Plant Life and Environment Dynamics

Prateek Srivastava
Ambrina Sardar Khan
Jyoti Verma
Shalini Dhyani *Editors*

Insights into the World of Diatoms: From Essentials to Applications

Springer

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Prateek Srivastava • Ambrina Sardar Khan •
Jyoti Verma • Shalini Dhyani
Editors

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Foreword

The diatoms are algal forms that are known for creating a castle of glass. They have a unique type of cell wall composed of silica. But for Leeuwenhoek, who devised a model of microscope with 300-fold magnification, it would not have been possible to see these minute organisms and their beautiful ornamentation. These ubiquitous organisms are the major primary producers in all aquatic ecosystems and moist places, and hence form the base of food web on which the higher trophic levels are dependent. They are well-known surrogate for the past climatic events because they settle at the bottom of lakes and oceans after death and remain intact because cell walls are readily preserved well along with their intricate microscopic details owing to their siliceous nature. As consumers of 20–25% of the global CO_2 they are important for the study of present climate.

Of recent, the science around diatoms has been concentrated mostly in Europe, Russia, North America, and Japan. As more and more samples were examined the workers left reference slides, material and publications as a record of their work. These are preserved in museums and institutions around the world. Major collections from the nineteenth century can be found in Philadelphia, Vienna, Berlin, Antwerp, Stockholm, Edinburgh and London. Diatom flora and taxonomy has been the major area of interest In India. Other perspectives have received scarce attention.

The initiative Diatoms: Biology and Applications is indeed an effort to generate interest in this less explored organism. This volume contains valuable information on the fundamentals of diatom biology and its multifarious applications to current as well as general issues. The included chapters throw a panoramic view of the world of diatoms touching upon biological aspects such as pigment composition and ecological and environmental facets such as climate change, ocean acidification and impact assessments. The book also takes into account varied applications of diatom research prevalent around the world such as nanobiotechnological utilization and forensic applications. I congratulate the editors for their collective wisdom in selecting a suitable theme for this volume. The information emerging from this volume will create interest in the young minds pursuing research as a career in national and international research institutes, universities and colleges. It will also attract research laboratories looking for fresh areas of research or unique organism models. It will be equally handy for planners, policymakers and managers of water for domestic and industrial use, be it rivers, lakes, lagoons and reservoirs.

I am quite convinced that the effort will spark and ignite minds when it reaches the bookshelves and e-books of individuals and libraries. It is a beginning to reach out and probe the scientific minds.

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Prakash Nautiyal

Preface: The World of Diatoms

We often come across partially or completely submerged rocks in streams and rivers covered by golden brown slimy films. These biofilms are composed of microscopic eukaryotic organisms commonly known as the diatoms. These wonderful organisms though are not only restricted to streams and rivers but are abundant in most aquatic ecosystems such as lakes, ponds, wetlands, oceans and even in soils with adequate moisture content.

The word diatom comes from the Greek dia, meaning “through”, and temnein, implying “to cut”, literally meaning “cut in half”, as they consist of two overlapping and interlocking units of their frustules. Diatoms are eukaryotic, unicellular and autotrophic organisms which are characterized by unique cell walls made up of silica (hydrated silicon dioxide). The frustules of diatoms are intricately sculptured and ornate. It is due to their enchanting beauty of their cell walls that these organisms have been labelled as “jewels of the sea”. They have been systematically placed in the stramenopile clade of the SAR supergroup and are recognized as members of Bacillariophyceae or Bacillariophyta.

The chloroplasts of diatoms contain chlorophylls a and c along with carotenoids such as fucoxanthin: the pigment responsible for the characteristic golden brown colour. These tiny organisms are the major component of the phytoplankton communities of the oceans and constitute approximately half of the organic material found in the oceans. They are responsible for the production of roughly 25–30% of oxygen globally which equals the contribution of the rainforests combined. They are known to be more energy efficient than their counterparts with organic cell walls. Moreover marine diatoms essentially sequester considerable amount of carbon dioxide from the atmosphere.

Fossil evidences trace back the origin of diatoms to early Jurassic Period though molecular clocks indicate the appearance of diatoms to Triassic period. The emergence of diatoms caused a major shift in the ocean carbon cycle with increased carbon locking in dead diatom cells. Genome sequencing of diatoms such as *Thalassiosira pseudonana* and *Phaeodactylum tricornutum* has thrown light on the unique secondary endosymbiotic origin of diatoms. These genomic studies also revealed several biochemical features of diatoms which are similar to that of organisms of the animal kingdom.

Diatoms have been extensively used in the water quality estimation of aquatic ecosystems such as lakes, rivers, wetlands, etc. and also in paleolimnological reconstructions. They are robust ecological monitors and have been used in assessment purposes throughout the world. The Water Framework Directive has recommended the utilization of diatoms in water quality monitoring programmes. The widespread use of diatoms for ecological health assessment of ecosystems has led to the generation of indicator and sensitivity value of several diatom species. Diatom indices are increasingly being used to evaluate the state of aquatic ecosystems. Recently the potential of terrestrial diatoms for ecological monitoring has also been explored.

Commercial applications of diatoms have a long history. Diatomaceous earth, the fossilized remains of diatoms, has been used in explosives, filtration systems, pest control, agriculture, etc. The unique way of deposition of silica by diatoms in their frustules has widespread applications in nanotechnology such as biosensors, bioimaging, drug delivery, etc. Diatoms have been extensively used in forensics and biofuel production.

The domain of diatom research has tremendous potential. From unravelling secrets of evolution to climate change mitigation, insights into the world of diatoms are expected to uncover evolutionary enigmas and enhance their commercial applications.

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Noida, Uttar Pradesh, India — Ambrina Sardar Khan
Prayagraj, Uttar Pradesh, India — Jyoti Verma
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Coming up with this book on diatoms would not have been possible without the contributions of our hardworking and dedicated authors. It was due to their willingness to contribute even after being engaged with their tight academic schedules. We wish to extend a deep sense of gratitude to all our authors. We profoundly appreciate and deeply acknowledge several eminent persons from the academia such as scientists, scholars and teachers who extracted time from their otherwise busy schedules, critically reviewed the manuscripts and provided us with their precious comments which led to a substantial enhancement in quality. We truly appreciate their cooperation and good understanding in meeting our rather strict paper submissions and review deadlines. We deeply admire the invaluable suggestions of Professor Prakash Nautiyal, HNB Garhwal University, for improving the content of our chapters. His distilled vision throughout the compilation of this book alleviated our challenges. Fundamental questions on the subject matter raised by Dr. Durgesh Kumar Tripathi, Amity University, during the book compilation deserve special appreciation. We are in praise of our research scholars who have worked relentlessly to check for typological errors and formatting issues. We wish to complement the production team members of the publication house for guiding us throughout the compilation of the book. Dr. Akanksha Tyagi and Mr. Jayesh Kalleri, Springer Publications, need special mention for allowing us operational flexibility in several areas. In spite of the fact that we have put in our best efforts to avoid any mistakes, there is a possibility of residual errors. Each chapter included in this book was finalized with primary responsibility of author and co-authors. The editors have gone through all the chapters included and reviewed them meticulously following international standards including ethics of publication. We are open to receive critical comments from readers for the improvement of our book. Last, but in no way the least, we wish to thank our family members from the core of our hearts for their understanding, patience and support for completion of this enormous task.

Prateek Srivastava
Ambrina Sardar Khan
Jyoti Verma
Shalini Dhyani

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About the Editors

Prateek Srivastava received a PhD in Botany from the University of Allahabad. His research areas are freshwater ecology and phycology. He has been awarded several research projects from reputed funding agencies of India viz. Ministry of Science & Technology, Ministry of Environment and Climate Change and Science and Engineering Research Board, most of which have focused on diatoms and other river algae. Two students have been awarded with the PhD degree under his guidance. He has been teaching ecology, biodiversity and phycology to UG and PG students since 2008. He has worked as an assistant professor in Amity University, Noida, from 2010 to 2017 and is presently working in the Department of Botany, University of Allahabad. He has more than 35 publications to his credit which include research papers in peer-reviewed international and national journals, book chapters, conference proceedings, etc. He has organized and attended several national and international seminars and workshops.

Ambrina Sardar Khan has completed her PhD in Environmental Science from the University of Allahabad in the year 2010. Since then, she has been working as an Assistant Professor in Amity University, Uttar Pradesh, and is engaged in UG and PG teaching. She taught various subjects like Geo-environmental and meteorological Sciences, Environmental Law, audit and policies, Disaster management and planning and Environmental pollution to the students of Master's in Environmental Sciences and M.Tech Environmental Engineering. Her areas of interest are Air and water quality monitoring, ecotoxicology, Nutritional and health risk assessment and Sustainable urban development. She had published two books namely *Disaster Management and Preparedness* with CBS publication and *Health & Environment: Key Factors for Sustainable Urban Development: Changing cities with challenging issues* with Lambert Publication. She is actively engaged in R&D and has published several research papers in various reputed journals.

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She has published more than 35 publications in peer-reviewed international and national journals, 7 book chapter and 10 reports. She has attended and presented more than 30 research papers in national and international seminars and 12 workshops. She has visited many countries as a recourse person and young researcher (Germany, Thailand, Malaysia and Nepal). She is a member and fellow of many national and international prestigious societies. She is a reviewer and member of the editorial boards of national and international journals. She has supervised a PhD student of Amity University as a Co-Supervisor (awarded) and one student enrolled for PhD under her supervision.

Shalini Dhyani is Senior Scientist with Critical Zone Group of Water Technology and Management Division in CSIR-NEERI, India. She is South Asia Regional Chair for IUCN CEM (Commission on Ecosystems Management) (2017–2020). She is IPBES Lead Author Global thematic assessment on Sustainable Use of Wild Species (2018–2021) and was lead author for IPBES Asia Pacific regional assessment of biodiversity and ecosystem services in Asia Pacific (2015–2018). She did her doctoral work from Forest Research Institute, Dehradun. Presently, she is involved in a multidisciplinary long-term Critical Zone research for understanding the functioning and impact of groundwater-dependent ecosystems and APN-IGES, Japan project on developing plausive alternate scenarios for Bhitar kanika, India. Dr. Shalini has worked on Biodiversity Inclusive impact assessment of important developmental projects across India and has also contributed to many NGT and Judiciary projects. Her work in Upper Ganga catchment focuses on understanding the role of riparian buffers for ensuring river health and role of phytochemicals in giving special property to river water. She has worked in Indo-EU projects on decontamination of soil and water using techno-ecological solutions. She was jury member for innovation prize programme on climate change adaptation (CCA) at scale (A@S) 2019 by IMC, UK, for Nepal. Dr. Shalini was awarded "IUCN-CEM Chair Young Professional Award" at World Parks Congress 2014 in Sydney, Australia, for her excellent research on Himalayan ecosystems. Dr. Shalini is a recipient of various national as well as international financial grants viz. UNEP, UNESCO, GIZ, FAO, IUCN, UNU, European Union-LEANES, Rufford SGP, DST, APN, etc. She has more than 60 national and international publications and many invited popular science talks to her credit.

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Abbreviations

β-car	β carotene
βTCP	β-tricalcium phosphate
AD	Alzheimer's disease
aDDS	Advanced drug delivery systems
AEAPTMS	3-aminopropyl trimethoxysilane
AMD	Age-related macular degeneration
APTES	3-aminopropyl triethoxysilane
Ax	Antheraxanthin
BQE	Biological quality elements
Chl a	Chlorophyll a
Chl b	Chlorophyll b
Chl c	Chlorophyll c
Chls	Chlorophylls
CWM	Conjunctive water management
Cx	β-cryptoxanthin
CxE	β-cryptoxanthin-epoxide
DD	Diadinoxanthin
Ddx	Diadinoxanthin
DE	Diatomaceous earth
DMAPP	Dimethylallyl diphosphate
DOX	Doxorubicin
DPOR	Dark operated protochlorophyllide oxidoreductase
DSNs	Diatomite silica nanoparticles
Dt	Diatoxanthin
Dtx	Diatoxanthin
FCP	Fucoxanthin-chlorophyll-protein
FTIR	Fourier transform infrared
Fx	Fucoxanthin
GGPP	Geranylgeranyl pyrophosphate
HEP	Hydroelectric projects
hMSCs	Human pluripotent stromal cells

HPQR	High-pressure rapid release
HTL	Hydrothermal liquefaction
HTU	Hydrothermal treatment
IDE/S	Index of Saprobity Eutrophication
IDG	Generic Diatom Index
IPCC	Intergovernmental Panel on Climate Change
IPP	Isopentenyl pyrophosphate
IPS	Specific Pollution Index
LHC	Light harvesting proteins
LPOR	Light operated protochlorophyllide oxidoreductase
LSPR	Localized surface plasmon resonance
MEP	Methylerythritol phosphate
MEV	Mevalonate
MG63	Hypotriploid human cell line
MGNREGS	Mahatma Gandhi National Rural Employment Guarantee Scheme
NPQ	Non-photochemical quenching
NPs	Nanoparticles
Nx	Neoxanthin
PDS	Phytoene desaturase
PEG	Polyethylene glycol
PH	Powerhouse
PL	Photoluminescence
PSY	Phytoene synthase
PTX	Paclitaxel
SDGs	Sustainable Development Goals
SERS	Surface-enhanced Raman spectroscopy
siRNA	Small interfering RNA
SLA	Sustainable Livelihoods Approaches
SPU	Signal processing unit
TDI	Trophic Diatom Index
TEMPO	2,6,6-tetramethylpiperidine-*N*-oxyl
USEPA	U.S. Environmental Protection Agency
VDE	Vx de-epoxidases
VOCs	Volatile organic compounds
Vx	Violaxanthin
WFD	Water Framework Directive
WHO	World Health Organization
ZEP	Zx epoxidases
Zx	Zeaxanthin

Chapter 1
Photosynthetic Pigments in Diatoms

Abhishek Sharma, Prishita Singh, and Prateek Srivastava

Abstract In this review, we present current knowledge of diatom photosynthetic pigments, along with some fresh insights into their physicochemical properties, biological role, biosynthetic processes, economic issues, and industrial relevance. Photosynthetic pigments are important bioactive molecules in the food, cosmetics, and pharmaceutical sectors.

Diatoms have distinct pigment composition which is even far different from those found in plants. The pigments present in diatoms are not only responsible for capturing solar energy during the process of photosynthesis, but they also show antioxidant with great role in the photoprotective processes. The chief light-harvesting pigments present in diatoms are chlorophyll a, chlorophyll c, and fucoxanthin; besides them, they also have collection of carotenoids like β-carotene, xanthophylls, diadinoxanthin, violaxanthin, diatoxanthin, and zeaxanthin having photoprotective functions and are generally produced during xanthophyll cycle as reaction intermediates. Commercially, these pigments have great potential application in food additives, pharmaceutics, and cosmetic industries; besides, these pigments are also being used in the field of medicine as remedy and diagnostics. In recent times, these diatoms have emerged as a great source of these bioactive compounds in various industries. A brief overview of the photosynthetic pigment of diatoms and their potential application in commercial field is presented in this review.

Keywords Photosynthetic pigments · Fucoxanthin · Chlorophyll · Diatoms · Biosynthesis pathways · Antioxidant

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1.1 Introduction

Diatoms are photoautotrophic microalgae which may be colonial or unicellular in arrangement, classified as protists of the group of the Bacillariophyta, and initiate various aquatic food chains and serve as important components of coastal and upwelling environments. The phylogenetic origin of diatoms is found to be different from other green micro and macro plants (Armbrust 2009). The diatoms developed from a secondary endocytobiosis of a eukaryotic host and a eukaryotic red alga. They can be found in a variety of environments, including marine, freshwater, and five terrestrial habitats. Various species thrive in typically hostile conditions, such as very acidic ecosystems and thermal water bodies, where temperatures preclude most other living forms from growing. Frustules, which are made of two valves, are exceptionally tough siliceous cell walls found in diatoms (Martin-Jézéquel et al. 2000). Diatoms are present in the environment and in items manufactured using diatomaceous earth, such as cleaning agents, paints, and some types of match heads. Diatom communities are highly sensitive to changes in abiotic conditions and are highly sensitive to environmental change compared to fish and macroinvertebrates (Pandey et al. 2017); hence, they are routinely used for biomonitoring purposes in both lotic and lentic environments. The most prominent unique feature of the photosynthesis apparatus in diatoms is the pigmentation of the light-harvesting apparatus and the thylakoid macrodomain organization. Diatoms are extremely important ecologically since they contribute roughly 20–25% of the world's primary production (Field et al. 1998; Sarthou et al. 2005). When compared to the total quantity fixed by the terrestrial rainforest combined, diatom photosynthetic activity accounts for 40% of marine primary production (Armbrust 2009). Diatoms are characterized by the brown color which originates from a high content of fucoxanthin being bound to "light-harvesting proteins" (LHC) in an equal or even higher ratio than chlorophyll (Chl) a (Gelzinis et al. 2015). The light-harvesting system of diatoms consists of the so-called "fucoxanthin-chlorophyll-protein" (FCP) complexes. Besides fucoxanthin (Fx), which represents the main light-harvesting pigment of diatoms, the xanthophyll cycle pigments "diadinoxanthin" (DD) and "diatoxanthin" (Dt) are additionally present. Different pools of Fx exist within the light-harvesting system, and one of these pools shows light absorption up to 550 nm, thus permitting the use of green light for photosynthesis (Szabo et al. 2010), which cannot be captured by the chlorophylls and other xanthophylls.

These pigments are not only responsible for capturing solar energy to carry out photosynthesis but also play a role in photoprotective processes and display antioxidant activity, all of which contribute to effective biomass and oxygen production. Diatoms are organisms of a distinct pigment composition, substantially different from that present in plants.

Pigments are chemical compounds which provide different colors to the organisms and the parts including flowers, corals, and even animal skin color. They reflect only certain wavelengths of visible light making them appear "colorful." More important than their ability to reflect light, pigments have become widely recognized as a source of unique bioactive compounds having potential use in the industrial, pharmaceutical, and medical fields. They are also rich in bioactive compounds like naviculan having antiviral activity (Lee et al. 2006) and amino acid derivative domoic acid, which have neuroexcitatory effects (Perl et al. 1990), and also contain few nucleotides which show traces of cytotoxic and blood platelet inhibitory activity (Prestegard et al. 2009).There is a wide range of beneficial diatom cell components like lipids and pigments; the amount of which can be influenced by abiotic stressors or genetic changes in metabolic pathways for various applications.

Some unusual pigments have also been reported in few diatoms like *Haslea karadagensis* which have quite different absorption maxima from those of marennine; its similar bioactivity has come to be called marennine-like (Gastineau et al. 2012). Furthermore, the three carotenoids, namely, β-carotene, diadinoxanthin (Ddx), and diatoxanthin (Dtx), are known to play an essential part in photoprotection, while violaxanthin (Vx), antheraxanthin (Ax), and zeaxanthin (Zx) may also be involved. This article focuses on the photosynthetic pigments mentioned above, which are necessary for diatom existence and are widely exploited in numerous sectors.

Although studies in this topic have been conducted for many years, there are still many things that need to be investigated further. The information presented below summarizes current knowledge about photosynthetic pigments found in algae, their biosynthesis processes, cell localization, economic characteristics, and industrial significance.

1.2 Pigment Localization in the Diatom Cell

The diatom cells have either a couple of little chloroplasts or one huge chloroplast (Lavaud 2007). In diatoms, Granal stacking is missing, for example, the thylakoid layers do not show distinction into Granal and stromal lamellae (Gibbs 1970) and contains the colors answerable for the retention of light for photosynthesis. These thylakoid films are organized into gatherings of three approximately stacked lamellae which range through the entire length of the chloroplast (Pyszniak and Gibbs 1992). The association of LHC proteins shows contrasts based on pigmentation when contrasted with LHCs of the higher plants (Gundermann and Büchel 2014).

Fx is present in lot amount in FCPs than the carotenoids present in LHCII; the molar Chl/carotenoid proportion ratio is practically 1:1 and 14:4, separately (Beer et al. 2011; Papagiannakis et al. 2005). Whenever it ties to the protein, Fx goes through outrageous bathochromic shifts, and since it relies unequivocally upon the extremity of the protein climate, a few populaces can be recognized, i.e., Fx red, Fx green, and Fx blue (Premvardhan et al. 2008, 2009, 2013). In diatoms, the Ddx pool is heterogeneous. As of late, three distinct pools of diadinoxanthin cycle shades were proposed. Two of these are bound to extraordinary antenna proteins inside Photosystem I and FCP, individually, and since their turnover is extremely low, they assume no immediate part in the Ddx cycle (Lohr and Wilhelm 2001). The protein-bound diadinoxanthin cycle colors would take an interest in the nonphotochemical extinguishing (NPQ) component, while the lipid-related ones would basically play a cell reinforcement work, searching 1O2 and peroxylipids. Pool of Ddx is all the more firmly associated with a protein-restricting site, which should contrast from the one involved by the Ddx present in low light circumstances (Alexandre et al. 2014). Thylakoid films of diatoms, likewise, contain other xanthophyll like Vx, Ax, and Zx (Lohr and Wilhelm 1999, 2001). In any case, these carotenoids collect just under unambiguous circumstances, e.g., during long haul brightening areas of strength for with. In addition, it has been demonstrated the way that Vx can be either an immediate or a circuitous (through the arrangement of Ddx) forerunner of Dtx.

1.3 Structure and Properties of Pigments of Diatoms

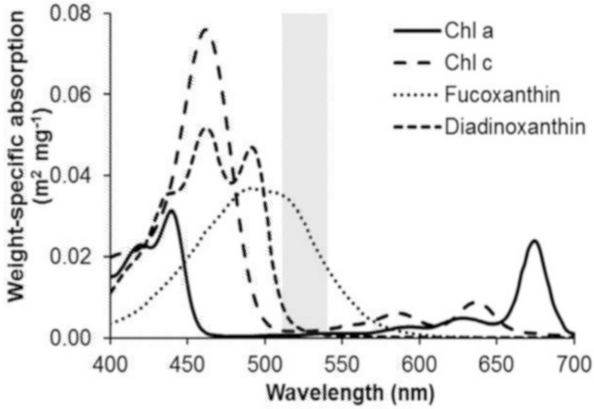
There are two kinds of pigments present in diatoms, i.e., chlorophyll and carotenoids, which are involved in photosynthesis and photoprotection. Chlorophylls trap light energy mostly blue and red wavelength of the electromagnetic spectrum which are used in photosynthesis. Chlorophylls, a light-absorbing green pigment, contain a polycyclic, planar tetrapyrrole structure having central metal ion magnesium in coordination complex. The Chl c pigment is found in diatoms, and the phytyl chain is absent in majority, because of which they are highly conservative structural motifs of the Chl (Zapata et al. 2006). Carotenoids act as accessory light harvesting pigments which capture light energy and feed it to the photochemical reaction center and protect it against photooxidative damage (photoprotection). They are comprised of xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons and contain no oxygen).

Chlorophylls absorb maximum light in the violet-blue and red part (Chl a) of the spectrum, but it also displays strong absorption in the yellow-orange (chlorophyll c) parts of the spectrum and thus are optically separated from carotenoids. Photosynthetic pigments are readily identified by their absorption in the visible portion of the electromagnetic spectrum, i.e., from 400 to 700 nm. Chl a absorption peaks at 430 and 662 nm and shows less peaks due to xanthophylls (480 nm) and Chl c (580, 620 nm). The major Chl c absorption peak at 450 nm is weakly visible, being hidden by xanthophylls and Chl a absorption (Fig. 1.1).

1.3.1 Chlorophyll

There are different kinds of pigments present in the photosynthetic organisms, but only two forms of Chls are found in diatoms, i.e., Chl a and Chl c. Chl a is present dominantly and plays a major role in photosynthesis by converting photochemical energy in majority of photosynthesizing organisms, while Chl c (as like Chl b in

Fig. 1.1 Showing absorption spectra of different pigments found in diatoms (Mulders et al. 2014)



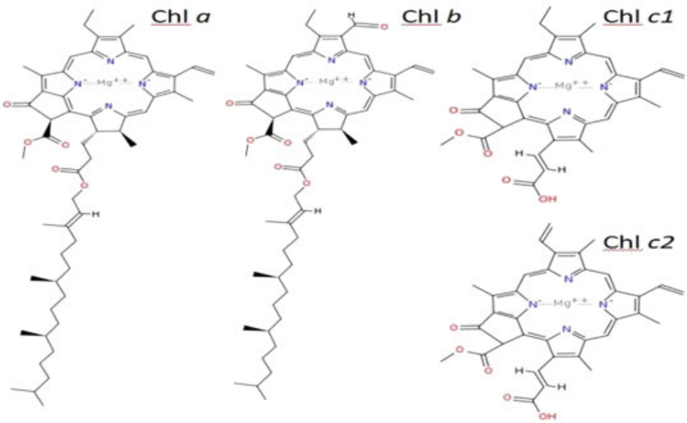


Fig. 1.2 Showing molecular structure of different chlorophyll molecules

plants) mainly acts as an accessory pigments which adequately participates in photosynthesis (Fig. 1.2).

Molecules containing four pyrroles forming a macrocycle (e.g., a porphyrin ring) are classified as closed tetrapyrroles. Chlorophylls (Chls) are conjugated, closed tetrapyrroles to which a cyclopentanone ring has been also added. Tetrapyrroles pigments play essential roles in photosynthesis, in the absorption of sunlight and its conversion into chemical energy, finally used to reduce CO_2. This energy conversion is the foundation for autotrophy in some prokaryotes (e.g., cyanobacteria), eukaryotic algae, and plants. Chlorophyll is found to have natural food coloring, antioxidant, as well as antimutagenic properties. According to estimations, the total natural production of Chls in the biosphere is around 109–1012 tons per year, in which majority of Chls are produced by photosynthetic marine microorganisms (Grimm et al. 2006; Hosikian et al. 2010). Among different sorts of Chls c present in diatoms, the most widely recognized are Chl c1 and c2. The particular construction of a Chl c acquires changes in the retention range to create areas of strength for a (blue) assimilation band in examination with a feeble band in the red district. The proportions of band I (at ~630 nm) to band II (at ~580 nm) are >1 for Chl c1-like chromophores, ~1 for Chl c2-like chromophores, and <1 for Chl c3-like chromophores.

1.3.2 Carotenoids

Carotenoids are a group of nonnitrogenous yellow, orange, or red pigments (biochromes) that are almost universally distributed in living things like plants and diatoms. There are essential types: the hydrocarbon class called carotenes and the oxygenated class called xanthophylls. There are seven forms of carotenoids that had been observed in diatoms where carotenes are represented by β-car and xanthophyll is represented by Fx, Dtx, Ddx, Zx, Ax, and Vx.

All the derivatives of these pigments include isomers and degraded products, which may be found in the cell, but all the trans isomers are present most abundantly and are functionally more active (Fig. 1.3). The possible cause of carotenoid instability could be the occurrence of a conjugated polyene chain in carotenoids, which may be responsible for their oxidation and E/Z isomerization due to heat, light, or chemicals. Membrane physical properties are affected by the structures of carotenoids in cis and transform, which are distinctly allocated in the membrane. The presence of carotenoids changes permeability for tiny molecules and the oxygen, which is related with their protective activity (Subczynski et al. 1991). In contrast to chlorophylls, carotenoids cannot be easily detected by the regular pigment analysis methods because they often get broken down due the destruction of their alternating double bond converting them into colorless compound (Lohr and Wilhelm 2001). Carotenoids generally shows absorption between the range of 400 and 500 nm, and their absorption properties is mainly defined by the conjugation length and the type of the functional groups attached to ionone rings which terminates the polyene chain (Zigmantas et al. 2004). In diatoms, the main light-harvesting carotenoid is Fx, but minor amounts of a 19′-butanoyloxyfucoxanthin-like pigment have also been found in *Thalassiothrix heteromorpha*, a diatom species (Kim et al. 2012). Fx has an allenic link, a conjugated carbonyl, a 5,6-monoepoxide, and acetyl groups, all of which contribute to the molecule's unique structure and spectral features. Its broad absorption band (between 460 and 570 nm) covers much of the gap left by chlorophyll in the green region, unlike other carotenoids. Diatoms also have the β-car, as well as two asymmetrical xanthophylls, Ddx and Dtx, which have an acetylenic group at one of the ionone rings. Vx, Ax, and Zx are three more xanthophylls that may be present (Lohr and Wilhelm 2001). It has also been found that these carotenoids assemble only at times like long-time exposure with strong light.

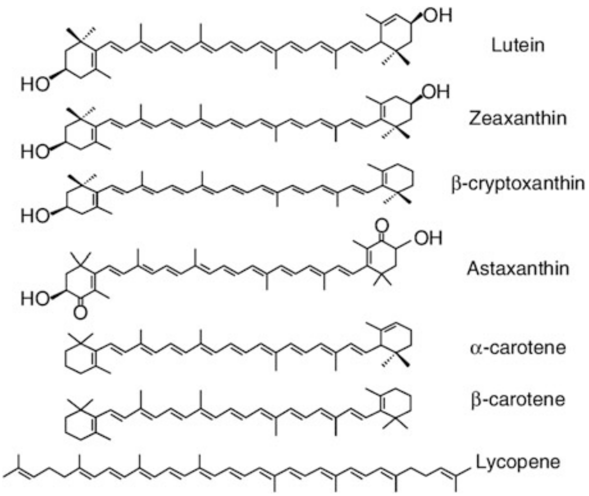


Fig. 1.3 Structures of various pigment derivatives

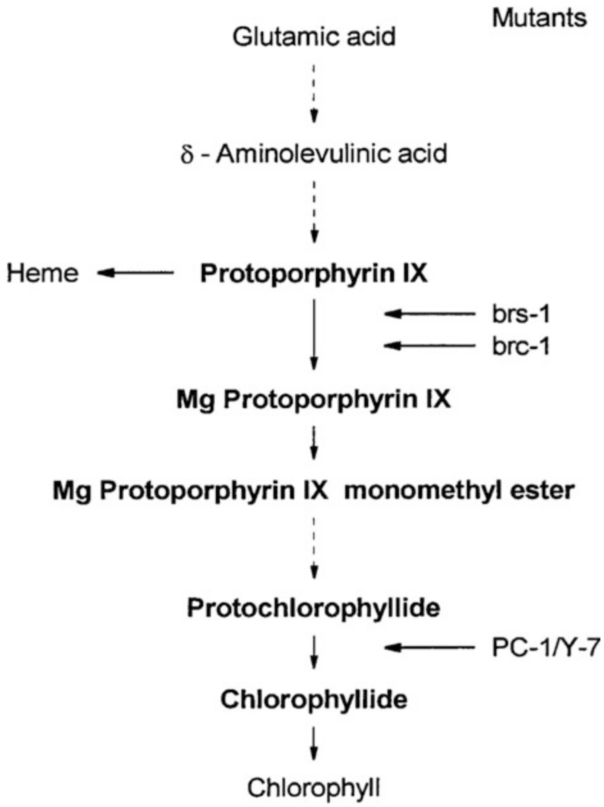
1.4 Biosynthetic Pathways of Pigments

1.4.1 Chlorophyll: Biosynthesis

The chlorophyll synthesis pathway has been extensively explored in higher plants and some algae groups, although it has been poorly investigated in diatoms (Kuczynska et al. 2015). However, all photosynthetic organisms share the same basic characteristics (Fig. 1.4). Chl production requires three universal steps: the creation of aminolevulinic acid, its transformation into Mg-porphyrins, and protochlorophyllide conversion to Chl (Grimm et al. 2006).

The first step depends on the cyclization of tetrapyrrole, the introduction of Mg leading to the formation of diviny-PChlide, and its reduction into PChlide a. Next, photo-independent PChlide oxidoreductase (DPOR) and photodependent enzyme (LPOR) catalyze the hydrogenation of PChlide to Chlide, which promotes the formation of Chl a in a further step. Several isoforms of LPOR have been found in some diatom species (Hunsperger et al. 2015). The final step is the insertion of phytol residues associated with the MEP pathway, which is also used for carotenoid formation. The molecular structure of Chl c may indicate that PChlide is a precursor of biosynthetic pathways where oxidation and dehydration are essential, but the enzyme that performs these steps has not been reported (Porra 1997). In general, the facts about Chl biosynthesis and the enzymes that catalyze each step are not clearly understood yet.

Fig. 1.4 Biosynthetic pathway of chlorophyll pigment



1.4.2 Carotenoid: Biosynthesis

Carotenoid biosynthesis occurs by two pathways which are methylerythritol phosphate (MEP) and mevalonate (MEV). Their occurrence is not well understood, but a few studies show that it depends on the growth rate (Cazzonelli and Pogson 2010). The products of both the pathways are dimethylallyl diphosphate (DMAPP) and its isomer, isopentenyl pyrophosphate (IPP) (Stauber and Jeffrey 1988). The next steps on the pathway to lycopene synthesis are the conversion of DMAPP to geranylgeranyl pyrophosphate (GGPP), which is catalyzed by GGPP synthase; then to phytoene by phytoene synthase (PSY); afterwards to ζcarotene, which is catalyzed by phytoene desaturase (PDS); and, finally, the product of ζcarotene desaturase (ZDS), which is lycopene (Bertrand 2010). Lycopene as a long and straight atom is cyclized by lycopene βcyclase (LCYB) to βvehicle, having two βionone rings at the two closures of the yield. In the following stage, xanthophyll is first shaped, and this response requires hydroxylation. Nonetheless, a quality encoding βcarotene hydroxylase (BCH) was not found in the diatom genome, and another that resembles LUT1 has been proposed as a hypothetical catalyst to make the development of Zx from βvehicle conceivable (Coesel et al. 2008). Two further light-reliant and reversible responses lead to Vx development by means of the moderate item Ax. Both are catalyzed by Vx deepoxidases (VDEs) in high light circumstances; however, switch responses are catalyzed by Zx epoxidases (ZEPs) in low light or in obscurity. Vx, on the other hand, is formed from Zx by β-cryptoxanthin (Cx) and β-cryptoxanthin epoxide (CxE) (Lohr and Wilhelm 1999). Further progress leading to the assembly of Fx, Ddx, and Dtx remains ambiguous due to the lack of information on the chemicals involved at the same time. Nevertheless, two models of potential conversions from Vx to Fx have been introduced so far. The main model proposed Vx as a precursor of Ddx, Dtx, and Fx (Lohr and Wilhelm 2001) with a response of Vx propagating to Fx through Dtx and Ddx. This theory was affirmed tentatively utilizing norflurazon, which hinders carotenoids, and was utilized after the aggregation of Vx. A rise in Fx level can be seen in low light. Another model depending on hypothesis about compound properties of these neoxanthin (Nx) and xanthophylls was viewed as an antecedent of Fx and Ddx (Dambek et al. 2012). The arrangement of Fx requires two adjustment steps: the ketolation of Nx and acetylation of a fucoxanthinol may occur, and, to help one of these theories, the distinguishing proof of the chemicals is vital. The most impressive methodology for this is to search for qualities which encode the proteins of interest on information basis. Notwithstanding, LCYB imparts amino corrosive character to NXS up to 64% which takes part in Nx creation, albeit no LCYB-like NXS in earthy-colored ocean growth was distinguished (Mikami and Hosokawa 2013). It is essential to uncover the entire xanthophyll biosynthetic pathway due to the numerous valuable chances to additional examinations and furthermore to plan transgenic creatures with an expanded xanthophyll level (Fig. 1.5).

1.5 Commercial Use of Photosynthetic Pigments

1.5.1 Fucoxanthin

Fucoxanthin ($C_{42}H_{58}O_6$) is a commercially important carotenoid. Diatoms, along with brown seaweeds, have been extensively utilized for in vitro and in vivo production of FX using different strains by modifying various metabolic and environmental factors (Bauer et al. 2019). FX has attracted significant interest in the past few decades because of its versatile functionality that includes antioxidant, anticancer, anti-inflammatory, and anti-obesity effects (Gammone and D'Orazio 2015). Due to its greater potential for preventing disease (better than β-carotene and astaxanthin), its uses in the nutraceutical and cosmetic industry and, consequently, the demand for FX are increasing (Lourenço-Lopes et al. 2021). The market and prices are burgeoning for FX produced from diatoms. Studies demonstrate that diatoms produce at about ten times more FX per gram of DW than any brown alga. The main goal is to increase the number of such useful products along with the economy of the process. However, the main challenges are to select/identify a strain of diatom that can produce consistent biomass and biomolecules under varying