 5-*Closterium* sp2, 6-*Cosmarium constrictum*, 7-*Cosmarium formosulum*, 8-*Fragillaria sp*,9-*Synedra tabulata*,10-*Nitzschia obtusa* ,11-*Anomoeoneis* sp, 12-*Pitophora* sp, 13-*Nitzchia hungarica* ,14-*Navicula* sp,15-*Pinnularia lundi*,16- *Pinnularia eburnean*

Plate 5. Photomicrographs of some of algae species during study period

1- *Oedogonum* sp, 2-*Stigeoclonium tenue*, 3-*Aphanothece microscopica*, 4- *Gyrosigma sp*, 5-*Melosira sp*, 6-*Navicula sp*,7-*Calothrix sp 1*, 8-*Calothrix sp2*, 9- *Hydrocoleum sp*,10-*Nostoc carneum*,11-*Nostoc linckia*

Table 1. Physico-chemical properties of domestic solid waste soil

Parameters	Unit	Site1	Site2	Site3	Site4	Site5	Site6	Site7	Site8	Site9	Site10
pH	–	6.4 ± 0.07	6.9±0.2	7.4±0.3	**5.6±0.1**	6.4±0.04	5.7±0.08	**5.6**±0.3	6.5±0.5	5.7±0.1	**7.5±0.3**
Electrical conductivity (EC)	µs/cm	**97±0.04**	**160±0.35**	156±0.27	143±0.23	155±0.73	123±0.27	156±0.2	132±0.22	152±0.26	123±0.22
Moisture content (%)	%	35±0.32	37.2±0.46	45.4±0.4	51.6±0.31	57.4±0.03	**58.4±0.01**	55±1.02	**34±0.98**	43±0.93	39±0.86
Water holding capacity (%)	%	**31±0.58**	48±0.02	39±0.01	**60±0.02**	59±0.01	56±0.72	52±0.08	50±0.09	45±0.12	35±0.34
Bulk density		3.1±0.02	1.4±0.01	2.1±0.01	**3.4±0.37**	**0.86±0.03**	2.1±0.02	2.1±0.05	3.0±0.06	0.89±0.34	2.0±0.08
Organic matter	%	1.6±0.02	**2.67±0.19**	2.13±0.01	0.87±0.01	1.95±0.02	0.97±0.01	2.1±0.12	**0.86±0.23**	1.56±0.06	0.98±0.45
Total Nitrogen	%	**0.78±0.02**	0.63±0.01	0.52±0.02	**0.07±0.01**	0.52±0.05	0.54±0.01	0.53±0.12	0.08±0.34	0.54±0.45	0.53±0.43
Available phosphorus	mg/kg	3.7±0.1	5.8±0.1	5.5±0.15	**2.6±0.1**	6.6±0.15	4.8±0.20	5.4±0.32	2.8±0.40	**6.7±0.34**	4.9±0.14
Available Potassium	ppm	185±0.57	187±0.57	**191±0.57**	**141±1.0**	171±0.57	157±1.0	192±0.32	**141±0.22**	172±0.34	157±0.27

Table 2. Abundance of algae on polythene bags of solid domestic sewage disposal sites (Total number of individuals ×10^{-3}/cm^2)

		Sites									
Sl. no.	Algal species	1	2	3	4	5	6	7	8	9	10
	Cyanophyceae										
1	*Anabaena spiroides*	-	-	5.27	-	-	-	-	5.82	5.27	6.23
2	*Anabaena* sp	5.6	-	-	3.4	1.0	-	2.4	-	7.3	-
3	*Aphanothece microscopica*	-	-	5.27	-	8.26	-	9.24		-	5.82
4	*Arthospira plantensis*	-	-	5.27	-	24.8	4.41	1.99	11.5	9.24	
5	*Calothrix fusca*	-	-	7.03	-	-	-	-	-	1.99	11.5
6	*Calothrix marchica*	29.3	-	-	21.50	9.65	4.41	-	7.13	-	-
7	*Hydrocoleum sp*	1.75	-	-	-	9.37	-	3.26	4.33	-	7.13
8	*Lyngbya cinerescens*	7.03	-	-	-	-	-	3.42	3.62	3.26	4.33
9	*Nostoc linckia*	2.93	-	-	-	3.38	-	2.93	-	3.42	3.62
10	*Nostoc carneum*	3.23	-	-	13.73	7.5	4.15	-	8.12	2.93	-
11	*Oscillatoria earlei*	10.5	1.71	10.85		4.84	9	-	-	-	8.12
12	*Oscillatoria limnetica*	8.67	-	-	6.25	-	-	3.83	7.91	-	-
13	*Oscillatoria okeni*	8.16	49.65	2.45	18.92	-	13.55	-	-	3.83	7.91
14	*Phormidium calcicola*	12.3	8.66	5.53	31.34	-	-	11.13	-	-	-
15	*Phormidium lucidum*	2.93	-	-	-	30.5	-	-	27.23	11.13	-
16	*Spirullina major*	17.8	-	-	-	-	2.46	2.54	-	-	27.23
	Chlorophyceae										
17	*Chlorella sp*	9.87	-	-	-	-	11.12	13.55	15.7	2.54	-
18	*Closterium* sp1	-	-	0.58	-					13.55	15.7
19	*Closterium* sp2	-	-	-	-	3.27	-	0.35	6.63		
20	*Cosmarium constrictum*	2.74	-	4.22	24.8	1.03	-	0.72		0.35	6.63
21	*Cosmarium formosulum*	-	-	-	7.37	5.19	-	22.77	21.15	0.72	
22	*Oedogonium sp*	6.83	4.16	-	-	16.20		1.02	-	22.77	21.15
23	*Pitophora* sp	-	-	10.38	5.11	23.84	5.91	-	-	1.02	-
24	*Scenedesmus quadricauda*	1.78	-	-	11.15					-	-
25	*Stigeoclonium tenue*	-	1.59	-	22.77	11.48	-	-	-		
26	*Spirogyra* sp.	11.48	-	-	-	10.58	-	3.87	-	-	-
	Bacillariophyceae										
27	*Anomoeoneis sp*	4.99	20.91	3.22	1.34	6.83	4.16	-	-	-	-
28	*Fragilaria sp*	-	1.01	-	10.75	19.82	-	3.91	7.28	-	-
29	*Gyrosigma sp*	12.25	-	-	-	5.73	4.92	-	-	-	6.10
30	*Navicula dicephala*	10.70	-	6.20	-	3.23	-	-	9.38	-	-
31	*Navicula minuta*	-	4.94	9.34	11.99	-	-	4.58	12.00	-	9.38
32	*Nitzchia hungarica*	5.19	-	22.77	21.15	9.35	-	-	12.95	-	11.22
33	*Nitzchia intermedia*	4.99	-	-	2.03	0.99	-	-	12.62	-	12.95
34	*Pinnularia dolosa*	4.99	20.91	3.22	1.34					-	12.62
35	*Pinnularia lundi*					-	-	21.15	10.38		
36	*Synedra tabulata*	-	1.59	-	22.77					21.15	10.38

Table 3. Density of algae on polythene bags of solid domestic sewage disposal sites (Total number of individuals ×10^{-3}/cm^2)

		Sites									
Sl. no.	Algal species	1	2	3	4	5	6	7	8	9	10
	Cyanophyceae										
1	*Anabaena spiroides*	-	-	2.93	-	-	-	3.38	1.70	-	6.83
2	*Anabaena* sp	3.23	-	-	2.93	1.55	-	0.38	-	6.83	-
3	*Aphanothece microscopica*	-	-	1.76	-	6.18	-	7.43	-	-	1.78
4	*Arthospira plantensis*	-	-	2.34	-	2.34	1.06	1.03	3.18	1.78	-
5	*Calothrix fusca*		-	20.66	-	-	-	-	-	15.67	2.05
6	*Calothrix marchica*	5.83	-	-	1.90	16.21	3.09	-	1.02	-	-
7	*Hydrocoleum sp*	2.38	-	-	-	3.32	-	1.07	8.44	-	16.20
8	*Lyngbya cinerescens*	2.93	-	-	-	-	-	6.67	10.75	3.07	4.99
9	*Nostoc linckia*	3.67	-	-	-	6.22	-	2.96	-	2.05	1.04
10	*Nostoc carneum*	16.71	-	-	11.54	4.08	3.12	-	2.12	12.25	-
11	*Oscillatoria earlei*	5.3	5.18	5.56	-	5.18	2.24	-	-	-	12.25
12	*Oscillatoria limnetica*	4.52	-	-	22.45	-	-	1.34	11.99	-	-
13	*Oscillatoria okeni*	8.68	5.77	28.2	21	-	7.47	-	-	12.99	3.07
14	*Phormidium calcicola*	4.3	2.3	2.7	1.6	-	-	15.18	-	-	-
15	*Phormidium lucidum*	10.58	-	-	-	3.32	-	-	0.67	4.99	-
16	*Spirullina major*	3.24	-	-	-	-	13.94	1.07	-	-	4.99
	Chlorophyceae										
17	*Chlorella sp*	3.78	-	-	-	-	2.56	2.78	1.78	2.54	-
18	*Closterium* sp1	-	-	0.67	-	-	-	-	-	0.97	0.43
19	*Closterium* sp2	-	-	-	-	0.78	-	0.65	0.54	-	-
20	*Cosmarium constrictum*	4.16	-	15.18	11.73	21.15	-	0.85	-	5.19	1.03
21	*Cosmarium formosulum*	-	-	-	4.62	16.21	-	1.02	2.09	16.20	-
22	*Oedogonium sp*	5.45	3.56	-	-	7.94	-	4.56	-	23.84	16.20
23	*Pitophora* sp	-	-	0.66	6.12	3.23	2.34	-	-		-
24	*Scenedesmus quadricauda*	2.67	-	-	13.82	-	-	-	-	-	-
25	*Stigeoclonium tenue*	-	3.26	-	6.24	10.74	-	-	-	-	-
26	*Spirogyra* sp.	3.68	-	-	-	11.39	-	5.83	-	-	-
	Bacillariophyceae										
27	*Anomoeoneis sp*	1.15	1.78	1.84	2.05	10.61	1.79		-	-	-
28	*Fragilaria sp*	-	21.9	-	5.9	5.10	-	1.53	10.20	-	-
29	*Gyrosigma sp*	4.45	-	-	-	3.23	2.78	-	-	-	5.73
30	*Navicula dicephala*	6.78	-	3.67	-	4.23	-	-	12.00	-	-
31	*Navicula minuta*	-	2.67	3.72	2.63	-	-	6.31	12.48	-	2.78
32	*Nitzchia hungarica*	13.7	-	-	0.78	0.98	-	-	4.20	-	0.99
33	*Nitzchia intermedia*	-	5.55	4.52	7.92	-	-	-	-	-	0.99
34	*Pinnularia dolosa*	16.20	2.09	1.02	5.83	-	-	-	-	-	3.87
35	*Pinnularia lundi*	-	-	-	-	-	-	0.98	4.97	-	-
36	*Synedra tabulata*	-	3.91	-	3.67	-	-	-	-	6.78	4.99

Table 4. Frequency of algae on polythene bags of solid domestic sewage disposal sites (Total number of individuals ×10^{-3}/cm^2)

		Sites									
Sl. no.	Algal species	1	2	3	4	5	6	7	8	9	10
	Cyanophyceae										
1	*Anabaena spiroides*	-	-	66.67	-	-	-	-	25.00	33.33	
2	*Anabaena* sp	25.00			33.33	100.00		25.00		66.67	
3	*Aphanothece microscopica*	-	-	66.67	-	33.33	-	100.00	-	-	33.33
4	*Arthospira plantensis*	-	-	33.33	-	66.67	-	-	66.67	66.67	66.67
5	*Calothrix fusca*	-	-	33.33	-		-	-	-	25.00	66.67
6	*Calothrix marchica*	100.00	-	-	66.66	33.33	33.33	-	33.33	-	-
7	*Hydrocoleum sp*	33.33	-	-	-	-	-	100.00	33.33	25.00	25.00
8	*Lyngbya cinerescens*	33.33	-	-	-	-	-	33.33	33.33	100.00	33.33
9	*Nostoc linckia*	100.00	-	-	-	66.67	-	33.33	-	33.33	100.00
10	*Nostoc carneum*	83.33	-	-	75.00	33.33	83.50	-	33.33	33.33	-
11	*Oscillatoria earlei*	58.33	56.66	50.00	-	33.33	33.33	-	-	-	33.33
12	*Oscillatoria limnetica*	50.00	-	-	66.67	-	-	77.67	33.33	-	-
13	*Oscillatoria okeni*	66.67	33.33	33.33	50.00	-	33.33	-	-	33.33	100.00
14	*Phormidium calcicola*	75.00	66.67	50	50.00	-	-	33.33	-	-	-
15	*Phormidium lucidum*	66.67	-	-	-	33.33	-	-	100.00	33.33	-
16	*Spirullina major*	41.67	-	-	-	-	33.33	33.33	-	-	33.33
	Chlorophyceae										
17	*Chlorella sp*	21.53	-	-	-	-	7.63	75.00	66.67	50.00	-
18	*Closterium* sp1	-	-	22.22	-	-	-	-	-	66.67	50.00
19	*Closterium* sp2	-	-	-	-	66.67		66.67	66.67	-	-
20	*Cosmarium constrictum*	68.33	-	41.67	46.33	33.33	-	33.33	-	100.00	-
21	*Cosmarium formosulum*	-	-	-	42.33	33.33	-	66.67	100.00	100.00	33.33
22	*Oedogonium sp*	50.00	100.00	-	-	33.33	-	100.00	-	-	-
23	*Pitophora* sp	-	-	44.44	71.9	33.33	66.67	-	-	61.9	-
24	*Scenedesmus quadricauda*	66.66	-	-	58.33	-	-	-	-	-	-
25	*Stigeoclonium tenue*	-	66.66	-	33.33	33.33	-	-	-	-	-
26	*Spirogyra* sp.	66.7	-	-	-	67.00	-	70.28	-	-	-
	Bacillariophyceae										
27	*Anomoeoneis sp*	41.58	50.00	41.58	45.33	66.67	33.33	-	-	-	-
28	*Fragilaria sp*	66.67	-	-	-	56.79	66.50	-	-	-	50.00
29	*Gyrosigma sp*	75.00	-	-	-	67.00	50.00	-		50.00	
30	*Navicula dicephala*	67.00	-	50.00	-	100.00	-	-	50.00	-	-
31	*Navicula minuta*	-	50.00	41.58	31.58	-	-	50.00	100.00	-	100.00

32	*Nitzchia hungarica*	100.00	-	25.00	50.00	56.79	-	-	25.00	-	25.00
33	*Nitzchia intermedia*	42.33	-	-	33.33	33.33	-	-	33.33	-	33.33
34	*Pinnularia dolosa*	33.33	50.00	33.33	50.00	-	-	-	-	-	25.00
35	*Pinnularia lundi*	-	-	-	-	-	-	75.13	41.50	-	-
36	*Synedra tabulata*	-	66.67	-	66.67	-	-	-	-	33.33	33.33

Table 5. Bivariate correlation analysis of the physico-chemical and biological parameters using Pearson correlation coefficients Correlations

	pH	EC	MC	WHC	BD	OC	TN	AP	K	TCS	TCHS	TBS
pH	1											
EC	.364	1										
MC	-.**518***	.281	1									
WHC	**-.524***	**.559***	.**788****	1								
BD	-.330	-.**556***	-.297	-.390	1							
OC	**.894****	.376	**-.545***	-.397	**-.560***	1						
TN	**.560***	-.231	-.365	**-.537***	-.413	**.642****	1					
AP	**.616****	**.499***	.092	.074	-.**871****.**729****		.**688****	1				
K	.**900****	.018	-.**652****-.**715****-.296	**.893****	**.820****	.**612****	1					
TCS	.373	.129	-.053	-.056	**-.635****.**557***		**.745****	**.761****	.**532***	1		
TCHS	**.507***	.172	-.407	-.292	-.127	**.500***	.095	.125	.403	.078	1	
TBS	.**474***	.209	-.408	-.285	-.029	.446	-.029	.031	.340	-.089	.**856****	1

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).
TCS-Total cyanobacterial species,TCHS-Total chlorophycean species,TBS-Total bacillariophyceae species

Table 6. Diversity indices of algae

Sl.No	Sites	Shannon- Wiener Diversity Index (H)	Simpson's dominance index (D)	Pielou's evenness index (J)
1	Site 1	1.3 ± 0.15	0.43 ± 0.08	0.55 ± 0.07
2	Site 2	**2.31 ± 0.24**	**0.14 ± 0.03**	**0.88 ± 0.01**
3	Site 3	1.59 ± 0.06	0.21 ± 0.02	0.86 ± 0.03
4	Site 4	1.45 ± 0.13	0.20 ± 0.03	0.87 ±0.005
5	Site 5	**0.65 ± 0.71**	**0.67 ± 0.34**	**0.43 ± 0.35**
6	Site 6	0.65 ± 0.28	0.62 ± 0.18	0.56 ± 0.24
7	Site 7	0.92± 0.16	0.43 ± 0.08	0.75 ± 0.10
8	Site 8	0.82 ± 0.21	0.56 ± 0.14	0.68 ± 0.15
9	Site 9	0.66± 0.23	0.47± 0.16	0.62± 0.10
10	Site 10	0.73± 0.28	0.24 ± 0.03	0.54± 0.07

Discussion

A rich colonisation of 36 species were found on polythene bag surfaces in solid waste dumping sites. Cyanophyceae (16), chlorophyceae (10) and bacillariophyceae (10) species were found to be colonised on polythene bags surface. Soil pH of domestic solid waste dumping site ranged between 5.6-7.4, slightly acidic to slightly alkaline. In the present study, highest pH was recorded in site 10 and lowest pH was recorded in site 4 and 7. Soil pH showed positive correlation with total chlorophycean species and total bacillariophyceae species. Among the soil properties, pH is certainly the most important factor determining the flora and fauna composition. In culture media optimal pH for the growth of cyanobacteria ranges from 7.5-10, with a lower limit of 6.5-7.0.However, in soil culture experiments, soils having slightly alkaline reaction were more favourable, while in natural environments cyanobacteria prefer neutral to alkaline pH. The development of soil acidity is generally believed to be associated with the base unsaturation caused by leaching out of bases and genesis from base-poor acidic rocks. The dissolved or free acidic substances, such as sulphuric acid and ferric and aluminium sulphate, accentuate acidity in acid sulphate soils (Dominicand and Madhusoodanan 1999). Soil pH is an important factor in determining the composition of algal communities. Acidic soil conditions are not considered ideal for blue green algae (Brock, 1973; Hahn and Kusserow, 1998), however, present study revealed rather high abundance and density of cyanophyceae. Among the

blue-green genus, *Oscillatoria, Lyngbya* and *Nostoc* were the dominant forms in most of the sites in the present study.

The pearson correlation coefficients indicated that the algal species colonised on polythene bags during June 2012- July, 2013 was mainly influenced by soil pH, soil electrical conductivity, moisture content, bulk density, organic matter,water holding capacity, total nitrogen, available phosphorus and available potassium. Algal colonisation on polythene bags surface are believed to be influenced by organic carbon,total nitrogen,available phosphorus and available potassium. The present study indicated that pH is positively correlated with distribution of algae on polythene surface consistent with the observation of Brady and Weil (2005) who reported that a pH range from 6.0 to 8.3 enhanced the nutrient availability for the growth of plants. A change in pH beyond this limit inhibited the availability of nutrients for the plants as soil tied up large quantities of nutrients and thus would not be available for plants, even though they remain in the soil (Charman and Murphy, 2000). Soil pH less than 6.0 increases the solubility of aluminium, manganese and iron, which can be toxic (Gardner et al., 2003).

In the present study, soil organic carbon showed positive correlation with total cyanobacterial species present on the polythene surfaces. Presence of humic substances enhance algal population in soil without acting as direct source of nutrients (Lee and Bartlett, 1976). Another closely related observation was that organic matter content increases the algal incidence due to higher moisture content of the soil (Friedman and Galum, 1974). The ameliorative effect of cyanophycean algae on soils are well documented by Kaushik and Subhashini, (1985). Due to their nitrogen fixing capability, cyanobacteria have a role in soil improvement (Roger and Kulasooriya, 1980). A common factor of microalgal community structure among the sectors of the study area was the dominance of cyanobacteria. The colonization ability of *Oscillatoria acuminatus*, *Arthospira gomontiana, Nostoc muscorum* was confirmed by its presence in a good number in the six study sites. It has been observed (Fritsch, 1907; John, 1988) that Cyanophycean algae represent the major component of the terrestrial microalgal vegetation in tropical regions, whereas Chlorophyceae are the dominant forms in temperate regions.

Cyanophyceaen members showed positive correlation with total nitrogen, available phosphrus and available potassium present in soil of domestic solid waste dumping site. Soil algae can also bind with Na^+ and K^+ ions and thus reduces the soil salinity (Subhashini and Kaushik, l98l). Algae are also reported to bring down the level of oxidizable matter in soil especially sulphate and iron content (Aiyer *et al.,* 1971). Many algae have been found to be capable of solubilizing insoluble phosphate in the soil (Bose *et at*., 1971). The concentration and quality of nutrients are probably more important in the blue-green algal diversity. Availability of phosphates and nitrates are important factors that favor the abundance of cyanophyceace in wetlands (Zancan *et al.,* 2006). The N:P ratio has great influence on cyanobacterial abundance (Hoyos *et al*., 2004). Total number of blue green algal isolates was found positively correlated to the amount of total N and P in soils as observed in the present study. The total number of blue green algal isolates showed negative correlation with organic carbon. This is in conformity with the report that low carbon favors richness of cyanobacteria in soils (Ohtani *et al*., 2000).

were reported by Suseela and Toppo in waste water Algal colonization on polythenes , (,.*et al* Sharma ;(2007 gen found that fifteen algal (2007) Suseela and Toppo .(2014)era viz., *Chaetophora*, *Coleochaete scutata*, *C. soluta*, *Aphanochaete*, *Gloeotaenium*, *Oedogonium*, *Oocystis*, *Oscillatoria*, *Phormidium*, *Chroococcus*, *Aphanothece*, *Fragillaria*, *Cocconeis*, *Navicula* and *Cymbella*.found that (2014) ,.*et al* Sharma *Phormidium tenue, Oscillatoria tenuis, Navicula cuspidat,a Monoraphidium contortum, Microcystis aeruginosa, Closterium costatum* and *Chlorella vulgaris* grown on degrading polythene bags as a substratum in the Lucknow cityrich ,In the present study . *Oscillatoria* with ,bags of domestic solid waste dumping site carry colonisation of algae were found on polythene found to colonise also were *Phormidium* and *Lyngbya* thick mat of ,*Oscillatoria* Besides .dominant genera the being useful in surfaces in the solid wastes is anticipated to be The algae found on polythene .during rainy days quite often and also in the bidegaradation of (1979,James and Evison ;1971 ,Cairns and Dickson) monitoring programmebio .(2014 ,.*et al* Sharma , 2017 *,.et al* Kumar) polythene

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RESEARCH ARTICLE

Efficient biodegradation of low-density polyethylene by cyanobacteria isolated from submerged polyethylene surface in domestic sewage water

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Abstract

Two dominant cyanobacterial species, *Phormidium lucidum* and *Oscillatoria subbrevis*, isolated from submerged polyethylene carry bags in domestic sewage water were found to be capable of degrading low-density polyethylene (LDPE) sheets efficiently. The FT-IR, SEM, NMR, CHN content, thermal, and tensile strength of PE were monitored for structural, morphological, and chemical changes of PE. The CHN analysis corroborated about 4% carbon utilization by the cyanobacterial species from the PE. The rapid growth of cyanobacterial species on the PE surface suggested that the microorganisms continued to gain energy from the PE. The reduction in lamellar thickness, weight, and crystallinity of the cyanobacterial-treated PE pointed to an efficient biodegradation process without any pro-oxidant additives or pretreatment. Alteration in bond indices computed from FT-IR spectroscopy revealed changes in functional group and side chain features indicating biodegradation. The enhanced laccase and manganese peroxidase activity corroborated the biodegradation. The ^{13}C-NMR spectroscopy of the PE is consistent with short branching providing further evidence of biodegradation. Scanning electron microscopy and optical microscopy exhibited large grooves on the surface suggesting significant disruption of polyethylene structure.

Keywords Biodegradation · Cyanobacteria · Domestic sewage water · FT-IR · NMR

Abbreviations

LDPE	Low-density polyethylene
FT-IR	Fourier transform infrared
SEM	Scanning electron microscopy
NMR	Nuclear magnetic resonance
CHN	Carbon, hydrogen, nitrogen
APHA	American Public Health Association
TGA-DSC	Thermogravimetry-differential scanning calorimetry
ASTM	American Society for Testing and Materials

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Introduction

Polyethylene is a ubiquitous commodity for packaging purposes throughout the world. Carry bags made of polyethylene due to its low cost and durability have myriad applications in day-to-day life such as their role in packaging, transportation of textiles, manufacturing laboratory instruments, and automobile components (Arutchelvi et al. 2008). The recalcitrant nature of polyethylene due to high molecular weight, complex three-dimensional structures, and hydrophobic nature causes these polyethylene bags resistant to natural environment. They are used as landfills and are usually thrown to natural water bodies (Shah et al. 2009; Nanda et al. 2010). Consisting of carbon and hydrogen polymers, polyethylene is remarkably resistant to biological decay; it can be degraded to some extent by sunlight and oxygen resulting brittleness and loss of tensile strength without proportionate loss of mass, while degradation by mechanical forces may merely lead to smaller pieces (Potts 1984). Currently, both marine ecosystem and urban areas are beset with problems of polyethylene disposal posing severe environmental threats (Caruso 2015). Durability and undesirable accumulation of synthetic polymers in the natural

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ecosystem and habitats continue to be major concerns. Though bioremediation is considered a potential tool to reduce the adverse effects, plastic waste recycling has largely remained an unsuccessful outcome (Shah et al. 2008; Ojo 2007; Bonhomme et al. 2003).

Algae are known to colonize on such polyethylene material submerged in waste water by mucilaginous secretion of extracellular polymeric substances (EPS) (Suseela and Toppo 2007; Sharma et al. 2014; Sarmah and Rout 2017). Colonization of microbial communities on polyethylene surfaces depends largely on the environmental factors, which may provide an ideal substratum for the colonization (Pathak and Navneet 2017). The polyethylenes thrown into water bodies being exposed to the sunlight are broken into small pieces by bacterial and algal attachment (Seneviratne et al. 2006). Direct degradation of PE by microorganisms utilizing only the polymer as sole carbon source has been documented (Roy et al. 2008). Biodegradation of polyethylene is a natural process aided by microorganisms such as bacteria, fungi, actinomycete, or algae (ASTM 1993; Ghosh et al. 2013; Qi et al. 2017; Ahmed et al. 2018). The degradation rate of polyethylene depends on the crystallinity, surface treatment, additives, molecular weight, and surfactants. Further, enzymes, both extracellular and intracellular types, play a crucial role in biological degradation (Gu 2003). While polyethylene biodegradation by bacteria, fungi have been quite extensively studied; studies related to the potential of algae have received only meager attention (Suseela and Toppo 2007; Kumar et al. 2017). Previous research dealt with biodegradation potential of PE by *Anabaena spiroides*, *Scenedesmus dimorphus*, and *Navicula pupula* (Kumar et al. 2017). Selection of microorganisms for biodegradation of polyethylene is crucial. The microorganisms with enhanced capacity to produce oxidative and lignolytic enzymes are more efficient in the biodegradation of polyethylene (Nayak and Tiwari 2011). We conjectured that dominant algae growing on polyethylene substrata utilizing the polymer carbon in oligotrophic conditions could be ideal candidates for test experiments. Thus, it was deemed fit to explore the efficacy of cyanobacteria as polyethylene biodegrading agent. Accordingly, the present work deals with PE biodegradation potential of two cyanobacterial species, *Phormidium lucidum* and *Oscillatoria subbrevis*, obtained from algal colonized submerged polyethylene substrates in domestic sewage water. The mechanism of biodegradation has been expounded by spectroscopic, morphological, mechanical, and thermal studies. The ASTM (2000) standards were followed for the present study. The ASTM D 5338-98 has been chosen for the tests in determining the aerobic biodegradation of polyethylene materials under controlled composting conditions. The aqueous test method, Test Method D 5247 (specific microbe test), which uses pure microbial cultures to assess the biodegradability of materials based on weight loss has been used in the present study. For ascertaining degradation, the standards used were as follows: ASTM D 3593 Test Method (FTIR and NMR), ASTM D 638 Test Method (SEM), ASTM D 638 Test Method (tensile properties), and ASTM D 5247 Test Method (weight loss).

Materials and methods

Materials

PE strip preparation

PE sheets of 20-μ thickness were dried under ambient conditions and cut into strips (1 cm × 1 cm), washed with 70% ethanol followed by distilled water.

Isolation of cyanobacteria

Domestic sewage water was collected in clean polythene bottles; samples were transported to the laboratory, stored at 4 °C, and analyzed within few hours after arrival in the laboratory. Standard protocols were followed for domestic sewage water analysis (APHA 2005). Algal colonized submerged polyethylene substrates were collected from sewage water of Silchar town in the state of Assam, India (Fig. 1a, b). The samples colonized on waste water submerged polyethylene carry bags were collected and observed under microscope, and two dominant species were screened for monoculture development. The algal samples were centrifuged, pellets were homogenized in sterilized BG-11(N+) medium, and the suspension was placed onto agar petri plates by pour plate method. The plates were incubated for 15 days under continuous illumination (2000 lx) at 24 ± 1 °C (Rippka et al. 1979). The colony developed in the agar media has been maintained in liquid BG-11(N+) medium (Table 1). The algal samples were observed under microscope and identified by diagnostic morphological features (Prescott 1952; Desikachary 1959).

Biological treatment of PE

Based on dominance over the occurrence of other algae on submerged polyethylene bags in domestic sewage water of Silchar town, two cyanobacterial species, *Phormidium lucidum* and *Oscillatoria subbrevis*, were selected for PE biodegradation assay. The PE strips were sterilized before incubation. In each conical flask, 400 ml of BG-11(N+) media with minimal carbon source and 4 ml of cyanobacterial culture were added. Ten pieces of pre-weighed PE strips (1 cm × 1 cm) were aseptically transferred to the flasks with mild shaking (Fig. 2). PE strips in BG-11 medium with no cyanobacteria added served as an abiotic control and that with

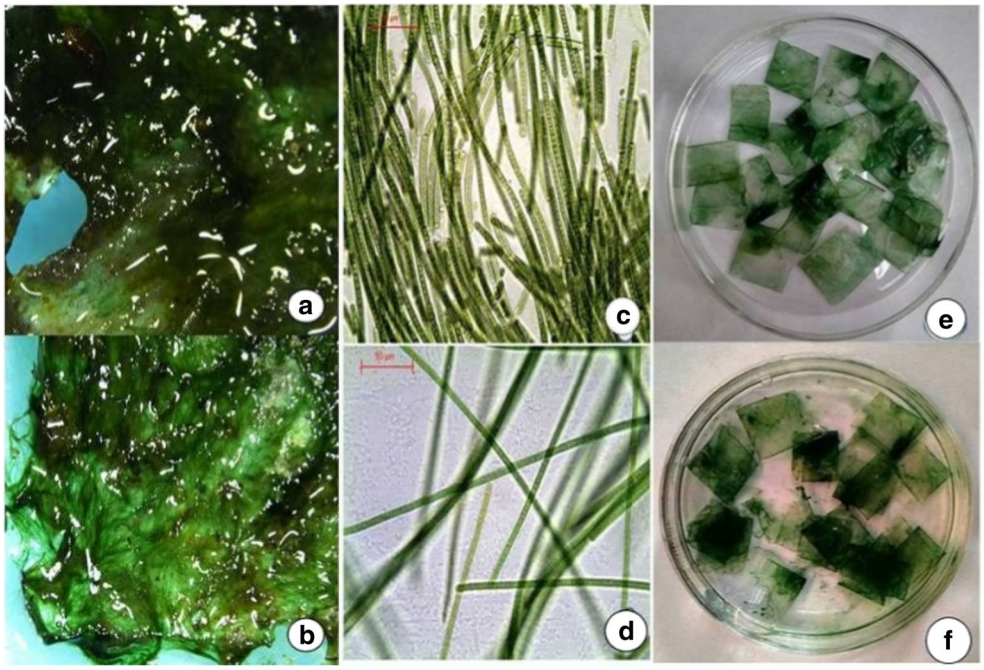

Fig. 1 **a**, **b** Algal colony on PE surface. Photomicrographs of **c** *Phormidium lucidum* and **d** *Oscillatoria subbrevis*. **e**, **f** Algae colonizing on test PE strip

only cyanobacteria, and no PE strips were set up in parallel as a biotic control.

Biodegradation studies

Optical microscopy

The changes on the surface of PE were monitored at × 80 magnification with an optical reflection microscope (StereoZoom Leica S8 APO).

SEM analysis

The surface morphology of the PE strip was observed through scanning electron microscopy (JEOL equipment, Japan, Model: JSM 6390 LV). The Pt-coated PE strips were placed on the sample holder and scanned at magnifications of × 1000, × 3000, × 5000, and × 10,000.

Table 1 The composition of the BG-11 medium per 1000 ml of distilled water

Ingredients	Amount (g)
Sodium nitrate	1.500
Dipotassium hydrogen phosphate	0.0314
Magnesium sulfate	0.036
Calcium chloride dihydrate	0.0367
Sodium carbonate	0.020
Disodium magnesium EDTA	0.001
Citric acid	0.0056
Ferric ammonium citrate	0.006

Final pH after sterilization (at 25 °C): 7.1

FT-IR spectroscopy

The structural changes in PE surface were analyzed by a Nicolet (USA) equipment (Model: Impact 410) at a resolution of 1 cm^{-1}, in the frequency range of 4000–400 cm^{-1}. Relative absorbance intensities (I) of the ester carbonyl bond at 1740 cm^{-1}, keto carbonyl bond at 1715 cm^{-1}, terminal double bond (vinyl) bond at 1650 cm^{-1}, and internal double bond at 908 cm^{-1} to that of the methylene bond at 1465 cm^{-1} were evaluated (Albertsson et al. 1987): keto carbonyl bond index (KCBI) = I_{1715}/I_{1465}, ester carbonyl bond index (ECBI) = I_{1740}/I_{1465}, vinyl bond index (VBI) = I_{1650}/I_{1465}, internal double bond index (IDBI) = I_{908}/I_{1465}. The crystallinity of the PE strips was measured using the formula: crystallinity (%) = $100 - [(1 - (I_a / 1.233I_b) / 1 + (I_a/I_b)) \times 100]$; I_a and I_b are the intensities of absorption bands at 1474 and 1464 cm^{-1}, respectively (Zerbi et al. 1989). The constant 1.233 corresponds to the relations of intensity bands of fully crystalline PE.

CHN analysis

The percentage of carbon, hydrogen, and nitrogen was measured using 0.1 g of dried, ground, and homogenized PE sample using a CHN Analyzer (Perkin Elmer, USA Model: 2400 Series 2).

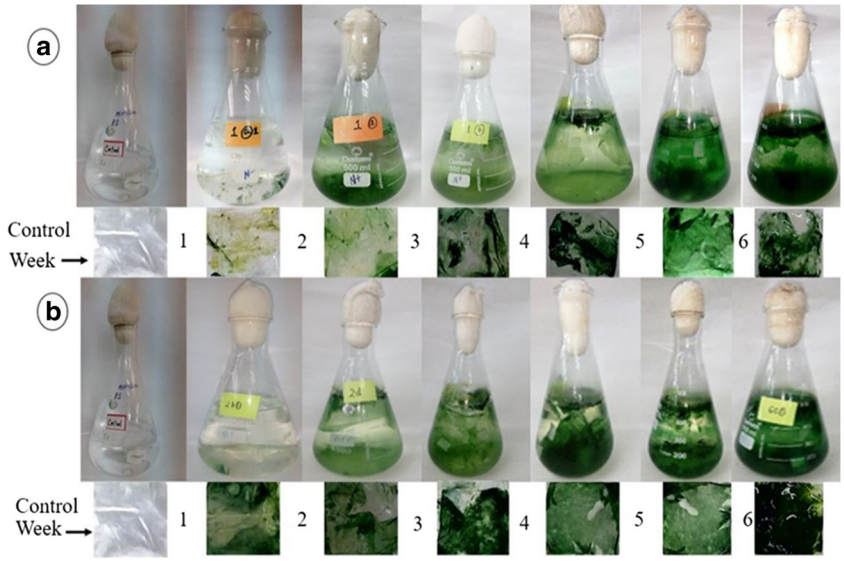

Fig. 2 Biological treatment of PE strips with **a** *Phormidium lucidum* and **b** *Oscillatoria subbrevis*

TGA-DSC analysis

Melting point (T_m) of the 0.1-g PE strips and enthalpy changes were analyzed by a Shimadzu Thermal Analyzer (TGA-DSC) (Model: TGA-50 & DSC-60). The lamellar thickness, L_c ($L_c = 2\sigma e/\Delta h$ (T_{m0}/T_{m0}-T_m), was estimated from the melting point following a modified procedure (Hoffman et al. 1976).

Tensile property

The tensile strength, percentage of elongation at break and modulus of elasticity, and the extension of the material under load were measured by a universal testing machine (Model: KUT-100 (E) UTNSRL).

Cyanobacterial growth and PE degradation

The growth of cyanobacteria in BG-11 only and on PE strip was measured separately by estimating the chlorophyll *a* on each alternate day for a period of 6 weeks (Kobayasi 1961). The growth curves and specific growth rates (μ h^{-1}) were calculated in log period as per Myers and Kratz (1955). The total carbohydrate (Spiro 1966) and protein (Herbert et al. 1971) content were analyzed. The extracellular polymeric substances of the cyanobacterial species were estimated by the method of Underwood et al. (1995). The weight changes of PE strips were determined using an electronic balance (Mettler Toledo RS 231C).

NMR spectroscopy

The PE strips were analyzed using ^{13}C-NMR spectroscopy on a JEOL equipment (Japan Model: ECS-400). For analysis, PE strips of both control and treated were dissolved in 1,2,4-trichlorobenzene in 10-mm tubes at 120 °C and a few drops of dimethyl sulfoxide were added as an internal lock. Hexamethyldisiloxane was used as chemical shift reference. Spectra were recorded in complete decoupling mode under the following conditions: pulse interval, 1 s; pulse delay, 5 s; spectral width, 500 Hz; number of accumulations, 1500–2000; number of data points per spectrum, 8000.

Enzyme activity

A 1-ml culture from both cyanobacterial treatments was added separately to 1 ml of 2 mM guaiacol and 3 ml of 10 mM sodium acetate buffer (pH 4.6). The reaction mixture containing guaiacol and sodium acetate was incubated at 30 °C for 15 min, and the absorptions were recorded in a UV-Vis spectrophotometer at 450 nm. One unit of laccase activity was defined as amount of enzyme required to hydrolyze guaiacol during incubation period. For manganese peroxidase, a similar procedure was followed with 1 ml of H_2O_2 added (Papinutti and Martinez 2006).

Data availability All data generated or analyzed during this study are included in this published article.

Results and discussion

Domestic sewage water quality and cyanobacteria

The temperature of natural water bodies is an important parameter for cyanobacterial growth. The water temperature was recorded to be 32–34 °C during the summer when algal

colonized polyethylenes were collected. The color of the waste water was black. The values of BOD, DO, and COD were 600 ± 0.34, 1.2 ± 0.04, and 1502 ± 0.32 mg/l, respectively. The total alkalinity was 10 ± 1.4 mg/l, free CO_2 was 64 ± 0.13 mg/l, and TDS was found to be 560 ± 0.18 mg/l. The suspended solid was 50 ± 0.54 mg/l. The chloride and calcium contents were found to be 168 ± 0.67 mg/l and 68 ± 0.13 mg/l, respectively. The sulfate, nitrate, and magnesium concentrations were 214 ± 1.6 mg/l, 12 ± 1.5 mg/l, and 30 ± 0.23 mg/l, respectively. The ammonia and phosphate contents were 30 ± 0.45 mg/l and 70 ± 0.45 mg/l, respectively. The physicochemical characteristics of the domestic sewage water of Silchar town were rather similar to those reported recently (Sarmah and Rout 2017).

The colonization of *Phormidium lucidum* and *Oscillatoria subbrevis* on PE surface and photomicrographs of isolated cyanobacteria are given in Fig. 1a–d. The species, *Phormidium lucidum* and *Oscillatoria subbrevis*, were found to be profusely distributed in submerged polyethylene surface of domestic sewage water with the latter recorded as the largest genus forming mat-like colonization in submerged polyethylene bags. The genera, *Phormidium* and *Oscillatoria*, were also found to occur on PE surfaces elsewhere. In a recent field-based study conducted in oligotrophic water bodies of Lucknow, Uttar Pradesh, 15 algal genera, viz., *Chaetophora*, *Coleochaete scutata*, *Coleochaete soluta*, *Aphanochaete*, *Gloeotaenium*, *Oedogonium*, *Oocystis*, *Oscillatoria*, *Phormidium*, *Chroococcus*, *Aphanothece*, *Fragilaria*, *Cocconis*, *Navicula*, and *Cymbella*, were found to be colonized on the surface of polyethylene (Suseela and Toppo 2007). Several species of algae, *Phormidium tenue*, *Oscillatoria tenuis*, *Navicula cuspidata*, *Monoraphidium contortum*, *Microcystis aeruginosa*, *Closterium costatum*, *Chlorella vulgaris*, and *Amphora ovalis*, were found to colonize on waste polyethylene materials in various ponds, lakes, and water bodies of Kota City in Rajasthan (Sharma et al. 2014).

Optical microscopy

The algae growing on PE surface in the field were observed under the microscope to check the attachment of microorganisms to the polyethylene surface (Arutchelvi et al. 2008). The colonization of *Phormidium lucidum* and *Oscillatoria subbrevis* on the experimental PE (Figs. 1e, f and 3) started after 1 week of inoculation which is attributed to initial degradation leading to insertion of hydrophilic groups to the PE strips as microorganisms prefer hydrophilic surface (Vasile 1993).The cyanobacterial species growing on the polyethylene surfaces were firmly attached on the PE surfaces and are not removable by water jet. It is presumed that the cyanobacterial colonization on the PE surface occurred utilizing the polymer as carbon source (Kumar et al. 2017). The cellular contents (carbohydrates and protein) of the species

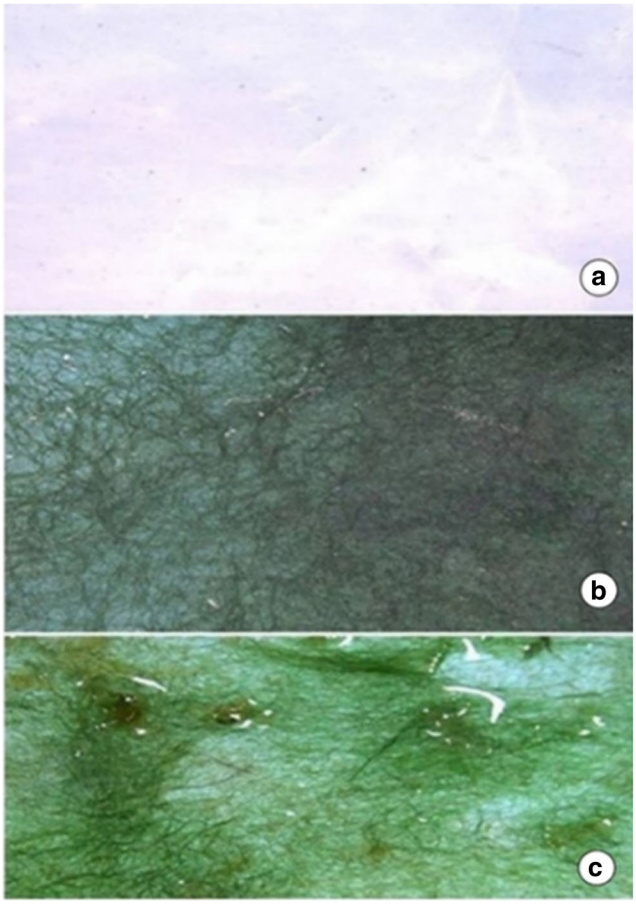

Fig. 3 Surface morphology of PE strip. **a** Abiotic control. **b** *Phormidium lucidum*-treated PE. **c** *Oscillatoria subbrevis*-treated PE strip after 6 weeks

growing on polyethylene surface were found to be higher relative to the biotic control. Accordingly, the specific growth rate (doubling time) of the cyanobacteria on polyethylene surfaces was also found to be higher than that of the biotic control. This provided clear evidence of PE carbon utilization by the cyanobacteria. Studies on degradation of polypropylene and bioriented polypropylene in the field condition have shown that microorganisms adhered the surface of the samples to initiate biodegradation (Longo et al. 2011). The species belonging to the families Chlorophyceae, Cyanophyceae, and Bacillariophyceae are the common occurrence on the surface of the polyethylene bags (Kumar et al. 2017).

SEM analysis

The adhesion of algae to the PE surface is a fundamental prerequisite for biodegradation. High surface hydrophobicity of polyethylene surface limits the alga colonization process. Surface erosion, formation of pits, and cavities on the surface of the treated PE were observed after 6 weeks of treatment (Fig. 4a_1, a_2, b_1, b_2, c_1, c_2). The surface of the strips treated by *Oscillatoria subbrevis* registered relatively higher damage

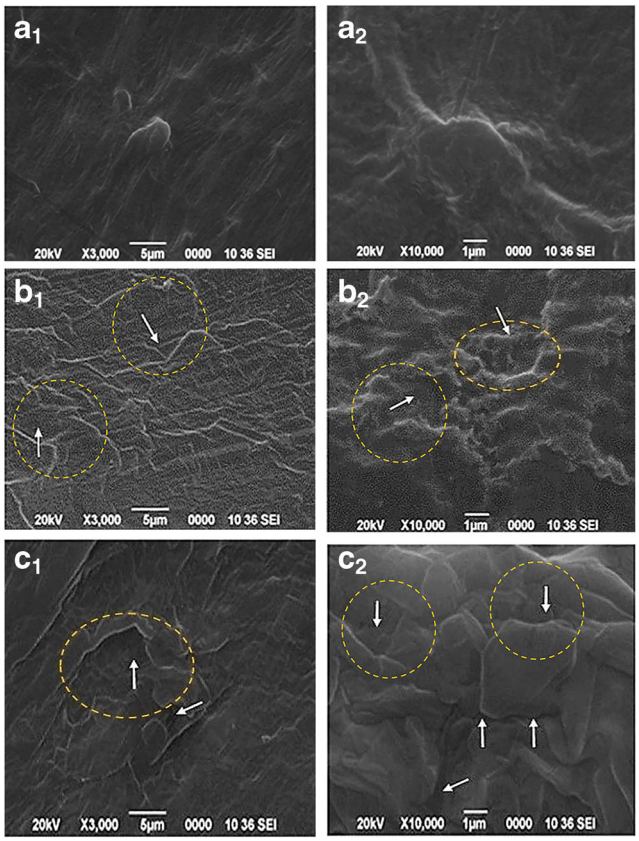

Fig. 4 Scanning electron micrographs of the surface of abiotic control PE strip (a_1, × 3000) (a_2, × 10,000) and PE strip after incubation with *Phormidium lucidum* (b_1, × 3000) (b_2, × 10,000) and *Oscillatoria subbrevis* (c_1, × 3000) (c_2, × 10,000) (treatment with BG-11 media and 2% SDS)

than that of *Phormidium lucidum*-treated strips. The *Anabaena spiroides* and *Navicula pupula* colonization on polyethylene surface exhibited rather similar surface features (Kumar et al. 2017). The SEM images provided evidence for the breakdown of the PE into its monomeric components (Sanin et al. 2003). The grooves and cracks confirmed the fragility of the PE strip on treatment with the cyanobacterial strains. Comparison with the control sheet clearly attested degradation. The grooves on the surface testify carbon utilization from the surface of polymer which upset the uniform branching of polymer matrix (Manzur et al. 2004). The corrosion of PE surface by cyanobacteria was scattered, not uniform, indicating that the amorphous region of the polymer was more susceptible to cyanobacterial adhesion and degradation. It is generally believed that cyanobacterial attack is mainly limited to surface or near-surface accessible particles (Albertsson et al. 1994).

FT-IR spectroscopy

FT-IR spectroscopy has been used to assess the biodegradation of polyethylene strips. Spectra of control PE strip displayed a number of absorptions reflecting the complex nature of the PE (Fig. 5a). Variations were noted in the intensity of bands in different regions when incubated with *Phormidium lucidum* and *Oscillatoria subbrevis* (Fig. 5b, c). Significant changes were noted for both the cyanobacterial species. The intensity of some peaks (2364 cm^{-1}) reduced more in *Oscillatoria subbrevis* whereas those at 2326 and 2850 cm^{-1} became sharper in the treated sample than those in the control. The peak observed at 2919 cm^{-1} in *Phormidium lucidum-treated* strips attributed to free OH combination while a new peak at 1633 cm^{-1} corresponding to C=O in *Oscillatoria subbrevis* adduced support to depolymerization activity of the cyanobacterial isolates. A peak at 1369 cm^{-1} in the control PE strip usually observed for 1000C short branching is found missing in the treated PE strips presumably owing to cyanobacterial degradation (Blitz and McFaddin 1994). The FT-IR spectrum of *Phormidium lucidum*-treated PE showed peak at 3422 cm^{-1} assigned to υ_{OH} mode indicating the occurrence of alcohol.

The peak at 1629 cm^{-1} for υ_{CO} mode originated from the presence of carboxylic acid group. For *Oscillatoria subbrevis*-treated PE, peaks at 667 and 468 cm^{-1} confirmed the presence of nitrogen-containing bio-ligands. The υ_{CO} peak at 1633 cm^{-1} is concordant with the presence of carboxylic group. The FT-IR signatures of ester, keto, vinyl, and internal double bond were quite diagnostic, and the corresponding indices validated PE biodegradation (Albertsson et al. 1987; Gardette 2006). The KCB, ECB, VB, and IDB indices were all found to be higher in the treated *Phormidium lucidum* case. A relatively higher value of the KCB and EBC of the cyanobacterial-incubated PE strips has been attributed to enzymatic activity of the organisms (Albertsson et al. 1994) (Fig. 6). However, when incubated with *Oscillatoria subbrevis*, the bond indices KCB, ECB, and VB though registered an increase, the IDB got reduced. In a previous study involving bacteria, *Arthrobacter* sp., an increase in the bond indices was noted while that with *Pseudomonas* sp., the IDB index was reported to be lower (Balasubramanian et al. 2010). The keto and ester carbonyls have been reported as major degraded products in the presence of enzyme oxidoreductase (Karlsson and Albertsson 1998). The formation of double bonds in the treated PE strip may have been effected by Norrish type II photochemical reaction (Albertsson et al. 1987; Chiellini et al. 2003). The crystallinity of PE strips was found to decrease up to 2% and 62% after incubation with *Phormidium lucidum* and *Oscillatoria subbrevis*, respectively. The free radical driven chain scission may have disrupted the crystalline order (Restrepo-Flórez et al. 2014). The cyanobacteria, in the present case, are believed to access the amorphous regions of the polymer most (Roy et al. 2008; Khabbaz et al. 1999; Patani and Sorrentino 2013). Cyanobacterial interaction may enhance the surface hydrophilicity of the PE by the formation of additional groups such as "carbonyl" that can be utilized by

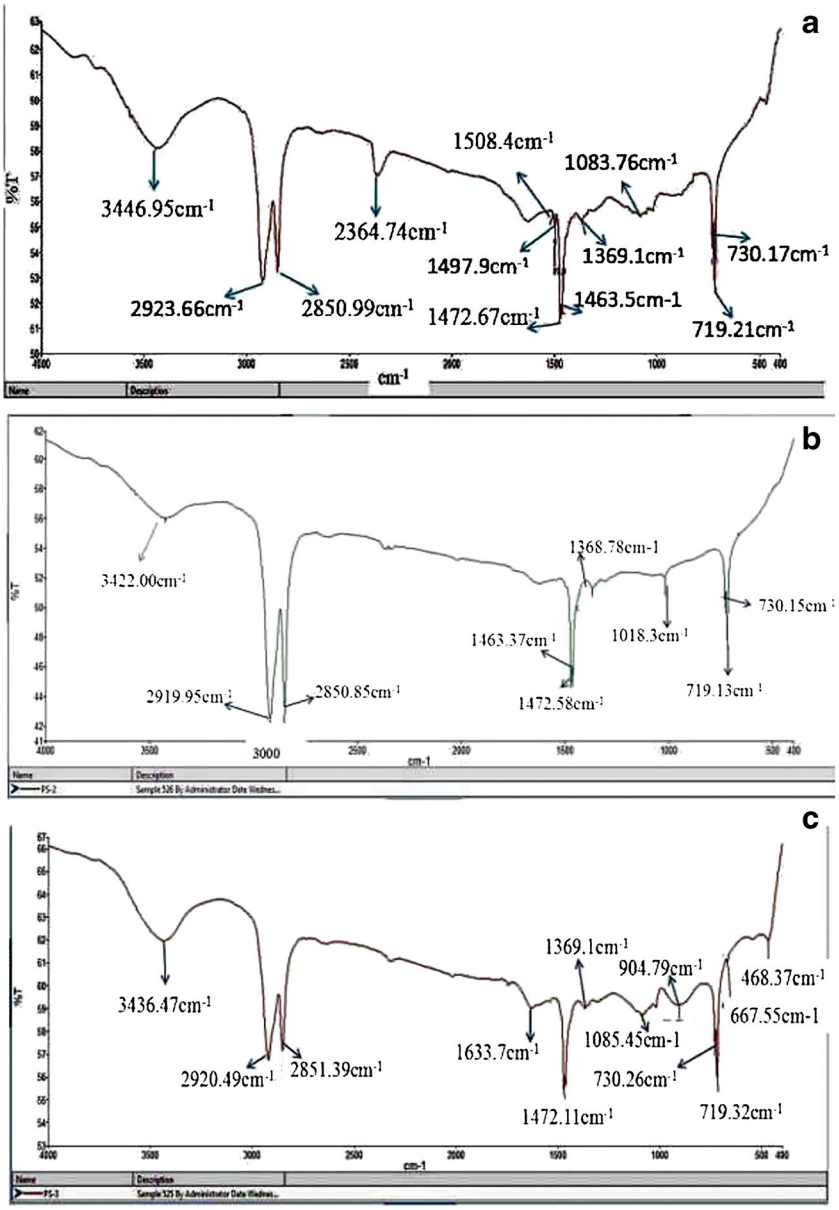

Fig. 5 FT-IR spectra of PE. **a** Abiotic control. **b** *Phormidium lucidum* treated. **c** *Oscillatoria subbrevis* treated

the microorganisms (Albertsson 1980; Ibiene et al. 2013). The carbonyl index increased considerably for both the algal-treated PE strip, a key marker for biodegradation. It is pertinent to mention herein that 2% SDS buffer solution has been

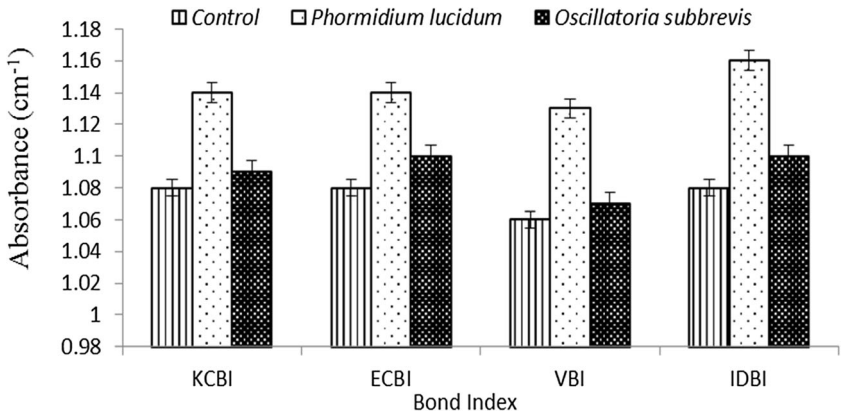

Fig. 6 Carbonyl and double bond indices as determined from FT-IR data of pre- and post-treated (6 weeks) PE

used for washing the PE strips before recording the FT-IR spectra to remove any extraneous substances such as microbial metabolites or culture media from the strips (Gu 2017).

CHN analysis

Carbon analysis after 6 weeks of incubation revealed the percentage of carbon in the control PE strips to be 84%. The extent of carbon utilized by *Phormidium lucidum* from the PE strip was 3% whereas *Oscillatoria subbrevis* utilized 4%. For polyethylene treated with bacteria, *Achromobacter denitrificans* have shown 2% of carbon being utilized from polymer by the bacteria after long exposure (Devi et al. 2015). The hydrogen and nitrogen in control PE strip were 14.50% and 0.12%, respectively. The treated ones showed hydrogen and nitrogen to be about 15% and 0.1%, respectively. Low nitrogen availability (0.12%) is a limiting factor for microbial growth. The adhesion mechanism of the cyanobacteria to PE surface may be attributed to the low carbon availability in the medium and confirms its ability to use the PE as a carbon and energy source (Awasthi et al. 2017a).

TGA-DSC analysis

Melting points for the treated PE strips were found to be slightly lower than those for the control (Fig. 7a). For *Phormidium lucidum*, onset temperature was 121.56 °C (heat enthalpy − 42.17 mJ) (Fig. 7b). For *Oscillatoria subbrevis*, the onset temperature was 121.37 °C (heat enthalpy − 74.41 mJ (Fig. 7c).The results from the present study demonstrated that heat release was relatively much higher in *Oscillatoria subbrevis*. For biodegradation of polyethylene by the bacteria *Aspergillus* sp., a reduction in melting point by 1.2 °C has been noted (Raaman et al. 2012). The melting and crystallization temperature of PE treated by *Phormidium lucidum* is found to be higher than that treated by *Oscillatoria subbrevis* in the present experiment. Formation of high molecular weight substances in *Phormidium lucidum*-treated case is believed to be one primary reason for this (Farukkawa et al. 2006). The TGA traces (Fig. 8a) of cyanobacterial-treated PE strips showed that initial decomposition temperature of the control PE strip decreased from 88 to 20 °C (*Phormidium lucidum*) and to 18 °C (*Oscillatoria subbrevis*), respectively. The weight loss versus temperature curve (Fig. 8a) showed a marked decrease in thermal stability of the treated PE strips. Similar decrease in the onset temperatures and corresponding weight loss were also observed in fungus-treated polyethylene films, though, in the presence of pro-oxidant (Corti et al. 2010). The lamellar thickness (L_c ~ 110 nm) for control PE strip following cyanobacterial exposure got reduced to 97.78 nm and 54.45 nm for *Phormidium lucidum* and *Oscillatoria subbrevis* treatments, respectively. The results are congruent with the observed trends in the melting points and may be explained by invoking the cyanobacteria's quest for energy with concurrent cleaving and rearrangement of chemical bonds.

Tensile property

The changes in the tensile strength and elongation at break due to cleavage of the polymer chain by the cyanobacterial species were monitored. The cyanobacterial-treated PE strips expectedly registered a reduction in tensile strength to ~ 7 from 11.8 MPa. The elongation break of *Phormidium lucidum*- and *Oscillatoria subbrevis*-treated PE strips was observed at 254 and 243 compared to 299% of extension of control strip. A reduction of 42.5% tensile strength of PE, 31.5% reduction in elongation, and 28.8% reduction in modulus of elasticity for polyethylene were noted earlier following biodegradation (Devi et al. 2015).

Growth study and PE degradation

Cyanobacterial growth on test PE got initiated from the first week of treatment itself. Cyanobacterial growth in PE strips was studied with reference to the biotic control (without PE strips, Fig. 8b). The growth of cyanobacterial species on PE was found to be higher in terms of chl *a* by about 3.5 μg/ml from that of the respective biotic control at the end of 42 days. The fast growing *Phormidium lucidum* showed specific growth rate (μ) in biotic control and on PE strips to be 0.134 ± 0.12 (generation time, $G = 178.25 \pm 0.45$ h) and 0.123 ± 0.12 ($G = 182.21 \pm 0.23$ h), respectively. The fast growing *Oscillatoria subbrevis* showed specific growth rate (μ) in biotic control and on PE strips to be 0.158 ± 0.23 (generation time, $G = 151.34 \pm 0.67$) and 0.143 ± 0.23 ($G = 156.24 \pm 0.37$), respectively. This can be attributed to the acclimatization of cyanobacteria in the PE strips with the degraded carbon source from the polyethylene (Arutchelvi et al. 2008). The algal growth increased with time and after a period of 6 weeks found to level off. In the present case, the BG-11 medium used had citric acid, ferric ammonium citrate, EDTA, and Na_2CO_3 as sources of minimal carbon, and hence to annul the effect of such chemicals on the polyethylene substratum, control experiments were simultaneously performed with the BG-11 medium alone (Gu 2017). The enhancement of cellular contents (carbohydrates and protein) of the species growing on PE surface was also observed. The carbohydrate content on PE and biotic control for *Phormidium lucidum* were 245 ± 0.26 μg/ml and 240 ± 0.56 μg/ml, respectively. The carbohydrate content on PE and biotic control for *Oscillatoria subbrevis* were 377 ± 0.26 μg/ml and 370 ± 0.56 μg/ml, respectively. The protein content of *Phormidium lucidum* on PE and biotic control were 214 ± 0.12 μg/ml and 210 ± 0.56 μg/ml, respectively. The protein content of *Oscillatoria subbrevis* on PE and biotic control were 240 ± 0.12 μg/ml and 230 ± 0.56 μg/ml, respectively.

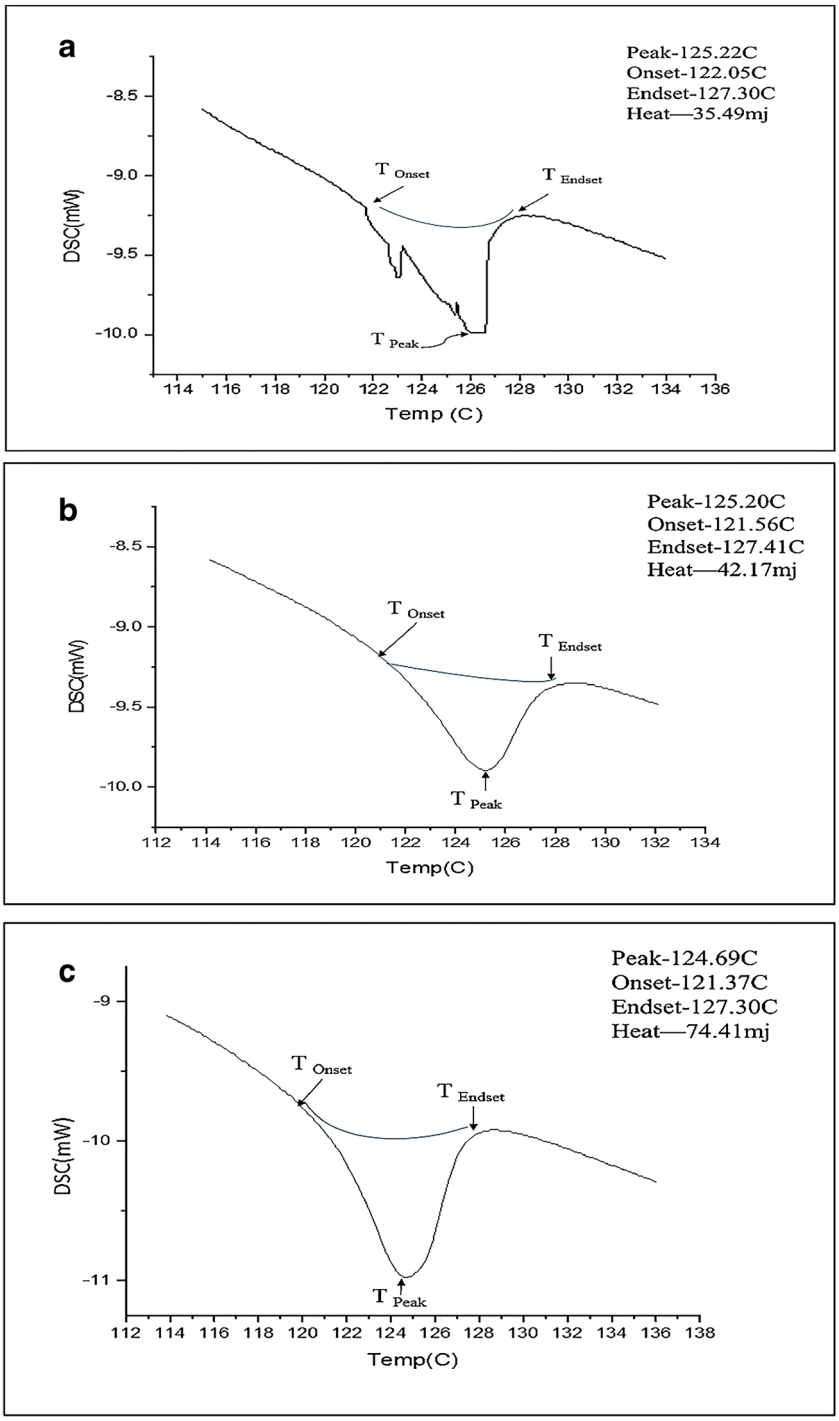

Fig. 7 Melting points of PE. **a** Abiotic control. **b** *Phormidium lucidum* treated. **c** *Oscillatoria subbrevis* treated

Studies also revealed that gram-negative bacteria could easily adapt to the environment rich in polyethylene and similar polymers. Since cyanobacteria are gram-negative, it is reasonable to conclude that they colonize the PE surface utilizing the polyethylene as partial carbon source (Dey et al. 2012). The structure of cell wall of gram-negative bacteria comprising either homopolysaccharides or heteropolysaccharides is known to impart mechanical stability enabling adhesion and cohesion on the polymeric surface and negotiate dynamic environmental conditions (Garrett et al. 2008). The cyanobacterial colonization on the polyethylene surface was observed to be maximum at the end of log phase. Extracellular polysaccharide (EPS) is believed to have aided in intercellular adhesion of cyanobacteria to the polyethylene surface (Dunne 2002). In the present study, EPS of cyanobacterial species were estimated to be 19.99 and 22.43 μg/ml for *Phormidium lucidum* and *Oscillatoria subbrevis*, respectively. The rapid growth of cyanobacteria on

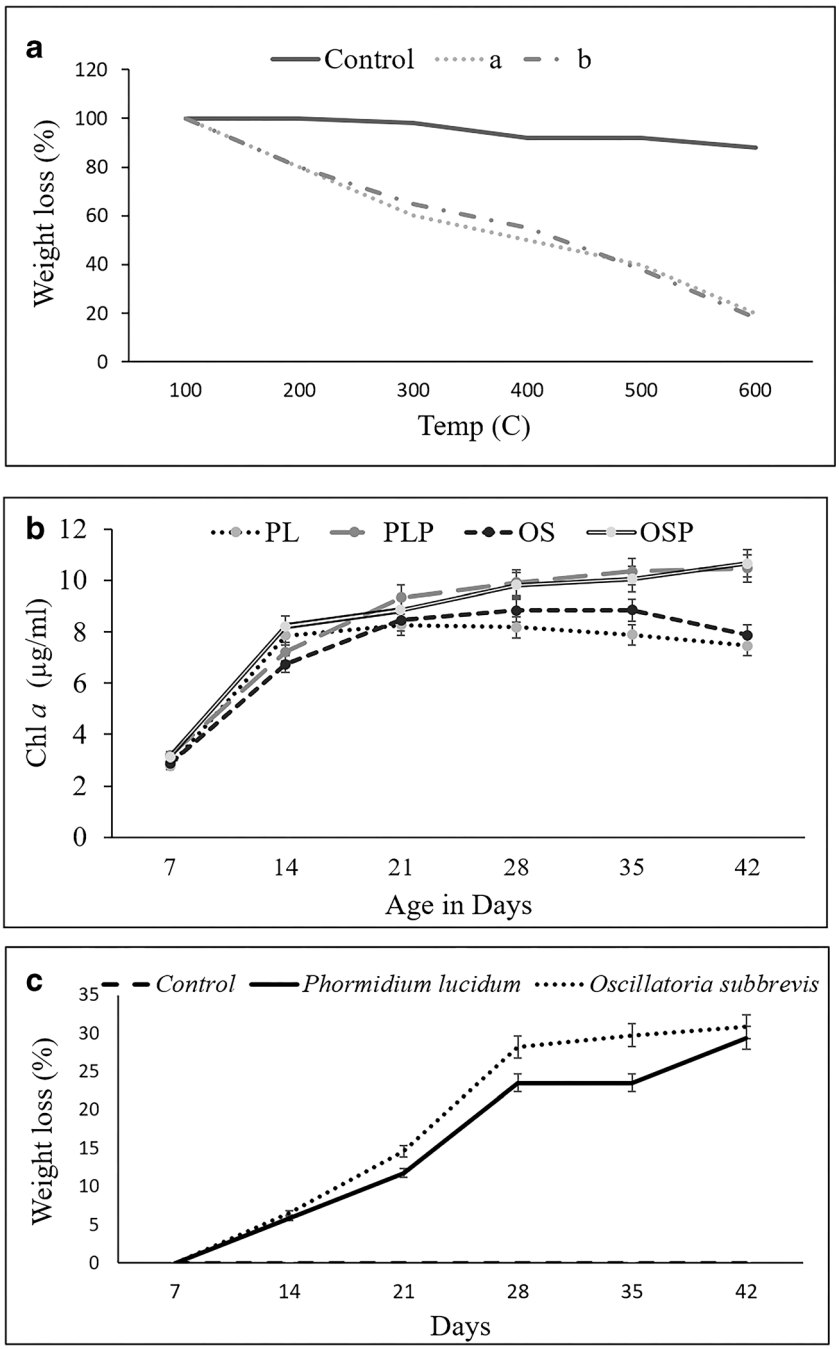

Fig. 8 **a** Change in the thermal behavior of PE due to cyanobacterial exposure (abiotic control, (a) *Phormidium lucidum*, (b) *Oscillatoria subbrevis*). **b** Growth pattern of algae on PE for a period of 6 weeks. **c** Percentage of weight loss of PE after incubation with algae, *PL *Phormidium lucidum*, PLP *Phormidium lucidum* on PE, OS *Oscillatoria subbrevis*, *OSP Oscillatoria subbrevis* on PE

the PE may be attributed to metabolizable compounds from the polymers (Koutny et al. 2006a). It is assumed that a significant amount of low molecular weight compounds is released to aqueous media from the PE strips (Koutny et al. 2006b).

The degradation for PE strips by the cyanobacterial species was monitored weekly by weight loss measurements. Weight loss of polyethylene is an important and well-recognized indicator of biodegradation. The highest reported weight loss so far by any microorganism appears to be ~ 60% after 3 months (Rajandas et al. 2012). In the present case, the weight loss associated with the cyanobacteria, *Phormidium lucidum*- and *Oscillatoria subbrevis*-treated PE strips, was found to be about 30% after 42 days (Fig. 8c). In the abiotic control (without inoculation), no significant weight loss was observed, thus ruling out any effect of the nutrient chemicals of the culture medium on the polyethylene substratum. Previous study involving polyethylene degradation by *Anabaena spiroides*, *Scenedesmus dimorphus*, and *Navicula pupula* recorded weight loss up to 8% only (Kumar et al. 2017). The reduction in weight of cyanobacterial-treated PE strips in the present study vis-a-vis the dual control used in the experiment unequivocally represents significant biodegradation by the tested

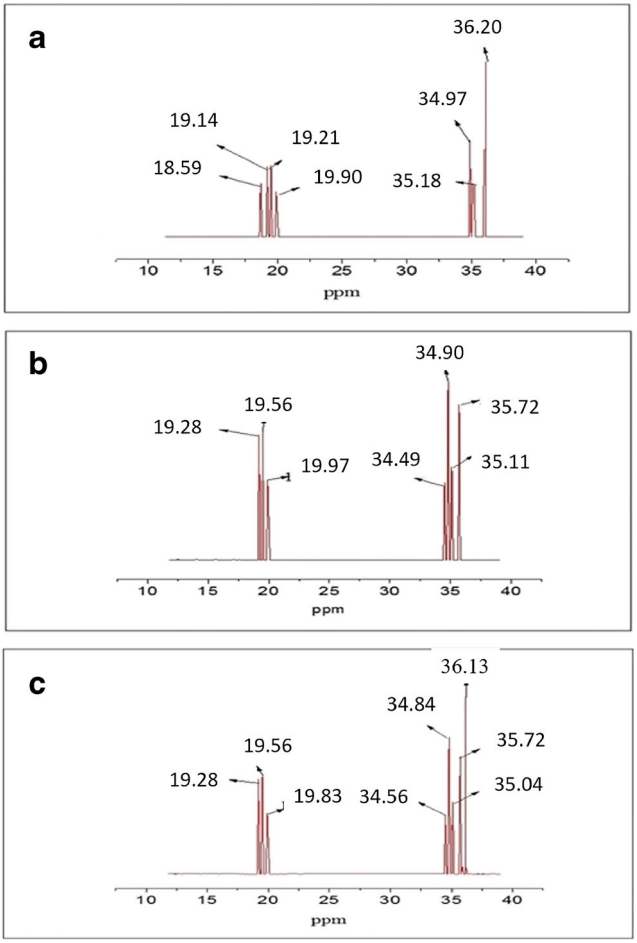

Fig. 9 ^{13}C-NMR of PE. **a** Abiotic control. **b** *Phormidium lucidum* treated. **c** *Oscillatoria subbrevis* treated

cyanobacteria (Awasthi et al. 2017b). In a recent study, about 81 ± 4% and 38 ± 3% weight loss was observed for LDPE strips and pellets, respectively, by *Enterobacter* sp. and *Pantoea* sp. bacterial consortia screened from plastic garbage processing area (Skariyachan et al. 2016).

NMR spectroscopy

The ^{13}C-NMR spectroscopy (Fig. 9) of the cyanobacterial-treated PE vis-à-vis the untreated PE ones (abiotic control) revealed microstructural changes in the polyethylene. The absorption peaks centered at 20 ppm in the control and treated PE strips are believed to have originated from common plastic additives like phosphoric acid esters. The carbon signals observed at 35 (multiplet) and 36.20 ppm in the control PE got altered slightly both in terms of position and multiplicity in the treated ones (Fig. 9b, c). Multiplet character of the signal at 34.5 ppm in the treated polyethylene can be assigned to carbonyl group of the acid moiety (Brandolini and Hills 2000). This observation may be correlated with formation of ester as end product and initiation of short branching due to degradation. It is quite plausible that degraded polyethylene furnished an organic soluble fraction implying a carbon uptake process linked to the metabolic pathway of the cyanobacteria (Miyazaki et al. 2012; Balasubramanian et al. 2014). Formation of carboxylic acid and other byproducts of polyethylene degradation is believed to facilitate carbon assimilation (Arnaud et al. 1994).

Enzymatic activity

Activity of laccase (0.009 IU/ml) was higher as compared to manganese peroxidase (0.0075 IU/ml) after 6 weeks treatment for both the cyanobacteria. High molecular weight polymer limits the enzymatic reactions leading to biodegradation. Two key mechanisms are believe to be operative; one is the reduction of molecular weight and the other oxidation of molecules (Yoon et al. 2012). The breakdown of large polyethylene molecules is in fact believed to have been initiated by enzymatic action accompanied by molecular weight reduction and enhancement of keto carbonyl index in the present case (Santo et al. 2012). Microorganisms are known to produce necessary oxidative and degradative enzymes and assimilate the polymeric carbon into their biomass (Hadad et al. 2005; Tribedi and Sil 2013). We conclude that cyanobacterial enzymes present in the liquid phase of the media interact with the macromolecules available at the surface of the PE strips triggering biodegradation (Chinaglia et al. 2018).

Conclusion

Spectroscopic, enzymatic, thermal, mechanical, and morphological studies in relation to dual experimental control clearly demonstrated efficient polyethylene biodegradation by the cyanobacteria. The fast growing, readily available and easily isolable cyanobacteria are capable of effectively colonizing on the PE utilizing carbon without any pro-oxidant additives or pretreatment. The results are of significance for development of biodegradation protocol for PE using freshwater nontoxic cyanobacteria which are more efficient besides being convenient, easy to handle, and less hazardous as compared to other bacteria or fungi. Under natural conditions, these cyanobacterial species have the potential to degrade polyethylene even more efficiently furnishing a tangible alternative solution to polyethylene waste management.

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Compliance with ethical standards

There is no research involving human participants and or animals.

Conflict of interest The authors declare that they have no conflict of interest.

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ALGAL COLONIZATION ON POLYTHENES AND EVALUATION OF BIODEGRADATION POTENTIALS

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

By

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To

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Chapter 10

General Discussion

Polyethylene are well known for packaging films, as well as in making commercial polyethylene bags. They degrade very slowly in comparison to pro-oxidant containing polyethylene. The degradation of polyethylene depends on the presence of impurities, carbonyl and hydroperoxides groups introduced during manufacturing processes. Indiscriminately dumping of polythenes into the sewage water and landfills are known to emit dangerous methane and carbon dioxide gases during their decomposing stages as well as highly toxic leachates (Simmons, 2005). It effectively blocks sewerage pipe lines, litters agricultural lands, canals, rivers and oceans. They are not biodegradable or take incredibly long time to break down into powdery plastic dusts which contaminate the soil and the water adversely affecting all life forms (Stevens, 2001). Algae are known to colonise on polyethylene surfaces submerged in waste water and domestic solid waste dumping site. Study of growth of algal species on such polythene substrata are important in the context of biodegradation of polythene. The physico-chemical properties of natural water bodies

are important for algal growth. In pursuance of the objectives of the present research, a total of 122 algal species belonging to 41 genera under 4 classes were found to be distributed on the polyethylene surfaces in Silchar town during the present study. Cyanophyceae were found to be the dominant class with 57 species spread over 13 genus. Chlorophyceae represented by 15 genus with 25 species. Bacillariophyceae were also found to colonise on polyethylene surfaces represented by 2 genus and each with 1 species. The species *Oscillatoria* was the largest genus with 29 species found to form mat like colonisation. Highest algal diversity was observed in Link road 1st (Site 1) during premonsoon season with maximum Shannon-Wiener diversity index (H=2.81 ± 0.15), minimum Simpson's dominance index (D=0.035 ± 0.14) and maximum Pielou's evenness index (J=0.98 ± 0.01). The algal distribution in Club road during post monsoon was observed to be least diverse with minimum Shannon Wiener diversity index (H=0.98 ± 0.14), maximum Simpson's dominance index (D=0.70 ± 0.03) and minimum Pielou's evenness index (J=0.26 ± 0.33).

Pearson's correlation coefficients calculated between various physico-chemical properties of water and total Cyanobacterial species present on submerged polythene bags in domestic sewage water drains have been presented in Table 4.29. Water pH has positive correlation (**r=0.942**, p<0.01**) with total Cyanobacterial species. BOD has positive correlation with total Cyanobacterial species (r = **0.311****, p <0.01) in the domestic sewage water. DO has positive correlation (r=0.656**, p<0.01) with total Cyanobacterial species. Total alkalinity has positive correlation (r=**0.923****, p<0.01) with total Cyanobacterial species. Sulphate, nitrate and calcium has a positive correlation (r=**0.585**, 0.446**, 0. 440**)** with total Cyanobacterial species.

The soil pH has negative correlation with total Chlorophyceae species (r= **-0.992****, p<0.01). BOD has negative correlation with total Chlorophyceae species (r=**-0.226***, p<0.05). Total alkalinityhas negative correlation with total Chlorophyceae species (r=-0.995**, p<0.05). Suspended solid (SS) has negative correlation with total Chlorophyceae species (r=**-0.974****, p<0.05).Nitrate has negative correlation with total Chlorophyceae species (r=**-0.475****, p<0.05). Free CO_2 has negative correlation with total Chlorophyceae

species (r=**-0.670****, $p<0.05$). All the physico chemical parameters were found to be negatively correlated with total Bacillariophyceae species.

Cyanobacterial population reached maximum number during pre-monsoon and decreased thereafter. *Oscillatoria limosa*, *O. princeps*, *O. subbrevis*, *O. tenuis*, *O. willei*, *Nostoc carneum*, *N. linckia*, *Phormidium lucidum*, *Cylindrospermum muscicola* and *Lyngbya diguetii*were found to be the most dominantspecies during pre-monsoon period on submerged polythene surface in sewage water. The physico-chemical properties of sewage water were found to influence the cyanobacterial colonisation on the polythene surface. The free CO_2 and pH value of sewage water were found to favor the cyanobacterial growth on the polythene surface. During monsoon period, *Oscillatoria limosa* and *O. princeps* were found to mat-like colonisation on the polythene surface. Sometimes, *Lyngbya diguetii* were found to colonise on the polythene surface during post-monsoon. In-between, pre-monsoon and monsoon, when the dissolved oxygen were found to be low, some *Oscillatoria* and *Phormidium* were found to dominate on the polythene surface.The value of pH, free CO_2, phosphate, nitrate and chloride concentration of sewage water were found to be responsible for the colonisation of blue-green algae on the polythene surface (Sarojini 1996; Tarar and Bodhke, 2002). The higher value of nitrate in sewage water are attributed to the colonisation of blue-green algae on the polythene surface (Jarousha, 2002).

Algae shows various forms that can acclimatize to different habitats through varying growth rates with variable genomes, different genes under different environmental conditions (Manoylov, 2014). Most of the cyanobacteria are covered by thick gelatinous sheath and some of the thallus morphology are also inconspicuous due to very fine thread like trichome with insignificant cross walls (Banerjee and Pal, 2017). Morphological characterization of cyanobacteria on the basis of trichomes, presence or absence of sheath, heterocyst present or absent, terminal or intercalary heterocyst, diffluent sheath forming free or floccose or soft mucilaginous thallus are necessary keys that leads to species identification. Morphological characterization play an important role in next level of taxonomy such as chemotaxonomy and phylogenetic studies of the cyanobacteria. In the present study, morphological characters of the isolates with cultural developmental history were found to be very helpful to avoid wrong identification of the isolates. A total of 33

algal isolates were taxonomically assigned to 13 genera and were identified as *Anabaena*, *Anabaenopsis*, *Aphanothece*, *Calothrix*, *Chlorella*, *Cylindrospermum*, *Fischerella*, *Hapalosiphon*, *Lyngbya*, *Nostoc*,*Oscillatoria*, *Phormidium*, and *Westiellopsis*. Maximum number of isolates were belonged to the genus *Anabaena* (6), followed by *Nostoc* (5), *Calothrix* (5), *Oscillatoria* (4), *Cylindrospermum* (2), *Westiellopsis* (2), and *Lyngbya* (2). The cultural and growth studies of isolates were performed in the present study. The maximum growth rate was revealed has by *Oscillatoria subbrevis* (E2) ($0.158\mu d^{-1}$) followed by *Anabaena oscillatoriales* (E30) ($0.157\mu d^{-1}$). The generation time was maximum in *Anabaena anomala* (E23) (277.86h) and minimum in *Calothrix* sp. (E20) (109.69h).

The biochemical constituents of algal isolates from polythene surface submerged in domestic sewage water showed isolates contain high cellular constituents of chla, carotenoids, protein, carbohydrate, vitamin C, lipid, phycobiliproteins, total phenolic content, polyphenol content and total flavonoid content. Significant differences were observed in biochemical constituent species wise. The characteristic morphological and physiological attributes of the species might be ascribed to typical physico-chemical properties of domestic sewage water. It has been reported that the cellular composition of cyanobacteria depend on the nature of strains, physiological state of the isolates and the nutrient conditions of environment from where they have collected (Vargas *et al.,* 1998; Subhashini *et al.,* 2003; Rosales *et al.,* 2005; Smith and Schindler, 2009). Phycobiliproteins content of the algal isolates were found to be direct relation with the environmental condition of species where from they were isolated. Grossman *et al.,* (1993) opined that environmental condition of species might alter the composition and abundance of phycobiliproteins. In the present study, the physic-chemical parameters were at variance in all the five sites. This, we believe, might have caused a variation in the total phycobiliproteins in the species studied.

The presence of enzymatic and non-enzymatic antioxidants in algal isolates clearly demonstrated its role against oxidant and other free radicals. The occurrence of enzymes viz., catalase, and peroxidaseand glutathione reductase in the algae are key factors to its adaptation to extreme environmental conditions (Mukund *et al.,* 2014). The algal isolates

showed the inhibition percent to DPPH radical scavenging activity, hydroxyl radical scavenging activity and total antioxidant activity. The algal isolates are believed to have developed defense against photo-oxidative damage by various antioxidative mechanisms to detoxify and remove highly reactive oxygen species (ROS) by producing several oxidative and radical stressors such as phenolic compounds and carotenoids (Tsao and Deng, 2004). As the algae were screened from submerged polythene surface in sewage water, it is anticipated that it might have gradually developed a system of either accumulating or releasing intra- or extracellular compounds to cope with the stress (Grossman *et al*., 1993; Ward and Singh, 2005, Paliwal *et al*., 2017). In the present study, the algal isolates were found to be rich in carotenoid content. The algal isolates were also revealed the percent inhibition of antioxidant activity. It is assumed that the presence of those carotenoids in algal isolates may be responsible for the antioxidant activity (Matsukuwa *et al.,* 2000). Algalisolates are found to be rich in various other natural compounds and pigments such as chlorophyll, phycocyanins, phenolic compounds, carotenoids, vitamins. Algal antioxidants have important role in regulating various diseases such as cardiovascular diseases, anti- inflammatory and immune protective, enhancing eye health, and increasing muscle strength. They are also effective in providing defence and antioxidant mechanism system via enzymatic and non-enzymatic antioxidants. The enzymatic and non-enzymatic antioxidants contain several mettalo-isoenzymes that can neutralise the harmful effects of ROS (Ahmad *et al.*, 2000; Sharma *et al.*, 2017).

In the present study, a total of 31 species of algae were isolated based on collection from polythene surfaces in domestic sewage water and solid waste dumping site. Five species of cyanobacteria based on dominance over occurrence for biodegradation of polyethylene. The cyanobacterial species, *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum* and *Cylindrospermum muscicola* were selected for polyethylene degradation. The products of degradation were tested by SEM, FTIR, NMR, tensile strength, and CHN analysis. In the present study, polyethylene degradation were observed in minimal carbon BG11 media. It was observed after 42days of experiment, the cyanobacterial species were grown better on $1cm^2$ polyethylene surface than BG11 liquid media. From the present study, it was evident that the cyanobacteria isolated from polythene surface in sewage water and solid waste dumping site are capable of utilizing

polyethylene as nutritional sources. In a recent study, capabilities of cyanobacteria in polyethylene degradation has been demonstrated (Kumar *et al.,* 2017).Studies also revealed that gram-negative bacteria could easily adapt to the environment rich in polyethylene and similar polymers.Since, cyanobacteria are gram-negative, they are found to be colonise on the polythene surface and utilizing the polyethylene as carbon source (Dey *et al.,* 2012).The structure of cell wall of Gram-negative is simple, they comprise of either homopolysaccharides or heteropolysaccharides. These molecules impart mechanical stability and are pivotal to adhesion and cohesion on the polymeric surface, and evasion from harsh dynamic environmental conditions. They consolidate the biofilm structure (Garrett *et al.,* 2008). The cyanobacterial colonisation on the polyethylene surface were observed to be maximum at the end of log phase. Extracellular polysaccharide of cyanobacteria helps in intercellular adhesion of cyanobacteria to the polyethylene surface ((Dunne, 2002). In the present study, EPS of cyanobacterial were measured and found to be 19.99, 22.43, 16.17, 21.44, 34.46µg/ml, respectively, *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum* and *Cylindrospermum muscicola*. Large surface area and organic content of polythene combined with cyanobacterial cell wall secretions render polythene surface more hydrophobic enabling fast colonisation (Sivan, 2011; Kumar *et al.,* 2017).

The rate of PE biodegradation was determined by weight loss in minimal carbon BG11 media. The present study revealed about 30% degradation of polyethylene for the tested species i.e., *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum* and *Cylindrospermum muscicola.* Previous studies reported 8% of weight reduction for PE by few cyanobacterial species over a period of 120 days (Kumar *et al.,* 2017). However, the present study suggested 27-30% of degradation for PE by the cyanobacterial isolates. The cyanobacterial isolates were found to grow better on polythene than BG11 media. The production of cellular constituents were found to be maximum on the polyethylene grown cyanobacteria. The pigment, protein and carbohydrate contents were found to be higher on polythene grown cyanobacterium than the biotic control (Shang *et al.,* 2009; Koutny *et al.,* 2006a). The enhanced production of cellular contents under minimal carbon source is considered as defensive mechanism of the cyanobacterial isolates (Paliwal *et al.,* 2017).

The FT-IR spectra of treated PE strip demonstrated several peaks in the range of 500-3446 cm^{-1} in comparison with the control PE. The FTIR spectra of treated PE also showedminor structural variation (peaks between 730-1500 and 2364-3446cm^{-1}) in comparison with the control.Present study confirmed that PE treated with cyanobacterial species underwent major structural changes which are a direct indication of biodegradation (Corti *et al.*, 2010; Esmaeili *et al.*, 2013) by the cyanobacterial species.

There were considerable differences in the percentage of elongation of treated PE after 42days by cyanobacterial isolates in comparison with the control. The tensile strength (TS) and elongation at break (EAB) for the control PE were found to be 11.8±0.12% MPa and 299±0.002%, respectively. The TS and EAB of treated PE by cyanobacterial species were found to be 6.7 ± 0.43 % and 243 ± 0.43 % after 42days.The reduction in tensile strength for tested PE in comparison with the control PE samples indicates structural changes of PE by the cyanobacterial species. All the findings related to tensile strength analysis of the degraded polymers are in accordance with the previous reports by Lee *et al.*, (1991); Orhan and Büyükgüngör (2000); Jakubowicz *et al.*, (2011).

After 42days of incubation in BG11 carbon minimal media, erosion, formation of pits and cavities were apparent on the surface of PE. However, in the present study, control PE revealed no erosion.The attachment of cyanobacterial species on the polyethylene surface is regarded as one of main criteria for biodegradation mechanism which were revealed by optical microscopy (Das and Kumar, 2015). The presence of cracks and cavities on the PE surface are considered as break-down the complex polyethylene form into its monomeric forms (Manzur *et al.*, 2004). The deformities on the polyethylene surface was interpreted in terms of enzymatic activities by the cyanobacterial species (Bhatia *et al.*, 2014).

Laccase and manganese peroxidase activities were measured for polyethylene degradation by the cyanobacterial species. Activity of laccase (0.009IU/ml) was found to higher as compared to manganese peroxidase (0.0075IU/ml) after 42days treatment. In biodegradation of polyethylene by cyanobacterial species, there are two key mechanism are believe to be operative, one is the reduction of molecular weight and second oxidation of molecules (Yoon *et al.*, 2012). The break-down of large polyethylene molecules is in fact believed to have been initiated by enzymatic action accompanied by molecular weight

reduction and enhancement of keto-carbonyl index (Santo *et al.,* 2012). Microorganisms are known to produce necessary oxidative and degradative enzymes and assimilate the polymeric carbon into their biomass (Hadad *et al.,* 2005; Tribedi and Sil, 2013). Cyanobacterial enzymes present in the liquid phase of the media interact with the macromolecules available at the surface of the polythene strips triggering biodegradation (Chinaglia *et al.,* 2018). Carbon analysis after 42 days of incubation revealed the percentage of carbon in the control PE to be 84%.The extent of carbon ultilised by the cyanobacterial species from the treated PE was around 4% for the cyanobacterial species. The adhesion mechanism of the cyanobacteria to polyethylene surface may be attributed to the low carbon availability in the medium and confirms its ability to use the polyethylene as a carbon and energy source (Awasthi *et al.,* 2017a).The NMR spectra of PE control have revealed some absorption peak centered at 20ppm in is believed to have originated from common plastic additives like phosphoric acid esters. The absorption peaks at 35.1ppm and 35.7ppm, conspicuously absent in the control, are due to esters and formation of some new -CH_2 group indicating the formation of ethyl propanoate. Multiplet character of the signal at 34ppm in the treated polyethylene can be assigned to carbonyl group of the acid moiety. The enhanced intensity of peak at 34.7ppm in the treated polythene suggested short chain branching (Brandolini and Hills 2000).The degraded polythene provides an organic soluble fraction implying a carbon uptake process linked to the metabolic pathway of the cyanobacteria (Miyazaki *et al.,* 2012; Balasubramanian *et al.,* 2014). Formation of carboxylic acid and other byproduct of polyethylene degradation reduces the molar mass of the polythene and facilitate carbon assimilation (Arnaud *et al.,* 1994).

Based on the research undertaken and analysis of the results, we furnish herein some generalized conclusions:

- A total of 122 algal species were found to colonize on the polythene surface in sewage water and domestic solid waste dumping site. The colonization pattern were found to anticipated that algal communities may use the polythene as a carbon source, and the submerged polythene in sewage water and domestic solid waste dumping site certainly serves as substratum for colonization.
- Factors like phosphate, nitrate, ammonia, temperature of the sewage water play an important role in colonization of algae on the polythene surface. As for the algal colonization on the polythene surface in domestic solid waste the organic carbon, total nitrogen, available phosphorus and potassium of soil were found to have significantrole.
- A total of 31 species of algae were isolated as pure cultures.
- The algal species isolated from submerged polythene surface in domestic sewage water are demonstrated to be a rich source of carbohydrate, proteins, lipids, vitamin C, phycobiliproteins, total phenolic, total flavonoids, carotenoid, and antioxidants.
- Some of the isolated algal species cultivated in untreated municipal sewage water without any additional nutrients afforded remarkable growth and biomass production. The cyanobacterial species, *Oscillatoria subbrevis* and *Nostoc carneum* were found to be capable of sequestering the nutrients from the sewage water. The lipid rich green alga, *Chlorella ellipsoidea* was able to significantly sequester nitrate and phosphate, increase the DO level, and lower the TDS well below the permissible limit. This also demonstrate that the algal species are capable of efficiently remediate sewage water, mitigate carbon dioxide as it grow proficiently in polluted water.
- The green alga, *C. ellipsoidea* was found to produce higher percentage of lipid in sewage water relative to the control medium may be exploited for its feasibility in biofuel generation.
- The antioxidants produced by the algal species in sewage water is anticipated to be of significance in pharmaceutical, food and cosmetic applications.

- Employing sewage water to harvest algae for production of value added chemicals could thus serve as an integrated approach for manifold applications.
- Spectroscopic, enzymatic, thermal, mechanical and morphological studies in relation to dual experimental control clearly demonstrated efficient polyethylene biodegradation by the cyanobacterial species.
- The cyanobacterial species, *Phormidium lucidum, Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc carneum* and *Cylindrospermum muscicola* were found to be efficient in biodegradation of LDPE polyethylene.
- The cyanobacterial species are capable of effectively colonising on the LDPE polythene surface utilising carbon without any pro-oxidant additives or pretreatment.
- The results of FT-IR and NMR spectroscopy corroborated the presence of alcohol and carboxylic acids as the degradation end products of polyethylene.
- The faster growth of the cyanobacterial species on the polyethylene surface is associated with greater weight loss and thinning of PE.
- The amorphous regions of the polyethylene are more easily degraded and that small crystals are likely to be consumed by the cyanobacterial species, but the rate of consumption of the smaller crystals were not investigated.
- It might be of interestto test the polyethylene under natural conditions.These cyanobacterial species have the potential to degrade polythene even more efficiently furnishing a tangible alternative solution to polythene waste management.
- The future investigations will likely to throw more mechanistic insights to the problem of polyethylene biodegradation. Isolation and identification of the enzymes able to oxidize and break polyethylene chains is a primary goal to elucidate the mechanisms of degradation of polyethylene. In the present research, laccase and manganese peroxide enzyme activity were monitored.
- Another important area of future research is the identification of biodegradation pathway involved inside cyanobacterial species.