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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Jyoti Verma • Shalini Dhyani
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ISSN 2730-6755 ISSN 2730-6763 (electronic)
Plant Life and Environment Dynamics
ISBN 978-981-19-5919-6 ISBN 978-981-19-5920-2 (eBook)
https://doi.org/10.1007/978-981-19-5920-2

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

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.

Prayagraj, Uttar Pradesh, India Prateek Srivastava
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Acknowledgements

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.

Jyoti Verma is working as an Assistant Professor, Department of Zoology, CMP PG College, University of Allahabad. She has worked with Indian Institute of Technology, Kanpur, as a Project Scientist in GRBMP, World Bank project, and Biodiversity expert in E-FLOW Project of World Wildlife Fund India supported by HSBC. She has also completed many Environmental Impact assessment reports of Northeastern states. She has completed a research project on diatoms of Indian subcontinent funded by Indian Government Agencies. Presently she is working on a UGC Start-up grant project on Diatom Biodiversity of Ken-Betwa Rivers. She has received UGC-Women PDF Fellowship Award 2011, CSIR-International Travel Fellowship 2012, Young Scientist award in Oral Presentation 2016, International Travel Fellowship DST 2018, Young Indian Diatomist 2018 and Young Environmental Scientist Award 2018 at JNU, New Delhi.

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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P. Srivastava et al. (eds.), *Insights into the World of Diatoms: From Essentials to Applications*, Plant Life and Environment Dynamics,
https://doi.org/10.1007/978-981-19-5920-2_1

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

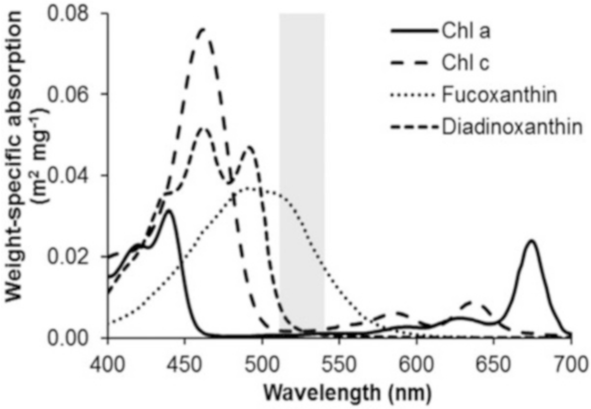

Fig. 1.1 Showing absorption spectra of different pigments found in diatoms (Mulders et al. 2014)

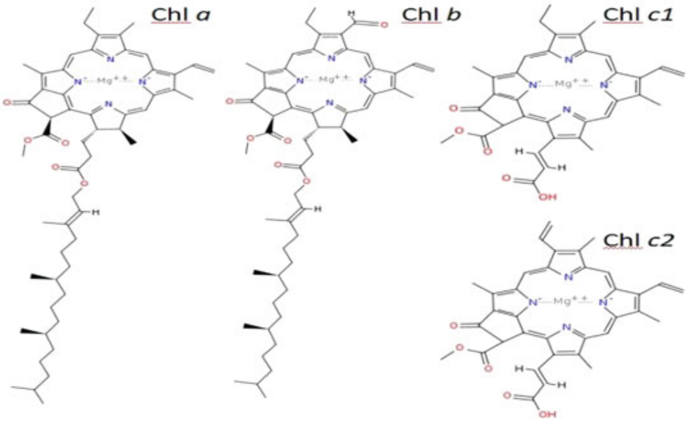

Fig. 1.2 Showing molecular structure of different chlorophyll molecules

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.

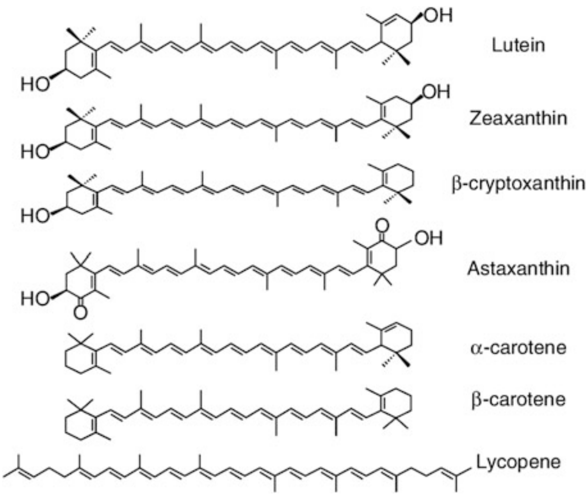

Fig. 1.3 Structures of various pigment derivatives

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.

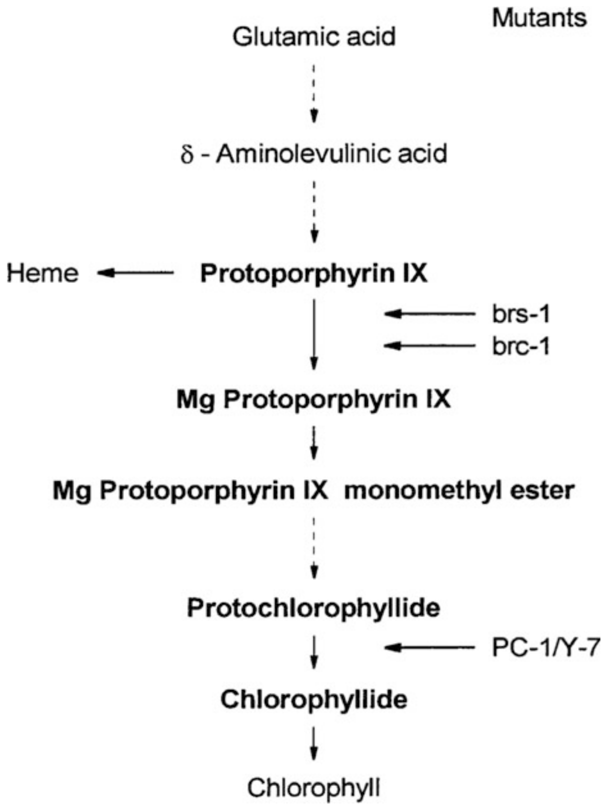

Fig. 1.4 Biosynthetic pathway of chlorophyll pigment

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

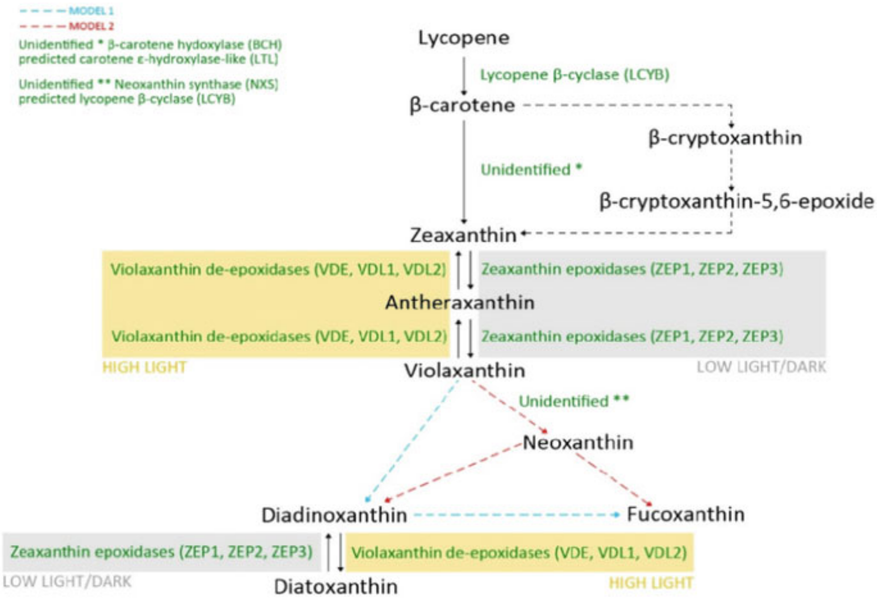

Fig. 1.5 Image showing biosynthetic pathway of photosynthetic carotenoids in the diatom from lycopene to fucoxanthin and diatoxanthin (Kuczynska et al. 2015)

conditions in outdoor cultivation. The methods to culture diatom at a commercial scale exist; however, there are certain limitations like identification and isolation of the best diatom strains for FX production, standardization of protocols to obtain pure cultures, and optimal nutrient requirements in a photobioreactor-based production system. Further, more studies are required focusing on reducing the input cost during downstream processing to obtain high quality and quantity of FX at reasonable price and purity. In addition, to efficient culture methods, critical information of the pathways involved in producing bioactive algal metabolites is required, including the identification of genes that could be used for genetic engineering. The current focus of algal engineering is on obtaining transformants with higher lipid accumulation to change the fatty acid composition. Optimization of cultivation conditions, microalgal engineering, and epigenomic reprogramming of algal strain can increase FX production. Not only FX but also intermediates have potential commercial application (Fig. 1.6).

1.5.2 Diatoxanthin and Diadinoxanthin

Diatoxanthin are a type of carotenoids which dissipate energy by means of nonphotochemical quenching (Lavaud et al. 2004). These carotenoids have great application in the food, cosmetic, and pharmaceutical industries; they also possess

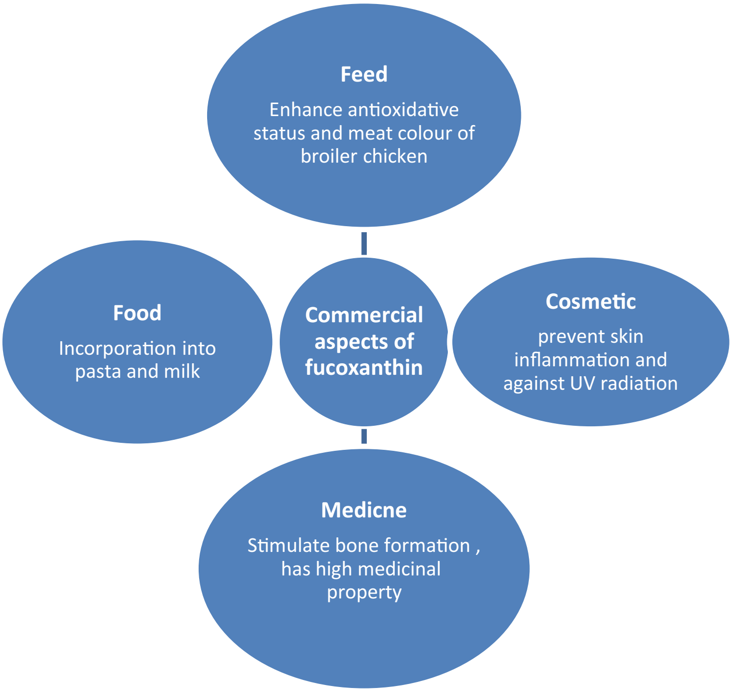

Fig. 1.6 Image showing commercial applications of fucoxanthin pigment

neuroprotective effects (Pangestuti and Kim 2011). Diatoms display diadinoxanthin cycle in which interconversion between epoxidized diadinoxanthin and epoxy-free diatoxanthin occurs. Two diatom-specific carotenoids are found in the diadinoxanthin cycle, an important mechanism which protects these organisms against photoinhibition caused by absorption of excessive light energy. This unicellular alga is a cosmopolitan marine pennate diatom. Since both diadinoxanthin and diatoxanthin occur only in few algal groups like diatoms, these pigments might be considered as diatom-specific carotenoids.

1.5.3 Zeaxanthin

Zeaxanthin is a carotenoid molecule that has antioxidant potential with several health benefits such as reducing the risk of age-related macular degeneration, glaucoma, and cataracts. *Phaeodactylum tricornutum*, a diatom, synthesizes zeaxanthin by zeaxanthin epoxidase and zeaxanthin de-epoxidase which have been proposed from the available genome sequence. These two enzymes may be involved in the two different xanthophyll cycles which operate in *Phaeodactylum tricornutum*.

1.5.4 Lutein

Lutein is a natural antioxidant and has drawn interest for its health-promoting functions. Lutein has potentials in free radical scavenging for skin health and can also prevent age-related macular degeneration (AMD) and Alzheimer's disease (AD) (Roberts et al. 2009). Absorbed lutein can accumulate in human retina, filter blue light, and, thus, protect eyesight. Orange-yellow fruits like mango and green leafy vegetables like broccoli are dietary sources of lutein .The marigold flower is the main source of natural lutein and lutein esters (Breithaupt and Schlatter 2005). However, there are some drawbacks of this source, such as mandatory harvesting in specific seasons and time-consuming petal separation. Other sources containing lutein also have such disadvantages as low concentration (corn residues, leafy green vegetables) and low bioavailability (egg yolk, crustaceans). The production of lutein from microalgae may avoid these troubles. Microalgae like diatoms can accumulate considerable biomass concentrations and accumulate lutein under suitable culture conditions. Even as compared with marigold-originated lutein, lutein in microalgae exists in free form. If in the coming future we became able to develop techniques to extract lutein from diatoms, it will prove to be beneficial to us by commercial aspect and medical level.

1.5.5 β-Cryptoxanthin

β-Cryptoxanthin has provitamin A activity. This carotenoid can supply one vitamin A molecule based on structure. This is likely due to the bipolar nature of *β*-cryptoxanthin. *β*-cryptoxanthin is associated with lower rates of lung cancer and improved lung function in humans. In tissue culture, *β*-cryptoxanthin has a direct stimulatory effect on bone formation and an inhibitory effect on bone resorption. However, the process of β-cryptoxanthin esterification in diatom is rarely investigated, and their roles in plants and animals are not well determined yet. In addition, the role of β-cryptoxanthin that involved therapeutic and immune-enhancing properties in human should be investigated further. Though β-cryptoxanthin is synthesized in our body, due to its medicinal values, we have to increase its consumption during diseases and for that we have to increase its productivity. Although they are present in plants with high amount, to fulfill our requirements, we have to find the measure to enhance its productivity for commercial purpose, and the best way is extraction of beta cryptoxanthin from diatoms. These carotenoids can also be found in diatoms, so there is a need to devise some cost-effective and time-saving methods to extract them from diatoms.

1.5.6 Chlorophyll: Commercial Aspects

Earlier chlorophyll were chiefly extracted from green leaves; however, emerging tools, techniques, and methods have been developed that rely on diatoms, cyanobacteria, or microalgae (Tong et al. 2012; Kong et al. 2014; Wrolstad and Culver 2012; Humphrey 2004; Heydarizadeh et al. 2013). It was discovered that the amount of chlorophyll taken from a given algae species was greatly dependent on its growth stage. Microalgae collected during the stationary growth phase were shown to have substantially more chlorophyll a than those extracted during the logarithmic phase (Schumann et al. 2005) (Fig. 1.7).

1.5.7 As Food Colorants

To increase the marketability of the products, special care is taken during food processing to retain and/or restore the green color of Chls and to avoid the formation of their less attractive colored and/or less healthy breakdown products in all

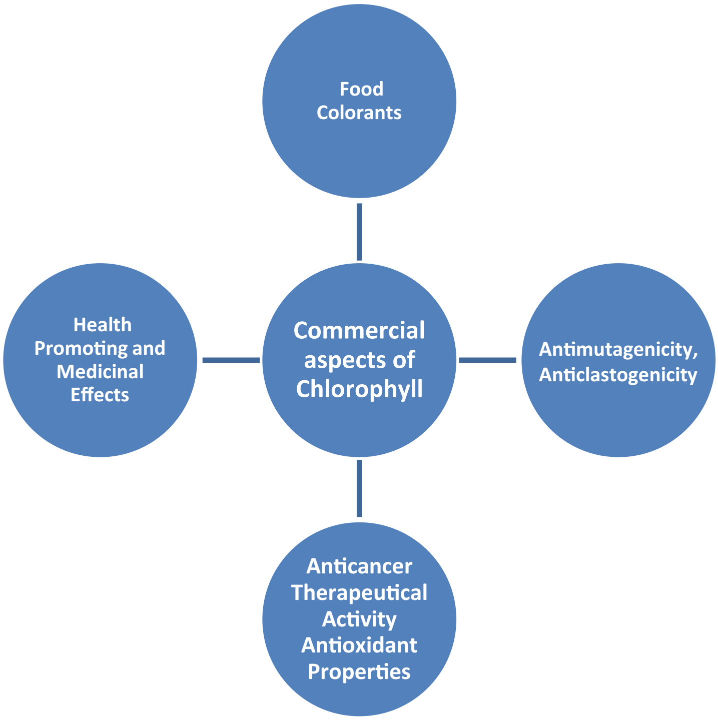

Fig. 1.7 Image showing commercial applications of chlorophyll pigment

commodities containing Chls (either inherently or as color additives or as medicinal products). One key issue that restricts usage of Chls as direct food colorants is that the central Mg is easily lost during processing (Arnold et al. 2012). This can be solved by replacing this ion with other metals within the macrocycle resulting in more stable Chl-metal complexes. The other limiting factor is the high hydrophobicity of the pigment molecule imparted by its long hydrophobic phytol chain (derived from phytol—C20H39OH) and by a fifth ring (cyclopentanone) in the macrocycle (Tumolo and Lanfer-Marquez 2012). Chemical modification of these groups can increase the water solubility of Chl derivatives and provides water-soluble food colorants.

1.5.8 For Health Promoting and Medicinal Effects

Effects of chlorophylls and their derivatives first of all in humans. The role of dietary Chl metabolites and derivatives in animals and humans is reviewed in detail elsewhere (Ma and Dolphin 1999; Ferruzzi and Blakeslee 2007; Ulbricht et al. 2014; Nagini et al. 2015). Ideally, medicinal studies with Chl derivatives should use a single compound with verified purity and/or with stable and well-characterized composition (Dashwood 1997; Chernomorsky 1994). However, often this is not the case. Most studies on, for instance, cancer-related research of Chl derivatives used the relatively cheap, stable, commercially available, and water-soluble food-grade Cu-chlorophyllin, the composition and purity of which was often not standardized (Dashwood 1997; Chernomorsky 1994).

1.5.9 For Antioxidant Properties

Most neurodegenerative and inflammatory diseases, cancer, diabetes mellitus, atherosclerosis, reperfusion injury, aging processes, etc., can be associated with excessive formation of free radicals resulting in oxidative stress and/or impaired antioxidant defense system of the organism. However, disease may be prevented, or the symptoms or effects may be alleviated by the therapeutic use of different antioxidants. It is well-established that Chls and their derivatives (especially Na-Cu-chlorophyllin) (Ferruzzi et al. 2002a, b; Lanfer-Marquez et al. 2005; Kumar et al. 2001, 2004) have antioxidant properties (Ferruzzi and Blakeslee 2007). They act as effective scavengers for reactive oxygen species (ROS), e.g., singlet oxygen (Nakamura et al. 1996; Kamat et al. 2000), hydroxyl radical (Boloor et al. 2000), and hydrogen peroxide (Kumar et al. 2001), and they also inhibit lipid peroxidation both in vitro and in vivo in splenic mice lymphocytes (Kumar et al. 2004) and ex vivo in mice brain, liver, and testis (Kamat et al. 2000). Several natural Chl derivatives were shown to inhibit hydroperoxide formation by lipid peroxidation during the exposure of linolenic acid to ferric nitrilotriacetate in the dark: Chl a had the strongest antioxidant activity.

1.5.10 For Photodynamic Therapy (PDT)

Several Chl precursors, analogs, derivatives, and metabolites (e.g., 10-hydroxypheophytin a) are being used as photosensitizers in medicine for PDT of cancer. During PDT, direct and selective tumor cell destruction is obtained by selective accumulation and light-activated ROS-mediated photo toxicity of photosensitizing agents within tumor cells and/or the surrounding vasculature. During this process, excited photosensitizers transfer their excitation energy to surrounding molecules (e.g., to oxygen) to produce singlet oxygen and other ROS and free radicals. In addition to substantial phototoxicity, photosensitizers used in PDT should preferably have low or no dark toxicity and low uptake by normal (non-cancer) cells. One of the major advantages of Chl derivatives in PDT—for instance, when compared with Photofrin, a hematoporphyrin is widely applied in PDT—is that they absorb better penetrating light (wavelengths above 650 nm) and can be, thus, used to treat larger and more deeply seated tumors (Rapozzi et al. 2009). In addition, they may be used to detect tumor cells by fluorescence (You et al. 2011).

1.6 Chemoprevention, Antimutagenicity, Anticlastogenicity, Antigenotoxicity, and Anticancer Therapeutic Activity

Several in vitro and in vivo data indicate that natural Chls (both Chl a and Chl b) and their most important dietary derivatives like chlorins, pheophytins, etc. (Chernomorsky et al. 1999; Ferruzzi et al. 2002a, b) but also Na-Cu-chlorophyllins (used in most studies) can have antimutagenic, mutagen trapping, antigenotoxic, anticlastogenic, and anticarcinogenic effects and can modulate xenobiotic metabolism both in simple model organisms (e.g., Salmonella), in animals (e.g., *Drosophila*, mice, and rats), and in humans (Nagini et al. 2015; Chernomorsky et al. 1997, 1999; Park and Surh 1998; Breinholt et al. 1999; Jubert et al. 2009). Chlorophyll and its derivative, Na-Cu-chlorophyllin, is found to prevent liver cancer in adults exposed to the carcinogen aflatoxin (Egner et al. 2003; Jubert et al. 2009); hence, Chl derivatives (especially chlorophyllin) are available as dietary supplements with anticarcinogenic/chemopreventive effect.

However, some authors have found that the tumor-preventive effects of chlorophyll and their derivatives can be explained by their absorption and their observed postabsorptive chemo-preventive effects on enzymes and other processes (Tumolo and Lanfer-Marquez 2012; Egner et al. 2000; Castro et al. 2009).

1.7 Conclusion

This review summarizes the biosynthetic pathways and focusses on commercial aspects of the photosynthetic pigments in diatoms. It also exposes that chlorophyll and carotenoids, the major photosynthetic pigments present in diatoms, have a great range of applications. However, unmodified chlorophylls are too labile for most practical use, but some derivatives are used due to their coloring effect, tissue growth stimulating effect, antioxidant, and antimutagenic properties, and this chlorophyll can be potentially extracted from diatoms. Even though not all diatom chemicals are known, there is ongoing study to uncover, identify, and examine their properties due to the relevance of these creatures. In a species of marine diatoms *Haslea ostrearia*, a blue-colored water-soluble pigment has been isolated which has antioxidant, anti-microbial, growth-inhibiting, and allelopathic properties (Gastineau et al. 2014). This pigment itself shows the potential of diatoms and their less-explored pigments. Chlorophyll is a most valuable bioactive compound that can be extracted from diatom biomass. It has various uses for its antioxidant and antimutagenic properties; besides, it is commonly used as natural food coloring agent. Nowadays, chlorophyll is being frequently used in the medicine field as remedy and diagnostics. Chlorophyll molecules are used in cancer therapy as a pharmaceutical application. Their roles as modifier of genotoxic effects are becoming increasingly important, besides it being known to have multiple commercial uses.

Last but not least, it is worth mentioning that although diatoms are being extensively used for commercial purposes, the pigments of diatoms have received less attention. Various pigments are yet to be isolated from diatoms which can have possible commercial applications. This is since our knowledge about the photosynthetic pigment and their derivatives present in diatoms is still limited.

Conflict of Interest The authors confirm that this article content has no conflict of interest.

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Chapter 2
Impact Assessment: Diatom Flora of Free-Flowing and Fragmented Stretches of Serially Impounded Bhagirathi River (Indian Himalayan Region)

Sandeep Kumar and Prakash Nautiyal

Abstract Diatom flora is studied in the free-flowing stretch and hydropower-impacted riverine stretches of the serially impounded Bhagirathi R. (the source tributary of the holy Ganga) in the Indian Himalaya. Diatom samples were obtained from a reference station located upstream (u/s) of Maneri stage-1 dam in the free-flowing stretch. Five stations were selected in the fragmented sections of the river receiving either low flows (downstream d/s dams) or regulated flows (d/s power houses).The sampling was performed at monthly interval during October 2016 to September 2017. Species richness (SR), floral composition, and abundance were determined to pinpoint the impacts. The reference station witnesses low SR (39) compared to the modified stretch (64) indicating accumulation of new species, hence the modified flora. However, in comparison to the past 120 species, the SR was very low. β-diversity (0.42) with higher species turnover respectively suggests moderate similarity in flora and species replacement from reference to fragmented stretches. Among the floral elements, araphids record an increase by 6.3% in the modified stretches. The number of abundant species (>10% abundance) increases from reference (5) to the modified section (14). Major species, viz., *Nitzschia palea*, *N. paleacea*, and *C. placentula* var. *euglypta*, recorded abundantly in the modified sections have ecologic preferences for low oxygen and elevated level of nutrients (eutrophic state) indicating more degradation and hence deterioration in these sections.

Keywords Benthic diatoms · Taxonomic richness · β-diversity · Bhagirathi R. · River fragmentation

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P. Srivastava et al. (eds.), *Insights into the World of Diatoms: From Essentials to Applications*, Plant Life and Environment Dynamics,
https://doi.org/10.1007/978-981-19-5920-2_2

2.1 Introduction

Benthic (periphytic) diatoms form the major component of producer community in the mountain rivers (Nautiyal et al. 1996a, b). They respond rapidly in number and composition to the current velocity (Soininen 2005) and changing level of nutrient enrichment (Karthick et al. 2013). These are among the biological quality elements (BQE) established by the European Water Framework Directive (WFD) for assessing the ecological health of water bodies in order to achieve the goal of ecological sustainability.

Ecological preferences of the diatom species led to the development of different indices. The Trophic Diatom Index (TDI), Index of Saprobity-Eutrophication (IDE/S), Specific Pollution Index (IPS), and Generic Diatom Index (IDG) are among the most common indices used for the assessment of trophic state, degradation eutrophication, and pollution, respectively, in the aquatic ecosystems. Besides, diatom species richness is commonly used to assess the habitat degradation (Wang and Wu 2005).

Diatom flora has been extensively studied to assess the impact of hydroelectric projects (HEP) on the river ecosystems (Thomson et al. 2005; Rouf et al. 2009; Wu et al. 2010; Tang et al. 2013; Bergey et al. 2017; Goldenberg-Vilar et al. 2021; Shibabaw et al. 2021). Few such studies exist in the Indian Himalayan region, Sharma et al. (2018) in the regulated sections of the Tons and Yamuna R., and Sharma et al. (2021) in the Tons river.

The present study reports the composition of benthic diatom flora, its distribution, and abundance levels in the HEP-modified stretches of the Bhagirathi R. The comparison of the floral elements was made between the present studies from Maneri to Tehri and earlier nearer to Tehri to pinpoint the changes in the floral elements and hence impacts.

The knowledge of the floristic composition and distribution in the HEP-modified sections, as well as the deviation from previous studies conducted on natural state of the river, will aid decision-makers in the conservation of the holy river in its headwaters. Furthermore, the present study meets the ultimate objective of the National Plan for Conservation of Aquatic Ecosystems (NPCA) of the Indian Government for the water resources (GoIMoEF 2019).

2.2 Materials and Methods

Bhagirathi R., the source tributary of the Ganga, originates from the Gomukh glacier (5299 m a sl) in the Indian Himalayan region. It traverses 196 km distance (Mathur 1991) and join the sister tributary Alaknanda at Devprayag forming Ganga R. onwards. Currently, the lower 121 km section of the river is serially impounded by four dams (Fig. 2.1) for generation of hydropower (2794 MW). As a result, the reduced flows exist in the stretches below each dam, while stretches below the

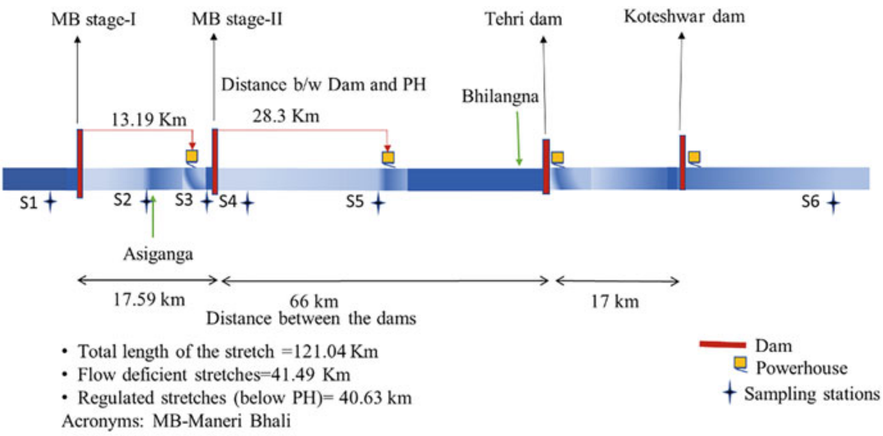

Fig. 2.1 Schematic chart showing series of dams, fragmented stretches, and sampling locations. Reference station lies in the free-flowing stretch indicated by sky blue color; stations S2 to S6 lie in the fragmented stretches indicated by faint blue (flow deficient); mixed dark and faint blue (regulated stretches). The royal blue color has reservoir condition. Distance between the stations was calculated in Google Earth

powerhouse (PH) receive regulated discharge according to the peaking requirements. These stretches receiving modified discharge were sampled at five locations. One reference station carrying unhindered flow representing a pristine section, located distantly u/s, was selected in the river to compare the flora.

Collection of the samples was done at monthly interval during October 2016 to September 2017. The submerged cobble substrate was taken from the riverbed at a depth of 15–30 cm. Three representative samples were scraped and brushed from an area of 3 cm^2 in different flows that form microhabitats (two samples from each: fast, medium, torrential). The samples were brought to the laboratory where processing (acid peroxide treatment) and preparation of the permanent Naphrax mount were carried out. The slides were then examined under the BX-40 Trinocular Olympus microscope (× 15 wide-field eyepiece) attached with condenser and PLANAPO ×100 oil immersion objective and NIKON digital imaging system. Examination of 200–250 valves per slide was done to generate species count. Identification was based on standard literature (Lange-Bertalot 2001; Krammer and Lange-Bertalot 1991; Lange-Bertalot and Krammer 2002; Krammer 2002, 2003; Krammer and Lange-Bertalot 1986a, b, 2000, 2004a, b, c, 2007a, b; Metzeltin and Lange-Bertalot 2002; Metzeltin et al. 2005; Werum and Lange-Bertalot 2004).

Total valves recorded for each flow was summed up to obtain the total count. The relative abundance of floral elements was computed, and the taxa exceeding the share of 10% were considered for comparison. Kruskal-Wallis test was performed for determining significant difference in flora of different stretches using STATISTICA ver.12 software. Cluster analysis (Ward's method) was performed to classify the stretches according to flora using Community Analysis Package (CAP) ver. IV. Components of the β-diversity (turnover and nestedness) were determined between reference and modified stretches using vegan package in R programming. The species accumulation curve was designed using species diversity and richness ver. 4.5 software.

2.3 Results and Discussion

2.3.1 Flora

The diatom flora of the hydropower-modified stretches of the Bhagirathi R. presently contains 1.72% centric and 98.27% pennate elements. Flora consists of 64 species of 20 genera and 5 families (Tables 2.1 and 2.2). In the total flora, centric element was represented by only one species. It belongs to the family Thalassiosiraceae and genera *Cyclotella*. The rest (63 species of 18 genera) were pennate elements. They constitute 4, 4, 8, and 3 genera from family Fragilariaceae (araphids), Achnanthaceae (monoraphids), Naviculaceae (Symmetric 3 and Asymmetric biraphids 5), and Bacillariaceae (biraphids), respectively. *Synedra* (5 species) among the araphids and *Achnanthes* (4 species) among the monoraphids were the species-rich[1] genera. *Navicula* and *Gomphonema* (6 species each) were the species-rich genera among biraphids constituting the bulk of biraphids flora. The pattern of species-rich genera was *Navicula* = *Gomphonema* < *Cymbella*, the remaining being *Synedra* < *Nitzschia* < *Achnanthidium* < *Achnanthes*.

Centric diatoms have very rare occurrence in the Himalayan rivers (Ormerod et al. 1994; Juttner et al. 1996; Verma and Nautiyal 2010). Nautiyal and Nautiyal (1999) reported only one species of *Cyclotella* in the lotic stretch of Ganga and two species in the reservoir section of the Ganga foothills in the Himalaya.

Among the pennate diatoms, the araphids accounts for the 18.6% of the flora. This was quite similar with the 17.5% in the Alaknanda River, the sister tributary of Bhagirathi R. (Nautiyal and Nautiyal 1999) and relatively higher than the central highland rivers (10% in Ken, Paisuni, Tons) of India (Verma and Nautiyal 2010). The share of araphid flora in the other Asian countries was low: 9.4–16.7% (Ohtsuka 2002). The monoraphids and biraphids, respectively, account for 16.96% and 63.8% of the flora and were also reported to be the dominant category in the glacier-fed rivers (Nautiyal et al. 1995; Nautiyal and Nautiyal 1996) and streams of Himalaya (Nautiyal et al. 2004).

2.3.2 Impact on Flora

The flora is compared to previous studies and the reference free-flowing stretch to better understand the impact of serial impoundment. Verma (2008 unpublished)

[1] Genera harboring the largest number of species.

Table 2.1 Flora from the natural and HEP-impacted Bhagirathi R.

Family	Diatom genera	PS	RS	FS
Centrale				
Thalassiosiraceae	**Centric**			
	Melosira	1		
	Cyclotella	2		1
Pennales				
Fragilariaceae	**Araphids**			
	Diatoma	5	2	3
	Synedra	8	5	5
	Ceratoneis			1
	Fragilaria	2		1
Achnanthaceae	**Monoraphids**			
	Achnanthes	1		
	Achnanthidium	17	5	10
	Planothidium	3	1	1
	Cocconeis	3	2	2
Naviculaceae	**Symmetric Biraphids**			
	Adalfia	2		
	Fallacia	1		
	Navicula	20	3	9
	Hipodonta	1		
	Luticola			1
	Pinnularia	1	1	1
	Sellophora	1		
	Stauroneis	1		
	Diploneis	1		
	Asymmetric Biraphids			
	Cymbella	14	8	6
	Encyonma	3	2	2
	Encyonopsis	2		1
	Gomphonema	10	5	9
	Reimeria	2	1	1
Bacillariaceae	*Nitzschia*	17	3	8
	Hantzschia		1	1
	Surirella	1		
	Amphora	1		1
	Total genera	**25**	**13**	**19**
	Total species	**120**	**39**	**64**

PS past studies, *RS* reference stretch, *FS* fragmented stretches

Table 2.2 Diatom flora natural and fragmented stretches of the Bhagirathi R. during 2016–2017

	PS	RS	MS
Centric			
Melosira Agardh	+		
Cyclotella Kutzing	+		+
C. meneghiniana Kutzing	+		
Araphids			
Diatoma anceps Ehrenberg	+		
D. mesodon (Ehrenberg) Kutzing	+	+	+
D. tenuis Agardh	+		+
D. tenuis var. *minus* Grunow	+		
D. vulgare Bory de Saint- Vinccent	+	+	+
Synedra ulna var. *mediocontracta* Forti	+		
S. dorsiventralis Muller	+		
S. inaequalis var. *jumlensis* Kutzing		+	+
S. ulna var. *amphirhynchus* (Ehrn) Grunow	+		
S. u. var. *aquaelis* Kutzing	+	+	+
S. u. var. *contracta* Ostrup	+		
S. ulna Ehrn	+	+	+
S. ulna var. *oxyrhynchus* Kutzing	+	+	+
S. rumpens Kutzing	+	+	+
Staurosirella mutablis Smith			
S. pinnata Ehrn			
Fragilaria capucina var. *vaurcheriae* (Grunow)	+	+	+
F. capucina	+		
Ceratoneis arcus var. *amphioxys* (Rabenhorst) Brun			+
Monoraphids			
Achnanthes. Crenulate Grunow	+		
Achnanthidium conspicua Myer	+		+
A. microcephala (Kutzing) Grunow	+	+	+
A. exigua var. *exigua* Grunow	+		+
A. e. var. *contracta* Torka	+		
A. pseudowazi Carter	+		+
A. subatomus Hustedt	+	+	+
A. marginulata Kutzing	+		
A. Helvetica Hustedt	+		
A. minutissimum var. *minutissimum* (Kutzing) Czarnecki	+	+	+
A. m. var. *affinis* Kutzing	+		
A. m. var. *robusta* Hustedt	+		
A. m. var. *jackii* (Robenhorst) Lange-Bertalot and Ruppel	+	+	+
A. m. var. *scotica* (Carter) Lange-Bertalot and Ruppel	+		+
A. pyrenaicum (Hustedt) Kobavasi	+	+	+
A. sphacelate Carter	+		
A. subhudsonis Hustedt	+		

(continued)

Table 2.2 (continued)

	PS	RS	MS
A. holistica Hustedt	+		
Cocconeis pediculus (Schumann) Cleve	+	+	+
C. placentula var. *euglypta* (Ehrenberg) Grunow	+	+	+
C. p. var. *lineata* Ehrn	+		
Planothidium lanceolata Lange-Bertalot	+	+	+
Planothidium lanceolata var. *elliptica* (Lange-Bertalot)	+		
Symmetric Biraphids			
Hipodonta sp.	+		
Navicula radiosafallax Lange-Bertalot	+	+	+
Navicula sp.			+
Navicula vitabunda Hustedt	+		
N. heimansiodes Lange-Bertalot	+		
N. krammerae Lange-Bertalot	+		
N. broetzii Lange-Bertalot & Reichardt	+		
N. capitellata Cleve-Euler	+		
N. radiosafallax Lange-Bertalot	+	+	+
N. reichardtiana Lange-Bertalot	+	+	+
N. venata Kutzing			
N. capitoradiata Germain	+		+
N. cryptocephala Kutzing	+		+
N. cryptotenella Lange-Bertalot	+	+	+
N. cryptotenelloids Lange-Bertalot	+	+	+
N. densilineolata Lange-Bertalot	+		
N. notha Wallace	+		
N. rostellata Kutzing	+		
N. seminulum Gruonw	+		
N. tripunctata (Mullar) Bory	+		
N. alineae Lange-Bertalot	+		
N. caterva Hohn & Hellerman	+		
Diploneis puella (Schum) Cleve	+		
Fallacia Kutzing	+		
Sellophora	+		
Luticola mutica (Kutzing) Mann			+
Adalfia sp.			
Adalfia. Muscora Kociolek & Reviers	+		
Stauroneis anceps Ehrenberg	+		
Pinnularia sp.	+	+	+
Asymmetric biraphids			
Cymbella vulgata Krammer	+		
C. excisa Kutzing	+		+
C. excisa var. *angusta* Krammer	+		
C. excisa var. *procera* Krammer	+	+	+

(continued)

Table 2.2 (continued)

	PS	RS	MS
C. *excisa* var. *excisa* Krammer	+	+	
C. excisa var. nova	+	+	
C. laevis Kutzing	+	+	+
C. orientalis Krammer	+		+
C. tropica Krammer	+		
C. tumida (Brebisson) Van Heurck	+	+	+
C. turgidula Grunow	+	+	+
C. turgidula var. *bengalensis* Krammer	+	+	
C. turgidula var. *venezolana* (Krammer)	+	+	
C. aspera (Ehrenberg) Cleve	+		
Encyonema minutum Hilse ex Rabenh	+	+	+
E. jemtlandicum var. *venezolanum*	+		
E. silesiacum (Rabenhorst)	+	+	+
Encyonopsis leei Krammer	+		+
E. l. var. *sinensis* Metzeltin & Krammer	+		
Denticula kuetzingii Grunow	+		
Gomphonema gracile Ehrenberg	+		
G. angustatum (Kutzing) Robenhorst	+	+	+
G. angustum Agardh	+	+	+
G. clavatum Ehrenberg	+		+
G. intricatum Kutzing	+	+	+
G. lanceolatum Agardh			+
G. minutum Agardh	+		
G. olevaceaum (Hornemann) Ehrenberg	+		+
G. olivaceum var. *olivaceoides* (Hustedt) *Lange-Bertalot*			+
G. parvulum Kutzing	+	+	+
G. pumilum (Grunow) Reichardt and Lange-Bertalot	+	+	+
G. pumilum var. *rigidum* Reichardt & Lange-Bertalot	+		
Nitzschia denticula Grunow	+		
N. dissipata Kutzing	+		+
N. fonticola Grunow	+	+	+
N. gracilis Hantzsch	+		
N. acuta Hantzsch	+		
N. tabellaria Grunow	+		
N. amphibia Grunow	+		+
N. inconspicua Grunow	+		
N. frustulum (Kutzing) Grunow	+	+	
N. linearis Agardh	+		
N. minima Meister	+		
N. amphibia var. *intermedia* Mayer	+		+
N. palea Grunow	+		+
N. pseudofonticola Hustedt	+		+

(continued)

Table 2.2 (continued)

	PS	RS	MS
N. paleacea Grunow	+	+	+
N. tabellaria Grunow	+		+
N. tenuis Smith	+		
Reimeria sinuate Gregory	+	+	+
R. uniseriate Guerrero & Ferrari	+		
Surirela angusta Kutzing	+		
Amphora inariensis Krammer	+		+
Hantzschia		+	+

PS past studies, *RS* reference stretch, *FS* fragmented stretches

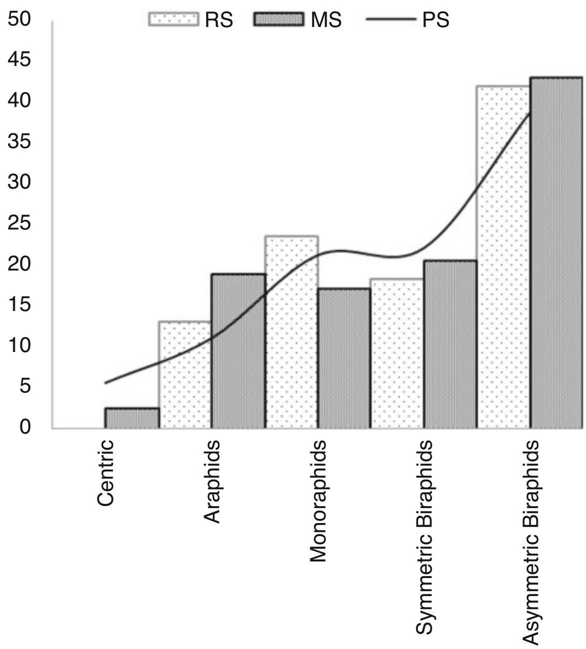

Fig. 2.2 Percentage composition floral categories in the reference fragmented and free-flowing (past) stretches in the Bhagirathi R.

reported 109 species belonging to 24 genera and 6 families in the natural flowing at Tehri before THDC became functional. Hence, flora tends to decline in the fragmented stretches, but the decline was insignificant (K-W test $p < 0.05$). Araphid and biraphids share increases from 11.1% to 18.64% and 50% to 52.3%, while monoraphids have decreased from 21.3% to 16.94% (Fig. 2.2). The increased araphid share is attributable to the prolonged water retention in low flow stretches, which are ideal for araphids because they lack a raphe system for attachment and hence mostly planktonic (Kooistra et al. 2009).

Compared to the reference station 39 species of 15 genera, 5 families, the species richness increases to 64 species in the fragmented stretches. This increase is due to

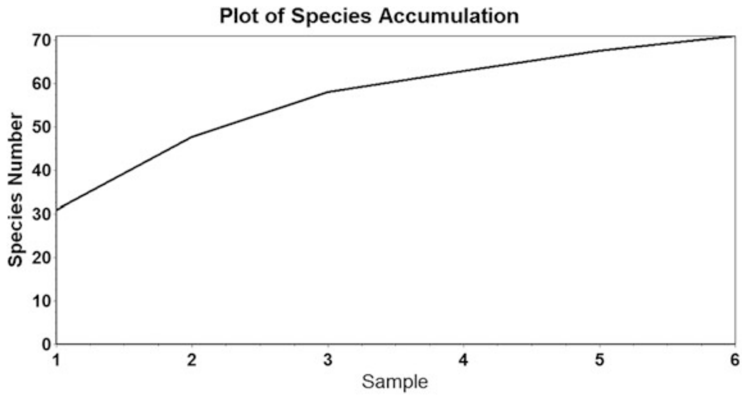

Fig. 2.3 Species accumulation curve showing increasing number of species at d/s stations

the accumulation of new species at the d/s stations (Fig. 2.3). The β-diversity (0.42) indicates moderate level of similarity in diatom flora between reference and fragmented stretches. However, the higher turnover (59.2) suggests the replacement of diatom species, while nestedness (40.7) indicates low richness differences. The cluster further verify the moderate similarity in flora between reference and fragmented stretches, while the past flora was quite different, hence grouped separate as an outlier (Fig. 2.4).

The pattern of species-rich genera in the modified section was quite identical. However, the reverse pattern in the remaining genera (*Achnanthidium* < *Achnanthes* < *Synedra* < *Nitzschia*) signifies the low flow along with some sort of changes in the nutrient level in the modified stretches favorable for *Synedra* and *Nitzschia*, respectively. Growns and Growns (2001) reported *Synedra* as indicator genera in the fragmented stretches of the Australian rivers as it possesses both attached and planktonic species (Gell et al. 1999) and hence recommended as useful indicators in future monitoring studies. Nautiyal et al. (2004) recorded *Navicula* (50) followed by *Achnanthes* (49) *Cymbella* (39) as species-rich genera in the Alaknanda-Ganga river system. Sharma et al. (2021) reported *Cymbella* > *Achnanthidium* > *Navicula* in the impounded and natural sections of river Tons.

Significant difference was observed in flora (K-W test $p < 0.05$) between reference location u/s Maneri (1300 m asl; 37 species) and the earlier study[2] ~15 km upstream of Tehri (600 m asl; 109 species). These locations are ca. 100 km apart, showing a fall of 700 m. The richness is low at Maneri, which increases down near Tehri. The floral elements increase in the middle and lower sections of the rivers (Nautiyal and Nautiyal 1999; Verma and Nautiyal 2009).

[2]Much before commissioning of Tehri dam.

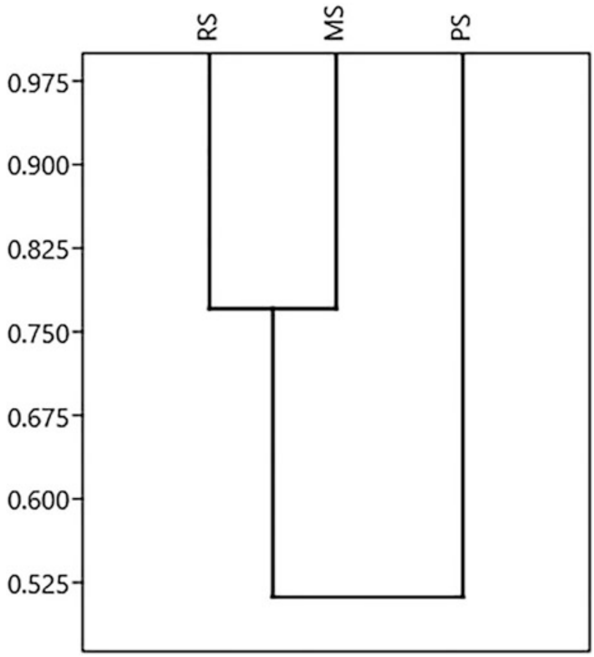

Fig. 2.4 Classification of stretches (Bray-Curtis clustering) based on diatom flora

2.3.3 Abundance

The most abundant taxa reflect the ecological state of any aquatic ecosystem (Kelly et al. 2008). Fourteen diatom species were recorded to be abundant (>10% relative abundance) in the modified stretches of the Bhagirathi R. (Table 2.3). The number of abundant species was surprisingly much more than the reference stretch where only five species were recorded: a notable shift.

This study demonstrates that flow reduction provides ideal conditions for new species that did not occur previously (Suren and Riis 2010). The present study records *Nitzschia palea* as new species appearing in the modified sections. The abundant taxa, viz., *Nitzschia palea*, *N. paleacea*, and *C. placentula* var. *euglypta* have van Dam ecologic preferences for low oxygen and elevated level of nutrients (eutrophic state) indicating more degradation in these sections. Rakowska (2001) and Noga et al. (2014) recorded *Nitzschia palea* and *Cocconeis placentula*, abundantly in the polluted rivers. The other taxa *S. ulna* and *S. ulna* var. *oxyrhynchus* require moderate oxygen level, occasionally aerophilic, tolerant-N-autotrophic, and eutrophic taxa. The presence of these taxa in the modified stretches indicates deteriorating state of the Bhagirathi River ecosystem in present times.

Table 2.3 Dominant taxa (>10% composition) forming assemblages in natural and modified stretches of the Bhagirathi River during 2016–2017

	Reference	Regulated
1	*A. minutissimum* var. *minutissimum*	*A. minutissimum* var. *minutissimum*
2	*R. sinuata*	*R. sinuata*
3	*A. pyrenaicum*	*A. pyrenaicum*
4	*A. subatomus*	*A. subatomus*
5	*G. parvulum*	*G. parvulum*
6		***E. minutum***
7		***C. placentula.* var. *euglypta***
8		***C. laevis***
9		***S. ulna.* var. *oxyrhynchus***
10		***E. silesiacum***
11		***N. palea***
12		***S. ulna***
13		***N. paleacea***
14		***D. tenuis***

Acknowledgments The author SK acknowledges the UGC-CSIR for SRF (NET) fellowship. Both the authors SK and PN thank the Head of the Department of Zoology for extending lab facilities.

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Web Resources

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https://earth.google.com/

Chapter 3
Use of Multivariate Techniques for Quick Assessment of Hydropower Impacts on the Producer Community in Himalayan Rivers with a Note on Required Sample Size

Prakash Nautiyal and Tanuja Bartwal

Abstract Multivariate techniques have been often used to compare and classify sampling sites. Here, it was used to make a quick assessment of the impact caused by flow modifications for Vishnuprayag and Srinagar hydroelectric projects on the producer communities constituting first trophic level of the Alaknanda River in Himalaya. For this study, the diatom communities were sampled during October, November, and February from flowing sections at three stations in each HEP; one u/s of dam, other two d/s of dam and powerhouse (PH). Species count was generated for each station. Both nonmetric multidimensional scaling (NMDS) and cluster analysis revealed two and three groups of locations, respectively, for 3- and 12-month data set. In case of the former, free-flowing S3 and deep column S5 were forming a subcluster, but no reasons were evident, as these were diametrically opposite locations, making it difficult to interpret. In case of the latter, the grouping was more reasonable as S2–S3 exhibit higher similarity, while S5 is distinct and S1 remained as an outlier. The study suggest that there is rapid change in diatom community structure disrupting River Continuum Concept (RCC), possibly due to modified hydrological regimes in longitudinally placed locations of the river resulting in separate subgroups. Analysis with large and small (data deficient) sample size shows differences in NMDS and cluster groupings, which appear to be irrelevant, demonstrating poor efficacy. Larger sample size was found to make the analysis more robust.

Keywords Alaknanda · Hydropower · Diatom community · Multivariate techniques

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P. Srivastava et al. (eds.), *Insights into the World of Diatoms: From Essentials to Applications*, Plant Life and Environment Dynamics,
https://doi.org/10.1007/978-981-19-5920-2_3

3.1 Introduction

Univariate, bivariate, and multivariate statistical techniques are generally used to study biotic communities. Of these, NMDS, PCA, CCA, and RDA are commonly used ordination techniques (Anderson et al. 2006; Nautiyal & Mishra 2013; White et al. 2016; Zhao et al. 2019; Ko et al. 2020).These techniques have been used to study drivers of diatom community structure in unimpacted (Soininen et al. 2004; Virtanen & Soininen 2012; Verma et al. 2016) and human impacted regions (Hlubikova et al. 2014; Nautiyal et al. 2015; Quevedo et al. 2018; Mutinova et al. 2020).

River Alaknanda is impacted by a series of two hydroelectric projects within a distance of ~140 km, as dam and power house modify the river flow in this stretch and disrupt the continuum. We presume that the community will be impacted in contrast to expected gradual change in community (RCC; Vannote et al. 1980). The purpose of this study is quick assessment (Chessman et al. 1999) of the river ecosystem using multivariate techniques. Nonmetric multidimensional scaling and cluster analysis are used to capture the changes in diatom community in a river serially impacted by hydroelectric projects. NMDS is used to compare the sampling sites in a two-dimensional plot in which sites with similar community structure are located close together compared to those far apart. Cluster analysis was used to classify sampling sites based on similar or dissimilar species abundance. This study can be useful in the management of river ecosystem health. In order to know the robustness of the statistical analysis, a 12-month data set for all other stations except S1 and S2 was subjected to NMDS and cluster analysis.

3.2 Study Area

Alaknanda is one of the major tributaries of the river Ganges. The river is impacted by two hydroelectric projects: one in headwaters, Vishnuprayag hydroelectric project (VHEP), and another in lower stretch, Srinagar hydroelectric projects (SHEP). The following six sampling locations were selected for this study: S1, 1 km u/s from the VHEP with steep slope; S2, d/s of the VHEP flow-deficient section; S3, 53 km d/s of VHEP power house (PH); S4, 30 km d/s from S3, considered as free-flowing section; S5, 1.5 u/s from the tail of SHEP impoundment; and S8, the station below the PH carrying regulated discharge from the SHEP.

3.3 Methods

Two types of samples were obtained: 3-month (small) and 12-month (large) data set. These sets differ in containing 3-month data from S1 to S8 compared with 3/4 months for S1 and S2 (not accessible) and 12 months for S3 to S8. Diatoms samples were collected by scraping 3×3 cm^2 area of cobbles from the sampling sites. Two replicates of samples were collected in sampling vials. Acid-peroxide treatment was used to clean the diatom frustule. Permanent slides were prepared using Naphrax (RI 1.74) and observed under BX 40 Trinocular Olympus microscope. Diatom count data was generated for NMDS and cluster analysis using CAP ver. 4.0. Ward's method (Bray-Curtis measure) was used to record distances and classify the stations. In order to detect robustness of results, small and large data sets were compared.

3.4 Results and Discussion

Multivariate and ordination techniques have been used to study composition of diatom flora, diversity, macroinvertebrate assemblages (Mishra & Nautiyal 2017), and fish fauna (Nautiyal et al. 2014) in the Ganga river from source in the Himalaya to Upper Gangetic Plains and in the Bundelkhand Plateau rivers. This study moves a step forward to make quick assessment of rivers modified by hydroelectric projects, using such statistical techniques. Nonmetric multidimensional scaling and cluster analysis of small data set creates two clusters (Figs. 3.1a and 3.2a). Since results are similar, the interpretation is described for cluster analysis. Of the two major clusters, one comprised of S1, S2, S3, and S5 and the other of S4 and S8. Within the first cluster, S1 and S2 are distant from each other and from S3 and S5 as well. The second cluster comprising of S4 and S8 are far distant. The continuum is breaking after S3 as it is more similar to S5 rather than S4. However, there is no reason for similarity as S3 is below the PH and S5 is u/s of the dam beyond impoundment. Apparently, there seems to be no similarity in physiochemical conditions at S3 and S5 in general and flow. Higher flows at S3 facilitate flushing of sediments compared to S5, where flushing is compromised due the tail of impoundment (a deep-water column created for SHEP). Substratum also differs, coarse hard at S3 compared to soft smaller sediments at S5. In a separate study, van Dam ecological values indicate different ecological categories: polyoxybiontic O_2, mesotrophic, and neutrophilic at S3 compared to moderate O_2, eutrophic, and alkaliphilic at S5.

The stations S4 and S8 are more similar as they are free-flowing stretches even though they are located in a considerable distance from the PH, respectively. Further, they are below PH, and both experience perturbed flow regimes due to peaking requirements of VHEP and SHEP. While flow regimes were responsible for the similarity between S4 and S8, the same does not appear to be the case among S3 and S5 (Figs. 3.1b and 3.2b). This suggests that there is ambiguity in the results as cluster

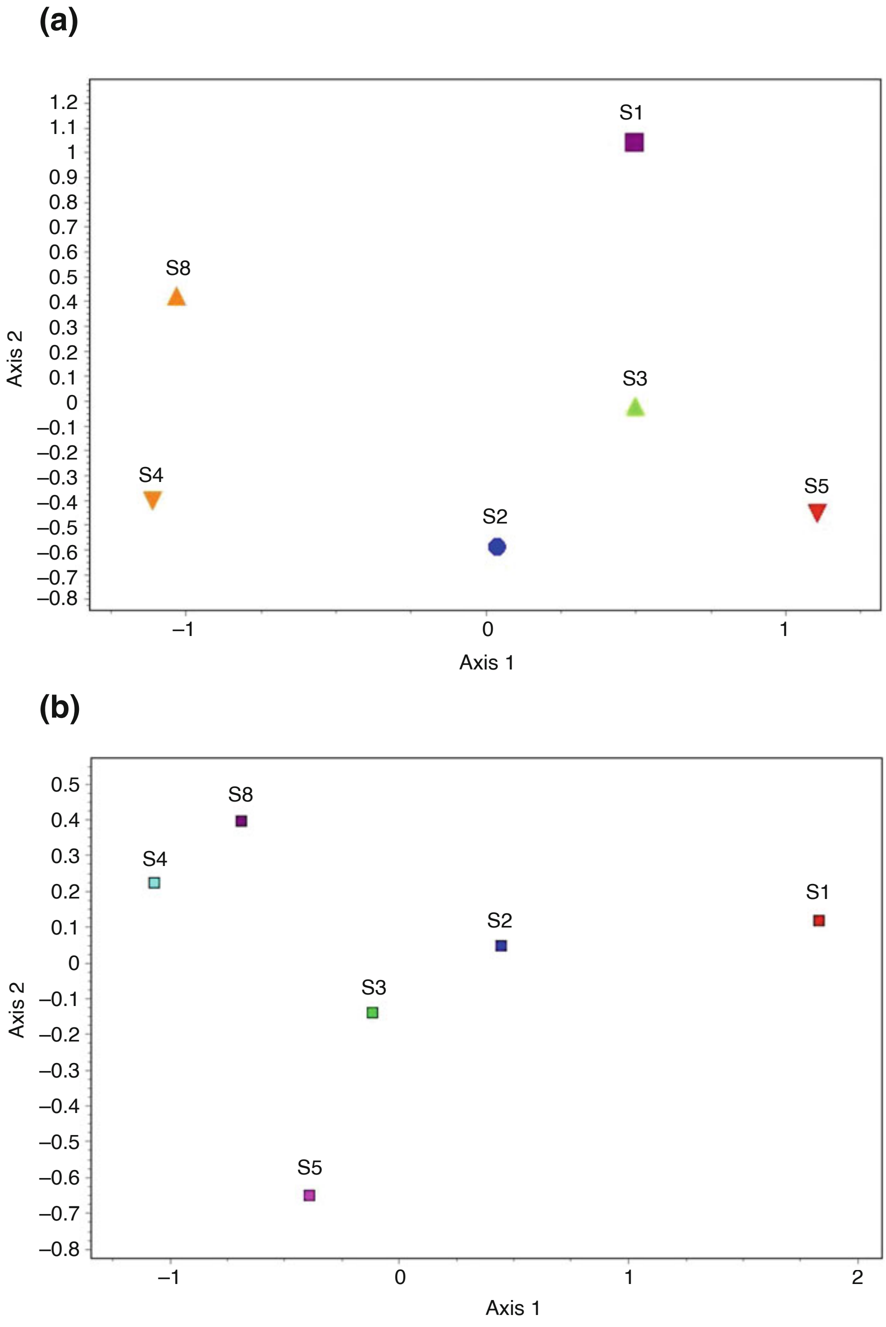

Fig. 3.1 (**a**) Nonmetric multidimensional scaling (MDS) plots for similarity/dissimilarity of diatom community between sampling sites based on small sample size (stress: 0.03). (**b**) Nonmetric multidimensional scaling (MDS) plots for similarity/dissimilarity of diatom community between sampling sites based on large sample size (stress: 0.004)

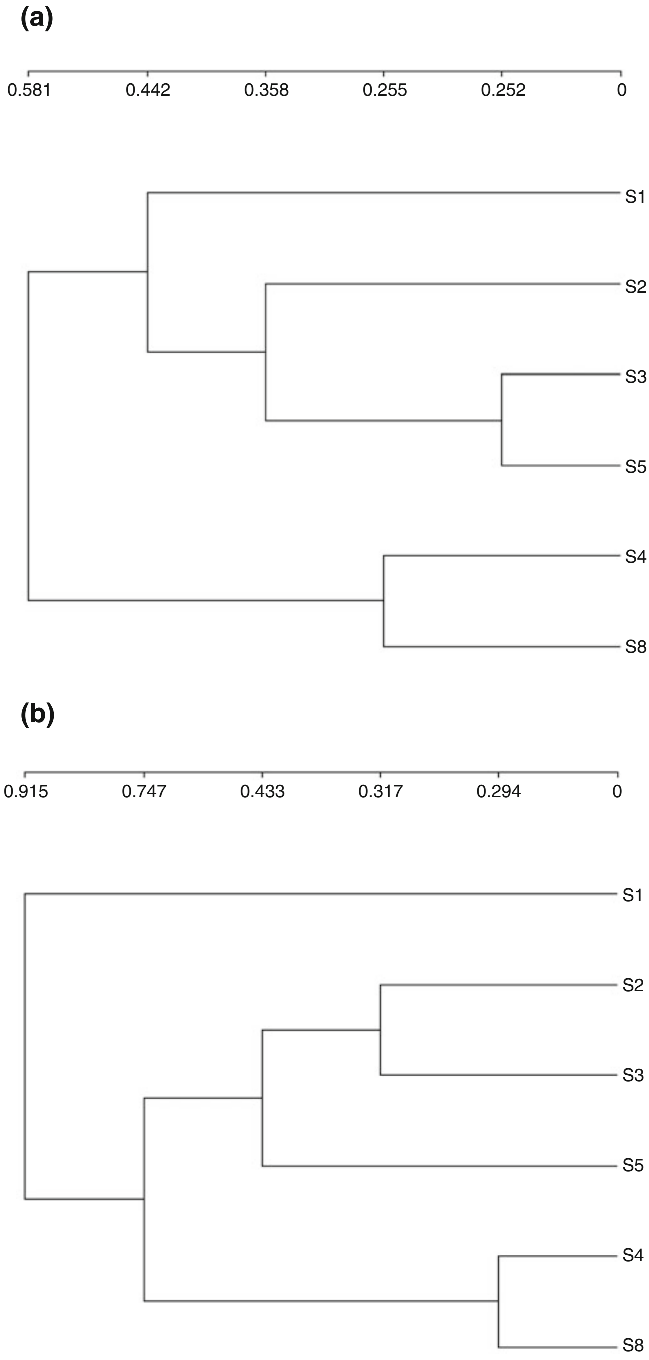

Fig. 3.2 (**a**) Cluster analysis for similarity/dissimilarity of diatom community between sampling sites based on small size. (**b**) Cluster analysis for similarity/dissimilarity of diatom community between sampling sites based on large sample size

analysis is showing the similarity between S3 and S5 despite the abovesaid differences between the stations. Fore et al. (1996) also reported that the PCA did not detect clear difference between most and least disturbed site.

However, there is difference in clusters when larger-sample size was analyzed. Both NMDS and cluster analysis techniques revealed three groups of locations: first S1 totally distinct from the rest as an outlier (possibly due to scanty data—3 months), second S2–S3–S5, and third S4–S8. The second cluster has two subgroups one comprising S2–S3 exhibiting higher similarity, while S5 is distinct but distantly similar to S2–S3. These groups and subgroups instead of one group with similar subgroups occurring in a longitudinal fashion clearly demonstrate disruption of the RCC. Analysis with large and small sample size shows differences in NMDS and cluster groupings, which appear to be irrelevant, demonstrating poor efficacy. Larger-sample size was found to make the analysis and result more robust.

3.5 Conclusion

The study suggests a rapid change in diatom community composition (disruption of RCC), attributable to modified horological regimes. Thus, the producer community is seriously affected by hydropower development. This study also shows poor efficacy of small data sets for present study. Large data set was more efficient.

Acknowledgments Authors are thankful to GBPINHSED, Kosi Katarmal, for providing financial assistance under NMHS, and the Head of the Dept. of Zoology, HNB Garhwal, for providing lab facilities.

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Chapter 4
Alpine Lake Environments and Psychrophile Diatoms Around the World with a Particular Emphasis on Turkish Glacial Lakes

Cüneyt Nadir Solak, Paul Hamilton, Łukasz Peszek, Małgorzata Bąk, Elif Yilmaz, Korhan Özkan, and Nesil Ertorun

Abstract Cold environments are considered extreme biomes and historically thought to be barren and unproductive. However, investigations show that some of the coldest regions in the world represent unique biodiversity hotspots. In this review, the prominent diatom communities of alpine lakes and ponds across different parts of the world, including the Arctic, Antarctic, and alpine regions, are evaluated. Glacial lakes in western Asia (Turkey) are specifically examined as a proxy for extreme cold aquatic systems between the primary mountainous areas of the Alps and the Himalaya. Biogeographically, Turkey is a subtropical region with two prominent glacial ecozones (southern Taurus and northeastern Anatolian Mountains). In this study, glacial lakes in the Kaçkar Mountains, northeastern Anatolia, are evaluated noting the importance of genera with smaller diatom taxa including *Genkalia*, *Psammothidium*, and *Eunotia*. The presence of genera like *Genkalia*

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P. Srivastava et al. (eds.), *Insights into the World of Diatoms: From Essentials to Applications*, Plant Life and Environment Dynamics,
https://doi.org/10.1007/978-981-19-5920-2_4

and cold environment species like *Psammothidium subatomoides* highlight the isolation of alpine biomes in subtropical regions. Broad biogeographic distributions of biological diversity from polar to alpine are considered, and a number of taxa within *Eunotia* and *Psammothidium* are recorded for the first time in this region.

Keywords Antarctica · Arctic · Kaçkar Mountains · *Psammothidium subatomoides* · Swiss Alps

4.1 Alpine Environments and Biomes

Extreme biomes have hidden mysteries that intrigue our sense of curiosity and understanding. Few get to see these environments, and even fewer have the chance to study them. Of the extreme biomes, glacial, Arctic, Antarctic, and alpine ecozones are globally the most prominent and significant to Earth's well-being. The capture and control of surficial water supplies hinges on the availability of water over Milankovitch orbital cycles of global cooling and warming (glacial and interglacial) (Bennett 1990; Huybers and Curry 2006). Through historical time, humans and human cultures have passed through or established living communities in cold biomes, although to-date sustainable living in these environments is limited. The most notable example of humans interacting with extreme cold environments is the migration of *Homo sapiens* from Africa to northern Asia (Russia) and then migrating across the Bering Strait, between the Chukchi and Bering Seas, to North America (Fladmark 1979; O'Rourke and Raff 2010). Paleo-Inuit and Indigenous Peoples of the North established living communities that survived and even thrived in cold environments across the Arctic. The successful survival of these early peoples and cultures is with the understanding that to survive means knowing the living conditions and biota of cold environments and using them in a sustainable way. Likewise, isolated cultures living in alpine ecobiomes around the world have an understanding of their restricted conditions (Rhoades and Thompson 1975; Viazzo 1989). Early cultures recognized the importance of living in cold environments without disturbing or with limited disturbances to the ecosystem. This knowledge, historic (traditional) understanding of cold extremes, is now becoming available to society at large and will become more incorporated into our scientific knowledge of extreme cold systems.

Data from the World Bank (2021) highlights the fact that mountains cover 25% of the Earth's land surface, supply freshwaters to more than 3.5 billion people, and maintain 12% of the human population. Alpine environments are found on every continent where continental uplift and volcanic activity occurred. The Antarctic is an extreme cold environment which is primarily ice coved and not considered to have alpine environments distinct from the rest of the continent. The prominent alpine ecobiomes are found across the Andes (South America), Rocky Mountains (North America), Alps (Europe), Eastern Rift Mountains (Africa), and Himalayans (Asia) (Figs. 4.1 and 4.2). In the western part of China, significant additional alpine ecobiomes are found in the Kunlunshan, Tianshan, Taihangshan, Qilianshan, and

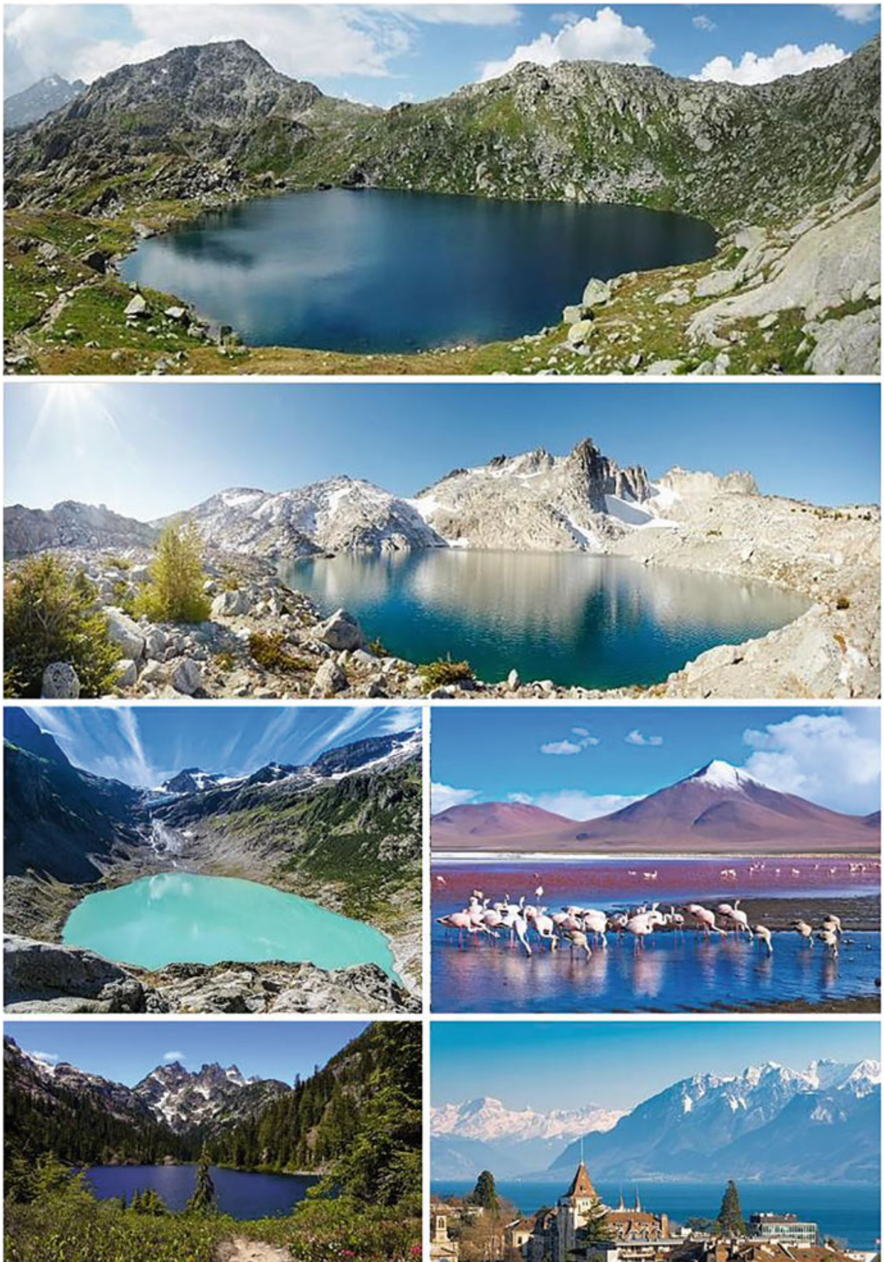


Fig. 4.1 Selected high alpine, alpine, and peri-alpine lakes and ponds. Top: Lake Orsirora (Switzerland), a high alpine cirque lake with sapphire blue (dark blue) waters surrounded by moss, grass, sedge, and boulder terrain. Second row from top: the Enchantments, Washington State, USA, a high alpine cirque lake with blue-green-colored waters surrounded by exposed

Hengduanshan mountains. The southern Alps of New Zealand have alpine ecobiomes, although the notable waterbodies associated with the Southern Alps are alpine and below treeline. Indeed, there are countless number of alpine lakes, ponds, and waterways around the world. WIKIpedia (2021) lists 40 famous alpine lakes, and it is no surprise that the majority is from the extensive Asian mountains with historical links to global trade and the Silk Road (lakes: 15, Pakistan; 7, India; 2, Nepal).

Alpine ecozones are identified as high-altitude regions above treeline (Pechlaner 1971). This ecozone is geographically characterized by steep slopes with high runoff (seepage, streams, and rivers) and variable conditions of unstable to stable biological growth. Water is retained in sections of flattened terrain (e.g., cirques or flatted meadows) across the alpine zone, and in these areas biological communities have more sustainable living conditions. Although sustainable, alpine wetlands, pond, and lakes are considered extreme biomes (Catalán et al. 2006; Hinder et al. 1999). In this chapter, alpine ecobiomes are separated into to three categories: (1) high alpine (high altitude cirques and valley meadows) (above treeline, >2500 m), (2) alpine (forested slopes and valleys) (1500–2500 m), and (3) peri-alpine (lakes and wetlands in the foothills of mountains) (below treeline, <1500 m).

In shallow waters, ice forms to the bottom sediments with no living life in the ice, except microbes. In these shallow frozen waters, sediments below the ice maintain a vast diversity of life which annually reappears in the warm seasons with primary production again feeding the food web. In deeper alpine lakes, with ice-covered water, the long winter seasons have extended thickened ice (up to 2 m) and snow cover which minimizes light availability for low lotic biological productivity (Ventelä et al. 1998). During the shortened growing season, there is an elevated level of solar radiance with altitude, which supports lotic productivity across the food web (Billings and Mooney 1968; Rose et al. 2009).

4.2 Cryoconite Holes

Small holes, water-filled depressions, and westage areas are commonly observed on the surface of glaciers. These cryoconite holes take their name from the presence of sediment-cryoconite or cold rock dust (Wharton et al. 1985). Arctic explorer A. E.

Fig. 4.1 (continued) bedrock and ion salt precipitate deposits (white). Third row from top, left: Trift Lake (Switzerland), high elevation alpine lake with turquoise (floured)-colored waters surrounded by a mix of exposed bedrock and vegetation. The sediment flour comes from the glacial stream at the top of the picture. Fourth row from top, right: Lake Colorada (Bolivia), a high alpine lake with extensive plankton and benthic invertebrate productivity (red colorations) associated bird and mammal wildlife. Bottom left: Unnamed Lake (Washington State, USA), an alpine lake with surrounding tree cover. Bottom right: Lake Lucerne (Switzerland), a large peri-alpine lake with a distinct limnion-layered structure. Image Copyright license agreements acquired from Gettyimages.com, 123rf.com, and share images from unsplash.com

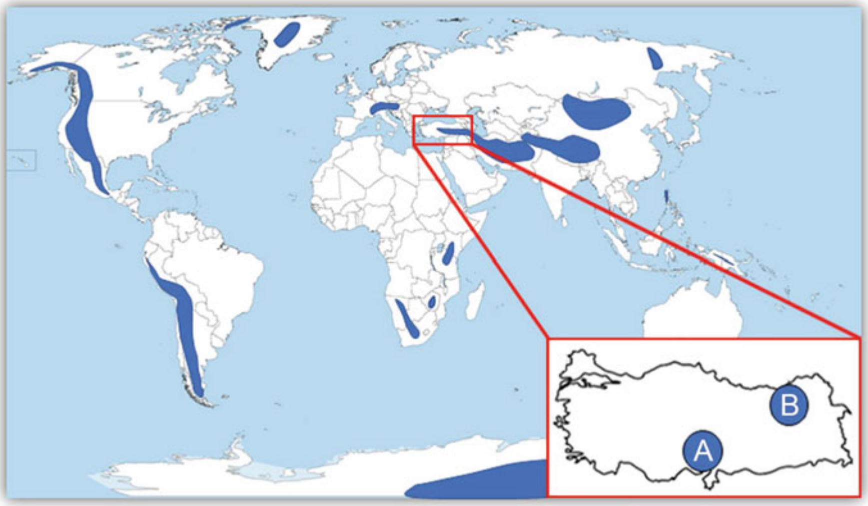


Fig. 4.2 Antarctic, Arctic, and high alpine mountain areas at different latitude zones. Insert map of Turkey ((**a**) southern Taurus Mountains in Turkey; (**b**) northeastern Anatolian Mountains). Modified base map from Google, Creative Commons CCO Licence, GNU Free Document Licence

Nordenskjöld created this term in his first trip to the Greenland Ice Cap in 1870 (Leslie 1879). He chose to name it from the Greek: *kruos* (ice) and *konis* (dust) (Gajda 1958). Wharton et al. (1985) noted that cryoconite holes may result partly from biothermal energy released by algal metabolism and microorganisms. Further, biological life in cryoconite holes could play a role in the colonizing of newly exposed habitats following glacial retreat. The holes can be in different sizes (less than 1 cm to 1 m in width) and rarely deeper than 60 cm (Steinböck 1936; Von Drygalski 1897). They have been reported throughout the world (in Greenland by Poser 1934; Svalbard by De Smet and Van Rompu 1994; Canada by Adams 1966; Antarctica by Wharton et al. 1985; glaciers of the Rocky Mountains by McIntyre 1984; Ecuador by Chamorro et al. 2021; and Himalayas by Takeuchi et al. 2000).

Previously, glaciers were considered as unproductive environments. However, this coldest of Earth's biomes has biodiversity hotspots of psychrophiles (Anesio and Laybourn-Parry 2012; Buda et al. 2020). Living organisms in these holes have been investigated since the 1930s (Charlesworth 1957; Gerdel and Drouet 1960; Mueller et al. 2001; Steinböck 1936; Wharton et al. 1981). In a comprehensive study, Mueller et al. (2001) compared southern (Canada Glacier) and northern (White Glacier) cryoconite holes in Antarctica. They reported that coccoid cyanobacteria were dominant in the southern cryoconite holes, whereas desmids were dominant in northern cryoconites. Further, there were a greater number of taxa observed in the Canada Glacier cryoconite. Regarding the diatoms, Chamorro et al. (2021) found 278 diatom taxa in 54 surface holes in the Antisana Glacier (Ecuador), and they reported that cryoconite holes are sites of high diversity. Moreover, the diatom *Muelleria cryoconicola* was described from the Commonwealth Glacier in Taylor Valley, Antarctica (Van de Vijver et al. 2010).

4.3 Alpine Lakes and Ponds

Although the African continent is relatively flat and dry, it has some of the oldest mountain ranges including extensive alpine ecobiomes. The Barberton Greenstone belt in South Africa, with an estimated age of 490 million years, is the oldest mountain range in the world (De Ronde and De Witt 1994). Other South African ranges (Waterberg, 2.7 billion years; Magaliesberg, 2.4 billion years; Pilansberg, 1.2 billion years) compliment the ancient continental formation of Africa. Mount Kenya, at 9 degrees south latitude, is an old mountain range (ca. 3 million years) which has been extensively eroded across today's glacial interglacial cycles forming a rugged topography with high altitude alpine lakes and ponds (Eggermont and Verschuren 2007). In the nearby Rwnnzori Mountains, close to the Equator, most of the alpine lakes are at high altitude (>3500 m) with glacial meltwater inputs in exposed bedrock catchments and scattered alpine terrestrial vegetation (Eggermont et al. 2007). Biological studies on African alpine lakes are limited (Barker et al. 2001).

The majority of freshwater lakes in South America were created through glacial activity and tectonic-volcanic events in the alpine areas of the Andes or in the surrounding foothills (Llames and Zagarese 2009). Lake Titicaca with an elevation of 3,812 m can be considered an alpine lake, although it is below treeline in the foothill's region (peri-alpine) and water temperatures do not fall below zero. Lake Titicaca is one of South America's largest lakes at 190 km long and up to 80 km wide. Historically, the lake has undergone significant water level changes over glacial and interglacial cycles (Fritz et al. 2007), and more recently (<2500 yBP), the land experienced human settlement. Evapotranspiration, caused by intense light, wind, and regional climate, accounts for ca. 90% water loss. Lake Titicaca has been investigated by different diatomists (Frenguelli 1939; Servant-Vildary 1991; Tapia et al. 2003), and some new centric and araphid diatom taxa have been described, *Cyclotella andina*, *Pseudostaurosira decipens*, *P. sajamaensis*, *Staurosira kjotsunarum*, *Ulnaria titicacaensis*, and *U. macilenta* (Morales et al. 2012, 2014; Theriot et al. 1985). The highest alpine ponds (pools) in the world are present in the Andes (Tsarenko et al. 2021). Lake Licancabur (Chile/Bolivia) at 5916 m a.s.l. is the highest lake covering a 6000 m^2 area. The lake is slightly saline and may have some geothermal heat sources. Ojos del Salado (Chile/Argentina) is the highest pond (pool) in the world at 6390 m a.s.l. Ojos del Salado is 100 m in diameter and shallow with turquoise (floured) water color. Acamarachi Pool (Chile) at 5950 m a.s.l. was the 5th highest pond, last known to be 10–15 m in diameter and now may be extinct. The status of Cerro Walter Penck pool(s) (Argentina), 6th highest in the world, is uncertain although reported with possible high sulfur content (Scanu 2013). Other high alpine glacial Andean lakes include Tres Cruces Norte (Chile); Lagunas Palcacocha, Perolcocha, and Tullpacocha (Parque Nacional Huascaran, Peru); and

Lagunas Huarmicocha, Colorcocha, Challhuacocxha, and Purectishgo from the Huanuco Lima Region (Peru). Biological studies on high alpine lakes in South America are limited (e.g., Aszalós et al. 2020).

The North American Rock Mountains have uncounted high alpine and alpine lakes, ponds, and wetlands scattered across the western marginal regions of the United States and Canada. Crater Lake, in the state of Oregon (USA), is the 2nd deepest lake in North America and 9th deepest lake in the world. Crater Lake (alpine) freezes every year and is considered a glacial lake (glaciers on adjacent mountains) with minimal lake outflow. Evaporation, rainfall, and snow fall are the modulating factors for water retention (Redmond 2007). The lake is below 3000 m (1884 m) and in the tree zone. The largest alpine lake in North America is Lake Tahoe with a surface area of 490 km^2 at an elevation of 1898 m (Gardner et al. 2000). Lake Tahoe is the second deepest lake in the United States with a depth of 501 m. The lake is below 3000 m (1898 m) a.s.l. and within the tree zone. Lake Tahoe does not freeze over during the winter. Other notable glacial alpine lakes include Tenaya Lake (2484 m a.s.l., max. depth 35 m), Yellowstone Lake (2357 m a.s.l., max. depth 120 m), Moraine Lake (1885 m a.s.l., max. depth 14 m), and Lake Louise (1768 m a.s.l., max. depth 70 m). Tenaya Lake is an isolated glacial lake, while Yellowstone has extensive tourist activity, and lakes Louisa and Moraine maintain local communities plus tourism. The Volcanogenic Garibaldi Lake (1484 m a.s.l., 109 m mean depth) is a more undisturbed natural alpine lake, semi-isolated with minimal camping and hiking activity. A high alpine lake of note, Thousand Island Lake (3001 m a.s.l., max depth 27 m, USA) is an isolated lake with hiking activity, formed at the bottom of a cirque after glacial retreat (Schoenherr 1992). A lot of research has been conducted on North American alpine lakes (selected references presented in text below) with many new diatom species described (as an example, Bahls 2010).

The Tibetan plateau and Himalayan Mountains comprise a countless plethora of alpine lakes ranging from ultra-freshwater to salt (or soda) lakes. The average elevation of the Tibetan Plateau is 4300 m a.s.l., and most of the lakes are categorized as salt lakes with varying levels of mineral loading. Snowmelt and rain are the primary water sources for lakes on the plateau. The largest lake, Namtso (4718 m a.s.l.), is an old lake, formed during the Paleogene age, and although without major settlements, the lake is surrounded by pastoral lands and is considered one of the holiest by Buddhist pilgrims (Li et al. 2008). In addition to snow and rain, Namtso Lake also has glacial water inputs. Lake color and mineral content vary greatly across the plateau, with sapphire blue (dark blue) lakes (e.g., Namtso Lake) the most evident using Landsat Copernicus imagery (Google Earth 2021). Turquoise (flour lakes) are the next prominent and located within valleys with high glacial mineral sediment loading. Some examples of these flour lakes include Tong Tso (4399 m a.s.l.), Zhaxi Co (4421 m a.s.l.), Gopug Co (4723 m a.s.l.), Chagbo Co (4518 m a.s.l.), and Changtiao Lake (4954 m a.s.l.). Green-colored lakes (light to dark) are also scattered across the Tibetan Plateau, represented by Yibug Lake (4562 m a.s.l.), Pongyin Co (4735 m a.s.l.), and Serbug Co (4524 m a.s.l.). Both turquoise and green-colored lakes have notable precipitated salt deposits in and around the lake basin. Qinghai Lake (3198 m a.s.l.) is the largest alpine lake in China with an average depth of 21 m. The lake is located in a contained basin (endorheic) with high salt and sediment accumulation (Rongxin et al. 2018; Zhang et al. 2011). In this lake, five permanent streams supply 80% of the water (Rhode et al. 2010). Qinghai Lake has extensive agricultural activity and, in the past, has had religious significance.

The alpine lakes in Pakistan, in contrast to the Tibetan Plateau, are situated within the Himalayan Mountains and are freshwater. The highest elevational lakes in Pakistan are Pakistan Lake and Shimshal Lake; both are at altitudes over 4,755 m. Other notable high alpine lakes over 3800 m include: Ansoo Lake (4126 m a.s.l.), Barah Lake (4512 m a.s.l.), Dakholi Lake (4771 m a.s.l.), Ghanche Lake (4600 m a.s.l.), Hrkolong Lake (4126 m a.s.l.), Karambar (4272 m a.s.l.), Rush Lake (4693 m a.s.l.), and Sheosar Lake (4142 m a.s.l.) (WIKIpedia 2021). These glacial lakes have sparse to limited terrestrial vegetation, are clear to turquoise colored (floured), and are shallow. Another series of alpine lakes (2500–3800 m, a.s.l.) are above treeline but have more biomass and diversity in terrestrial vegetation compared to the high alpine lakes. These lakes from Pakistan include, Dudipatsar Lake (3800 m a.s.l.), Lulusar Lake (3410 m a.s.l., Mahodand Lake (2900 m a.s.l.), Payee Lake (2895 m a.s.l.), Pyala Lake (3410 m a.s.l.), Ratti Lake (3700 m a.s.l.), and Saiful Muluk Lake (3224 m a.s.l.) (WIKIpedia 2021). Biological studies, on high alpine lakes in Pakistan are few (e.g., Barinova et al. 2013).

The Alps are layers of rock of European, oceanic, and African origin (De Graciansky et al. 2011). The specific folding and fracturing of the rock, coupled with erosion, form the steep vertical peaks of the central Alps (Gerrard 1990). These formations create enhanced runoff with fewer high alpine lake environments. The prominent lakes of the Alps are at the foothills of these steep vertical peaks with lower elevations and referred to as peri-alpine lakes. Lakes Geneva (372 m a.s.l., average depth 154 m), Lugano (282 m a.l.s., average depth 134 m), Garda (62 m a.s.l., average depth 136 m), Zurich (402 m a.s.l., average depth 49 m), and Konstance (396 m a.s.l., average depth 90 m) are examples of peri-alpine lakes below treeline in the foothills of the central alps. Higher elevated alpine lakes like Resia Reschen (1499 m a.s.l.), Kratzberger (2122 m a.s.l.), and Lago di Vernago (1692 m a.s.l.) are still below treeline and with human settlements. Across the Central Alps, there are scattered pockets of cirque and shallow valley ponds and lakes. In the Parco Naturale Gruppo di Tessa region (South of Innsbrück, Austria), high altitude ponds and small lakes of note are evident (e.g., Egret lakes) which are <3000 m a.s.l. but above treeline with minimal terrestrial vegetation. The Parco dell'Alpe Veglia e dell' Aple Devero region of Italy has a mixture of high latitude ponds and lakes above treeline with turquoise (floured) and sapphire blue (dark blue) colored waters (e.g., Lago del Sabbione, 2460 m a.s.l.; Lago del Narèt, 2305 m a.s.l.). The biology and ecology of alpine lakes across Europe have been studied (references presented in text below). Many new diatom species have been described from the Alps; as an example, Hustedt (1943) described four species new to science from high mountain lakes around Davos Switzerland.

4.4 Turkey and Area

Turkey is located between temperate forest zone mountains (southern Taurus Mountains) and subtropical sclerophyllous forest zone mountains (northeastern Anatolian Mountains) (Nagy and Grabherr 2009; Solak et al. 2012, Fig. 4.2). The mean elevation is less than 500 m in the western Anatolia, about 1000 m in the central Anatolia, and over 3000 m in the mountanous area of eastern Anatolia. There are three main glacial areas: the Taurus Mountains, the Kaçkar Mountains, and some high mountains in Anatolia Plateau (Sarıkaya and Çiner 2019, Fig. 4.3). A number of investigations have studied the morphological and biological aspects of Alpine lakes in Turkey (e.g., Atıcı 2018; Aygen et al. 2009; Şahin et al. 2020; Yıldız et al. 2012; Yıldız-Gürbüzer 2016). For instance, the morphometrical features of selected lakes in Kaçkar and Soğanlı Mountains (northern eastern Anatolian mountains) have been evaluated by Sarı et al. (2015), and the reported max. depths of the lakes were between 0.5 and 49.0 m (mean 7.4 m), max. length of the lakes ranged between 25.2 and 497.4 m (216.8 m), and max. widths between 15.5 and 297.9 m (mean 135.0 m). At present, diatom assemblages of some glacial lakes have been studied. Among them, Atıcı (2018) reported *Cymbella affinis* as abundant in the Artabel Lakes of Eastern Blacksea. In Aygır Lake (also Eastern Black Sea), Taş (2016) noted *Achnanthidium minutissimum*, *Lindavia bodanica*, *L. comensis*, and *Diatoma mesodon* were abundant in the plankton.

4.4.1 Van and Urmia Lakes

The soda lakes Van and Urmia are recognized under 40 famous alpine lakes in the world (WIKIpedia 2021). The lakes are located in the "*Irano-Anatolian Mountains of Central Asia*" region, considered in the top 35 biodiversity hotspots of the world (Marchese 2015).

Van Lake, the largest lake in Turkey, is located at a high altitude (1648 m a.s.l.) in Eastern Anatolia. It is 450 m deep with a volume of 576 km^3, thus the largest soda lake and third largest closed lake in the world. This saline lake is defined by its elevated sodium and potassium levels, with a balance of bicarbonate and carbonate ions along with other alkaline earth ions, giving a Na-CO_3-Cl-(SO_4) chemistry (Reimer et al. 2009). The lake has a high specific conductance of 22.9–26.7 mS. cm^{-1} and a pH of 9.3–9.8. The presence of diatoms in sediment deposits and characteristics in terms of hydrology and water chemistry of Van Lake and associated rivers were detailed by Reimer et al. (2009). Van Lake is famous for a special type of sediment called microbialites, carbonate forming crusts (Kempe et al. 1991; Kempe and Kaźmierczak 2003; López-García et al. 2005). Unique in regard to these geochemical characteristics, Van Lake also hosts endemic species such as the pearl mullet *Alburnus tarichi* (Guldenstaedtii, 1814). About 80 years ago, Legler and Krasske (1940) described several diatom species from Van Lake: *Amphiprora*

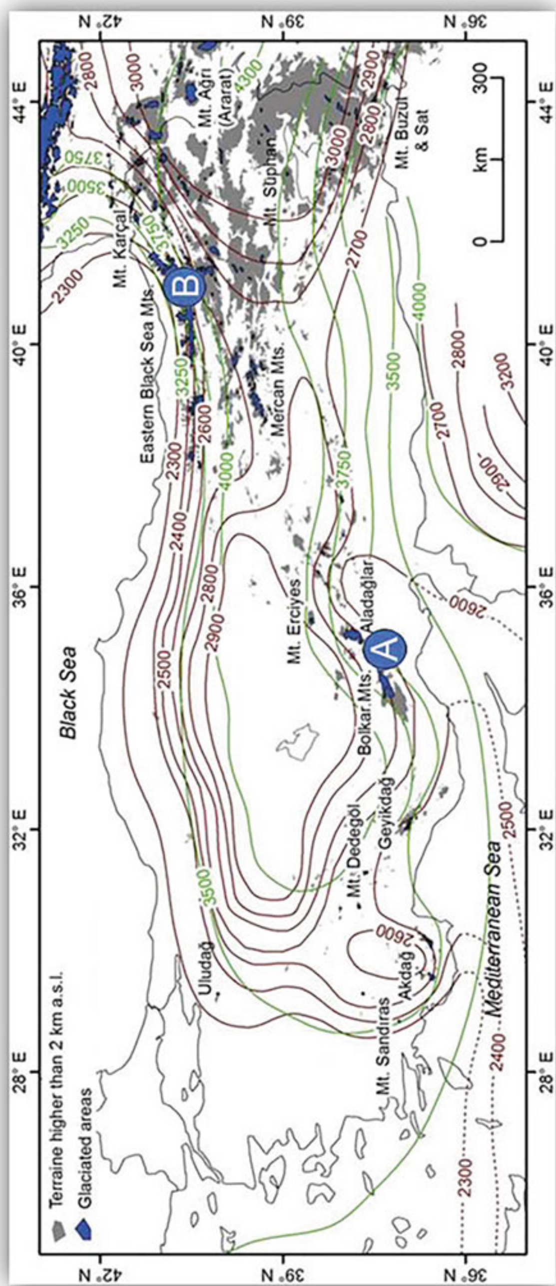


Fig. 4.3 Altitude map of Turkey showing high elevation terranes (grey, alpine environments) and glacial areas (blue, high alpine environments ((**a**) southern Taurus Mountains in Turkey; (**b**) northeastern Anatolian Mountains). With permission and modified from Sarıkaya and Çiner 2019)

paludosa var. *densestriata* (Syn. *Entomoneis densestriata*), *Rhopalodia musculus* var. *supresemicirculatus* (Syn: *Rhopalodia supresemicirculata*), *Nitzschia incognita*, and *Surirella invicta.* Other species have also been documented, including *Nitzschia vitrea*, *N. frustulum*, *N. frustulum* var. *subsalina*, *N. fonticola*, *N. inconspicua*, *N. kuetzingiana*, and *N. communis*, in a review of Van Lake phytoplankton and littoral diatoms species (Gessner 1957). The lake community has a selective emphasis for taxa from the orders Bacillariales, Rhopalodiales, and Surirellales. Some of the described taxa were later reinvestigated and imaged by Lange-Bertalot et al. (1996). Also, reports have been published on diatoms from the surrounding area (e.g, Solak et al. 2012). Recently, a new species from the genus *Nitzschia* was found in the lake (Solak et al. 2021). Additional investigations on benthic diatom assemblages of Van Lake show a potential presence of more endemic taxa (unpublished data).

Lake Urmia is the second largest hypersaline lake in the world, located in northwest Iran. Due to its unique biodiversity, the lake was declared a National Park, Ramsar Site, in 1971 and a Biosphere Reserve by UNESCO starting in 1976 (Khatami and Berndtsson 2013; Nhu et al. 2020). The elevation of the lake is 1278 m a.s.l. with a maximum water depth of 16 m and salinity range of 217–300 g L^{-1}. The pelagic zone of the lake is inhabited by brine shrimp (e.g., *Artemia urmiana*), bacteria, archaea, and phytoplankton (Eimanifar and Mohebbi 2007). Recently, the water quality of the southern part was investigated by Goshtasbi et al. (2021). They report that dissolved oxygen levels in the lake were quite low (2.99–3.37 mg L^{-1}), and intensive cyanobacteria blooms were present. Annually, the climate is semiarid with evaporation pressures on Lake Urmia. The lake basin contains 204,000 ha of agricultural land and gardens. Most of the agricultural pressures are linked to irrigation. The main sources for agricultural water are both surface and underground water (Nhu et al. 2020). A comparison of diatom assemblages between Van Lake and lakes from the Tibetan region shows no similarities. Yang et al. (2003) investigated forty lakes from the Tibetan region between 2797 m and 5180 m a.s.l. and reported *Achnanthidium minutissimum*, *Amphora libyca*, *Cymbella affinis*, *Nitzschia perminuta*, *Sellaphora pupula*, and *Staurosirella pinnata* as common. Moreover, *Anomoeoneis sphaerophora*, *Surirella peisonis*, *Mastogloia elliptica*, and *Navicymbula pusilla* were characteristic saline species. In contrast, *Berkeleya* sp., *Nitzschia incognita*, *Surirella invicta*, and *Rhopalodia supresemicirculata* are the characteristic saline taxa in Van Lake (unpublished data).

4.5 The Holocene

Extreme cold biomes, like the Arctic and Antarctic, have a short extant history of ecosystem development and sustainability, which comprises the latest interglacial period of the Holocene (Jersabek et al. 2001). There are exceptions where isolated regions in the Arctic survived the last glaciation with Arctic lakes extending back to previous interglacial periods (e.g., marine isotope stage (MIS 5)) (Crump et al. 2021;

Hughes et al. 2013). Biological records in the sediments of past interglacials are limited, and age models need further validation (Wilson et al. 2012). The Holocene epoch is divided into three stages, recognizing changing climate conditions during the melting and retreating of the glaciers and current climate state at the end of the Little Ice Age (Walker et al. 2018, 2019). Global alpine biomes were slow to develop during the Greenlandian stage (8300–11,700 YBP) gradually warming with glacial melt highlighted by evaporation regime changes. The development of temporary and permanent glacial and alpine waterbodies was extensive through this period with fluctuations in climate impacting biological productivity (e.g., Smol et al. 2005). The Northgrippian stage (8300–4200 YBP) documents an initial cooling followed by a drier period with accelerated glacial ice melt. The development of temporary glacial pond lakes likely declined during this stage with extended cold seasons and ice cover (e.g., Zhang et al. 2020). The Meghalayan stage (4200–60 YBP) represents periods of reduced precipitation across Asia and a colder climate (Walker et al. 2018). Alpine lakes and ponds have experienced dramatic water level changes, periods of drought, and pond/lake extinctions throughout the Holocene (Zhang et al. 2020). Through these stages of the Holocene, terrestrial biomes also developed and advanced or retreated quickly from low altitudes extending up mountain range slopes with the present-day tree line delineating the advancement of biome production up to the alpine. The initiation of the Anthropocene epoch (International Commission on Stratigraphy) proposed for some time in 2022/2023 will document continued warming of the global climate after 1950 with implications for changes in alpine ecosystems.

4.6 Chemistry

The primary elements impacting high alpine lakes and ponds are the dominant anions and cations in the region. These are coupled with other erosional elements from rock and soils to create a broad range of observed water color. Clear waters with low ion and inorganic elemental content have the classic dark to sapphire blue (dark blue)-colored waters in contrast to lakes with high ion and/or inorganic element loading creating the turquoise to green (floured) waters (suspended particles reflecting light). This water color is ultimately determined by type of local bedrock, erosional patterns, and water retention time in the waterbody. In lower peri-alpine lakes, organic matter further contributes to darken water color (particles absorbing light). Available nutrients and organic matter can further contribute to water color through the growth of plankton and littoral-related tychoplankton. This algae growth typically gives a green color due to the absorption of sunlight by chlorophyll. However, other color pigments, like pink (cyanobacteria secondary pigments) and golden brown (diatom lipids), can be observed in the plankton and on the bottom of ice covers (e.g., Frenette et al. 2008).

Nutrients are low in high alpine aquatic systems, with both nitrogen and phosphorus often limiting. In high elevation cirque and valley lakes above tree line, the source of nutrients is bedrock and soil, local biological activity with decaying organic matter, and atmosphere deposition through rain, snow, and glaciers. Studies from around the world have shown atmospheric nitrogen as a primary seed for this nutrient in high alpine aquatic primary production (e.g., Liu et al. 2015; Rogora et al. 2008; Wolfe et al. 2003). However, atmospheric N sources will vary depending on geographic location and global wind circulation conditions. Alpine biomes close to anthropogenic sources or regions of high lowland productivity (e.g., Alps in Europe) will have higher nitrate levels, while at the other extreme, more remote locations (Andes, South America) with minimal atmospheric N sources will have enhanced local cycling of organic nitrogen (Rogora et al. 2008). Glaciers are excellent sinks for the accumulation of nitrogen over long periods of time. Detailed studies show nitrogen sources from glaciers (long-term sinks) are more plentiful compared to snowmelt (annual sinks) (Slemmomon and Saros, 2012; Saros et al. 2010). On the Tibetan Plateau with paleo-marine sediments, the groundwater for Ximen Co Lake was found to be an important source of dissolved inorganic nitrogen relative to total phosphorus (Luo et al. 2018), ultimately driving this lake system toward phosphorus limitations. Phosphorus is consistently a limiting nutrient in alpine aquatic systems (Camarero and Catalan 2012; Tiberti et al. 2010). Since dissolved organic phosphorus (DOP) is not readily available in most alpine lakes, dissolved inorganic phosphorus (DIP) is the primary source for algal growth (Hudson et al. 2000). Like nitrogen, atmospheric sources of phosphorus through rain, snow, and glacial melt are substantial even in the Tibetan Plateau (Rongxin et al. 2018; Zhang et al. 2019). However, as illustrated above, nutrient loading and cycling in high alpine lakes varies globally. Since many alpine lakes are both N and P limited (Cook et al. 2020; Zhang et al. 2019), it is the specific colimitation interaction (not N/P ratio) between these nutrients and ions that will determine nutrient availability for primary productivity (Harpole et al. 2011). Wildlife plays a minimal role in nutrient cycling, although, as observed in Lago Colorada, Bolivia (Fig. 4.1), wildlife can have some specific influences on lake nutrient loading and cycling (Sarnelle and Knapp 2005).

The ionic and nutrient composition of high elevation lakes on the Tibetan Plateau (old marine seabeds) is heavily influenced by soils and bedrock (Li et al. 2021; Luo et al. 2018). Alpine lakes on the plateau are predominantly brackish to soda lakes (Ma et al. 2011) with nutrient interactions alleviating salinity limitation for bacteria and plankton growth (Jiang et al. 2007; Li et al. 2021; Yue et al. 2019). Therefore, salinity effects on phytoplankton composition are more complex than previously reported (Ferreira et al. 2019). These findings from the Tibetan Plateau further highlight the complexities of changing nutrient and ion-salt sources in directing stochastic and deterministic biological diversity and primary production.

The majority of high alpine lakes are oligotrophic, limited by light and nutrients (Liu et al. 2011; Mitamura et al. 2003). High alpine ponds and lakes also experience elevated UV levels (Caldwell et al. 1980) with deeper photic zone in clear waters due to nutrient limitations and low productivity (Scully and Lean 1994). Thus, these alpine lakes and ponds have poorly developed planktic and tychoplanktic communities associated with the littoral zone (Doyle and Saros 2005). The particulate-rich turquoise and green-colored lakes further reduce the photic zone, limiting phytoplankton productivity in the surface waters (Sommaruga 2015). In contrast, peri-alpine lakes have more complex planktic communities with a broad tropic structure. In a global examination of planktic assemblages in alpine systems (Europe, Chile, Ethiopia), Filker et al. (2015) observed a broad spectrum of taxa in the plankton with dinoflagellates more prominent in Europe, mixotrophic cryptophytes in Chile, and chlorophytes and heterokonts (including diatoms) in Ethiopia and Chile. Tolotti et al. (2006) also noted the prominence of dinoflagellates, cryptophytes and chrysophytes in European alpine phytoplankton. Other researchers have observed the importance, abundance, and diversity of chrysophytes in alpine and Arctic aquatic systems (Betts-Piper et al. 2004; Charvet et al. 2012; Duff et al. 1992; Kamenik and Schmidt 2005; Lotter et al. 1998). The green chlorophyte algae (including desmids) are present but not abundant in alpine lakes and ponds, unless elevated levels of nutrient are present (Lepori and Robin 2014; Oleksy et al. 2021; Priddle and Happey-Wood 1983; Tolotti et al. 2006).

With respect to alpine plankton ecology, factors controlling growth are mixed. Catalán et al. (2009), in a 235 alpine lake study in Europe, identified four factors controlling biotic growth in alpine systems: (1) lake size was linked to rotifers; (2) pH correlated to diatoms, (3) ice cover was associated with chydorids and planktic crustaceans, and (4) Trophic statue (structure) was linked to chironomids. Tolotti et al. (2006) in a similar 70-lake study observed catchment vegetation, geochemistry (acidity/alkalinity), and nitrate to be correlated with phytoplankton and trophic status linked to the zooplankton community. Other alpine lake systems, Tibetan Plateau, for example, have shown phytoplankton community diversity to be higher in more saline habitats (Lozupone and Knight 2007; Zhong et al. 2016). In another study, Marchetto et al. (2009) noted that lake size and catchment type correlated with phytoplankton. Jacquemin et al. (2019) also observed that small rocky catchments reduced functional richness of phytoplankton, while increasing total phosphorus increased the autotrophic component of the phytoplankton, and lower phosphorus levels encouraged mixotrophic communities. Anneville et al. (2005) studying peri-alpine lakes further noted the preference of mixotrophic communities with reduced phosphorus loading. Also, Lotter et al. (1998) observed that total phosphorus was linked to diatom growth but not with chrysophytes and cladocerans. In other studies, nitrogen has been identified as a controlling factor (Liu et al. 2015; Rogora et al. 2008; Tolotti et al. 2006). In peri-alpine lake systems, cyanobacteria can be dominant as blooms or even toxic blooms (Monchamp et al. 2019). The control of cyanobacteria blooms in lakes can be achieved simply by the reduction of carbon and nutrients (Dokulil and Teubner 2000). Monchamp et al. (2019) using sediment genetic markers in peri-alpine lakes found cyanobacteria phylogeny associations with air temperature and water column and little to no correlation to elevated levels of phosphorus and nitrogen. Finally, Vogt et al. (2021) studying 256 lakes across an elevational gradient document that protists showed decreasing richness and diversity with increasing altitude as well as a high proportion of region-specific specialists. In summary, many factors are selectively driving plankton productivity and diversity. First, altitude matters, and climate is driving many of the determining factors. Phytoplanktons are correlated to catchment size (including vegetative development) and nutrients. Higher nutrients align with autotrophic dominant communities, whereas lower nutrients associate with mixotrophic communities. Zooplanktons are correlated to lake size, climate, and phytoplankton composition.

Cyanobacteria play a significant role in benthic alpine aquatic biodiversity and productivity (Jungblut and Vincent 2017; Mez et al. 1998; Sommaruga and Garcia-Pichel 1999). As an example, the diversity of algae and cyanobacteria as bioindicators of Lake Nesamovyte (Eastern Carpathians, Ukraine) was investigated by Tsarenko et al. (2021), and they recorded 234 species in the lake. In alkaline environments, cyanobacteria are common and scattered on the substratum or in mats and sediment crusts. When nitrogen is limiting, nitrogen fixing cyanobacteria mats and crusts are observed in the lake benthos (Dickson 2000). Since climate and reduced levels of carbon and nitrogen can determine planktic cyanobacteria growth, it would be no surprise that benthic cyanobacteria are also under the control of these factors. Further, cyanobacteria are susceptible to UV exposure, and benthic crusts act as shields in addition to UV protection compounds to sustain communities (Castenholz and Garcia-Pichel 2012). In a novel study, it was demonstrated that selected cyanobacteria can migrate within crusts and mats in shallow waters, optimizing living conditions (Garcia-Pichel et al. 1994). Given the broad global distribution of cyanobacteria, it is not surprising that there is genetic similarity in cyanobacteria taxa between Arctic, Antarctic, and alpine ecoregions (Jungblut et al. 2009). The significance of biotic crusts in alpine lakes, especially ion-rich lakes, is yet to be determined.

Diatoms are a common assemblage in the plankton of oligotrophic to mesotrophic alpine to high alpine lake environments with depths >10 m. In shallower lakes and ponds, productivity is predominant in the benthos (Wunsam et al. 1995). In harsh polar and glacial environments, diatoms can also be found on ice in surface pools, under the ice as attached mats and in terrestrial environments with sporadic water exposure (Bashenkhaeva et al. 2015; Van Kerckvoorde et al. 2000; Vincent et al. 2000). The reported number of species in alpine environments varies depending on the criteria used to define alpine. A range from 109 to 719 taxa of diatoms, primarily benthic, have been reported (Clarke et al. 2005; Feret et al. 2017; Robinson and Kawecka 2005). Numbers on the higher side (350 plus) are currently in-line with species numbers reported from the Arctic (Antoniades et al. 2008; Foged 1974, 1981) and Antarctic (Verleyen et al. 2021).

Diatoms are well adapted to poor environments with limited nutrients and correlate with phosphorus (Bigler et al. 2005; Lotter et al. 1998; Murphy et al. 2010), nitrogen (Hobbs et al. 2011; Tolotti et al. 2007), pH (Koinig et al. 1998; Psenner and Schmidt 1992), salinity (Yang et al. 2003), silicates (Tolotti et al. 2007), and even UV light (Vinebrooke and Leavitt 1996). However, the statistical sensitivity of nutrient-diatom correlations for phosphorus (as an example) below detection limit or even <10 ug L^{-1} may not be effective at giving consistent relationships over spatial scales (Berthon et al. 2013). At present, there is no one environmental factor that can explain the growth of diatoms across globally spaced alpine systems, although chemical factors are prominent (Rivera-Rondón and Catalán 2020).

4.7 Diatom Biodiversity in Extreme Environments

Many studies in cold regions around the world have documented the biogeography and taxonomy of diatoms (e.g., Van de Vijver et al. 2004; Zidarova et al. 2016; Spaulding et al. 2021, in Antarctica; Foged 1974; Antoniades et al. 2008; Zimmermann et al. 2010; Pla-Rabés et al. 2016; Zgrundo et al. 2017, in the Arctic; Patrick and Freese 1961; Foged 1981; Bahls and Luna 2018, in Alaska; Skvortzow (1929, 1937, 1938a, 1938b), Lange-Bertalot and Genkal 1999; Laing et al. 1999; Reichardt 2009; Genkal et al. 2010; Kulikovskiy et al. (2012); Kulikovskiy, Kociolek, et al. 2015a; Potapova et al. 2014; Pestryakova et al. 2018; Potapova 2018, in Siberia; Rumrich et al. 2000; Morales et al. 2009; Blanco et al. 2013; García et al. 2021, in the Andes; Dickie 1882; Suxena and Venkateswarlu 1968; Jüttner et al. (2000, 2004, 2010, 2011, 2017, 2018); Bhatt et al. 2008; Van de Vijver et al. 2011; Krstić et al. 2013; Verma et al. 2017; Mohan et al. 2018; Wetzel et al. 2019; Radhakrishnan et al. 2020, in the Himalayan Mountains; Van de Vijver et al. 2010, in South African glaciers; Lin et al. 2018; Zhang et al. 2019, in China, and Levkov et al. 2005; Kawecka and Robinson 2008; Buczkó et al. 2010; Cantonati et al. 2012; Buczkó 2016; Feret et al. 2017; Heudre et al. 2019, in Europe).

4.7.1 Antarctica

The first notable diatom work on extreme environments was done by Reinsch (1879) in the Antarctic region, and then many diatom studies followed (*detailed in* Zidarova et al. 2016). In a bipolar study of the Antarctic and Arctic, Van de Vijver et al. (2004) examined the genus *Stauroneis* and described 40 new taxa. Van de Vijver et al. (2010) also worked on the genus *Muelleria* from Antarctic and South African glaciers and, in that study, observed more than 15 taxa with some of them new to science. Over the last two decades, Van de Vijver and his colleagues have conducted extensive work on maritime Antarctic and Antarctic diatoms with the description of many new taxa Verleyen et al. (2021). To further assist in diatom identifications, Zidarova et al. (2016) prepared a taxonomic monograph using LM and SEM images. More recently, Spaulding et al. (2021) established a website on Antarctic freshwater diatoms.

In a preliminary examination of 13 samples from the maritime Antarctic, South Shetland Islands (Robert, Ardley, Livingstone, Horseshoe, and Galindez Islands), we identified ca. 100 freshwater taxa from ponds and lakes. Abundant taxa included *Chamaepinnularia krookiformis*, *Navicula australoshetlandica*, *Planothidium australe*, *P. renei*, and *Psammothidium subatomoides.* Overall, most of the identified taxa are small forms belonging to the genera *Chamaepinnularia*, *Luticola*, *Microcostatus*, and *Psammothidium*, while also some big forms exist belonging to *Navicula*, *Nitzschia*, *Stauroneis*, and especially *Pinnularia*. Among the taxa, *Navicula gregaria, Nitzschia gracilis*, and *N. homburgiensis* were interesting because *N. gregaria* (for example) is documented from polluted inland waters in Turkey. *Nitzschia gracilis* and *N. homburgiensis* are not abundant; however, the taxa are also observed across different regions in Turkey.

4.7.2 Arctic

Early investigations on Arctic diatoms were done by Ehrenberg (1844), Lagerstedt (1873), and Cleve (1896). Cleve continued to study Arctic diatoms, marine and freshwater, from the circumpolar Arctic region into the early 1900s. Since then, a plethora of diatom research has been conducted on the Arctic biome considering everything from taxonomy to climate change (*detailed in* Antoniades et al. 2008; Smol et al. 2005). More recently, diatom assemblages in the Canadian Arctic Archipelago were archived in monographs by Antoniades et al. (2008), Zimmermann et al. (2010), and Bahls et al. (2018). Antoniades et al. (2008) investigated the diatoms of freshwater ponds and lakes in the Prince Patrick, Ellef Ringnes, and northern Ellesmere Islands from the Canada Arctic Archipelago and identified 362 taxa with 7 new species across 6 genera (Antoniades et al. 2008). They along with Bouchard et al. (2004) and others have documented the richness and abundance of small araphid and monoraphid diatoms in cold environments. In contrast, Zimmermann et al. (2010) examined a Pliocene–Pleistocene freshwater diatom flora on Bylot Island documenting the historical diatom flora from this extreme environment.

Despite the large area of Alaska, there are limited works about freshwater diatoms in the region (e.g., Bahls and Luna 2018; Hein 1990; Patrick and Freese 1961). Foged (1981) investigated 258 samples from 218 localities in Alaska and identified 987 taxa with the description of 9 species, 20 varieties, and 16 formae as new for science. The diatoms from Wrangell-St. Elias National Park in the southwest side of Alaska were more recently examined by Bahls and Luna (2018) with 139 taxa identified. Most of the Wrangell-St. Elias National Park diatoms (84%) were reported from Europe, and a rare taxon *Surirella arctica* was also noted.

Greenland, Iceland, Spitzbergen, and Franz-Josef Land represent real islands or island clusters of biogeological importance. Lagerstedt (1873) made the first diatom survey for Spitzbergen, and our examination of original material found many small species from the genera *Humidophila*, *Navicula*, *Eunotia*, *Staurosira*, *Staurosirella*, *Achnanthidium*, and *Planothidium*. Zgrundo et al. (2017) observed 96 taxa (excluding subspecies, varieties and formae) in aquatic habitats of northern Spitsbergen, including the smaller taxa *Achnanthidium minutissimum*, *Staurosirella pinnata*, and *Nitzschia alpina*. Foged (1974) examined 244 samples from Iceland and recorded 689 freshwater diatom taxa (excluding forms). In the Iceland study, there were

37 small araphid taxa, and 55 small monoraphids. Also, the diatom structure and diversity in Franz Josef Land Archipelago was investigated by Pla-Rabés et al. (2016), and *Diatoma tenuis* was the most abundant taxon with several circumpolar taxa observed such as *Chamaepinnularia gandrupii*, *Cymbella botellus*, *Psammothidium* spp., and *Humidophila laevissima*.

Historically, Skvortzow (1929, 1937, 1938a, 1938b) published extensive floristic surveys of diatoms from Siberia. Genkal et al. (2010) examined centric diatoms of some water bodies in northeastern West Siberia and noted circumpolar planktic diatoms: *Aulacoseira subarctica, Cyclotella arctica, C. comensis, Stephanodiscus invisitatus*, and *S. triporus*. Later, Genkal et al. (2012) identified the Pennatophyceae members of the region with unidentified taxa from the genera *Amphora, Cavinula, Eunotia, and Navicula*. Diatom assemblages from Yakutian lakes in northeastern Siberia were documented by Pestryakova et al. (2018) reporting 157 taxa. In addition, a monograph was prepared by Lange-Bertalot and Genkal (1999) from Vaygach, Mestnyi, Matveev Islands, and Yugorsky Peninsula in northwest Siberia. Lake Baikal is the largest freshwater lake in the World with about 10% of the planktonic algae recorded as endemic (Kozhov 1962, 1972). Genkal and Bondarenko (2006) also confirmed endemic diatom assemblages in the lake. In a volumetric treatise, Kulikovskiy et al. (2012) and Kulikovskiy, Lange-Bertalot, and Kuznetsova (2015b) documented 10 new genera and 382 new species from Lake Baikal. Additional taxa and some genera have also been described in separate articles (e.g., Kociolek et al. 2013, 2018; Kulikovskiy et al. 2016; Kulikovskiy, Gluschenko, Genkal, et al. 2020a; Kulikovskiy, Gluschenko, Kuznetsova, et al. 2020b; Kulikovskiy, Kociolek, et al. 2015a). Reichardt (2009) underlined that diatom studies across the Russian Arctic were limited with respect to the large area, and he described some new *Gomphonema* taxa (*G. demersum*, *G. jergackianum*, *G. marvanii*). Potapova et al. (2014) recently described a scattered selection of four new taxa, from the Yakutia region in Siberia. Potapova (2018) also documented the distribution of *Psammothidium* species by describing *Psammothidium onufrii* from northeastern Siberia.

Laing et al. (1999) investigated 23 lakes located near Norilsk, Siberia, and found the prominent diatom communities were very similar to Canadian and Fennoscandian lakes, supporting the findings of a circumpolar distribution in the Arctic diatom flora. Taxonomically, an interesting work was conducted by Paull et al. (2008) using small fragilarioid (*Staurosira* and *Staurosirella*) taxa from the Canadian Arctic, Siberia, and Fennoscandia; they found different morphological forms of *Staurosirella pinnata* sensu lato and *Staurosira venter* could be distinguished and that these groups (morphs/taxa) were distributed across the circumpolar Arctic.

4.7.3 Andes and Patagonia

The early diatom flora from Andean Patagonia was documented with drawings and species lists by Frenguelli (1924, 1936, 1942) and Frenguelli and Orlando (1956). However, diatom floras of the Andes have received little attention in comparison with North temperate freshwaters (Blanco et al. 2013). Rumrich et al. (2000) reported that many different cosmopolitan taxa, typical from Holarctic and temperate areas in Europe, were present in the Andes, while common European species were not. The epilithic diatoms from cloud forest and alpine streams in Bolivia were investigated by Morales et al. (2009), and they described two new species (*Fragilaria cochabambina* and *Achnanthidium sehuencoensis*) and underlined that totally 118 species were found and 42 of them were newly observed for the region. Some recently described taxa include: *Navicula venetoides*, *Pinnularia boliviana*, *Nitzschia sansimoni*, *Surirella striatula* var. *halophila*, and *S. moralesii* from a saline lake in Lopez Area (Argentina) (Blanco et al. 2013). García et al. (2021) also described four small species including *Pseudostaurosira australopatagonica*, *P. catalinae*, *P. tehuelcheana*, and *P. zolitschkae* from four shallow lakes in Santa Cruz Province on the east side (Argentina) of the Andes and one shallow lake from the Magallanes Region on the west side (Chile).

4.7.4 Himalaya and Tibetan Plateau

In the Himalaya, diatom studies began early with Dickie (1882), the results detailed in Suxena and Venkateswarlu (1968) with an extensive list of the diatoms. Compère (1983) also published a list of algae from Kashmir and Ladakh in Western Himalaya. Nautiyal, Kala, and Nautiyal (2004) reported 200 diatom taxa (including 77 of them as first records) from the Mandakini basin. Then, Nautiyal, Nautiyal, et al. (2004) investigated the streams in Garhwal region in India and documented 58 as first records for the area. Some years later, the distribution of diatoms within the family Acanthaceae from the Himalayan highlands was published by Verma et al. (2017). In 2008, Bhatt et al. (2008) reported the invasive species *Didymosphenia geminata* (Lyngbye) Schmidt as abundant in Western Himalaya.

After Himalaya was recognized as a hotspot (Jüttner et al. 2000), many new taxa were described. Taxonomic studies have centered around the genus *Oricymba* (3 new species), and the older *Gomphonema* (8 new species), along with *Odontidium* (4 new species). Additional species were also described from the genera *Achnanthidium*, *Cymbopleura*, *Navicula*, and *Synedra* (Table 4.1).

Table 4.1 A list of new diatom taxa described from the Himalaya region after 2000

Taxon	Length (µm)	Width (µm)	Reference
Achnanthidium coxianum I. Jüttner, L. Ector, and C.E. Wetze	8.1–12.9	2.5–3.0	Wetzel et al. (2019)
A. rostropyrenaicum Jüttner and E.J. Cox	18.0–24.5	4.3–4.5	Jüttner et al. (2011)
Cymbopleura emoda Jüttner and Van der Vijver	35.0–40.0	6.8–7.7	Van de Vijver et al. (2011)
C. gokyoensis Jüttner and Van de Vijver	20.1–21.7	2.7–3.7	
Eunotia igorii S. Krstic, Z. Levkov, and A. Pavlov	38.2–72.7	12.6–15.3	Krstić et al. (2013)
E. panchpokhariensis S. Krstic, Z. Levkov, and A. Pavlov	23.4–32.6	2.4–3.3	
E. paramuscicola Krstić, Z. Levkov, and A. Pavlov	9.5–29.0	2.0–3.3	
E. zechii Krstić, Z. Levkov, and A. Pavlov	20.3–55.3	3.1–5.6	
Gomphonema adhikharyi C. Radhakrishnan, Sudipta K. Das, Kociolek J, B. Karthick	28.0–34.0	5.0–6.0	Radhakrishnan et al. (2020)
G. chubichuense I. Jüttner and E.J. Cox	11.0–22.0	3.0–5.0	Jüttner et al. (2000)
G. pararhombicum Reichardt, Jüttner, and E.J. Cox	20.0–70.0	6.5–11.0	Jüttner et al. (2017)
G. nepalense Reichardt, Jüttner, and E.J. Cox	18.0–55.0	6.0–8.0	
G. nediense Reichardt, Jüttner, and E.J. Cox	19.0–31.0	5.0–6.0	
G. incognitum Reichardt, Jüttner and E.J. Cox	17.0–39.0	4.0–6.5	
G. makaluense Reichardt, Jüttner, and E.J. Cox	26.0–62.0	6.0–9.5	
G. saccatum Reichardt, Jüttner, and E.J. Cox	12.5–45.3	5.2–7.0	
G. sinestigma Reichardt, Jüttner, and E.J. Cox	17.0–22.0	3.0–4.0	
Navicula obtecta I. Jüttner and E.J. Cox	41.0–60.0	7.0–9.5	Jüttner et al. (2000)
Odontidium nepalese I. Jüttner, Gurung, Van de Vijver, and D.M. Williams	22.0–63.5	8.5–12.0	Jüttner et al. (2017)
O. himalongissimum I. Jüttner, D.M. Williams, and E.J. Cox	10.0–56.5	5.5–8.5	
O. longiovalum I. Jüttner, D.M. Williams, and E.J. Cox	10.5–61.0	6.0–10.0	
O. parvoapiculatum I. Jüttner, D.M. Williams, and E.J. Cox	12.5–38.5	6.0–8.5	
Oricymba japonica (Reichelt) Jüttner, E.J. Cox, Krammer, and Tuji	30.0–55.0	9.0–13.0	Jüttner et al. (2010)
O. latirotundata I. Jüttner and Van de Vijver	54.0–71.0	13.0–16.0	
O. subaequalis I. Jüttner, Krammer, E.J. Cox, Van de Vijver, and Tuji	33.0–43.0	9.0–11.0	
O. subovalis I. Jüttner, Krammer, and E.J. Cox	25.0–55.0	9.0–11.0	
Synedra inaequalis var. *jumlensis* I. Jüttner and E.J. Cox	37.0–48.0	7.5–11.0	Jüttner et al. (2000)

4.7.5 European Alps

High-altitude lakes from the European Alps have been well studied. Hustedt conducted many microscopic examinations of diatoms from Germany, Austria, and Switzerland, which included alpine waters (e.g., Hustedt 1931, 1943). More recently, a taxonomic study by Levkov et al. (2005) documented diatoms from the highlands of the Shara and Nidze Mountains (North Republic of Macedonia) and noted the common presence of smaller diatoms which included *Cavinula pseudoscutiformis, Eunotia incisa*, and the larger *Tabellaria flocculosa*. Also, diatom composition in low conductivity lakes (5.7–23.9 μS cm^{-1}) in Polish (Tatra Mnt.) and the Swiss Alps (Macun Lakes) were compared by Kawecka and Robinson (2008). They found *A. minutissimum*, *Eunotia exigua*, *Fragilaria capucina/gracilis* group, *Psammothidium marginulatum*, and *Tabellaria flocculosa* were the most frequent taxa along with *Psammothidium subatomoides* in streams within the Macun Lakes area. The diatoms of glacial lakes in the South Carpathians, Romania, were investigated by Buczkó et al. (2010) and Buczkó (2016), with *Aulacoseira* species well documented using LM and SEM images. Buczkó (2016) prepared a monograph with 119 taxa, noting that small *Psammothidium* species were very abundant and diverse in the study.

Cantonati et al. (2012) investigated springs from the southeastern Italian Alps and recorded *Achnanthidium pfister, A. pyreaicum, A. lineare, Gomphonema elegantissimum,* and *Nitzschia fonticola* in carbonate rheocrenes. Feret et al. (2017) sampled 62 natural lakes above 1300 m a.s.l. in the French Alps and noted *Achnanthidium*, *Encyonema*, *Encyonopsis*, *Denticula*, *Staurosirella*, and *Navicula* taxa to be prominent. Among the taxa, *Achnanthidium minutissimum*, *Encyonema minutum*, *Encyonopsis subminuta*, and *Denticula tenuis* were dominant. Of special interest, *Psammothidium abundans* an Antarctic species was reported in Longemer Lake (Vosges Mountain, France) by Heudre et al. (2019).

4.7.6 Taurus Mountains and Anatolian Mountains in Turkey

4.7.6.1 Primary Producers

Turkey alpine biomes are geographically located in a mid-zone between subtropical and temperate regions (Solak et al. 2012). At present, studies on the diatom flora of alpine lakes are still in progress, and we have limited knowledge on the biogeography and diversity. In the Black Sea Region, the planktic community, and ecological state of Aygır Lake in the Karagöl Mountains, was examined by Taş (2016); she reported 48 algae in the lake (Bacillariophyta (21), Chlorophyceae (10), Cyanobacteria (7), Ochrophyta (3), Euglenophyta (3), Charophyta (2), Cryptophyta (1), and Miozoa (1)). Atıcı (2018) observed 96 taxa (Bacillariophyta (58), Chlorophyta (17), Cyanobacteria (15), Euglenophyta (2), Pyrrhophyta (2), and

Cryptophyta (2)) in the Artabel Lakes. Subsequent ecological studies of the Artabel Lakes were conducted by Şahin et al. (2020) with 95 diatom taxa from 15 lakes in the region; they further reported that the lakes were not polluted.

4.7.6.2 Biodiversity in the Alpine Lakes of Turkey

The distribution of aquatic oligochaetes in high alpine lakes of the eastern Black Sea Region has been examined by Yıldız et al. (2012) with 28 taxa in 59 lakes. Yıldız (2011) also investigated the zooplankton fauna of some alpine lakes in Verçenik Valley (Rize, Turkey) and documented 29 species belonging to Rotifera (17), Cladocera (9), and Copepoda (3). Ten of the rotifera species have also been reported from Antarctic glacial lakes. Then, she studied alpine lake zooplankton faunas in the Kaçkar Mountains (northern eastern Anatolian mountains) and Aladağlar (southern Taurus), recognizing 28 species of Rotifera (20), Cladocera (5), and Copepoda (3) in the lakes (Yıldız-Gürbüzer 2016). Ustaoğlu et al. (2008) studied glacial lakes in the Uludağ Mountains and found 36 zooplankton, 38 benthic macroinvertebrates, and 8 vertebrates, with a Rotifera, *Microcodides hertha*, documented as a new record for the Turkish fauna. Zooplankton composition and abundance in Alpine Lake Eğrigöl (Antalya) was investigated by Aygen et al. (2009), reporting 41 species (Rotifera (30), Cladocera (8), and Copepoda (3)) in the lake.

4.7.6.2.1 Diatom Biodiversity

In Turkey, the dominant diatom genera include *Cymbella*, *Gomphonema*, *Navicula*, and *Nitzschia*. In contrast, the glacial lakes have a diverse collection of genera but different in dominance. The dominant genera include *Cavinula*, *Eunotia*, *Genkalia*, *Nupela*, *Psammothidium*, *Stauroneis*, and *Tabellaria*. Like diatoms from the Alps, taxa from these genera are not typical for the lowlands of Turkey. Moreover, a number of taxa in the alpine lakes of the Kaçkar Mountains are also present in the Antarctic and Arctic regions (e.g., *Cavinula pseudoscutiformis* and *Psammothidium subatomoides*). Species in the genus *Psammothidium* are rarely found in low elevation Turkish freshwaters (only *P. perpusillum* is reported in Taşkın et al. 2019). However, species in this genus exist with a high diversity in Turkish alpine lakes.

In total, about 150 species are currently recognized from alpine lakes and ponds in Turkey, with many more rare taxa still to be discovered. New records or common diatom taxa from the alpine lakes and ponds are listed in Table 4.2. The genus *Eunotia* has the greatest number of new or common species (7) followed by *Psammothidium* (7) and *Stauroneis* (5).

Five prominent taxa, *Tabellaria flocculosa*, *Caloneis vasileyevae*, *Cavinula pseudoscutiformis*, *Genkalia digitulus*, *and Genkalia boreoalpina* are present in Turkish alpine lakes (Figs. 4.4 and 4.5). *Cavinulapseudoscutiformis* is also present in Scotland and in the northern and alpine regions of Canada and the United States (Cvetkoska et al. 2014; Jüttner et al. 2021). Morales et al. (2007) also found

Table 4.2 New or common taxa recorded for alpine lakes and ponds across Turkey

Taxon	Length (µm)	Width (µm)
Caloneis vasileyevae Lange-Bertalot, Genkal, and Vekhov[a]	15.0–17.3	4.3–4.6
Cavinula pseudoscutiformis (Hustedt) DGMann, and Stickle[a]	6.0–11.1	6.0–10.0
Eunotia bilunaris (Ehrenberg) Schaarschmidt[b]	47.5–69.8	4.3–5.7
Eunotia boreoalpina Lange-Bertalot and Nörpel-Schempp[a,c]	12.0–30.9	4.5–5.2
Eunotia botuliformis F. Wild, Nörpel, and Lange-Bertalot[a]	10.2–34.1	3.7–3.8
Eunotia crista-galli Cleve[a]	23.9–27.4	4.3–6.2
Eunotia curtagrunowii Nörpel-Schempp and Lange-Bertalot[a]	20.4–29.8	7.0–8.9
Eunotia islandica Østrup[a]	21.0–38.7	7.3–10.0
Eunotia minor (Kützing) Grunow[c]	19.9–34.1	4.0–5.2
Eunotia subarcuatoides Alles, Nörpel, and Lange-Bertalot[a]	14.0–23.3	2.9–3.7
Eunotia valida Hustedt[b]	35.9–74.9	5.4–6.4
Genkalia digitulus (Hustedt) Lange-Bertalot and Kulikovskiy[a]	10.4–16.8	3.8–4.9
Genkalia boreoalpina Wojtal, C.E. Wetzel, Ector, Ognjanova-Rumenova, and Buczkó[a]	13.9–15.9	4.0–4.2
Psammothidium daonense (Lange-Bertalot) Lange-Bertalot[a]	8.8–17.9	5.5–6.8
Psammothidium didymium (Hustedt) Bukhtiyarova and Round[a,c]	6.9–9.0	3.6–4.0
Psammothidium helveticum (Hustedt) Bukhtiyarova and Round[a]	11.8–21.5	4.0–7.1
Psammothidium levander i(Hustedt) Bukhtiyarova and Round[a]	9.1–9.6	5.1–5.5
Psammothidium microscopicum (Cholnoky) S. Blanco[a,c]	5.7–8.0	3.6–4.2
Psammothidium rossii (Hustedt) Bukhtiyarova and Round[a]	9.0–15.9	4.9–6.2
Psammothidium subatomoides (Hustedt) Bukhtiyarova and Round[a,c]	7.3–11.2	4.6–5.0
Psammothidium ventrale (Krasske) Bukhtiyarova and Round[a]	10.6–11.0	4.8–5.2
Stauroneis agrestis J.B. Petersen[b]	31.2	7.0
Stauroneis intricans Vijver and Lange-Bertalot[a]	29.7–32.6	6.6–6.9
Stauroneis reichardtii Lange-Bertalot, Cavacini, Tagliaventi, and Alfinito [a]	39.9–43.2	8.7–8.9
Stauroneis subgracilis Lange-Bertalot and Krammer[a]	69.9–72.8	13.3–13.4
Stauroneis thermicola (J.B. Petersen) J.W.G. Lund[a]	14.2–16.8	3.3–4.0
Tabellaria flocculosa (Roth) Kützing[c]	23.5–26.5	8.0–9.1

[a] New record taxa
[b] Present
[c] Common taxa

C. pseudoscutiformis in high altitude mountains streams in Bolivia. Beside *C. cocconeiformis, C. pseudoscutiformis* has also been reported from Spitsbergen (Svalbard), by Zgrundo et al. (2017). *Genkalia* is another notable group in high altitude regions. The genus was described by Kulikovskiy et al. (2012) with the type species from Lake Baikal. *Genkalia* is identified by a solitary isopolar valve with

Fig. 4.4 Map of the Western Mediterranean and Black Sea region. Locations of Lake Urmia (Iran) and Lake Van (Turkey). Inset. Outlines of the lakes and satellite image showing water color and lake basin morphology. Scale bars on the figure. Modified base maps from Google, Creative Commons CCO Licence, GNU Free Document Licence

weak sigmoid raphes, and areolae are covered by fine membranes on the external face. Wojtal et al. (2014) noted that *Genkalia digitulus* (Syn. *Navicula digitulus*) is a common taxon in high altitude lakes of the Holarctic region and northern Europe. They also described an additional species *Genkalia boreoalpina* from Switzerland. Both *Genkalia* taxa exist in the Kaçkar Mountain lakes. However, the distribution of *G. boreoalpina* is still poorly known, along with *Caloneis vasileyevae*. The later species was described by Østrup from Denmark, currently reported as rare in different studies across Europe (Veselá and Johansen 2009; Wojtal 2013). In this study, *C. vasileyevae* was present but rarely found in the Black Sea glacial lake region. *Psammothidium daonense* was recorded as new for Turkish diatom flora. The taxon was originally described from Italy by Lange-Bertalot (1999). *Encyonema minutum* and *E. silesiacum* were commonly found in Turkish alpine lakes while very rare across other regions in Turkey. *Encyonema* taxa were quite abundant in Turkish glacial lakes while commonly reported to "exist (about %1)" in other waterbodies around the country (Solak et al. 2012). We believe that *Achnanthidium* and *Planothidium* populations need to be investigated further to document the forms.

Six new records of *Eunotia* are reported here, and two species are common. These taxa represent simple lunate shapes of moderate size (23–75 μm length range, Table 4.2, Figs. 4.6, 4.7, and 4.8). In the high Arctic, 18 species of *Eunotia* have been documented, although most species are rare with 2 species (*E. rostellata* and *E.*

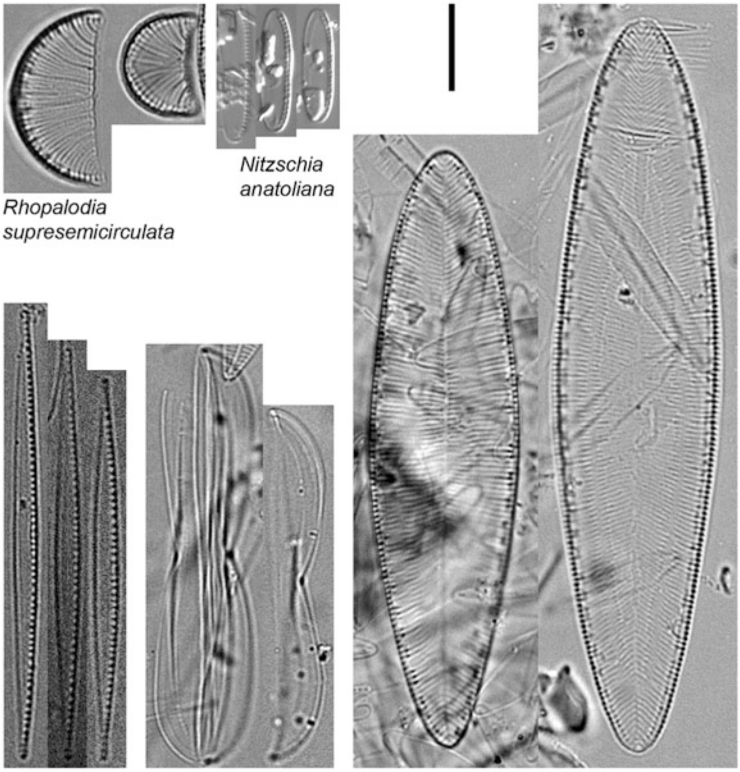


Fig. 4.5 Light microscope images of described soda taxa from Van Lake. Scale bar: 10 μm

boreotenuis) showing populations up to 3% relative abundance. In Turkey, *E. minor* and *E. boreoalpina* (both acidophilic taxa) have prominent population with 5–10% relative abundances. Although there are problems with the taxonomy of *E. minor* (Lange-Bertalot et al. 2011), this taxon is documented to have a Holarctic distribution that can live in higher altitude springs and headwaters within circumneutral to acidic waters. *Eunotia crista-galli*, *E. islandica*, and *E. boreotenuis* are noted from extreme Arctic environment, while *E. curtagrunowii*, *E. subarcuatoides*, and *E. valida* have Holarctic distributions and can occur in spring and higher altitude headwaters under circumneutral to lower pH, with low nutrient conditions (Lange-Bertalot et al. 2011). *Eunotia minor* and *E. boreoalpina* are also documented as acidophilous mire taxa observed in siliceous seepages and pool springs in the Italian Alps (Cantonati et al. 2012). *Eunotia* species (17) have also been commonly

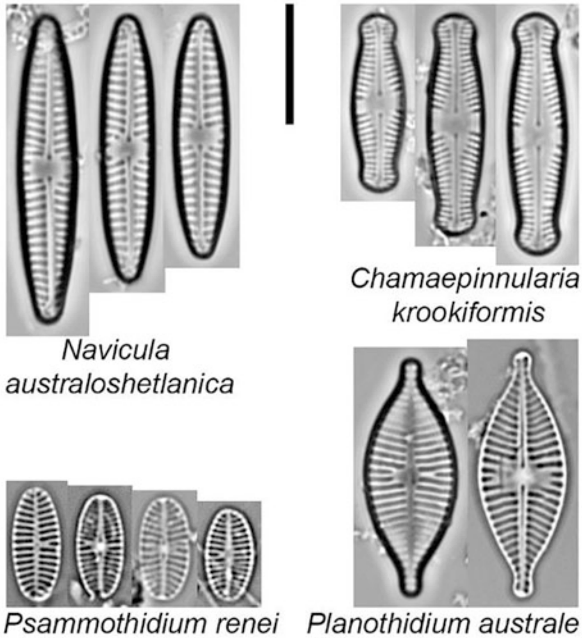
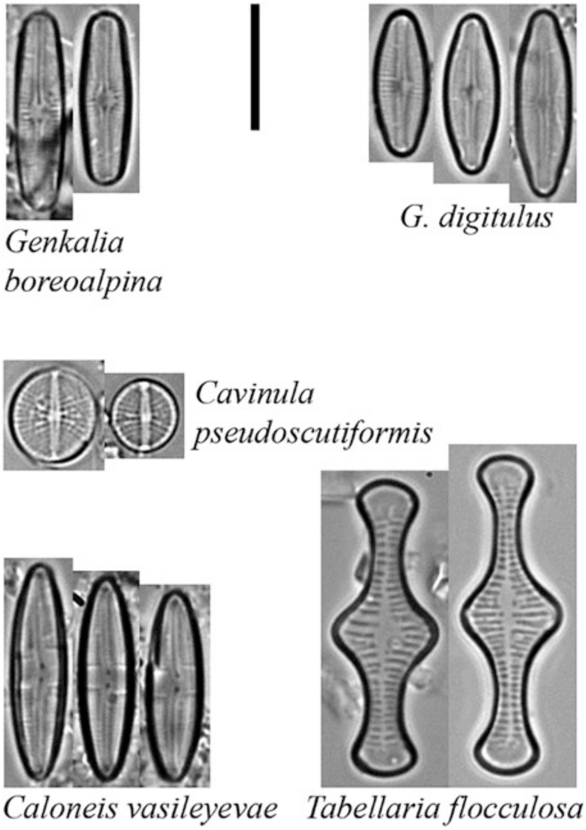


Fig. 4.6 Abundant taxa in the maritime Antarctic samples from the South Shetland Islands. Scale bar: 10 μm

observed in high altitude lakes in Macedonia, including *E. bilunaris*, and *E. minor* (Pavlov and Levkov 2013). In general, *Eunotia* species are regarded as good indicators of circumneutral to low pH and low conductivity (Krammer and Lange-Bertalot 2004; Rimet et al. 2007; Siver et al. 2004).

Monoraphid (one valve with raphe, one valve without a raphe) species are typically small forms that can be found in harsher environments. Eleven genera to date (*Achnanthidium*, *Eucocconeis*, *Gololobovia*, *Gogorevia*, *Karayevia*, *Lemnicola*, *Planothidium*, *Platessa*, *Psammothidium*, *Rossithidium*, *Skabitschewskia*) have been documented from extreme cold environments. *Psammothidium* species are not typically dominant but consistently found across cold study regions. The seven *Psammothidium* species documented from Turkey are similar in numbers to Antoniades et al. (2008) from the Arctic with 9 taxa (Table 4.2, Figs. 4.9, 4.10, 4.11, 4.12, 4.13, 4.14, and 4.15). More specifically, *Psammothidium didymium*, *P. helveticum*, *P. levanderi*, *P. subatomoides*, and *P. ventrale* are species found in both high alpine Turkish lakes and high Arctic freshwater systems. Surprisingly, Foged (1981) in his extensive study of diatoms from Alaska only found 5 *Psammothidium* (as *Achnanthes*) species with no matches to the species from Turkish alpine lakes. *Psammothidium helveticum*, *P. levanderi*, and *P. subatomoides* have also been recorded from Spitsbergen (Svalbard) (Zgrundo et al. 2017), while

Fig. 4.7 Light microscope images of five prominent diatoms from glacial lakes in Turkey. Four taxa are small motile diatoms, with one (*Tabellaria flocculosa*) littoral tychoplanktic taxon. Scale bar: 10 μm



Pla-Rabés et al. (2016) recorded 11 *Psammothidium* species from Franz Josef Land Archipelago with two similar taxa (*P. subatomoides*, *P. ventrale*) to the Turkish alpine lakes. Both *P. subatomoides* and *P. ventrale* are found in circumneutral to alkaline waters with moderate conductivity levels (species optima 82–97 μS cm^{-1}) and moderate dissolved organic carbon levels (ca. 2 mg L^{-1}) (Antoniades et al. 2008). Hofmann et al. (2013) further document that these taxa can be observed in low mountain ranges. *Psammothidium didymium* (Syn. *Achnanthes didyma*), *P. helveticum* (Syn. *A. helvetica*), and *P. levanderi* (Syn. *A. levanderi*) were found in low alkalinity lakes in Northeastern United States by Camburn and Charles (2000). Also, Spaulding et al. (2021) reported that *P. levanderi* occurred in İle da la Possession and Crozet Archipelago. The latter taxon and *P. didymium* were also tentatively given in the checklist of Great Lakes diatoms by Stoermer et al. (1999) (Figs. 4.16, 4.17, 4.18, 4.19, and 4.20).

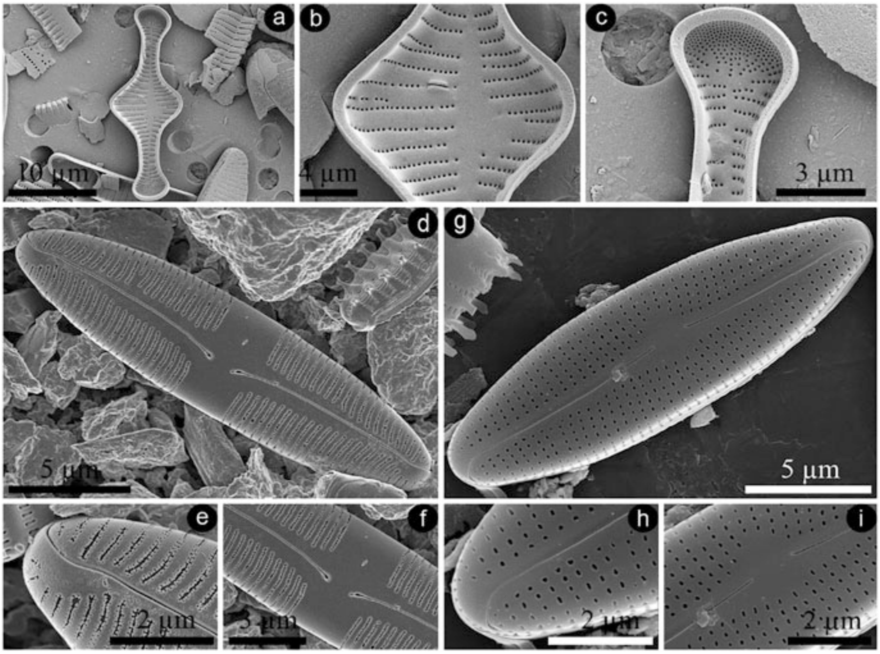


Fig. 4.8 Scanning electron microscope images of three prominent diatoms from glacial lakes in Turkey: *Tabellaria flocculosa, Caloneis vasileyevae, Genkalia digitulus*. (**a–c**) Internal views of *T. flocculosa*. (**b**) Shows the center with a special flattened tubular structure (the rimoportulae); (**c**) shows the end of the valve with a fine punctate apical pore field. (**d–f**) External views of *C. vasilevevae*. The central region lacks markings with a broadly curved slit (raphe) extending from end to end with an interruption in the middle. (**e**) Shows two rows of very small pores which are interrupted by unnatural cracks forming after the death of the diatom. (**g–i**) External views of *G. digitulus* showing raphe slits running down the middle and round to elliptic pore holes extending to both sides. Scale bars are presented on each figure

Among these prominent taxa, some preliminary observations on *P. subatomoides* show differences between the specimens of alpine glacial lakes and Antarctic lakes. A single sample from each region was examined, and 30 specimens of *P. subatomoides* in each sample was measured. The average size of Antarctic specimens was slightly smaller (Table 4.3, Fig. 4.21). In this comparison, specimens from a Swiss alpine lake (about 2400–2600 m. a.s.l.) and Turkish glacial lake (about 3000 m. a.s.l.) were on average larger than specimens from the Antarctic lake. Moreover, areolae (pore) sizes in the Turkish forms are larger than Antarctic forms, while areolae numbers are higher in Antarctic forms (6 areolae) compared to Turkish forms (4 areolae) in central area of raphe valves (Fig. 4.17). These could be different species which are difficult to separate or populations showing differ-

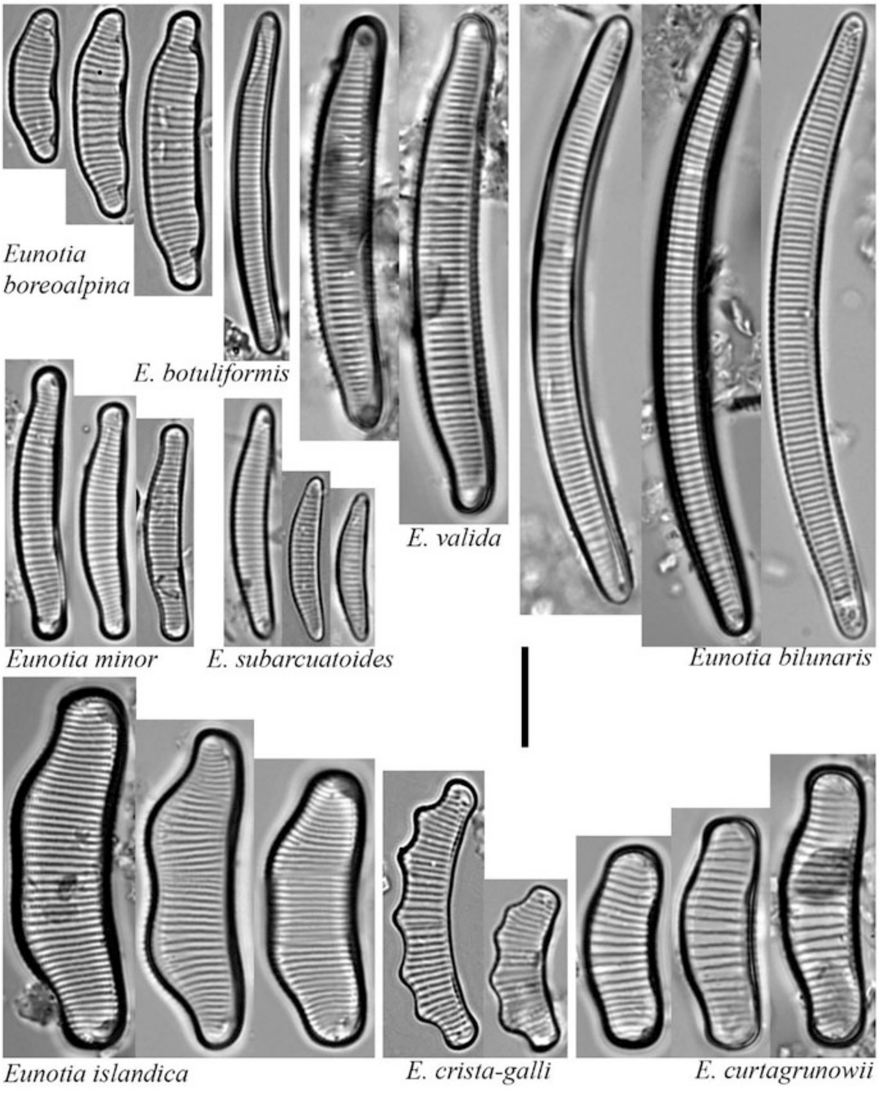


Fig. 4.9 Light microscope images of different *Eunotia* species found in Turkish alpine lakes. Scale bar: 10 μm

ences in phenotypic expression. What environmental differences between polar and mid-latitude extremes might control population size? Possible factors would include light regimes (photosynthetic active radiation, positive; UV light, negative), growing season temperatures, and duration of the growing season.

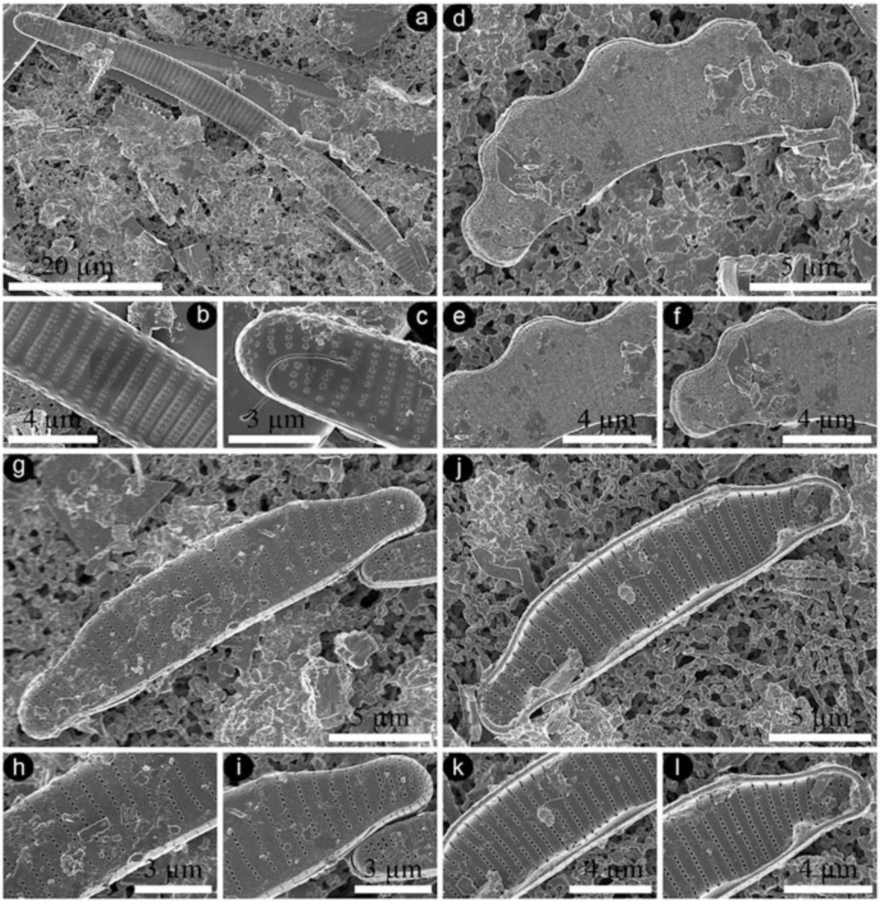


Fig. 4.10 Scanning electron microscope images of *Eunotia bilunaris, E. crista-galli,* and *E. boreoalpina*. (**a–c**) External views of *E. bilunaris*, whole valve, center and apex. (**d–f**) External views of *E. crista-galli*, whole valve, center and apex. (**g–i**) External valve surface of *E. boreoalpina*, whole valve, center and apex. (**j–l**) Internal views of *E. boreoalpina*, whole valve, center and apex. Scale bars on each figure

The genus *Stauroneis* represents larger taxa from extreme environments which are not common but show a high level of species richness. Van de Vijver et al. (2004) recognized 63 taxa from Antarctic and Arctic environments with an average maximum valve length of 75.5 µm compared to 36.5 for *Eunotia* species from Turkish alpine lakes. However, there is a broad range in *Stauroneis* valve sizes with polar species as small as 17 µm (maximum length). In Turkish alpine waters, the largest species (*S. subgracilis*) was 72 µm in length with the other species all less

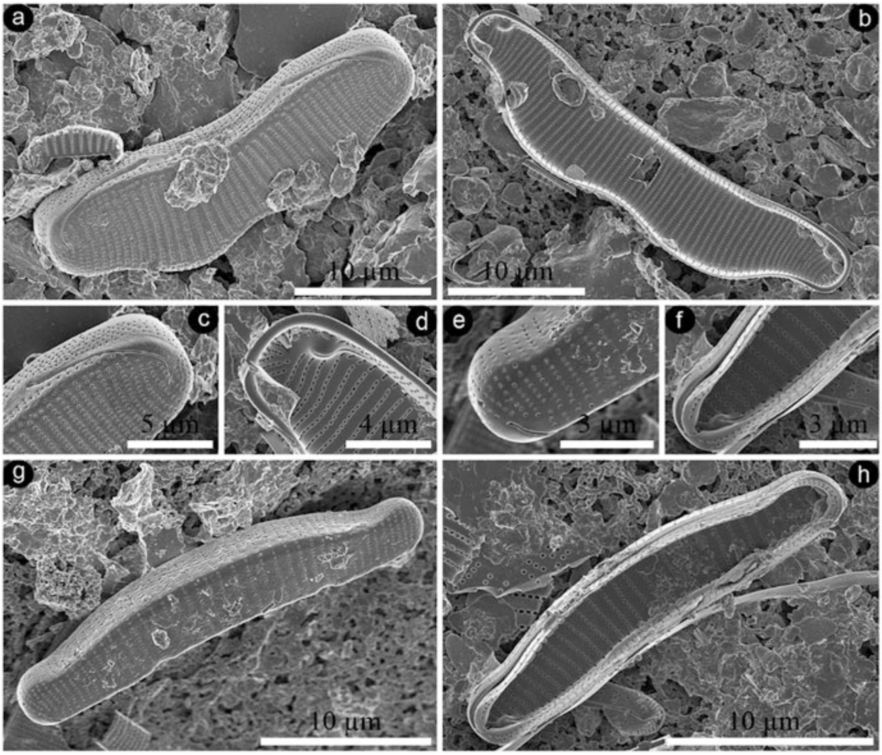


Fig. 4.11 Scanning electron microscope images of *Eunotia islandica* and *E. minor*. (**a**, **c**) External views of *E. islandica*, whole valve and apex. (**b**, **d**) Internal views of *E. islandica*, whole valve and apex. (**d**, **g**) External views of *E. minor*, whole valve, and apex. (**f**, **h**) Internal views of *E. minor*, whole valve, and apex. Scale bars on each figure

than 40 μm (Figs. 4.19 and 4.20). None of the species were abundant. The larger species, *Stauroneis intricans*, *Stauroneis reichardtii*, and *Stauroneis subgracilis* were found in the lakes as new records for the Turkish diatom flora. The smaller *Stauroneis thermicola* was also found in alpine environments from the North American Rocky Mountains (Bahls 2010). Four of the recorded species in Turkey are found across the Arctic, and one species (*S. subgracilis*) is found at both poles. *Stauroneis reichardtii* was originally described from Sardinia, Italy, by Lange-Bertalot et al. (2003) and then recorded from Jan Mayen Island, Norway (Van de Vijver et al. 2005). The higher link of Turkish alpine diatoms with Arctic species, compared to Antarctic species, indicates biogeographic dispersal and distribution patterns favoring the northern hemisphere (Figs. 4.22 and 4.23).

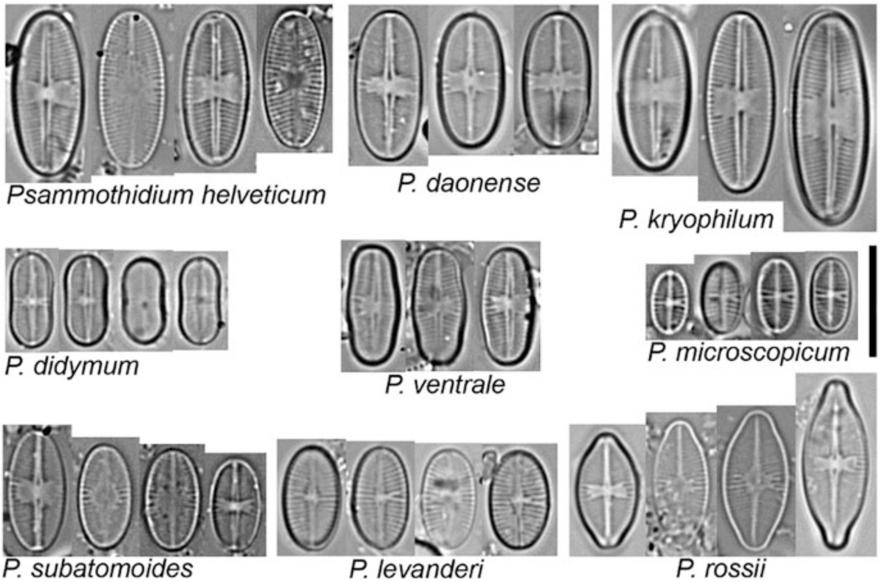


Fig. 4.12 Light microscope images of *Psammothidium* species from glacial lakes in Turkey. Scale bar: 10 μm

4.8 Environmental DNA and Cold Extremes

Environmental DNA (eDNA) analyses have exploded in the scientific literature over the last 10 years, and studies in alpine and Arctic regions have shown changing environments (climate) based on eDNA distributional patterns (Hou et al. 2014). In diatom research, eDNA studies on the Arctic Lake in Siberia found 163 verified diatom sequences, while 176 morphological species were identified by Hudson et al. (2000). Projected out to regional surveys, total species estimates would be in line with the works of Foged and others (>350 species). Within the generic complexes of *Staurosira* and *Staurosirella* (dominant polar taxa), a DNA study revealed that multiple haplotypes could be distinguished with a latitudinal and climate gradient (Stoof-Leichsenring et al. 2014, 2015). This research has direct implications for biogeographic studies, especially in alpine and polar biomes that have clear disjunct separation. In another study of recent and ancient sedimentary DNA, Stoof-Leichsenring et al. (2020) found that selected diatom genera are more resilient and able to adapt to environmental change. In extreme cold environments, this could suggest that the more prominent smaller monoraphid genera are better able to adapt to harsher conditions but may not have the same competitive advantage when other genera are not displaced by the environment. Rules of assemble structured by the

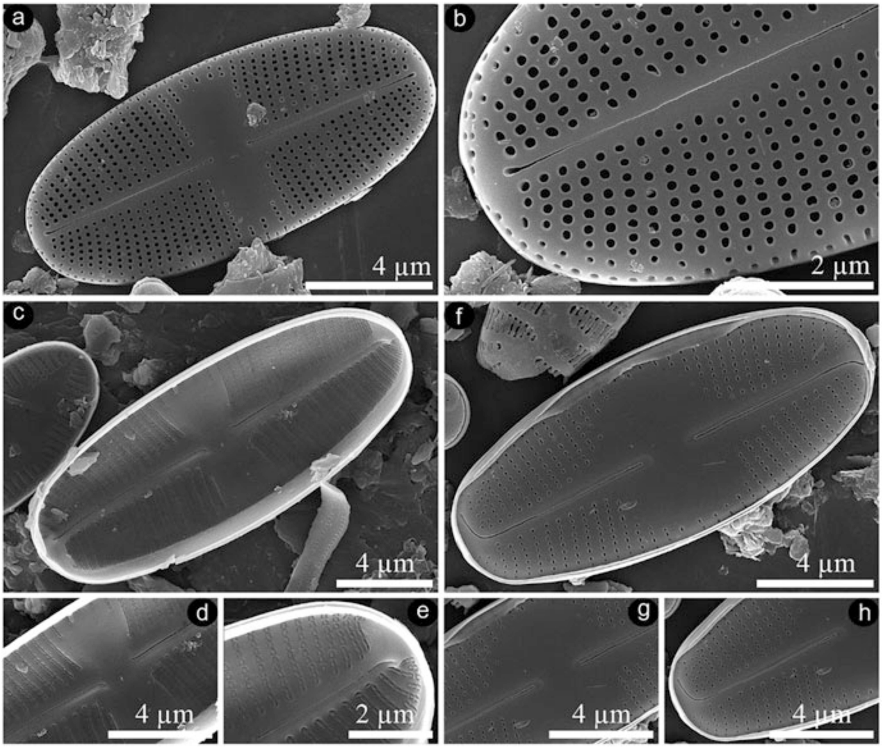


Fig. 4.13 Scanning electron microscope images of *Psammothidium daonense* and *P. helveticum.* (**a**) External view of *P. daonense*. (**b**) Internal view of *P. daonense*. (**c–e**) Internal view of *P. helveticum*. (**f–h**) External view of *P. helveticum*. Scale bars are presented on the plate

environment, with deterministic processing, may have significance when determining diatom assembles of alpine and polar biomes.

4.9 Trends in Biogeography for Alpine Lakes and Ponds

At present, there are no clear trends in global equatorial to polar biogeography when considering high alpine, alpine, and even peri-alpine environments. The somewhat random distribution of alpine biomes including differences in elevations and bedrock chemistry introduces another layer of factors pressuring stochastic and deterministic colonization processes. Further niche size has a very important role to play in determining the ultimate success of community composition. In this chapter, we

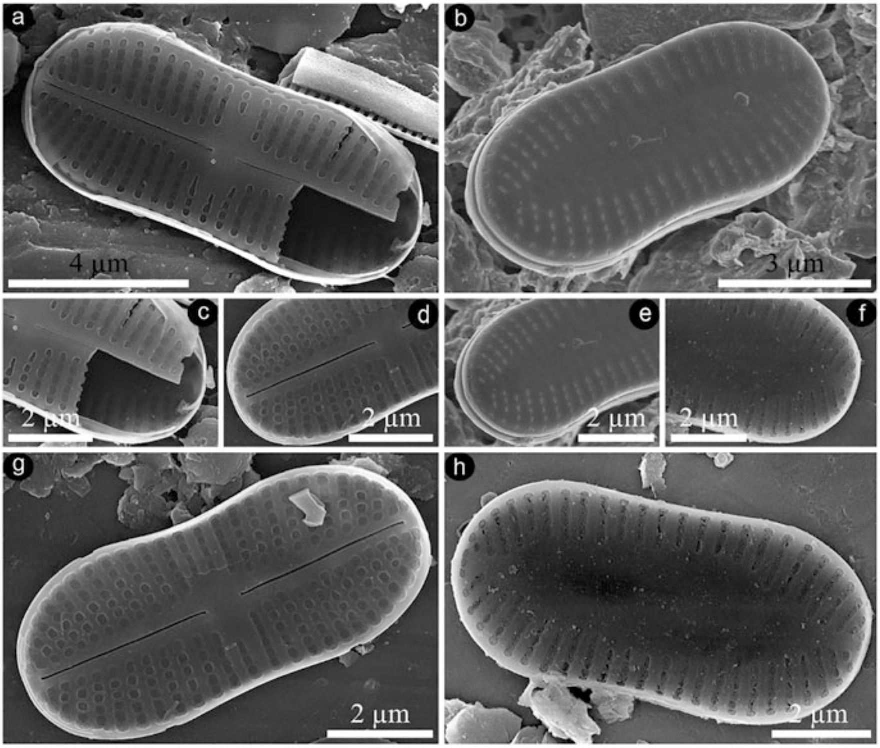


Fig. 4.14 Scanning electron microscope images of *Psammothidium didymium*. (**a**, **c**) External views of the raphe valve. (**b**, **e**) External views of the raphe valve. (**d**, **g**) Internal views of the rapheless valve. (**f**, **h**) Internal views of the rapheless valve. Scale bars are presented on the plate

examined the current state of the scientific literature and used diatoms as a proxy for community, regional systems, and broader scale biogeographic distributions. Diatoms tell us that high elevation alpine communities near the equator have some commonalities with polar environments. Many variables are impacting diatom community development, in decreasing order of importance: climate, physical factors, and chemical factors. In addition to natural factors, there are anthropogenic factors from atmospheric deposition of contaminants to regional agriculture which alter the biology. It is interesting to note that different forms of nitrogen will be deposited through atmospheric deposition in alpine biomes depending on distances from source, thus linking the impacts of nutrients directly with climate. Apart from the complexity of competing environmental factors, there are some trends that in the future can be explored. Under similar physical conditions: (1) species diversity decreases with increasing exposure to cold extremes (increasing latitude or eleva-

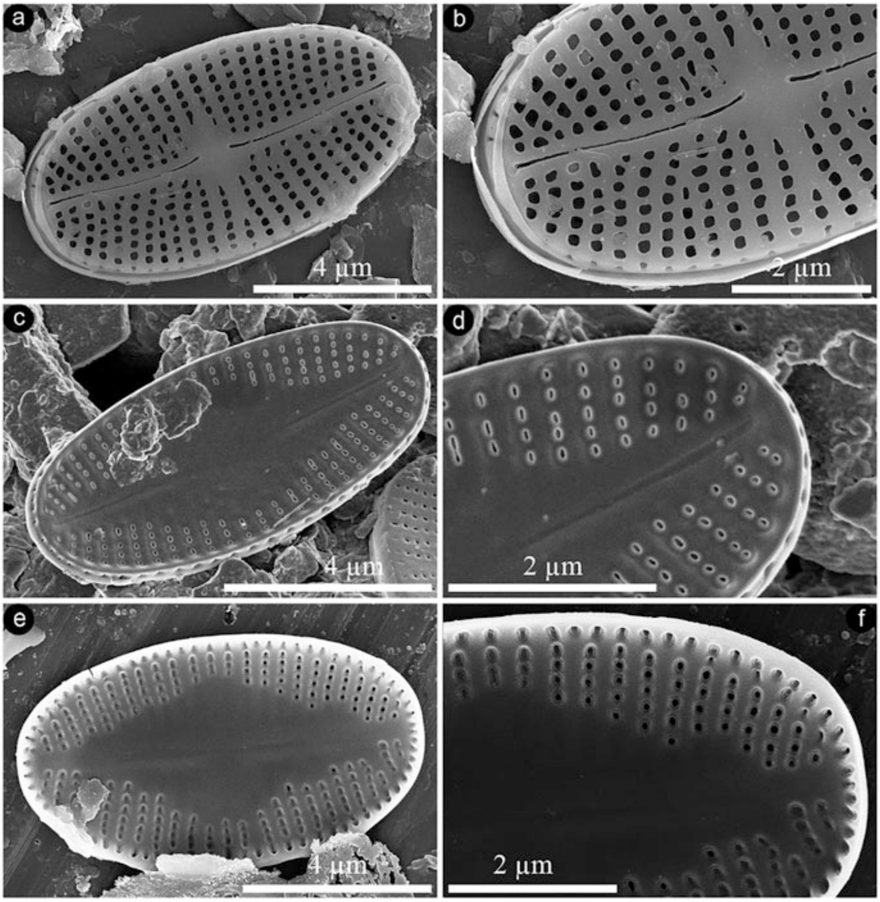


Fig. 4.15 Scanning electron microscope images of *Psammothidium levanderi*. (**a**, **b**) External views of the raphe valve. (**c**, **d**) External views of the rapheless valve. (**e**, **f**) Internal views of the rapheless valve. Scale bars are presented on the plate

tion); (2) prominent populations of smaller species, or smaller sizes of a single species, trend with increased exposure to colder environments; (3) in diatoms, the structural complexity of valve morphology decreases with increasing exposure to harsher (colder) environments; (4) again in diatoms, there is a trending change from biraphid species to monoraphid species and then to araphid species with increasing exposure to extreme conditions; (5)examples of species radiations (long-term projections in species evolution) are not documented from cold environments (i.e.,

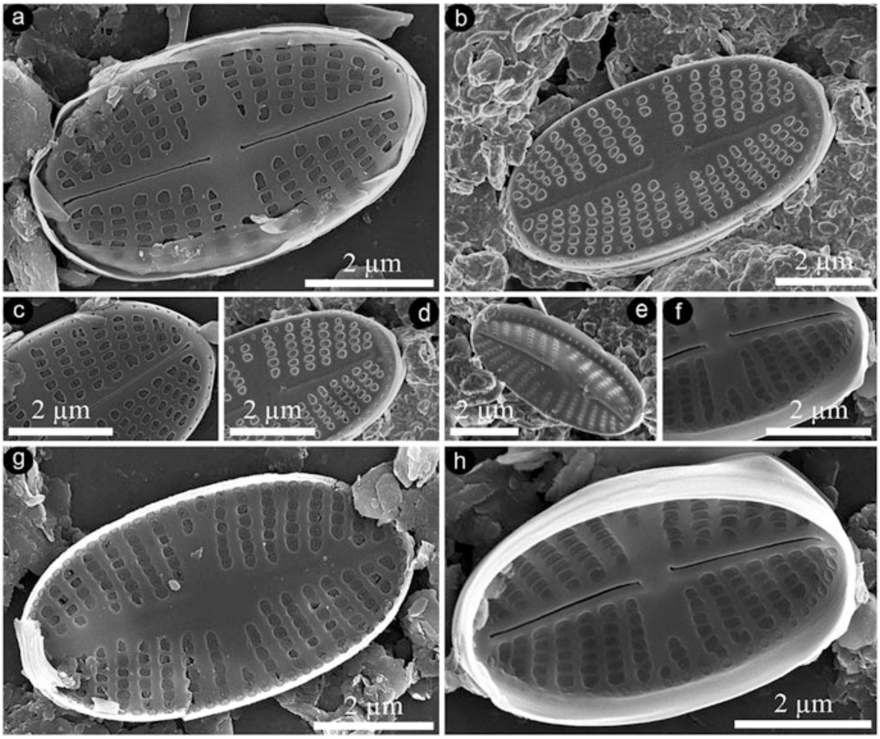


Fig. 4.16 Scanning electron microscope images of *Psammothidium microscopicum*. (**a**, **c**) External views of the raphe valve. (**b**, **d**) External views of the rapheless valve. (**e**) Shows slit-like areolae in the mantle. (**g**) Internal view of the rapheless valve. (**f**, **h**) Internal views of the raphe valve. Scale bars are presented on the plate

species surviving and radiating over glacial exposure cycles). Thus, extreme environments are constraining biodiversity and limiting evolutionary processes.

Although there are interesting trends in biogeographic distributions, noted exceptions should be considered. Foged (1981) conducted an extensive study of the diatoms from Alaska and documented 987 species, and varieties, beyond what might be expected. In another study of diatoms from Iceland, Foged (1974) documented 689 taxa which are in line with more recent taxa studies from the Arctic. These results challenge the overriding idea of decreasing diversity with increasing latitude. Foged examined more sample material (microscope slides from a single sample) and habitats than other research studies which could, in part, explain the higher recorded number of species. The justifications presented are still not enough to explain the high diversity of diatom taxa from Alaska. In the future,

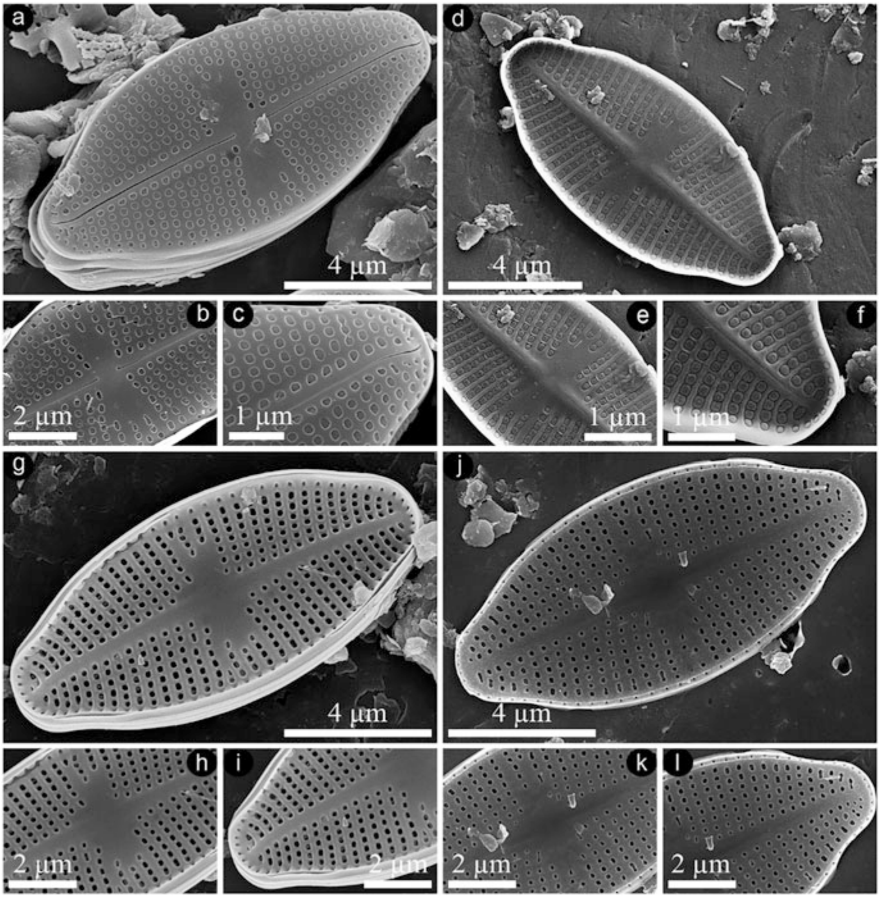


Fig. 4.17 Scanning electron microscope images of *Psammothidium rossi*. (**a–c**) External views of the raphe valve. (**d–f**) Internal views of the raphe valve. (**g–i**) Internal views of the rapheless valve. (**j–l**) External views of the rapheless valve. Scale bars are presented on the plate

can we find this level of diversity in alpine biomes with intense study? The answer is yes. Another interesting anomaly is paleo-sediment sea beds at high altitudes (e.g., Tibetan Plateau (Asia), Lake Urmia (Iran)). The paleo-sediments are a primary seed for ions and nutrients, creating a more insular and stable biome. Since the aquatic lakes and ponds are similar and diversity of habitats more limited, these alpine environments may be less diverse (alpha, beta biodiversity), although with more morphologically complex diatom taxa. However, high inorganic sediment loads in selected lakes will influence regional diversity. Finally, the presence of similar taxa

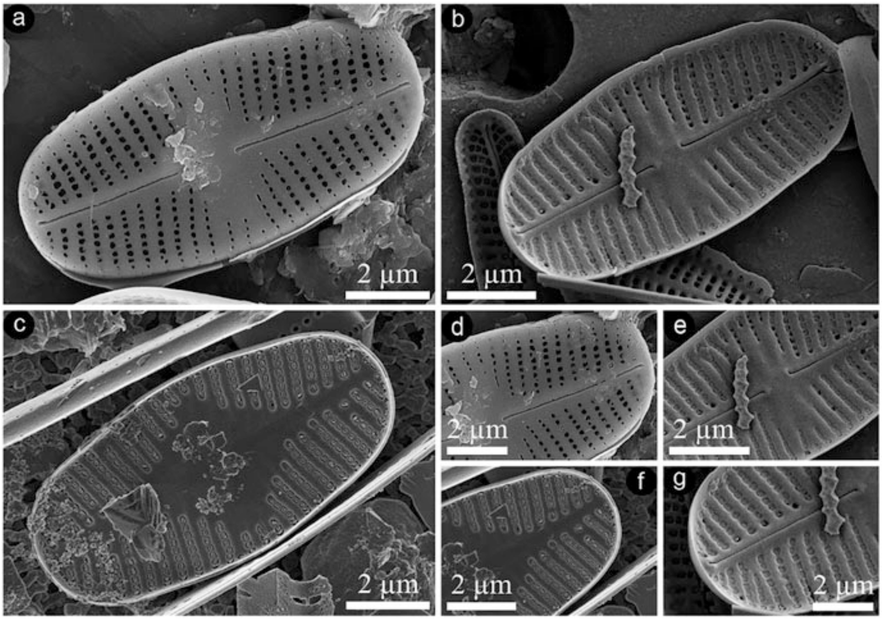


Fig. 4.18 Scanning electron microscope images of *Psammothidium ventrale*. (**a**, **d**) External views of the raphe valve. (**b**, **e**, **g**) Internal views of the raphe valve. (**c**, **f**) Internal views of the rapheless valve. Scale bars are presented on the plate

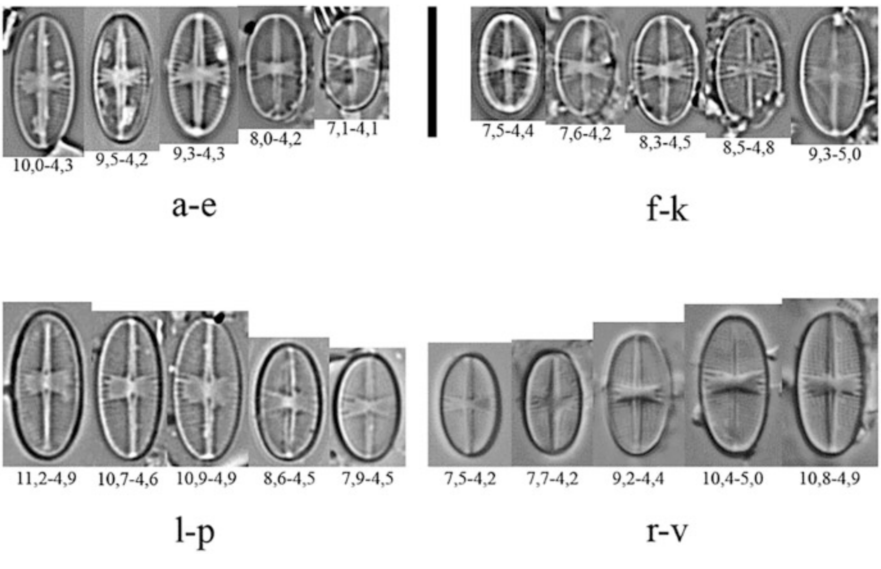


Fig. 4.19 Light microscope images of *Psammothidium subatomoides* in glacial lakes from maritime Antarctica (**a**–**e**, Robert Island; **f**–**k**, Horseshoe Island), Turkish glacial lakes (**l**–**p**), and Swiss alpine lakes (**r**–**v**). Scale bar: 10 µm

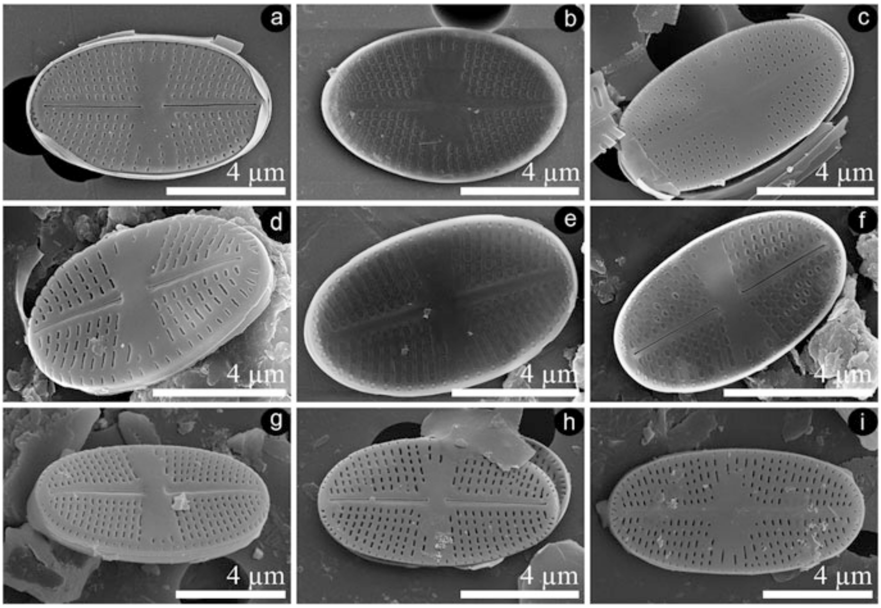


Fig. 4.20 Scanning electron microscope images of *Psammothidium subatomoides* in glacial lakes from maritime Antarctica (**a**–**c**), Turkish glacial lakes (**d**–**f**), and Swiss alpine lakes (**g**–**i**). Specimens of this species have two enclosing valves (shells) with different surface structures. (**a**, **d**, **g**, **h**) outside view of the valve with a raphe. (**f**) inside view of the valve with a raphe. (**c**, **i**) outside view of valve without the raphe. (**b**, **e**) inside view of the valve without a raphe. Scale bars: 4 µm

in equatorial alpine systems with aquatic freshwaters at both poles (Antarctic and Arctic) supports the idea that there is some level of connectivity between these distantly spaced biomes. The level and significance of this connectivity is yet to be determined, although genetics gives us the tools to examine this question.

4.10 Climate and the Anthropocene

Climate change will, in the future, greatly impact extreme environments, especially polar and alpine biomes. The Great Acceleration (1950s–1980s) has been documented with diatom proxies in Arctic (e.g. Gajewski et al. 1997; Kaufman et al. 2009) and alpine aquatic systems (e.g., Sommaruga-Wögrath et al. 1997). Due to their extreme environmental conditions (i.e., low temperatures and strong solar radiation) and simple trophic structure, lake and pond biodiversity will be more

Table 4.3 *Psammothidium subatomoides* specimen measures from three distinct regions: maritime Antarctic, Turkish glacial lakes, and Swiss Alps

	Antarctic_lakes			TR_glacial lakes			Swiss alpine lakes		
	L	W	L/W	L	W	L/W	L	W	L/W
1	10.0	4.3	2.3	10.7	4.6	2.3	9.4	4.8	2.0
2	9.5	4.2	2.3	10.9	4.9	2.2	9.2	5.1	1.8
3	9.3	4.1	2.3	10.2	4.7	2.2	7.2	3.8	1.9
4	9.2	4.0	2.3	10.3	5.0	2.1	9.2	4.9	1.9
5	9.1	4.1	2.2	10.1	4.8	2.1	9.6	4.6	2.1
6	9.3	4.3	2.2	9.9	4.9	2.0	7.3	4.2	1.7
7	8.6	4.5	1.9	10.0	4.5	2.2	10.0	5.0	2.0
8	8.1	4.1	2.0	9.7	4.4	2.2	10.4	5.2	2.0
9	8.0	4.2	1.9	8.9	4.5	2.0	6.3	3.8	1.7
10	7.7	4.2	1.8	9.3	4.5	2.1	7.9	4.0	2.0
11	7.1	4.1	1.7	9.2	4.3	2.1	9.2	4.5	2.0
12	9.3	5.0	1.9	8.9	4.6	1.9	9.6	4.1	2.3
13	8.5	4.8	1.8	8.4	4.5	1.9	10.4	5.1	2.0
14	8.2	4.4	1.9	8.1	4.3	1.9	8.0	4.3	1.9
15	8.3	4.3	1.9	7.3	4.6	1.6	11.1	5.5	2.0
16	8.4	4.4	1.9	11.2	4.9	2.3	8.8	4.6	1.9
17	8.3	4.5	1.8	8.9	4.9	1.8	9.0	4.6	2.0
18	8.3	4.6	1.8	8.5	4.9	1.7	8.7	4.6	1.9
19	8.1	4.5	1.8	8.1	4.6	1.8	11	5.8	1.9
20	8.0	4.3	1.9	7.9	4.4	1.8	6.5	3.8	1.7
21	8.1	4.7	1.7	7.9	4.5	1.8	9.1	4.3	2.1
22	7.6	4.4	1.7	9.2	4.1	2.2	10.6	4.8	2.2
23	7.7	4.7	1.6	8.1	4.0	2.0	7.8	4.3	1.8
24	8.0	4.1	2.0	10.1	4.9	2.1	10	4.7	2.1
25	7.9	4.5	1.8	8.3	4.6	1.8	9.3	4.6	2.0
26	7.9	4.4	1.8	9.1	4.3	2.1	6.7	3.9	1.7
27	7.7	4.4	1.8	8.6	4.5	1.9	10.7	5.2	2.1
28	7.6	4.4	1.7	9.6	4.9	2.0	10.8	5.2	2.1
29	7.6	4.2	1.8	7.8	4.1	1.9	8.1	3.9	2.1
30	7.5	4.4	1.7	7.8	4.6	1.7	8.7	4.4	2.0
Mean	8.3	4.4	1.9	9.1	4.6	2.0	9.0	4.6	2.0
Max	10.0	5.0	2.3	11.2	5.0	2.3	11.1	5.8	2.3
Min	7.1	4.0	1.6	7.3	4.0	1.6	6.3	3.8	1.7

Underlined valve lengths greater than 10 μm. All measurements are in μm

sensitive to environmental change (Arndt et al. 2013). Future changes in alpine lakes and ponds will be related to precipitation, glacial melt (Barker et al. 2001), and solar irradiance (Lean et al. 1995). Globally, the impact of climate change will vary among regions. Some regions, like the alps, will experience different levels of acidification

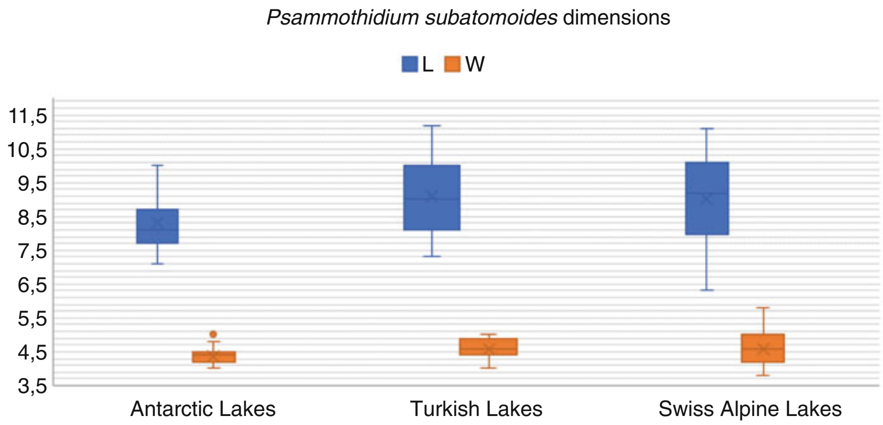


Fig. 4.21 The maximum, minimum, and mean lengths of *Psammothidium subatomoides* specimens from glacial lakes in maritime Antarctic, Turkish glacial lakes, and Swiss alpine lakes (*L* length; *W* width). Scale on *Y*-axis in μm

(Gąsiorowski and Sienkiewicz 2010; Sommaruga-Wögrath et al. 1997), and more isolated regions will be impacted by growing season and nutrification (Wolfe et al. 2003), while other regions will experience evapotranspiration and drought with waters becoming more salty (ion rich) (Barker et al. 2001; Calanca 2007).

In regions with enhanced nutrification through glacial melt, shifts in community structure will occur within phyla and between phyla, even with minor changes in nutrients (Saros et al. 2003). Specifically, there will be a shift from mixotrophic (e.g., cryptophytes) and oligotrophic communities (diatoms, chrysophytes) to meso- (all microbe groups) and eutrophic communities favoring chlorophytes and cyanobacteria. Under most impact scenarios (excluding drought), within phyla, there will be increases in species richness, biodiversity, and a shift to larger morphologically complex taxa. Over the last 100 years, the Arctic diatom flora has shown increasing richness and diversity with a trend toward larger taxa (Overpeck et al. 1997). At present, smaller taxa are still predominant. Similarly, the alpine lakes and ponds of Turkey also show a dominance of smaller species, although diatom survey studies are not extensive (Bouchard et al. 2004). Regarding the Turkish freshwater diatom flora, *Cymbella affinis*, *Navicula cryptotenella*, *N. tripunctata*, *Nitzschia dissipata*, *N. media*, *Pantocsekiella ocellata*, and *P. delicatula* are generally abundant taxa except in the Inner Anatolia glacial region (Solak et al. 2012). Due to the high conductivity, inland waters have different diatom compositions. For this reason, brackish species *Conticribra weissflogii*, *Craticula halophila*, *Craticula anatoliana*, *Ctenophora pulchella*, *Navicula recens*, *Navicula phylleptosoma*,

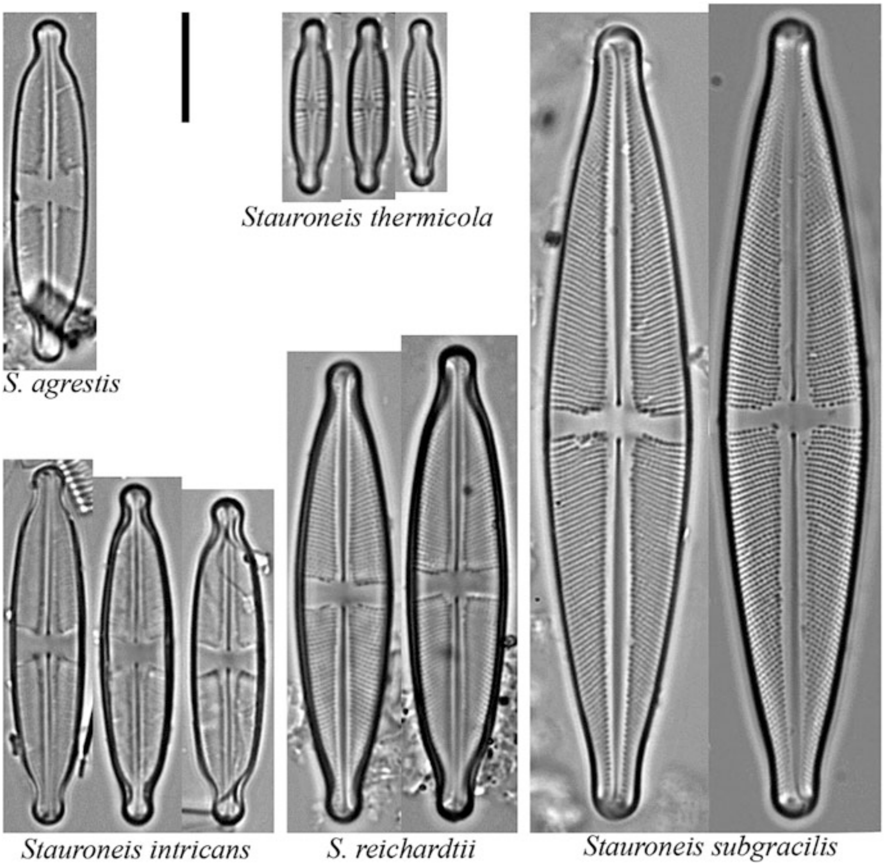


Fig. 4.22 Light microscope images of *Stauroneis* species found in glacial lakes in Turkey. Scale bar: 10 μm

Tabularia fasciculata, and *Halamphora coffeaeformis* are quite common in the region (Çetin et al. 2021).

The stress of human disturbance in alpine biomes, especially in peri-alpine and alpine systems, has been evident for a long period of time (Salmsao et al. 2018). Agriculture is the top stressor in alpine biomes across every continent, and alpine lakes on the Tibetan Plateau would be an excellent example. Additional environment stressors, but less disturbing, include hydroelectric development, mining, forestry, human settlements, and tourism. In the future, agriculture and other human disturbances will continue to be globally significant factors in peri-alpine and alpine aquatic systems. In more isolated (high alpine), typically smaller, and exposed aquatic systems, local conditions will continue to be a limitation, although atmospheric depositions of nutrients and contaminants (current and past captured in glaciers) will become more significant. Climate change is creating a large laboratory experiment to which many hypotheses will be tested.

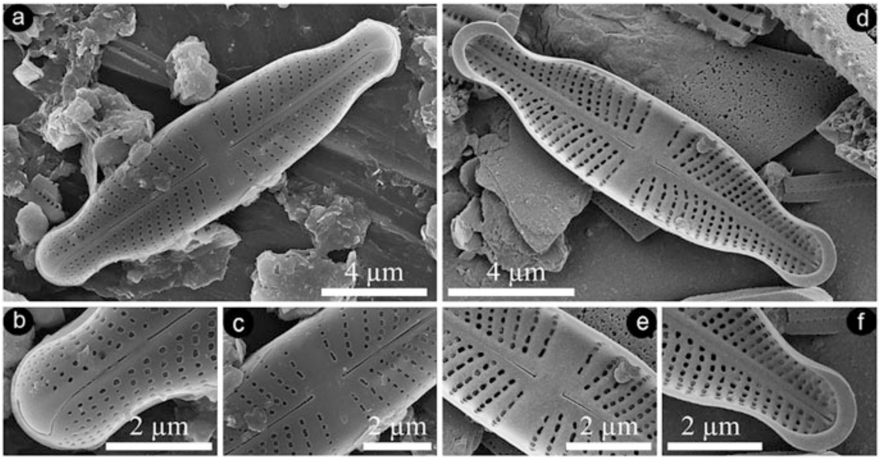


Fig. 4.23 Scanning electron microscope images of *Stauroneis thermicola*. (**a–c**) External views of the valve. Note the central space across the valve and the "?" shaped ends of the raphe slit at the poles. (**d–f**) Internal view of the valve. Note the thickened rim at each apex of the valve. Scale bars are on the images

Acknowledgments This research has been supported by Kutahya Dumlupınar University Scientific Research Projects Coordination Unit under grant number 2021-13. Also, the work was partly funded by the Polish Ministry of Science and Higher Education under the name of "Regional Excellence Initiative" in the years 2019–2022 Project No. 026/RID/2018/19 (ŁP). Antarctica samples were collected, and KÖ is supported by TÜBİTAK Projects (Grant no: 118Y330). This study was carried under the auspices of Presidency of the Republic of Turkey, supported by the Ministry of Industry and Technology, and coordinated by Istanbul Technical University (ITU) Polar Research Center (PolReC).

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Chapter 5
Ocean Acidification Conditions and Marine Diatoms

Sarah H. Rashedy

Abstract Ocean acidification doesn't just erode calcium carbonate shells. It can also slow the rate of diatoms to build their beautiful, intricate silica cell walls. Thinner walls mean lighter diatoms making the algae less able to transport carbon to the deep ocean. Diatoms are a key group of non-calcifying marine phytoplankton, responsible for ~40% of ocean productivity. Growth, cell size, and silica content are strong determinants of diatom resilience and sinking velocity; therefore, the effect of diatom species on ocean biogeochemistry is a function of its growth strategy, size, and frustule thickness. In natural environments, pH directly affects the diatom's growth rate and therefore the timing and abundance of species. Consequently, understanding impacts of ocean acidification on diatom community structure is crucial for evaluating the sensitivity of biogeochemical cycles and ecosystem services in the world's oceans.

Keywords Ocean acidification · Biogeochemistry · Diatoms · Growth · Frustule thickness

5.1 Introduction

Ocean acidification is a global threat to the world's oceans, estuaries, and rivers. It is projected to grow as carbon dioxide (CO_2) and continues to be emitted into the atmosphere at record-high levels. The oceans take up CO_2 from the atmosphere and are responsible for absorbing around a third of the CO_2 emitted by fossil fuel burning, deforestation, and cement production since the industrial revolution (Sabine et al. 2004). While this is beneficial in terms of limiting the rise in atmospheric CO_2 concentrations and hence greenhouse warming due to this CO_2, there are direct consequences for ocean chemistry. Ocean acidification describes the lowering of seawater pH and carbonate saturation that result from increasing atmospheric CO_2

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P. Srivastava et al. (eds.), *Insights into the World of Diatoms: From Essentials to Applications*, Plant Life and Environment Dynamics,
https://doi.org/10.1007/978-981-19-5920-2_5

concentrations. There are also indirect and potentially adverse biological and ecological consequences of the chemical changes taking place in the ocean now and as projected into the future (Ridgwell and Schmidt 2010).

Climate affects diatoms in complex ways. As the planet warms due to the increase in carbon dioxide, scientists predict that diatoms will decrease compared to other planktons such as coccolithophores and cyanobacteria (Tatters et al. 2013). In lakes and rivers, a changing climate alters river flow in many parts of the world. The frequency and severity of droughts and floods is changing, which influences diatom species and where they grow. Furthermore, climate controls circulation patterns and thermal stratification of lakes and oceans, which alter diatom species composition (Bach and Taucher 2019). Diatoms affect climate on a global scale. As diatoms photosynthesize, they absorb carbon dioxide from the atmosphere and release oxygen. Although diatoms are very small, they live in the vast oceans, the world over. The effect of fixation of carbon by diatoms and release of oxygen alters the chemistry of the atmosphere. It is well-known that diatoms play a vital role in pelagic food webs and elemental cycling in the oceans (Smetacek et al. 2012). Therefore, understanding the effects of global warming on diatom community structure is essential for considering the sensitivity of biogeochemical cycles and ecosystem services in the world oceans.

5.2 Ocean Acidification

Ocean acidification is the ongoing decrease in the pH value of the Earth's oceans, caused by the uptake of CO_2 from the atmosphere. Friedlingstein et al. (2020) stated that the rate of atmospheric CO_2 levels has persisted to increase and nearly tripled between the 1960s and 2010s. Oceans have mitigated this growth by absorbing about a quarter of CO_2 emissions between 1850 and 2019. As the amount of carbon dioxide in the atmosphere increases, the amount of carbon dioxide absorbed by the ocean also increases. This leads to a series of chemical reactions in the seawater which has a negative impact on marine life and ecosystem functioning (Vargas et al. 2022). As the ocean acidifies, the concentration of carbonate ions decreases. Calcifying organisms such as mussels, corals, and various plankton species need exactly these molecules to build their shells and skeletons (Fig. 5.1). Also, other marine organisms that do not have calcium carbonate shells or skeletons need to spend more energy to regulate their bodily functions in acidifying waters.

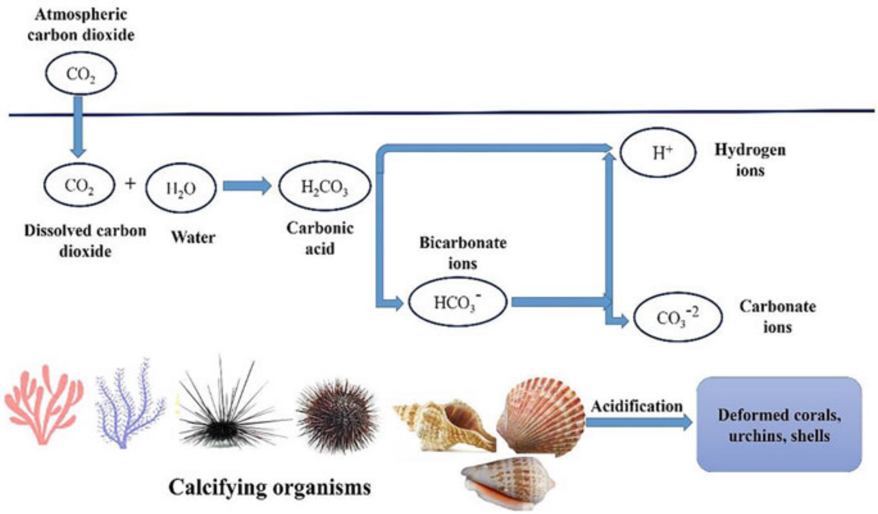

Fig. 5.1 Ocean acidification

5.3 Effects of Ocean Acidification on Marine Diatoms Community

Diatoms are among the most important and prolific microalgae in terms of both abundance and ecological functionality in the ocean. They are within the division of Bacillariophyta and serve directly or indirectly as food for many animals with an assessed contribution of almost 25% to global primary production (Tréguer and De La Rocha 2013). There are at least 30,000 of diatom species which differ in size and ranging from below 3 μm up to a few millimeters (Mann and Vanormelingen 2013). Diatoms are found as single cells or in chains in pelagic and/or benthic habitats and take free-living, surface-attached, symbiotic, or parasitic lifestyles (Mann and Vanormelingen 2013).

Diatom community composition could be affected by different environmental stressors in different ocean areas due to their massive relevance for the Earth system (Tréguer et al. 2018). There have been a number of research articles (Table 5.1) that studied the effects of future CO_2 on marine diatom communities in short-term incubations (Bach et al. 2019; Feng et al. 2009, 2010; Hare et al. 2007; Kim et al. 2006; Tortell et al. 2002, 2008). Also, short-term ocean acidification experiments were done with single species of cultured diatoms (Chen and Gao 2003; Li et al. 2012; Sobrino et al. 2008; Sun et al. 2011; Wu et al. 2010). Few others (Crawfurd et al. 2011; Tatters et al. 2012) have used experimental plans in which isolated diatoms were exposed to different CO_2 conditions for longer periods (more than three months). Most studies indicated that elevated CO_2 led to a measurable increase in phytoplankton productivity, promoting the growth of larger

Table 5.1 Effects of ocean acidification reported on marine diatoms community

Experimental condition	Effects	References
Field incubation experiment, phytoplankton assemblages exposed to CO_2 levels of 150 and 750 ppm (dissolved CO_2 ~3 to 25 μM)	Relative abundance of phytoplankton taxa fluctuated significantly between CO_2 treatments. Abundance of diatoms decreased by about 50% at low CO_2 relative to high CO_2. CO_2 concentrations could potentially impact competition among marine phytoplankton taxa and affect oceanic nutrient cycling	Tortell et al. (2002)
Surface water samples (5 m) were collected at 35 locations. Phytoplankton was concentrated by gravity filtration onto 2.0 mm pore size filters; the fraction of cellular HCO_3 and CO_2 uptake was measured	Diatom species composition is sensitive to CO_2 concentrations ranging from 100 to 800 ppm. Elevated CO_2 led to a measurable increase in phytoplankton productivity, promoting the growth of larger chain-forming diatom	Tortell et al. (2008)
Mesocosm setup and sampling, using different concentrations of CO_2 of 25, 41, and 76 kPa (250, 400, and 750 matm)	Two phytoplankton taxa (microflagellates and cryptomonads) and two diatom species (*Skeletonema costatum* and *Nitzschia* spp.) account for approximately 90% of the phytoplankton community	Kim et al. (2006)
Incubation of phytoplankton communities from two areas under conditions of elevated sea surface temperature and/or partial pressure of carbon dioxide (pCO_2)	Community composition was shifted away from diatoms and toward nanophytoplankton	Hare et al. (2007)
Shipboard continuous culture experiment (Ecostat) was used. Four treatments were tested: 12 °C and 390 ppm CO_2, 12 °C and 690 ppm CO_2, 16 °C and 390 ppm CO_2, and 16 °C and 690 ppm CO_2	Both elevated CO_2 and temperature resulted in changes in phytoplankton community structure	Feng et al. (2009)
Shipboard continuous culture experiment	After 18 days of incubation, increased diatom and *Phaeocystis* abundance. The major influence of high CO_2 was on diatom community structure, by favoring the large centric diatom *Chaetoceros lineola* over the small pennate species Cylindrotheca closterium	Feng et al. (2010)
Natural phytoplankton communities were bounded for 32 days in situ mesocosm with a pCO_2 gradient fluctuating from 380 to 1140 μatm. Nutrients were added to all mesocosms (N, P, Si)	Total diatom biomass was significantly positively affected by high CO_2 after nutrient enrichment. CO_2 effects on diatom biomass and species composition were weak during oligotrophic conditions but became quite strong above ~620 μatm after the nutrient enrichment. Ocean acidification in the subtropics may support the effectiveness of (large) diatoms and cause changes in diatom community composition	Bach et al. (2019)

chain-forming diatom. Future studies will be required to evaluate whether this is also the case for other types of algal communities from other marine systems (Table 5.1).

5.4 Impacts of Ocean Acidification on the Growth of Diatoms

Diatoms in different waters suffer from variations of light and temperature as well as fluctuations in seawater carbonate chemistry. It is predictable that the growing partial pressure of CO_2 (pCO_2) in seawater due to ocean acidification will reduce the cellular requirement of diatoms for energy and resources, therefore stimulating diatom growth and carbon (C) fixation (Tortell et al. 2008). Diatoms show diversified responses to ocean acidification; higher CO_2 concentrations are displayed to enhance (Gao et al. 2012b; Kim et al. 2006; King et al. 2011), have no effect (Boelen et al. 2011) or even inhibit (Li and Campbell 2013; Low-Décarie et al. 2011; McCarthy et al. 2012; Sugie and Yoshimura 2013) growth rates of diatom species. However, raised CO_2 in the ocean increases its availability to algae; the reduced pH can affect the acid-base balance of cells (Flynn et al. 2012). In addition, the higher CO_2 and reduced pH levels can interact with solar radiation and temperature, showing synergistic, antagonistic, or balanced effects (Gao et al. 2012a). Therefore, the mechanisms involved in the responses to ocean acidification of diatoms need to be further explored.

5.5 The Physiological Response of Marine Diatoms to Ocean Acidification

The responses of marine diatoms to ocean acidification are highly variable and species-specific as shown in Table 5.2. Diatoms work greatly efficient CO_2 concentrating mechanisms (CCMs) to reach a high ratio of carboxylation to oxygenation (Raven et al. 2011). They are resistant to high levels of UV radiation (Wu et al. 2012), preferable a low sensitivity to photoinactivation of PSII compared with other phytoplanktons (Key et al. 2010 and Wu et al. 2011), and positively exploit variable light (Lavaud et al. 2007).

Li et al. (2012) estimated the combined effects of ocean acidification, UV radiation, and temperature on the diatom *Phaeodactylum tricornutum* and grew it under two CO_2 concentrations (390 and 1000 μatm); growth at the higher CO_2 concentration increased non-photochemical quenching (NPQ) of cells and partially responded the damage to PS II (photosystem II) produced by UV-A and UV-B. The ratio of repair to UV-B-induced damage decreased with increased NPQ, reflecting induction of NPQ when repair dropped behind the damage, and it was higher under the ocean acidification condition, showing that the increased pCO_2 and lowered pH counteracted UV-B-induced harm. As for photosynthetic carbon fixation rate which

Table 5.2 Physiological response reported of marine diatoms

Diatom species	Type of response	References
Thalassiosira pseudonana	Unaffected	Crawfurd et al. (2011) Yang and Gao (2012) Wu et al. (2014) Shi et al. (2015) Hong et al. (2017)
Ditylum brightwellii	Unaffected	Riebesell et al. (1993)
Nitzschia spp.	Unaffected	Kim et al. (2006)
Chaetoceros brevis	Unaffected	Boelen et al. (2011)
Phaeodactylum tricornutum	Positive	Wu et al. (2010) Hong et al. (2017)
Chaetoceros mueller	Positive	Shi et al. (2019)
Navicula pelliculosa	Positive	Low-Décarie et al. (2011)
Pseudo-nitzschia multiseries	Positive	Sun et al. (2011)
Skeletonema costatum	Positive	Gao et al. (2012b)
Attheya sp.	Positive	King et al. (2011)
Navicula directa	Negative	Torstensson et al. (2012)
Thalassiosira weissflogii	Negative	Mejía et al. (2013)
Nitzschia palea	Negative	Low-Décarie et al. (2011)
Navicula directa	Negative	Torstensson et al. (2012)

increased with increasing temperature from 15 to 25 °C, the elevated CO_2 and temperature levels synergistically interacted to reduce the inhibition caused by UV-B and thus increase the carbon fixation.

5.6 Conclusions and Future Perspectives

Ocean acidification is known to reduce calcification of many calcifying organisms. Different diatom species may have entirely diverse responses to ocean acidification, mostly because of variances in species or phenotypes. The ocean acidification made changes in diatom competitiveness, and assemblage structure may change key ecosystem services; therefore, monitoring community abundance of diatoms over longer timescales is important to gain information on their responses to environmental changes.

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Chapter 6
Diatom Algae for Carbon Sequestration in Oceans

Bhaskar Venkata Mallimadugula and Adeela Hameed

Abstract Diatom algae are responsible for about 20–25% of primary production on Earth. They are said to have evolved ~200 million years ago (mya). Diatoms have a unique feature—a silica exoskeleton, giving them an advantage over the other phytoplankton. Diatoms play a vital role in regulating the nitrogen cycle in oceans and freshwater bodies. However, anthropogenic activity has adversely impacted diatom production and water ecology over the past 250 years. Their production may have decreased by as much as 40% in the last 50 years. The invention of mechanized trawlers enabled fishing on an industrial scale since these trawlers and other support vessels could travel long distances and spend more days out in the ocean. Thus, fish stocks plummeted, which directly affected the diatom population. Growing diatom algae is also considered the best solution to eutrophication. Diatoms take in CO_2 and nutrients like nitrogen and phosphorus, produce oxygen, and are also consumed by zooplankton and fish. Planting forests is considered a carbon sequestration solution. Similar is growing macroalgae in coastal waters. Diatoms in coastal waters keep it clean, enabling mangroves and sea grasses to grow. Dead diatoms sink to the depths of the ocean, together with other organic matter, and sequester carbon into the depths. This is called the ocean's biological pump. However, growing microalgae/phytoplankton is currently not being considered as a valid carbon sequestration solution. What is required is a thorough research into the world of oceans to understand in further detail the role of diatoms in increased ocean productivity and carbon sequestration.

Keywords Blue carbon · Carbon sequestration · Climate change · Diatom · Eutrophication · Fish carbon · Fisheries · Ocean biological pump · Whales

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P. Srivastava et al. (eds.), *Insights into the World of Diatoms: From Essentials to Applications*, Plant Life and Environment Dynamics,
https://doi.org/10.1007/978-981-19-5920-2_6

6.1 Introduction

Bacillariophyceae/diatom algae are the most prolific phytoplankton on Earth, responsible for about 20–25% of primary production (Marella et al. 2016). This accounts for 40–50% of primary production in the oceans (Fox et al. 2020). Annual diatom production has been estimated to be approximately 23 GtC/yr (Mann 1999). This may be compared to the tropical rainforest production of ~18 GtC/yr, agriculture production of ~8 GtC/yr, and anthropogenic carbon emissions of ~10 GtC/yr (Mann 1999). Thus, diatoms may be considered the most important photoautotrophs on Earth (Amin et al. 2012).

Diatoms are said to have evolved ~200 million years ago (mya) (Benoiston et al. 2017). Thus, they are the last phylum of phytoplankton to have evolved. *Cyanobacteria* evolved over 2400 mya and *Chlorophyceae* ~1700 mya. Dinoflagellates and coccolithophores evolved after that. Diatoms have a unique feature—a silica exoskeleton (Marella et al. 2016). This gives them an advantage over the other phytoplankton and is perhaps the reason for their evolutionary success. Diatoms are more nutritious and easier to digest than other phytoplankton forming the preferred diet for zooplankton and newborn fish. Diatoms are grown in shrimp hatcheries to feed the post larval shrimp for the first 3 days (Tam et al. 2021). Thus, the survival of newly hatched fish depends on diatom availability. We may conclude that diatoms are the most important phytoplankton/microalgae in oceans (Fox et al. 2020).

Dr. Bostwick Ketchum of Woods Hole Oceanographic Institute, USA, is quoted to have said, "***All fish is Diatom***." (Barber and Hiscock 2006)

Diatoms are also more complex than other phytoplankton. They require silica and micronutrients such as iron, zinc, boron, etc., for their complex metabolism (Marella et al. 2018). So, when the availability of silica and micronutrients reduces in absolute terms or in relation to the availability of macronutrients, the production of diatoms declines.

6.2 Advent of Technology and Decline in Diatom Production

Anthropogenic activity has adversely impacted diatom production and water ecology over the past 250 years, ever since the Industrial Revolution (IPCC 2018). This impact has accelerated in the past 100 years or so, and diatom production may have decreased by as much as 40% in the past 50 years. Industrial whaling and fishing, eutrophication due to flow of nutrients into the oceans, and building of dams, in addition to other activities, are the main reasons for the decline in diatom production (IPCC 2022).

Mechanized ships and boats were invented in the late eighteenth century and commonly used after the invention of the diesel engine in the 1890s. This enabled large-scale hunting of whales. By 1960, their population had already plummeted when an embargo on whaling was imposed by the United Nations. The International Whaling Commission had been set up in 1946 as the global body responsible for management of whaling and conservation of whales. Ever since, whaling was banned in the 1960s; whale stocks have recovered a little. (IWC 2022).

About 100 years ago, human population was ~2 billion. Now, it has reached ~8 billion. Back then, we grew enough food to feed the-then population, whereas, today, we are bound to grow food to feed this fourfold increase in population. This 400% increase in food production has been achieved utilizing new techniques like irrigation by building dams, fertilizers such as urea, phosphates, introducing hybrid varieties, modern machinery, efficient pesticides, etc. Agriculture production is about 8000 million tons per year as of now (Fakhr-ul-Islam and Karim 2019).

The invention of mechanized, i.e., diesel engine powered, trawlers enabled fishing on an industrial scale, since these trawlers and other support vessels could travel long distances and spend more days out in the ocean. Wild fish catch is estimated at about 100 million tons per year, whereas aquaculture production adds another 100 million tons. Investments in wild fisheries are a fraction of investment in agriculture and forests (IPCC 2018).

Industrial fishing depleted fish stocks in the oceans. Many species of fish were at risk of becoming extinct. However, measures are being taken to conserve the ocean fauna, such as fishing holiday during the spawning season, limits of catch, limits on the size of fish that may be caught, type of fishing gear that may be used, etc.

6.3 Interdependence in the Ocean Ecosystem

A key question is the impact of decline in whales and fish population on production of phytoplankton and zooplankton. Does the reduction in numbers of the predators increase or decrease the production of their prey? The first impression would be that when predator numbers are reduced, prey can grow rapidly. However, contrary to the first impression, reduction in predator population actually results in decrease in production of prey (Smetacek 2014). Predators digest and retain the carbon in the prey but excrete most of the macronutrients, like nitrogen and phosphorus, and micronutrients, like iron and zinc (Buskirk and Yurewicz 1998). These nutrients are the input required by the phytoplankton and is called the nitrogen cycle in water (Amin et al. 2012).

Thus, when the predator numbers decline in the oceans, nutrient cycling too slows down. As such, the nitrogen cycle rate decreases. Phytoplankton can grow based only on the fresh input of nutrients from land sources or physical upwelling of nutrients in the oceans. This is one of the reasons for decline in primary production in oceans, and most of this is related to the production of diatom algae (Benoiston et al. 2017).

In the twentieth century, there was an increase in the input of macronutrients into water bodies due to untreated and treated sewage, fertilizer runoff, animal dung, stormwater, etc. Increase in human and farm animal population resulted in increase in the production of sewage and dung. Growth in agriculture and in use of chemical fertilizers, mainly NPK fertilizers, also resulted in a splurge of macronutrients into lakes, rivers, and oceans (USEPA 2022).

Construction of dams across rivers, on the other hand, resulted in the reduction in the flow of water and silt into oceans. Dams divert water from the river to fields for agriculture, while silt is held back. Silt contains silica and micronutrients, like iron, zinc, and the rest. So, reduction in silt inflow in the oceans resulted in the decline in diatom production (Humborg et al. 2000).

6.4 Eutrophication

Eutrophication of coastal waters due to nutrient input from wastewater, both treated and untreated, fertilizer runoff, and stormwater is a major problem. Eutrophication is causing a decline in the amount of carbon sequestered in coastal water. Prevention of eutrophication will help increase quantum of carbon stored in coastal waters. (Jiang et al. 2018).

6.5 Carbon Sequestration in Oceans

How much carbon is sequestered in the oceans and the annual flux have not been quantified in any of the IPCC reports, such as the Report of WGIII AR6—Mitigation of Climate Change or the Report of WG1 AR5 Chapter 5: Changing Ocean, Marine Ecosystems, and Dependent Communities or Chapter 6: Carbon and Other Biogeochemical Cycles. (IPCC 2018).

One way to quantify the gross carbon sequestered on Earth is with reference to the oxygen in the atmosphere. All the oxygen in the atmosphere is produced during photosynthesis. There is no other natural process that produces oxygen on a large scale. UV radiation and lightning may cause some of the water vapor in the atmosphere to split. This releases some oxygen; however, some of it becomes ozone. So, these processes do not have a net impact on the amount of oxygen in the form of O_2 in the atmosphere. The mass of oxygen is ~23% of the total mass of the atmosphere. This is 1,200,000 Gt of oxygen. The ratio of carbon in the organic carbon produced during photosynthesis to the oxygen produced is 1 atom of C to 1 molecule of O_2. So, in terms of mass, it is 12:32, based on the atomic mass of C and O. Thus, for 1,200,000 Gt of oxygen, the carbon in organic carbon on land and oceans is ~450,000 GtC. The IPCC reports do not mention this figure, since no peer-reviewed papers have been published on this subject, and IPCC reports are based entirely on peer-reviewed papers.

The role of trees, especially tropical rainforests, such as the Amazon jungles, in sequestering carbon on land is well-known (Butler 2020). There is, unfortunately, less information and discussion on the role of diatoms in carbon sequestration in the oceans.

6.6 Role of Diatoms in Carbon Sequestration

Growing diatom algae is the best solution to eutrophication. Diatoms consume CO_2 and nutrients like nitrogen and phosphorus, produce oxygen, and are consumed by zooplankton and fish. So, carbon enters the food chain, and oxygen remains in the water, keeping it clean. This is the best solution to eutrophication. Diatoms out-compete the other algae, such as cyanobacteria (blue-green algae) and dinoflagellates (red tides). Therefore, algal blooms are prevented (Marella et al. 2016).

BGA and red tides are not good food for zooplankton and fish, whereas diatoms are the best natural food for these (Mann 1999).

Diatoms play a vital role in regulating the nitrogen cycle in oceans and freshwater bodies (Marella et al. 2016). They are the best feed for zooplankton, such as copepods, krill, etc.; small herbivore fish, such as Clupeidae family of fish, herring, sardines, and menhaden; and all the *oily fish,* such mackerel, anchovies, etc. These small fish are the feed for bigger fish, whales, etc. All animals in water that feed on diatoms recycle silica, nutrients, and micronutrients (Tam et al. 2021). Once biomass increases to a sustainable level, it will sustain itself. So, the cost of growing diatoms in oceans may have to be incurred for 25 to 50 years only.

Growing forests on land is considered a carbon sequestration solution. Similar is growing macroalgae in coastal waters (Fox et al. 2020).

Phrases such as ***Blue Carbon*** and ***Fish Carbon*** have been coined to describe sequestration in coastal waters using mangroves, sea grasses, corals, and growing fish in pens and cages, respectively. Dead diatoms also sink to the depths of the ocean, together with other biological/organic matter, and sequester carbon into the depths. This is called the ***Ocean Biological Pump*** (Honjo et al. 2014). However, growing microalgae/phytoplankton is currently not being considered as a valid carbon sequestration solution.

6.7 Organic Carbon in Oceans

There are no peer-reviewed papers on the estimates of total stock of organic carbon in the oceans. This would include live, dead, and fossilized biomass, i.e., bacteria, phytoplankton, zooplankton, fish, crustaceans, corals/dead corals, whales, hydrocarbons, methane, methane hydrates, etc. (Sigman and Hain 2012).

Gross biomass on land is estimated at ~450 to 600 GtC, by IPCC reports. It may be possible that a similar biomass exists in the oceans.

6.8 Blue Carbon

Growing diatoms in coastal waters keeps it clean, enabling mangroves and sea grasses to grow. Shallow waters are the spawning and nesting ground for fish and amphibians. They lay eggs in the shallow waters, and diatoms are the best feed for the newly hatched fish and tadpoles. Blue carbon is the carbon stored in shallow coastal waters by mangroves, sea grasses, and macroalgae (Macreadie et al. 2019).

6.9 The Way Ahead

The questions that remain are not hard to answer. However, what is required is a thorough research into the world of oceans. These massive water bodies supporting a rich biodiversity, biogeochemical cycles occurring within or out, nutrient transfer, historical peak and its theoretical maximum limit need to be understood in further detail so as to easily comprehend the role of diatoms in increased ocean productivity and carbon sequestration.

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Chapter 7
Diatoms: A Potential for Assessing River Health

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Abstract Diatoms are autotrophic, photosynthetic, and eukaryotic microalgae belonging to phylum Ochrophyta. The main function of diatoms is to convert dissolved carbon dioxide to oxygen in water. In aquatic ecosystems, diatoms are primary producers. Presence of diatoms in rivers is very common and of equal importance. River health assessment is assessing the health and quality of river. Diatoms add up to nutritional status, can be used as biomarkers, and are usually dominating at higher altitudes and in upwelling regions. For long time, physical and chemical monitoring is being done for river assessments. River ecosystems are prone to threat by human activities causing moderations in sedimentation delivery, flowing patterns, and even biodiversity loss. Diatoms, being a good bioindicator for quality of water and land use, can be used as a potential to assess the health of a river. Diatoms respond with change in nutrient availability, concentration of ions, and organic loading.

Keywords Diatoms · River health assessment · Water · Quality · Bioindicator

7.1 Introduction

Single-celled and photosynthesizing algae having siliceous skeleton are diatoms. They are present in fresh waters, marine waters, soil, and places that have adequate moisture content. Diatoms reproduce by cell division. Diatoms are not motile; their mobility occurs by the secretion of mucilaginous material with raphe (a slit like

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P. Srivastava et al. (eds.), *Insights into the World of Diatoms: From Essentials to Applications*, Plant Life and Environment Dynamics,
https://doi.org/10.1007/978-981-19-5920-2_7

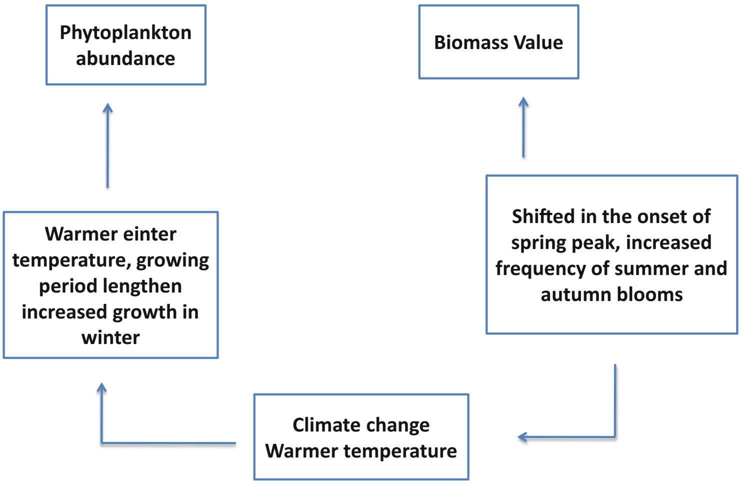

Fig. 7.1 Impact of environmental stressors on rivers

groove/channel). Diatoms are autotrophic and hence restricted to only 200 m down water depths called photic zone. Their cell is composed of transparent, opaline silica. Diatoms contain chlorophyll a and chlorophyll c content which are light-absorbing molecules. These molecules gather energy through the sun and by the process of photosynthesis turns into chemical energy. They can remove atmospheric carbon dioxide through carbon fixation. Long-chain fatty acids are produced by diatoms, and they are a crucial energy source for food web (zooplanktons to insects to fish to whales). Diatoms can be used as a bioindicator to know the health of aquatic systems such as rivers. Different species of diatoms have different tolerant ranges for environmental stressors like concentration of nutrient, suspended sediment, elevation, flow regime, and human interferences (Fig. 7.1). Hence, their presence aids in monitoring and assessing water's biotic conditions. Communities of diatoms demand specific environmental conditions and counter quickly to environmental change which employs them as cost-effective to assess the health of rivers (aquatic ecosystems) and human impacts. (Dalu and Froneman 2016). Fishes and macroinvertebrates have longer generation times as compared to diatoms. Quick response to change in environmental conditions by diatoms offers EWS (early warning systems) for increased pollution and restored habitat success. Their study is an important aspect for assessing and monitoring programs globally. Habitat history of surface water body can be identified by undisturbed core sediments from aquatic ecosystems (Amoros and Van Urk 1989; Cremer et al. 2004; Gell et al. 2005). Previous aquatic conditions may be assessed using diatoms on fishes and macrophytes (Venkatachalapathy and Karthikeyan 2015; Rosati et al. 2003; Yallop et al. 2009). Diatom's study can also help in inferring environmental changes in water bodies including marine, estuaries, and brackish water; however, in freshwater rivers and lakes, interpretations and techniques are highly challenging.

7.2 Health of Aquatic Ecosystems and Rivers

Two general methods for environmental conditions assessment in streams and rivers using diatoms are diatom index and IBI (index of biotic integrity) (Stevenson et al. 1999). Rimet et al. in 2012 observed that, in Europe, Australia, and America, development of many biotic indexes took place before 1999 (Rimet 2012). Some of diatom indexes were developed in Asia (Xue et al. 2019) In America, Europe, and Asia, the development and application of benthic diatom index of biotic integrity (BD-IBI) in ecosystem health assessment already took place (Ruaro and Gubiani 2013; Zalack et al. 2010). However, in China, BD-IBI is applied and shown good results in monitoring and assessing ecosystem health for the past few years (Tang et al. 2006; Tan et al. 2015).

Aquatic pollution not only includes organic and nutrient pollution but also metals and pesticides (Fig. 7.2). Policies are concerned with pollution and its environmental impact. Very few published papers established diatoms, hydrocarbons, and pesticides relationship (Schmitt-Jansen and Altenburger 2005; Debenest et al. 2008, 2009; Morin et al. 2009; Rimet et al. 2004; Rimet 2012). Ethiopian streams had shown advantage of diatoms in water courses that were severely affected. The stream showed presence of diatoms and no macroinvertebrates (Rimet 2012). Diatoms,

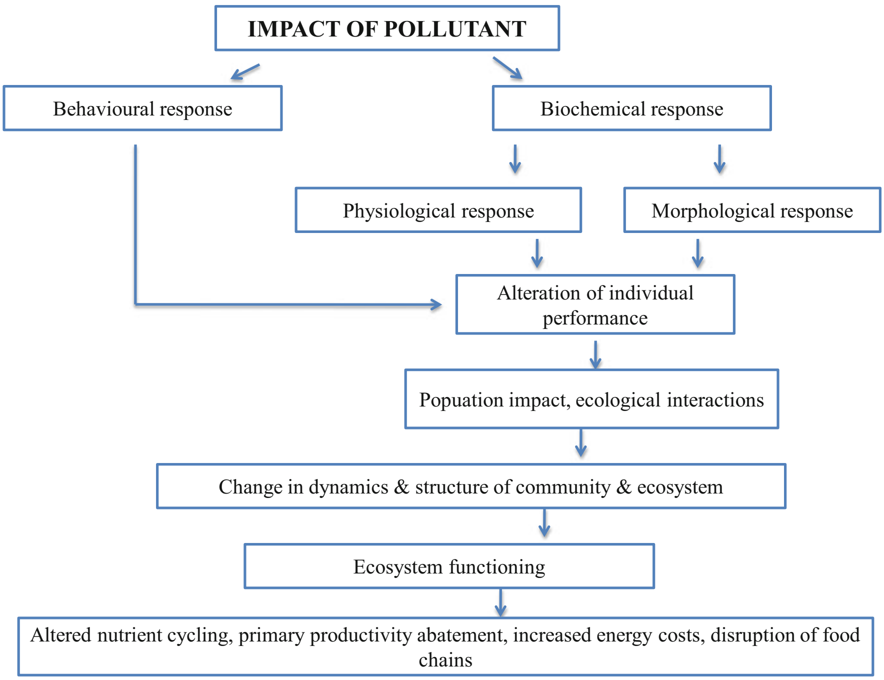

Fig. 7.2 Impact of pollutants on aquatic ecosystem

fishes, macroinvertebrates, and macrophytes were four bioindicators compared in one study (Hering et al. 2006).

Benthic algae, fishes, and macroinvertebrates have unique importance in riverine ecosystems' health conditions and are convenient for biological indices' sampling, identification, and calculations, and therefore they are commonly used in health assessments (Chessman and Royal 2004; Kennard et al. 2006; Qu et al. 2016).

With increased metal concentration in water and decreased measured biomass, chlorophyll a and cell density are observed (Hill et al. 2000; Ivorra et al. 2000; Gold et al. 2002; Morin et al. 2007; Raunio and Soininen 2007; de la Pena and Barreiro 2009). Some studies based on measuring mat thickness showed that the exposure of *Navicula pelliculosa* to Cd contamination prevents mat formations and reduced biomass (Irving et al. 2009; Rimet 2012). Freshwater organism and its biodiversity are sustained by rivers and streams as they are valuable ecosystem (Qu et al. 2016; Arthington et al. 2006).

Several decades ago, initial development of biological indices took place, and since then they are used in river health assessments (Norris and Hawkins 2000).

In the past, many approaches used single kind of aquatic organism for river health assessment on the basis of budget limitations and expert opinions (Barbour 1999; Boulton 1999). However, recent advancements and understanding of the relationships among three different aspects, viz., biological, physical, and chemical, lead to more detailed assessment and application of broad range of aquatic organism and ecosystem processes (Flinders et al. 2008; Wei et al. 2009; Bunn et al. 2010; Bae et al. 2011, 2014).

7.3 Diatoms in River Health Assessment

Two hundred 50 million years ago, during the Triassic period, diatoms arose suggested by molecular clock-based estimates (Sorhannus 2007), and the earliest well-preserved fossils of diatoms came from 190 million years ago, the early Jurassic period (Sims et al. 2006). Primarily, only cyanobacteria and green algae (slightly larger than bacteria) constituted phytoplankton before the arrival of diatoms (Armbrust 2009). The emergence of dinoflagellates and coccolithophorids (larger eukaryotic phytoplankton and diatoms) shifted the global organic cycling which initiated the decline in concentration of atmospheric carbon dioxide and increased oxygen concentrations (Fig. 7.3) (Katz et al. 2005).

The cell wall of diatoms is made with hydrated glass ($SiO2.nH2O$) essentially (Drum and Gordon 2003). The biogenic silicon cycling is controlled by diatoms in world's ocean such that each silicon atom entering the ocean incorporates into diatom cell wall (Strzepek and Harrison 2004) before getting buried on the sea floor (Treguer et al. 1995). Dead diatom's cell wall accumulates on the sea floor depending upon conditions as immense silica deposits up to 1400 meter thick. This was found on eastern Antarctic peninsula's island named Seymour (Sims et al.

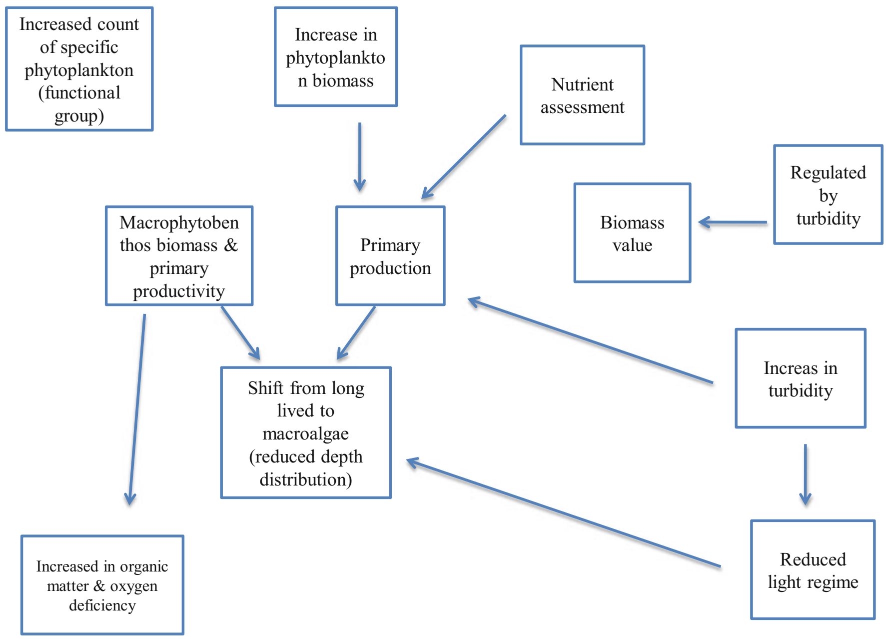

Fig. 7.3 Algae/diatoms as bioindicator

2006). Diatomaceous earth has numerous uses such as flea powder, insulations, and ingredients for toothpastes (Armbrust 2009).

7.4 Water Quality and River Health Assessment

Use of multi-metric bioindicators was recommended by freshwater scientists and European Water Framework Directive (Karr 1981) based on reference condition approach (Bailey et al. 1998) for assessing river ecological conditions and accounting natural heterogeneity of communities (Marzin et al. 2014).

Rivers have various functions to offer human beings, but human activities impact river's health which leads to poor conditions of rivers (Wang et al. 2019). The river suffered degradation through human influence directly and indirectly. Process and structure of natural aquatic ecosystem is adversely affected by channel modifications, flow regulations, and water pollution all throughout the world (Maddock 1999). River health concept was first introduced by USEPA in 1972 Clean Water Act which requires to maintain physical, chemical, and biological integrity of river (Wang et al. 2019).

The impact of ecological effects of water on aquatic biodiversity is direct, and therefore it is used as health indicator (Fryirs 2003). A powerful indicator named zooplankton is present in between fish (top-down regulators) and phytoplankton (bottom-up factors) in a food web and provides information on cost-effective and key measuring indicators for river to be of well ecological status (Hulyal and Kaliwal 2008; Jeppesen et al. 2011). The primary producer in a water body is single-celled phytoplankton which is sensitive to water environment change. They are important for monitoring water bodies biologically (Cardinale et al. 2002; Wang et al. 2019).

7.5 Bioindicators for River Health Assessment

Three types of indicators were recognized by Cairns and McCormick (1992), which are early warning indicators signifying impending health decline, compliance indicator signifying acceptable limit's deviation, and diagnostic indicators identifying deviation causes. The range of these above indicators is from different aspects of the physical and chemical habitat (Maddock 1999; Maher et al. 1999) to biological features of the inhabitants. Focuses of biological aspects are broad taxonomic group like water birds (Kingsford 1999), macroinvertebrates (Kay et al. 1999; Marchant et al. 1999), and diatoms. Various living organisms like algae, macroinvertebrates, fish, etc. are present in aquatic ecosystem habitats (Fig. 7.4) which are capable to tell

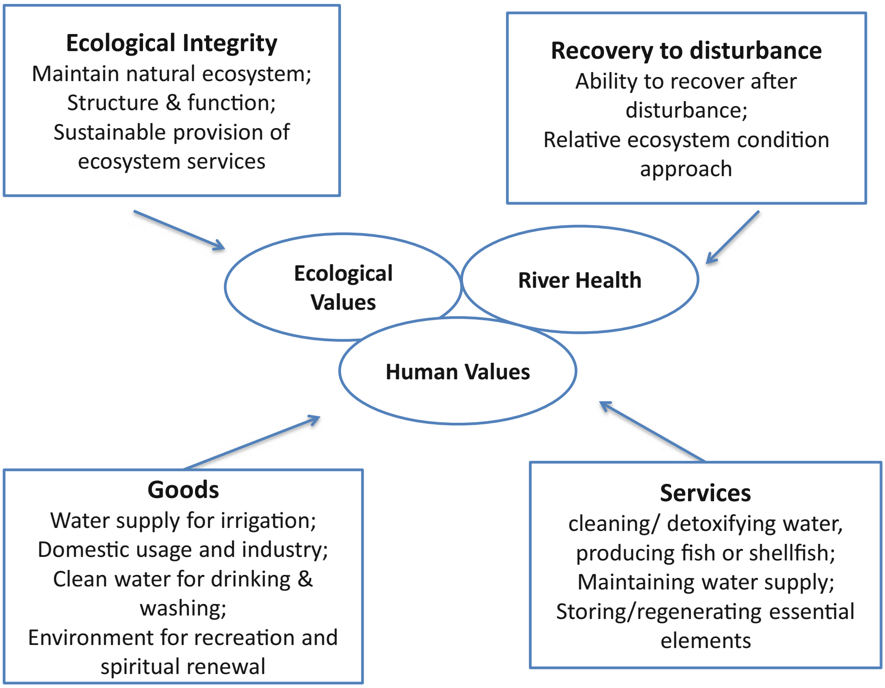

Fig. 7.4 River habitat

continuous and integrative characteristics of water quality. This is why they are considered as worthy bioindicators (Singh and Saxena 2018).

Nutrient levels in water are indicated by diatoms. Excess in these nutrient levels is one of the greatest threats in US streams. Higher nutrient levels increase algae productivity resulting in blooms. These blooms can reduce dissolved oxygen which eventually kills the fishes.

Population dynamics of aquatic ecosystem is directly affected with change in natural environmental conditions such as flow rate, dissolved oxygen, water temperature, and food resources. These population change, human activities, and pollution increase characteristic biological communities with differing ecosystems. Agricultural fertilizer runoff and sewage pollution causes eutrophication feeding plants and algae leading to their overgrowth.

Expert panel recommended DELPHI forecasting method as best way for selecting variables for water quality indices (Pinto and Maheshwari 2011). In Kenya, South Africa, Zimbabwe, and Zambia, implementation of diatoms-based biomonitoring programs were a success. In South Africa, this approach was also incorporated in the National River Health Program (Dallas et al. 2010) which now is the part of National Aquatic Ecosystem Health Monitoring Program. In South Africa, methodology standardization led the foundation for diatom sample's collection and analysis (Taylor et al. 2007). The program is anticipated to give alike results in African countries like Kenya, Zimbabwe, and Zambia, and these countries are in standardizing diatom methodology process; these protocols should take endemic diatom taxa into considerations (Dalu and Froneman 2016).

7.6 Future Perspectives and Conclusion

It is necessary to know how ocean ecology and biochemistry is affected by diatoms. Genomic sequencing of representative diatoms and its analysis can identify how these organisms can help in interpreting river health assessments. Next generation eco-genomic sensors monitor the sentinel species presence, its expression, and give the information about physiochemical properties that are biologically relevant. Monitoring genes that encode iron storage molecule ferritin continuously can provide information for biological availability of iron in surface waters and iron's presence in water (Sedwick et al. 2007). Concluding that diatoms use in biomonitoring has value in going relevant information to common problems about ecological conditions. This can be used for both short- and long-term biomonitoring for health and functioning of aquatic ecosystem.

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Chapter 8
Terrestrial Diatoms and Their Potential for Ecological Monitoring

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Abstract Diatoms have long been utilized as robust ecological indicators for aquatic ecosystems. Ecological data of aquatic diatoms have been well documented. Autecological and biotic indices have extensively used for ecoassessment of water bodies throughout the world. In spite of the fact that diatoms are quite abundant in terrestrial environments and respond quickly to soil environment fluctuations, ecological studies on these entities are substantially lacking as compared to their aquatic counterparts. Of late researchers have investigated certain aspects of soil diatom ecology from some parts of the world. Terrestrial diatoms have been found to be quite responsive to soil environmental conditions, anthropogenic disturbances and agricultural practices. This review attempts to assemble the diverse findings associated with the terrestrial diatoms and their response towards various stressors and explores the future prospects of soil diatom ecology.

Keywords Terrestrial diatoms · Soil microbiome · Agricultural practices

The diatoms are ubiquitous, highly successful microalgae (heterokonts) and constitute one of the most diverse groups on organisms on our planet. They are unique in possessing a highly ornamented cell wall made up of silica (frustules). Diatoms are unicellular eukaryotes mostly occurring as solitary cells, but colonial forms are common as well. An estimated 100,000–200,000 species are known with new species continuously added every year by scientists (Armbrust 2009; Mann and

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P. Srivastava et al. (eds.), *Insights into the World of Diatoms: From Essentials to Applications*, Plant Life and Environment Dynamics,
https://doi.org/10.1007/978-981-19-5920-2_8

Vanormelingen 2013). The frustule's morphology has formed the backbone of diatom taxonomy and systematics.

The pigment composition of diatoms is quite distinct as along with chlorophyll a and c; they contain a group of carotenoid pigments which are photoprotective in nature (Demmig-Adams and Adams 2000; Kuczynska et al. 2015; Fernandes et al. 2018). They are highly important photosynthetic organisms which account for approximately 20–25% of global oxygen produced via the process of photosynthesis (Field et al. 1998; Sarthou et al. 2005; Scarsini et al. 2019). By virtue of the fact that diatoms are highly sensitive to various environmental variables, these heterokonts have served as highly robust ecological indicators of aquatic ecosystems and have been extensively used in estimation of their ecological health for long (Dixit et al. 1992; Prygiel and Coste 1993; Van Dam et al. 1994; Prygiel et al. 1999; Datta et al. 2019; Maurya et al. 2020; Foets et al. 2020a, b). Ecological tolerance and sensitivity values of most of the abundant diatom taxa have been established, and several diatom indices have been developed throughout the world for assessment of freshwaters which have yielded promising and effective results (Lecointe et al. 1993; Prygiel and Coste 1993; Kelly et al. 1995; Rakowska and Szczepocka 2011; Tan et al. 2017; Antonelli et al. 2017).

As diatom biotic indices are extensively used worldwide for assessment, the ecological amplitudes of aquatic diatoms have been mapped comprehensively. A plethora of literature exists which take into account the tolerance of aquatic diatoms to environmental fluctuations and their environmental requirements (Watson and Kalff 1981; Peters 1983; Sprules and Munawar 1986; Cattaneo 1987; Tremblay et al. 1997; Cattaneo et al. 1997; Duarte et al. 2000; Vidal and Duarte 2000; Tsuda et al. 2003; Lavoie et al. 2006; Jones et al. 2014; Carayon et al. 2019; Passy 2007). In spite of the fact that diatoms are quite abundant in terrestrial environments, ecological studies on these entities are substantially lacking as compared to their aquatic counterparts (Falkowski et al. 1998). This could be attributed to the fact that diatom indices dedicated exclusively for soil assessments are completely lacking (Barragán et al. 2018). However, soil diatoms have exhibited considerable potential with respect to soil ecosystem assessments (Johansen et al. 2010; Zhang et al. 2020). Soil diatoms not only contribute significantly to organic carbon enrichment of soil ecosystems but also have a pivotal role in the soil formation and augmentation of stability in soil aggregates (Shein et al. 2016).

8.1 Soil Microbiomes

Soil microbiomes are one of the richest and most diverse communities of microorganisms on our planet (Jansson and Hofmockel 2018). These microbiomes consist of complex interactions of bacteria, viruses, fungi, archaea, and protists. These interactions have an array of consequences on nutrient cycling, determination of fertility of soil, and carbon sequestration (Prescott et al. 2019). Diatoms are an integral part of the soil microbiome and contribute significantly by enhancing organic carbon concentrations and stabilizing aggregates of soil particles.

8.2 Soil Diatoms and Environmental Factors

Soil diatoms are defined as those diatoms which are living on substrates moistened solely by atmospheric water contribution (Lund 1945, 1946; Hoffmann 1989). Though these communities seem to be widespread and ubiquitous like aquatic diatom communities, soil diatoms are rather scarce. Scanty researches made the use of soil diatoms more popular on the basis of two principle areas: first as indicators of anthropogenic disturbances in different ecosystems (Dorokhova 2007; Heger et al. 2012; Fazlutdinova and Sukhanova 2014; Vacht et al. 2014; Antonelli et al. 2017; Blanco et al. 2017) and the other one as hydrological tracers to assess terrestrial-aquatic connectivity (Pfister et al. 2009; Klaus et al. 2015; Martínez-Carreras et al. 2015; Coles et al. 2016). Barragán et al. (2018) showed that the terrestrial diatom communities are a dynamic biota that is ready-to-use for implementing quality assessment methods for soils. Other research also showed that the soil diatoms would be a potential bioindicator for soil environment assessment (Johansen et al. 2010; Zhang et al. 2020). Over the last few years, soil diatoms have received much attention (Johansen et al. 2010), such as detailed surveys in the subantarctic region (Van De Vijver and Beyens 1998; Van de Vijver et al. 2002; Gremmen et al. 2007; Moravcová et al. 2010), Greenland (Van Kerckvoorde et al. 2000), Italy (Zancan et al. 2006), Russia (Bakieva et al. 2012), Poland (Stanek-Tarkowska and Noga 2012; Stanek-Tarkowska et al. 2013, 2015, 2017; Noga et al. 2014), Spain (Blanco et al. 2017), and Luxembourg (Antonelli et al. 2017; Barragán et al. 2018; Foets et al. 2020a, b).

High sensitivity to environmental changes, the ease of collecting and the well-known autecology (Van Dam et al. 1994; Wu et al. 2017) of freshwater diatom communities, can be easily transferred to soil diatoms as well. Moreover, ecological studies of diatom communities on soils are also quite responsive as well as sensitive to several environmental variables such as soil moisture and pH (Lund 1945; Hayek and Hulbary 1956; Van De Vijver and Beyens 1998; Van Kerckvoorde et al. 2000; Van de Vijver et al. 2002; Souffreau et al. 2010; Antonelli et al. 2017). Foets et al. (2021) showed that the environmental parameters (soil moisture and pH) and disturbances caused by farming practices play a vital role in structuring terrestrial diatom assemblages (Heger et al. 2012; Stanek-Tarkowska and Noga 2012; Vacht et al. 2014; Antonelli et al. 2017; Foets et al. 2020a). Apart from these factors, diatom communities are also affected by the other parameters like organic matter, nitrogen, and carbon content in the soil (Gärtner 1996; Kokfelt et al. 2009; Nielsen et al. 2011; Binoy and Ray 2016; Stanek-Tarkowska et al. 2018; Foets et al. 2021).

According to the recent study of Zhang et al. (2020), some environmental parameters, such as MgO, OP, TP, and conductivity, were found to be significantly correlated with soil diatom communities (Battarbee et al. 2001; Epstein and Bloom 2005; Yallop and Anesio 2010; Levkov et al. 2013a, b, c).

8.3 Soil Diatoms and Anthropogenic Disturbances

Soil is a composite environment distinguished by a broad range of microorganisms, chemical compounds, and complex physical structure. Antonelli et al. (2017) hypothesized that soil diatom communities can serve as a representative of anthropogenic disturbance. In their study, they suggested that pH and land use factors influenced in structuring diatom communities, both in terrestrial and freshwater systems.

Urban soils and their biodiversity provide a variety of ecosystem services, which includes nutrient cycling, depollution, fertility, and carbon storage (Guilland et al. 2018; Santos et al. 2020). They are an important element in the urban ecosystem functioning, which impact by most from land use and pollution (Li et al. 2017). Moreover, urban soils are the supreme and perfect dumping ground for huge quantities of waste (Minaoui et al. 2021). These wastes contain many pollutants such as organic matter, fertilizers, pesticides, metals, plastics, and many other contaminants, which cause a degradation and changes of the soil structure as well as the quality of soil (Fierer et al. 2009; Compaore et al. 2019; Jilani and Rashid 2020), which further influence most of the soil microorganisms, their diversity, and functions. This becomes an important ecological issue (Fierer et al. 2009; Ibekwe et al. 2017; Guilland et al. 2018; Santos et al. 2020). However, a few groups of soil microorganisms such as microalgae especially diatoms remain poorly studied (Geisen et al. 2018; Guilland et al. 2018). These soil diatom communities are sensitive to many anthropogenic factors (VanLandingham 1968; Sukhanova et al. 2000; Berard et al. 2004; Zancan et al. 2006; Heger et al. 2012; Stanek-Tarkowska and Noga 2012; Uhr 2013; Antonelli et al. 2017). Some researchers studied in relation to different types of disturbance such as oil pollution (Dorokhova 2007), animal disturbance (Moravcová et al. 2010), agricultural activities (Zancan et al. 2006; Heger et al. 2012), and herbicides effect (Zurek 1981; Berard et al. 2004). However, unlike aquatic environments, only a few studies deal with heavy metals impact on soil diatom assemblages (Morin et al. 2012; Wanner et al. 2020) or terrestrial algal community (Dorokhova et al. 2005; Song et al. 2013; Emiliya and Zhemadukova 2017). Human activities also influence the distribution of diatom communities (Megharaj et al. 1998; Berard et al. 2004; Megharaj et al. 2000a, b; Vacht et al. 2014).

8.4 Soil Diatoms and Agricultural Practices

With Green Revolution serving the population rise, land has been considerably exploited, and numerous cultivation methods have been implemented inducing an accelerated decline in soil health. There has been implementation of a numerous restoration strategies of which one such assessment bioindicator lately used are the diatoms. They are responsive to the soil components such as pH, carbon, and nitrogen, effect of land use, farming, and tillage practices (Lin et al. 2013; Antonelli et al. 2017). Complementing to this study, Heger et al. (2012) observed the possible relation between diatom specificity and abundance in conventional and organic fields; Zancan et al. (2006) reported similar results on fields with intensive ploughing and herbicide and fungicide applications. Lands employing reduced tillage accounted greater species diversity contrasting the conventional practices (Stanek-Tarkowska et al. 2018; Stanek-Tarkowska and Noga 2012). Barragán et al. (2018) showed the species richness decline in the disturbed agricultural lands (Useldange region – 4 species) comparative to undisturbed forest and grasslands (Weierbach basin – 40 species) which attributes to influence the species composition of terrestrial diatom communities (Van Dam et al. 1994; Van De Vijver and Beyens 1998) further linking riparian zone to the stream on establishing rapid surface and subsurface hydrological relativity (Pfister et al. 2017).

Scientists, owing to previous data, advanced on deciphering the relation between diatom abundance and distribution with agricultural practices. Antonelli et al. (2017) observed the dominance of *Hantzschia amphioxys* (Ehrenberg) Grunow, *Nitzschia pusilla* (Kützing) Grunow, and *Hantzschia abundans* Lange-Bertalot in samples collected on farmland and grasslands associated with pH values (6.2 ± 0.8) and overall lower C and N soil content (C: 3.2 ± 2.8%; N: 0.3 ± 0.2%). According to Cullimore and McCann (1977), *Hantzschia amphioxys* shows resistance to different herbicides. There is abundance of *Eolimna minima*, *Nitzschia pusilla*, and *Pinnularia* cf. *obscura* in all samples. Similarly, Zancan et al. (2006) reported the abundance of *Hantzschia amphioxys* (Ehrenberg) Grunow in Cleve and Grunow and *Navicula pelliculosa* (Brebisson ex Kutzing) Hilse on vineyards, pasture, corn field, and abandoned land. The experiments revealed less species diversity in the disturbed sites. Lands with reduced tillage was abundant in *Amphora montana*, whereas *Stauroneis thermicola* and *Pinnularia obscura* dominated in traditional tillage samples; *Falacia monoculata* was found only in reduced tillage samples (Stanek-Tarkowska and Noga 2012). In general, comparatively, reduced tillage sites exhibited higher species diversity attributed to increased organic carbon facilitating soil water retainment and bulk density along with reduction in amount of readily dispersible clay (Stanek-Tarkowska et al. 2018; Stanek-Tarkowska and Noga 2012). Vijayan and Ray (2016) reported highest ecological parameters of diatoms found in the Lower Kuttanad soil region (high organic matter defining salinity and alkalinity), during Virippu season, at the seedling stage of the crop, whereas the lowest value in Kayal soils (characterized by silt loam – silt clay loam) during Puncha season at the seedling stage accounting to specific soil factors, crop seasons, and soil phosphorus in the wetland paddy soils. Stanek-Tarkowska et al. (2013) surveyed the cultivated soils of Podkarpacie Province, applied with ammonium nitrate and phosphorus-potassium mineral fertilizers, illustrating the dominance of *Sellaphora nana*, *Stauroneis borrichii*, *S. parathermicola*, and *S. thermicola.* Anticipating the conclusion behind the structuring of diatom communities, Foets et al. (2020b) and Zancan et al. (2006) explained that the diatom species abundant in undisturbed sites were sensitive comparative to the ones found on disturbed lands which display wide tolerance ranges.

Besides the spatial significance in the agricultural practices, terrestrial diatoms have been a focal point for their temporal aspects. Diatom species differ in population peaks temporally accounting to variation in environmental factors (Köster and Pienitz 2006) particularly factors determining soil moisture content (Foets et al. 2020b). These variations are dominant particularly in agricultural fields (Foets et al. 2020a) giving an insight into their seasonal patterns (Antonelli et al. 2017). It was observed that the diatom assemblage growth lacked any significant variation (Cantonati 1998) in forests and grasslands (Foets et al. 2020b).

In spite the fact that terrestrial diatoms hold a promising future in the field, many species remain ecologically unexplored leading to discrepancies. For instance, *Caloneis lancettula* was found abundantly in the samples from farmland and grassland even though classified as a very sensitive species, suggesting a probable adaptation of the species to grow on a disturbed land. Similarly, *Stauroneis parathermicola* lacked ecological data (Antonelli et al. 2017).

8.5 Conclusion

Terrestrial ecosystems are distressed globally reckoning to the detrimental impact of anthropogenic activities bringing about ecological instability and threat to extinction of organisms in near future residing in it, triggered by the global climate change. For the integrated evaluation and renewal of these ecosystems, biomonitoring techniques transformed to be an imperative tool.

Recently, soils have been surveyed for its ecological status chiefly owing to their biological, physical, and chemical variables. The result of current study suggested the possible link connecting these physical and chemical variables (i.e., soil bulk, soil density, organic matter, soil pH, moisture, land use, total C and N soil content, sulfate, phosphate) to the diatom species composition. The extent of diatom species abundance is found mainly dependent on the soil texture and moisture content. Lately, a few efforts have been made to define the autecology and implement water quality index (IPS) to assess the terrestrial diatoms. Nevertheless, there is an immediate need for the ecology exploration of these terrestrial diatoms and developing soil diatom indices.

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Chapter 9
Role of Diatoms in Forensics: A Molecular Approach

S. K. Pal, Nitika Bhardwaj, and A. S. Ahluwalia

Abstract Diatoms are the small, autotropic, eukaryotic organisms found abundantly everywhere in nature. They are popularly called jewels of the sea, due to the beautiful ornamentations present on their frustule wall. They are regarded as golden standards in solving drowning-related crimes in the area of forensic science. Diatoms act as a supportive tool in deciphering the case investigations, due to the fact that their small size and diverse nature in the environment make their entry inside the body of drowned victim easy. While conducting autopsy of a drowned victim, the presence of diatoms in the lungs and other distant organs like the brain, liver, and kidneys as well as in femur reflects light on the cause of death. The presence of diatoms in various tissues of drowned victim reveals that the case is of antemortem drowning, whereas, in postmortem immersion cases, negligible number of diatoms will be present in the body of the victim. Various methods have been proposed by many scientists for the digestion of inorganic and organic material of diatoms for their identification purpose. However, with the advancement in research, molecular approach became more reliable and specific in the world of forensic limnology. In this chapter, we have discussed the efficiency of molecular tool; this approach helps in the making of database which in future will help the taxonomists to preserve the species and generate a record for study. Diatoms with the help of DNA barcoding can successfully help in solving drowning-related crimes with more accuracy and low chance of contamination.

Keywords Diatoms · Antemortem drowning · Postmortem immersion · Forensic limnology · Molecular approach

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P. Srivastava et al. (eds.), *Insights into the World of Diatoms: From Essentials to Applications*, Plant Life and Environment Dynamics,
https://doi.org/10.1007/978-981-19-5920-2_9

9.1 Introduction

Diatoms (class Bacillariophyceae) are unicellular, photosynthetic eukaryotic microscopic algae widely present in every aquatic habitat. They are universally found in almost every moist substrate including soil, rocks, and aquatic plants either in the form of single cell or in colonies and sometimes as pseudo filaments (Bhardwaj et al. 2021). The colonies of the diatom cells are linked together with the help of mucus pads. In some circumstances, the mucus covers the entire diatom cell which gives them appearance of small seaweeds (Hendey 1973). Ehrenberg was the first scientist to study about diatoms in India (Gandhi 1957). Diatom cell is composed of silica (SiO_2) cell wall popularly known as frustule which has soap box-like appearance. They are comprised of chlorophyll a, c_1, and c_2 along with the carotenoid called fucoxanthin (Lee 2018). This fucoxanthin is responsible for communicating golden yellow color to the diatom cell; thus, they are also popularly known as golden brown algae. The presence of hard siliceous frustule in a diatom cell is the key identification feature of the class Bacillariophyceae. It comprises of beautiful geometric ornamentations which vary from cell to cell, thus helping in distinguishing particular type of diatom taxa. This ornamented frustule of the microalgae is not as stiff as calcite; rather, it is slightly flexible due to which they can undergo slight amount of deformation. The silica cell wall of diatoms has certain types of nanostructural impregnations such as areola, spikes, pores, stigma, and channels imparting unique patterns to the cellular surface (Almqvist et al. 2001).

Diatoms are capable of growing in various types of habitats such as freshwater, saltwater, terrestrial, damp places, ice, moist soil, etc. Generally, they are present as free-floating microscopic bodies in aquatic habitat, but some of them are attached to moist substrates like rocks with the help of stalks (Karthick et al. 2013). These microscopic algal species play huge role in the detection of water quality and paleoenvironmental reconstruction as their growth is very specific to certain parameters of their habitat such as pH, TDS, conductivity, temperature, light period, and other required nutrient availability. Studies conducted on diatom assemblages along with their species and relative abundance helps to denote the quality of water making them as an important indicator of environmental health (Round 1981; Reynolds 2006). They are regarded as sensitive agents toward organic matter, toxicants, and heavy metal contamination present in water bodies (Blanco and Bécares 2010; Morin et al. 2016). Due to this supreme quality, they are highly recommended as biomonitoring tool for the assessment of aquatic water bodies (Stevenson et al. 2012).

They show very close association with heterotrophic bacteria which mainly includes Proteobacteria, Bacteroidetes, and Actinobacteria (Gautam et al. 2017). Whenever a diatom cell dies, there frustule gets settled in the environment which is popularly termed as diatomaceous earth or diatomite (LeBeau and Robert 2003). There are more than 200,000 species of diatoms present in the environment making them diverse group of microorganisms (Saxena et al. 2022). Each diatom cell is composed of unique three-dimensional surface characteristic; there structure varies from rod-like, hexagonal, or circular as per the type of species (Amato 2010). Due to their diverse abundance, these microorganisms are responsible for 40% total marine primary productivity. Reproduction in diatoms occurs commonly by cell division; i.e., they undergo asexual reproduction. Cell division occurs mitotically along with transverse to the longitudinal axis of the individual cell. They are also capable of sexual reproduction by forming auxospores (Cupp 1943). Their population show increase in spring and autumn season as compared to winter and summer season (Trent 2004). The most common types of diatoms are pennate and centric. The centric diatoms are radially symmetrical, whereas pennate are elongated and bilaterally symmetrical in shape (Uthappa et al. 2018).

Diatoms play important role in global CO_2 fixation, making them suitable candidates in solving the problem of global warming (Aoyagi and Omokawa 1992; Chisti 2007). The outcome of the studies conducted by various researchers threw light on the relativity of diatom diversity residing in polar regions with severe cold condition in denoting global climatic changes undergoing in such regions (Bopp et al. 2005; Alvain et al. 2013). According to a report of geological survey, they are considered to produce 30% of gasoline which reflects their tendency for the production of biofuel also (Ramachandra et al. 2009). Their average tendency to form lipids in diatoms is more than any other microalgal species such as cyanobacteria, Ochrophyta, Chlorophyta, and other classes (Griffiths and Harrison 2009). According to the study conducted by Das et al. (2015), these microorganisms also serve as an important alternative source of aquaculture water remediation. Moreover, these microlagal species have been center of attraction for research in various fields of producing various forms of sustainable products like fine chemicals, medicinal drugs, biofuels, plastic, and food products (Lebeau and Robert 2003; Bozarth et al. 2009).

Apart from all the applications of diatoms in several fields like biotechnology, industrial, cosmetology, and medicinal, they have also shown their importance in solving drowning-related crimes in forensics. A branch named forensic limnology deals with deciphering of drowning investigations with the help of microalgae, i.e., diatoms. Their small size, siliceous cell wall, and wide abundance in and around waterbodies make them suitable evidence in forensic pathology (Pal et al. 2017).

9.2 Framework of Diatom Cell

Individual diatom cell has soap box-like structure; as discussed above, the outer wall is made up of silica known as frustule. This hard wall is resistant to environmental and chemical changes undergoing around it. The frustule has upper part and lower part called epitheca (epivalve/upper valve/mantle) and hypotheca (hypovalve/lower valve), respectively. These valves are interconnected with each other with the help of connecting bands called girdle bands. The upper valve is slightly larger than the lower, making their cell structure cell look like a box and lid. The frustule of diatom cell has beautiful three-dimensional patterns on it which are useful for the

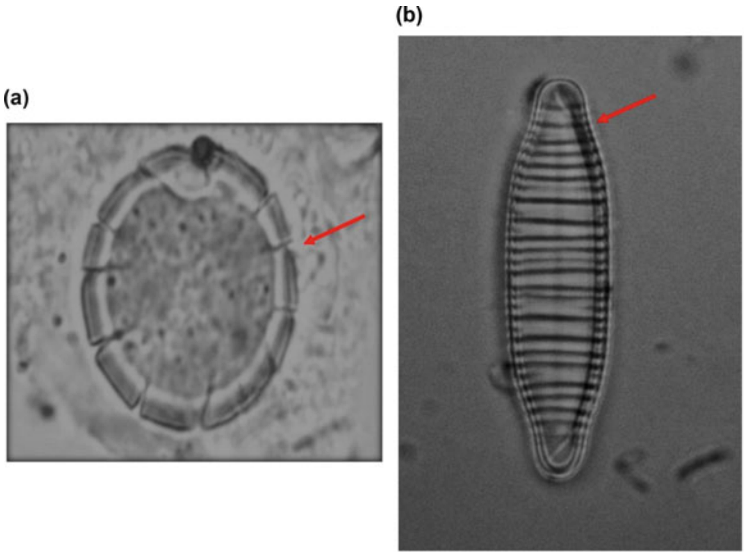

Fig. 9.1 Types of diatoms on the basis of their symmetry: (**a**) centric diatom, (**b**) pennate diatom

classification and identification of a particular diatom species (McLaughlin 2012). The process of biogenesis of both the valves and girdle bands are associated with the cell cycle. The valves are fabricated at the time of cell division, whereas the girdle bands are produced at the time of interphase. Both the valve and girdle bands are produced separately in a compartment known as silica deposition valves (SDV). Once their formation is complete, they get secreted out the cell through exocytosis and get accumulated on the cell surface at their respective positions (Heintze et al. 2020). On the basis of their symmetry, they are further classified as centric and pennate diatoms (Fig. 9.1). Pennate diatoms are bilateral in symmetry and have central spine-like structure called raphe which helps in their slight locomotion. Those pennate diatoms which contain raphe are termed as raphid diatoms. However, centric diatoms are radially symmetrical and lack the feature of raphe. Therefore, centric diatoms along with some pennate diatoms without raphe are called araphid diatoms (Williams and Kociolek 2007; Bhardwaj et al. 2021). According to reports, centric diatoms are abundantly found in marine water (Harwood and Nikolaev 1995).

9.3 Diatoms in Forensic

In the fields of forensic science, drowning-related crimes are very common and hard to solve. Forensic pathologist from the past had suffered a lot of problems as there are less or no relevant evidence to decipher drowning mysteries. Drowning is defined as respiratory failure of a person who accidently or forcibly gets submerged in liquid medium, commonly water. According to the recent reports of World Health Organization (2021), drowning is considered as third primary cause of deaths worldwide. Globally, about 236,000 deaths are occurring annually due to drowning. The diagnosis of cause and manner of death in such cases become difficult due to lack of evidence, or sometimes, due to prolonged overstay in water, the body gets putrefied. Forensic pathologists have to struggle in detecting the mode of death of victims recovered from water body. Experts in this field have to unravel all the possible circumstances to know the manner of death whether the case is of accidental or suicidal drowning or the body was dumped in water after homicide. It is not necessary that drowning only occurs in deep water bodies, but it can be possible in 5 cm–6 cm of fluid also. However, in such circumstances, other factors, such as narcotic influence, alcohol intoxication, epilepsy, head injury, cardiac arrest, etc., have to be considered carefully (Krstic et al. 2002). A special branch called forensic limnology has gained attention which comprises of application of microalgae especially diatoms in solving crimes related to drowning (Piette and Els 2006; Farrugia and Ludes 2011; Munro and Munro 2013). Diatom test is based on the principle that when a person is drowned in water body, he or she has the urge to respire; during this time of struggle, diatoms along with some other debris present in aquatic media enter inside the body of victims through body openings. The diatoms reach the lungs through respiration and get transported to several other organs through systemic circulation. These diatoms get penetrated in various organs such as the lungs, heart, spleen, kidneys, etc. However, their presence has been detected in distant organs like the brain and femur as well as in bone marrows. However, in cases where the victim was in an already dead condition and somehow his body was recovered from the water, the body will have a negligible number of diatoms. This implies the cause and site of death other than drowning and thus helps in deciphering the mystery (Krstic et al. 2002). The hard wall of diatom is resistant to the changing climatic conditions and chemical resistant and also remains intact in case of putrefaction. This makes them suitable candidates for corroborative evidence in drowning cases.

9.4 History of Diatoms in Forensic Science

Guy (1861) was the first scientist who elucidated about the entrance of aquatic debris inside the body of a drowned victim. In 1896, Hofmann was one of the scientists to detect diatoms from the lung fluid. Revenstorf (1904) solved the drowning mystery by using diatoms as corroborative evidence. Incze (1942) concluded the presence of diatoms in blood and parenchymatous organs and denoted that these microorganisms can enter travel to distant organs via the lungs. Thereafter, Tamaska (1949) showed the existence of diatoms in the bone marrow of the drowned victims as a sign of sure shot drowning. Thomas et al. (1961) suggested that diatoms act as reliable evidence to detect that victim has undergone a couple of breaths during submersion. Porawski (1966) denoted that these small microalgal species if present in the body organs or bone marrow of drowned victim reflect the cause of death to be antemortem drowning. Timperman (1972) described the fate of diatoms inside the body of victim in a way that, whenever a person drowns inside the water body, water along with its contents including diatoms enters inside the lungs. Once the lung cavity gets congested with the water media, it starts imparting pressure on the alveolar walls. This leads to rupturing of peripheral alveoli and thus water and other contents pass into blood stream. Diatoms are one of the main components of water which due to their high abundance and small size enters and gets settled in distant organ till the person stops respiring. Those diatoms which are able to creep into the organs of drowned victims are called diatom-associated drowning (DAD). Numerous scientists conducted several experiments on various organs such as the lungs, liver, sternum, femur, kidney, stomach, and brain (Matsumoto and Fukui 1993; Pachar and Cameron 1993; Taylor, 1994; Pollanen et al. 1997; Ludes et al. 1999; Hürlimann et al. 2000), and their results supported the utility of diatoms in solving such cases. A criterion of concordance has been set for the applicability and validity of diatom test. This rule states that a significant number of diatoms must be present while preforming diatom test before coming to any final conclusion (Pollanen 1998a, 1998b). Sidari et al. (1999) and Ludes et al. (1999) proposed that either 20 diatoms or 5 complete diatom frustules must be present per 100 l of pellet recovered from 10 g of sample from the body of drowned victim. Moreover, in case of diatoms present in the body of non-drowned victim or in post-mortem immersion cases, their quantity may be detected in the lungs but not in distant organs (Krstic et al. 2002; Kakizaki et al. 2018; Lunetta et al. 2013; Bortolotti et al. 2011). Overall, the quantitative (diatom density), qualitative (species), and morphological study of diatoms is a useful tool for making them supporting tool in drowning cases.

9.5 Why Only Diatoms as Supportive Evidence in Forensics

Earth's surface is full of microorganisms, but, apart from all of them, only diatoms are considered best supportive evidence by forensic experts in deciphering drowning cases. This is due to the major following reasons:

- There are diverse group of species with small size range which makes their entry inside the body organs feasible.
- The hard silica cell wall is resistant to the chemical changes while performing test in laboratory which helps in recovering of intact structure of diatoms from the respective body organ of drowned victim.
- In case of putrefied bodies or sometimes due to prolonged overstay in the water, many organs get blended, and only the bones are left behind. In such circumstances also, diatoms can be recovered and help in solving the case mystery.
- Their growth corresponds to certain specific parameters of the environment. Thus, by qualitative and quantitative analysis of diatoms from the body of

drowned victim and putative drowning site, the cause and site of death can be concluded successfully.
- A wide range of study has been done on the taxonomy of diatoms due to which these species are easy to identify and study.

9.6 Recovery of Diatoms from Postmortem Samples

While solving drowning cases, samples from the body of the deceased, most commonly soft tissues such as lungs, liver, heart, etc. or hard bones like femur and sternum, are sent for diatom test. These biological samples are analyzed in the laboratory, and various tests are performed for the recovery of the diatoms. For denoting the site of crime, water sample from the suspected drowning media is also used as reference material for the comparative study of diatoms. Several methodologies have been proposed for the efficient recovery of diatom frustule from the samples.

9.6.1 Acid/Chemical Digestion Method

The most common and oldest form of method is acid digestion or chemical digestion of samples. This method has been used globally and has proven successful in recovering intact diatom frustules from the samples (Singh et al., 2006). In this method, different strong chemicals, namely, nitric acid (HNO_3), hydrochloric acid (HCl), and hydrogen peroxide (H_2O_2) or (H_2SO_4) are used to digest the organic material present in the diatoms. This digestion helps in making the frustule view more elaborative for morphological study. Auer and Möttönen (1988) performed the study on 107 drowned victims. Thin strips of tissues, namely, lungs, liver, and kidney, along with the water sample were separated in a flask and allowed to boil by using distilled water. 10 ml of HNO_3 with 30% H_2O_2 was added to the samples, and the sample acid mixture was boiled carefully. Once the boiling was completed, this mixture was allowed to cool and washed with distilled water after frequent centrifugations at 3000 rpm. The final sediment was then analyzed microscopically. Ludes et al. (1994) conducted acid digestion test on 12 dead bodies in putrefied state. The putative drowning water sample and other soft tissues including lungs, liver, and kidneys were treated with HNO_3. This sample acid mixture was centrifuged at 2000–2500 rpm. After the completion of subsequent washings, the endmost residue was then used for microscopic observation. Pollanen (1998a, 1998b) solved cases of six homicidal drowning. The chemical digestion was performed by using HNO_3 (50 ml) on the bone marrow and femur (50 g) of the drowned victims in a clean flask. This mixture was then boiled for 48 h and then kept undisturbed for cooling. Again, washing of this mixture was initiated in Oporto River with the help of repeated centrifugations by using distilled water. The final pellet was then observed under phase contrast microscope. Gruspier and Pollanen (2000) solved the case mystery of five amputated legs recovered from the Lake Ontario, Lake Erie, and Niagara River. The digestion method was applied in the same manner as discussed above (Pollanen 1998a, 1998b). Krstic et al. (2002) executed chemical digestion by using H_2O_2 and H_2SO_4 along with saturated solution of permanganate on the tissue samples of laboratory rats, drowned corpse, and control samples to analyze the validity and utility of diatom test. Ago et al. (2011) studied nine cases of victims died in bathwater and one due to ischemic heart diseases in bathwater. The samples recovered from the body if the deceased was subjected to 10% formalin diluted with distilled water and kept undisturbed for few days. Thin sectioning of the tissues was done and washed with distilled water. Again, the samples were digested with H_2SO_4 and HNO_3, and qualitative and quantitative analyses of the diatoms recovered were analyzed under the microscope. Magrey and Raj (2014) suggested acid digestion as most relevant method to remove inorganic or organic material present inside the diatom cell. They solved 31 drowning cases happened in Jammu and Kashmir, India. They treated biological samples (sternum, femur, clavicle, and lungs) along with the suspected water sample with HNO_3 for extraction of diatoms. The cases were successfully solved with outcome of diatom test. Lin et al. (2014) performed acid digestion to solve 100 drowning cases. Diatoms were successfully recovered from the fluid of sphenoid sinus and lower lobe of the lungs. They used H_2SO_4 and HNO_3: HCl in the ratio of 1:3 for sphenoid fluid and lower lobe of lungs, respectively. Coelho et al. (2016) studied drowning deaths that happened in Oporto River from November 2012 to March 2014. The water sample was treated with 96% (W/W) of 20 ml of H_2SO_4 and tissue samples with 37% (W/W) of 20 ml HCl. The last residue left after repeated washing and centrifugations were place on clean slide and observed under microscope. Apart from utilizing acid digestion technique in biological samples recovered from humans, this method also became popular in extracting diatoms in veterinary context. For instance, Xu et al. (2011) conducted chemical digestion with HNO_3 on body organs (lungs, liver, kidneys, and bone marrow) of 56 rats. The experiment was performed by submerging rats in water to mimic drowning. The outcome of the test remained successful in denoting the manner and site of drowning by comparative study of diatoms recovered. Fucci et al. (2017) threw light on the recovery of diatoms from the biological organs of 10 different wildlife animals and putative drowning site. Also, with the advancement of research in forensic limnology, many modifications were made in this technique and its applications. Scott et al. (2019) conducted experiment to extract diatoms from nine different types of natural and synthetic clothing types. Wang et al. (2015) executed experiment on 20 minced kidneys which were thoroughly mixed with water-rich in diatoms. An improved version of acid digestion technique was suggested by Pal et al. (2021). They conducted a study on lungs and bone marrow extracted from the bodies of 66 victims died due to drowning. They ran a comparative test by using reverse aqua regia solution and traditional acid digestion method. In reverse aqua regia, HNO_3: HCl in the ratio of 3:1 was added to the biological samples, and the mixture was heated at 60–70 °C for 2 h, whereas in other method 50 ml HNO_3 was poured in sample and allowed to heat for 48 h at 60–70 °C on a hot plate. The comparative study of diatoms recovered by using both techniques showed reverse aqua regia to be more efficient. This technique proved time saving more conventional and efficient in high yield of diatoms with clearer and intact morphological features.

9.6.2 Limitations of Acid Digestion Technique

Being one of the most adopted methodologies, there were various limitations for the application of chemical digestion. For instance, working with strong acids such as HCl, HNO_3, and H_2SO_4 can lead to injuries or burns which can cause health hazards. Prolonged overstay of samples in such strong acid during the experiment can lead to destruction of diatom valves and thus can lead to false investigation. Additionally, repeated centrifugations at high rpm can also lead to contamination and loss of diatoms (Bhardwaj et al. 2021). In spite of all these drawbacks, this chemical digestion process with slight modification can give successful results suggested by Pal et al. (2021). Also, the chance of contamination can be avoided by using double distilled water during the washing of acid sample mixture and using clean and sterilized required equipment (Pollanen 1998a, 1998b).

9.6.3 Enzymatic Methods

Many scientists conducted extraction of diatoms by enzymatic methods for forensic consideration (Ludes et al. 1994; Kakizaki and Yukawa 2015; Kakizaki et al. 2018). Ludes et al. (1994) conducted diatom analysis on 12 corpses recovered from several water bodies of France. The various tissues retrieved from the body organs of the deceased victims were treated with 500 μl of proteinase K (10 mg/ml), 100 ml of 0.01Mof Tris-HCl, buffer solution (pH 7.5), and 2% of SDS. This mixture was incubated at 50 °C and left undisturbed. To this sample mixture the volume of solution was diluted by using 100 ml with distilled water. Then, centrifugations were performed at 3000 rpm for 15 min. The final residue was analyzed microscopically. Kakizaki and Yukawa (2015) performed an experiment on 20 lung samples recovered from 10 drowned victims. The digestion of the sample was done by using Qiagen Proteinase K, Qiagen Buffer ATL, and 5 NHCl. The tissue sample was minced properly and placed in polymethylpentene centrifuge tubes which consists of 6 ml buffer ATL and 1 ml of Qiagen Proteinase K. To check cross-contamination, negative control of 1 ml ultrapure water sample was used. Digestion of the sample mixture was done at 56 °C for 15–16 min, and then the sample was allowed to centrifuge. The final residue left was mixed with 13 ml of 5NHCl and heated at 75 °C. Once again, washing of the sample residue was done after centrifugation. At last ethanol was added to the final recovered residue, and further diatom analysis was done. Kakizaki et al. (2018) recovered diatoms from the body of 80 corpses retrieved from various aquatic sites. The enzyme used for this experiment was papain extricated from *Carica papaya*. The outcome of the digestion method was found to be efficient at 50 °C for 1 h at the concentration of 0.5 mg/ml. With the help of this enzymatic method, the authors were successful in solving drowning cases of 80 victims. Enzymatic method was found out to be better than chemical digestion, but its major drawback was that it was costly.

9.6.4 Microwave Digestion, Vacuum Filtration, and Scanning Electron Microscope

Hu et al. (2013) conducted a study by merging microwave digestion and vacuum filtration preceded by scanning electron microscopy. For the experiment, 20 ml water along with 2 g of thin strips of lungs, liver, and kidneys were digested in microwave MW000 with the addition of 6 ml of HNO_3 and 2 ml of H_2O_2 to the samples. The sample acid mixture was allowed to liquefy by rising the power for 5–10 min. This fluid was further subjected to vacuum filtration, and the results were automatically scanned by SEM. As compared to previously discussed methods of digestion, this method proved more efficient, rapid, and safer and has low chance of contamination. Zhao et al. (2017) solved the case mysteries of 128 drowned victims with the help of this method. From the comparative study of diatoms recovered from tissue samples and suspected drowning site's media, the case was successfully solved. Also, there findings threw light on the fact that, in case of antemortem drowning cases, the possibility of having diatoms in the lungs is 100% whereas 97% in other organs.

9.6.5 Soluene 350 Digestion

Matsumoto and Fukui (1993) performed an experiment which mimics the case of drowning. They took 5 g of various tissue samples from the rats and mixed them thoroughly with the water enriched in diatoms. The sample was allowed to centrifuge at 3000 rpm for few minutes. On the completion of centrifugation process, the pellet of the residue formed was transferred to 30 ml glass tube followed by the addition of Soluene 350. This sample mixture solution was set down in ultrasonic cleaner with full exposure to ultrasonic waves. To this sample solution, centrifugation at 3000 rpm for 5 min was initiated. The outcomes of the experiment conducted were better from the earlier traditional methods. Yoshimura et al. (1995) conducted study on the two victims whose bodies were recovered from the Yodo River, Japan. Thin sectioning of lungs, liver, and kidneys from the body of victims were taken for diatom test along with the water sample from the abovementioned river. Centrifugation of the sample was done by using Milli-Q water in order to avoid chance of contamination. The supernatant was discarded after the centrifugation was complete, and the pellet left behind was incubated after the addition of Soluene 350 into it. The incubation of the sample solution was kept undisturbed overnight. Further after the incubation of the sample was complete, it was again centrifuged for 60 min. The final residue was observed under microscope, and the analysis of the diatoms recovered helped to solve the caseworks of drowned victims. Sidari et al. (1999) reported that this technique works better on freshwater samples as compared to seawater due to the reason that the diatoms recovered from seawater has less amount of silica as compared to those recovered from freshwater sources.

9.7 Preparation of Diatom Slides

The diatoms recovered after the digestion process were used for the qualitative, quantitative, and morphological study. The residue left after final centrifugation was placed on clean glass slides. The permanent slide of the sample was made by mounting the material with high refractive index mountant such as Naphrax (~1.74), DPX (~1.520), or Euparol (~1.483), and then coverslip is placed over the sample, and the slide was allowed to dry in order to fix the sample. After fixation of the sample, permanent slides are ready to be observed under the microscope.

9.8 Molecular Approach in Forensics

Diatoms are microorganisms diversely present worldwide. The correct identification and classification of these microalgal species is a laborious task for the experts beyond species level due to their multiple morphological patterns within a population. The frustule of diatoms has very minute changes in ornamentations which makes them different from other species. In the fields of taxonomy, the proper identification of a particular microorganism is an important task to achieve. Thus, many scientists have supported the application of molecular biology in determining the type of diatom to species levels which helps forensic experts who deals with solving drowning-related crimes via diatoms (Babanazarova et al. 2010). In forensics also, molecular techniques have been gaining vast popularity over traditional methods and are widely adopted in research also with new innovations and successful outcomes (He et al. 2008; Kakizaki et al. 2012; Kermarrec et al. 2013; Kakizaki et al. 2018). The fundamental and most important aspect of molecular approach is that they only detect live cells of diatom having DNA present in the body organs of drowned victims. They are unable to detect those diatoms which lack DNA and usually found during cross-contamination. However, apart from molecular approach, none other method used in this field, especially microscopic techniques, can distinguish whether the diatom has been recovered from the live cell or came from any sort of cross-contamination. This can have direct effect on misleading of case investigation. DNA barcoding is the most suitable approach for the identification of diatom by using genetic sequence (Ratnasingham and Hebert 2007). It stands on the principle of standardizing short sequence of DNA which can be recovered and specified as distinctive identification marker for all the species on earth (Hebert et al. 2003). Hebert et al. (2003, 2004) was the first to formulate the term *barcoding* to assist identification. Various types of genes have been suggested and studied for the barcoding of diatoms like ribosomal RNA (16 s, 18 s), RUBISCO (ribulose1,5-bisphophate), cytochrome oxidase subunit1 (COI), silicon transporter (SIT), and ribosomal internal transcribed spacer (ITS) (Evans et al. 2007; MacGillivary and Kaczmarska 2011; Zimmermann et al. 2011). Hamsher et al. (2011) according to their performed study stated *rbcl* to be the best suitable gene for identifying different diatom genera. They also reported COI-5P to be the best gene locus for differentiation of red and brown algae. Pollanen (1998a) suggested another important target for the amplification, i.e., frustulin protein. This protein is found in the hard silica cell wall called frustule. It has no role in wall formation and is just a constituent of the frustule (Bowler et al. 2008). A relevant DNA barcode has must be short in order to get easily amplified. It should be flanked by conserved regions to make suitable universal primers. Moreover, it must contain the ability to identify diatoms to species level. To identify diatoms present in the human tissue with molecular approach, only those diatom-specific genes have to be amplified to get the correct results. A relevant reference database of diatoms helps in the screening of barcodes of diatoms. Quast et al. (2013) reported SILVA ribosomal RNA as the most substantial database which consists of 16S, 18S, and SSU sequences. Moreover, 16SrDNA helps to easily differentiate between human (eukaryotic 18SrDNA) and plant genome, thus helping in denoting the case study to be of antemortem or postmortem drowning (He et al. 2008). The 18SRNA gene has been broadly used as a barcoding marker. Zhao et al. (2017) used DNA barcodes from 18SrDNA at V7 region for differentiating 9 diatom species. The molecular approach of diatoms simply deals with designing of relevant primers, DNA extraction from the cells, PCR amplification, and sequencing. The primer for every species should be made carefully by focusing on conserved and hypervariable regions. The DNA extraction from the cell can be achieved by various methodologies such as 2 × CTAB (cetyltrimethylammonium bromide) method (Doyle 1990; Kumar et al. 2016). The PCR needs 10 μl of solution which consists of 5 μl of PCR reagent, 0.1 μl of suitable primer, 20 ng of DNA, and double distilled water. A standard condition for the initiation of PCR has to be made which includes various cycles of different temperatures and variable durations, such as 94 °C for 10 min then 28 cycles of 30 s at 94 °C and then again 30 s at 60 °C and 30 s at 72 °C followed by final cycle of 10 min at 72 °C. For electrophoresis technique, 2% agarose gel has to be used (Li et al. 2019). Once the final products from PCR have been made and recovered, they are sent to Sanger sequencing for further analysis. However, the PCR technique varies according to the gene of interest and categories of primers (He et al. 2008). Other molecular techniques like FISH (fluorescent in situ hybridization) (Ishii et al. 2004), qPCR (Ahlgren and Rocap 2012), etc. have been used to trace the footprints of known taxa. DNA barcoding has been regarded as an outstanding tool because of vast advantages. Some of them have been listed as follows:

- This method is time saving and more specific in their results.
- They promote recognition of new species.
- They are appropriate tool for large campaign such as Craig Venter's Global Ocean Sampling (Rusch et al. 2007; Desalle 2006; Rach et al. 2008).
- They aid in identification of complex morphological organisms which are unable to be identified by any other methods.

9.9 Application DNA from Diatoms Via Molecular Biology

Several limitations of various techniques used for extraction of diatoms have been discussed earlier in this chapter. Molecular approach due to its rapid high specificity tends to become more popular and efficient in the fields of forensic limnology for deciphering drowning case mysteries. Kane et al. (1996) solved a drowning case that happened in Japan by tracking picoplankton belonging to cyanobacteria with the help of 16SrRNA locus.

He et al. (2008) imitated the scenario of antemortem drowning by conducting an experiment of submerging 12 rabbits in Donghu Lake (China). DNA was extracted from the biological tissues of the drowned rabbits, and molecular approach was applied, and it was concluded that, by 16SrDNA gene locus amplification at 487 bp, the presence of plankton was there. This led to denoting the cause of death to be antemortem drowning. However, in postmortem category of rabbits, few numbers of planktons were detected only in two rabbits. This formed a strong base to detect the cause of death by using molecular tool with least number of errors. A suspicious case of drowning of 39 year-old lady had occurred in a well. The manner and cause of death became laborious to detect. The autopsy reports as well as diatom test showed no positive findings of diatoms. However, with the application of molecular technology, the amplification of 16SrDNA was observed, and the case was found out to be of antemortem drowning which helped efficiently to solve the case mystery. Reports of Suto et al. (2009) concluded the presence of specific bacteria, such as *Streptococcus salivarius*, *Streptococcus sanguinis* found in the throat, and *Aeromonas hydrophila*, in water samples can help to decipher the drowning mysteries with the help of molecular approach. Rutty et al. (2015) solved 20 cases of drowning-related casework with the help of molecular technology.

Rácz et al. (2016) suggested that detection of diatoms by using molecular tools would only brace up the autopsy diagnosis. Li et al. (2019) conducted study on 10 water sites in Nanjing section of Yangtze River for forensic application. They identified diatoms to genus level with the help of both optical and electron microscope and used 18SrDNA sequencing for the detection of species. The 18SrDNA has been used widely due to some major advantages such as that they are residing in all eukaryotic organisms. They contain numerous copies per genome and are very expressive due to which the molecular study can be done at RNA level also. They are a mixture of conserved as well as variable nucleotide due to which they are suitable for phylogenetic reconstruction at various level of taxonomy (Vinayak and Gautam 2019).

Jiang et al. (2020) made a diatom array based on molecular approach and denoted 169 diatom species from a wide range of aquatic reservoirs of China. They also invented an auxiliary sample preparation method for the isolation of DNA which helps in identification of diatoms in the body tissues of drowned victims as well as in water samples. They conducted their study on samples taken from pure culture of various diatoms, tissues from six drowned victim's body, and eight from environmental water sites like lakes, rivers, or ponds. While extracting diatoms from the pure cell cultures, they generated a protocol based on the structural and composition diatom cell wall. The hard silica cell is resistant to all lysis agents in fact to guanidinium isothiocyanate, although they successfully removed the pectin and polysaccharide present in the composition of cell wall by 0.5× TE buffer. Once the cell wall was lysed, the DNA present inside the diatom cell gets exposed for further treatment. A 15 ml of diatom culture was centrifuged and then 1 ml of ddH_2O was added to the pellet. After the completion of this step, the left residue was placed in 1.5 ml microtube and again centrifuged for 3 min at 12000 rpm. Later, the supernatant was discarded, and 50 mL of 0.5× TE (5 mM Tris–HCl and 0.5 mM EDTA pH 8.0) was added to the residue and kept for 30 min in water bath at 90 °C. Then, this sample was kept in an ice bath for few minutes to cool. Only 5 μl of the supernatant was sufficient as genomic DNA template for PCR in order to amplify 18SDNA. For human tissues, samples placed at −80 °C were first allowed to defrost at room temperature. Then 6 g of guanidinium isothiocyanate was added to 10 g of tissue sample. This mixture was homogenized properly and then transferred to 15 ml centrifuges tube. Incubation of the sample mixture was initiated at 65 °C for 1 h. Subsequent centrifugations were performed at 3000–4000 rpm for few minutes. Then, 4 ml of (0.1 M Tris–HCl (pH 8.0) + 2% SDS + 1 mM CaCl2 + 0.1 M NaCl) was poured in the sample mixture, and again homogenization of the mixture was done. To this sample mixture, Proteinase K was added, and repeated centrifugations were initiated with the addition of ddH_2O to the pellet. Finally, the mixture was transferred to a 1.5 ml microtube and again centrifuged for 14,000 rpm for 3 min. 500 mL 0.5 M Tris–HCl (pH 8.0), 10 mL 50 mg/mL 20 nm acid washed silica particles, 10 mL 10 mg/mL RNase, 30 mL 1500 U/mL DNase were added to the residue and vortexed. This mixture was administered for digestion at 50 °C for 1.5 h. Again, centrifugation was performed at 14000 rom for 6 min, and then guanidinium isothiocyanate was added to the pellet. Repeated washing and centrifugation were done by using ddH_2O. The final residue was mixed with 25 mL of 1X TE and placed in water bath at 90 °C for 30 min in order to extract the genomic DNA. Once the extraction was completed, the DNA was subjected PCR. The cycle of PCR was initiated in two rounds for the genomic DNA recovered from the human tissues, whereas a single round was sufficient for the DNA recovered from cultures. The amplification was based on 18srDNA by using specific primers and stable PCR conditions for 30 cycles. For the detection of the desired amplified products, 1%gel electrophoresis was performed. The diatoms were identified on the bases of records present in NCBI database. The hypervariable regions of the diatom were detected and further used for making diatom arrays. Finally, diatom array produced were subjected to hybridization technique. Liu et al. (2020) studied casework of 23 victims; out of which, 19 were of known drowning cases in various water bodies.

9.10 Controversies in the Validation of Diatom Test in Forensics

Diatoms have been acting as key players to solve drowning-related crimes. However, a large extent of controversies was also there which were against their application and validity in forensics. We are familiar with the fact that the presence of diatoms in the body tissues of drowned victim makes the case of antemortem drowning. However, there have been many cases where the victim died due to other reason than antemortem drowning, but there were diatoms present in their body. This leads to several research studies which criticized the application of diatoms as supporting evidence in solving such crimes. Diatoms can be present already in the body via air inhaled or foodstuffs (Hendey 1980; Krstic et al. 2002; Gordon et al. 1988; Spitz and Fisher 1973; Yen and Jayaprakash 2007), and their presence will alter the results of diatom test during the autopsy of a victim, thus leading to false judgment. Hürlimann et al. (2000) postulated that the diatom density keeps on decreasing by 10–100 while entering from water to lungs and then 100–1000-folds while travelling to distant organs. Thus, the number of diatoms inside the victim's body depends upon their abundance in the aquatic habitat where drowning occurs. Langer et al. (1971) reported that the presence of diatoms inside the lungs of a person can be found if he smokes low-quality cigars. While smoking the diatoms, fragments of small size can penetrate inside the lungs easily. Lunetta et al. (2013) reported that diatoms can enter inside the body of non-drowned victims through wounds or injuries or via strong hydrostatic pressure of water. Lunetta et al. (2013) stated that diatoms can also occur in the body tissues of non-drowned victims due to contamination of equipment used while performing autopsy or diatom test. Apart from several controversies, this test is still in great use. The diatom test should be conducted by taking full measures in order to avoid contamination or cross-contamination. Moreover, while performing the autopsy, external and internal signs of drowning should be observed with full care and then related to the outcomes of study which will help to reach the appropriate judgment.

9.11 Conclusion

Diatoms act as golden standards for denoting the cause and site of death in drowning cases. From the past many years, these microalgal species remained very helpful for forensic pathologists in deciphering complex cases of drowning-related crimes, even though in such investigations where the only left evidence is the skeleton of the victim or sometimes the body recovered from water is in extreme putrefied stage. In such circumstance, application of diatoms in solving the crime remained successful. Due to their small size and hard silica cell wall, they get easily penetrated inside the body organs of a victim and settle down there till the person dies and all undergoing vital processes stops. The efficiency of diatom test depends upon quantity of diatoms, number of diatoms recovered from organs, and suspected drowning medium. Diatom tests are gold standard in drowning cases. Diatoms, on the other hand, cannot penetrate inside the body in already dead condition if thrown in water to alter the crime scene because there is no breathing or circulation due to which concentration of these species will remain nil in the distant organs. Certain parameters, such as contamination from various sources, the number of diatoms in the drowning media, the number of diatoms retrieved from organs, etc., have been found to influence the efficacy of a diatom test. Despite some above-discussed criticisms regarding their validity, diatom tests are commonly acknowledged as a useful tool in solving the mystery of drowning deaths. As a result, it is hard to argue that diatoms are not the gold standard in forensics. Under proper supervision, the diatom test has been shown to be accurate in the examination of drowning-related cases. DNA barcoding in analysis of diatoms remained a sensitive and specific reliable method to differentiate between diatom communities or fining new species. The nuclear, mitochondrial, as well as chloroplast genome act as strong genome markers in order to identify several diatoms present in water bodies and thus have direct effect on aquatic health also. However, DNA database do not contain sufficient DNA sequence to cover all diatom community. The focus should be on making plant or diatom specific PCR primers in order to maintain limited homology between human tissue and diatom cell. This technique not only helps to find out the cause of death in forensic drowning cases but also helps in setting up diatom database for reference, and a suitable diatom mapping can be generated. In future, diatom analysis data may benefit from the application of more advancement in molecular approaches such as PCR, diatom mapping, automatic diatom identification and classification (ADIAC), and artificial intelligence with neural networks. These techniques are more reliable and provide better results as compared to the old traditional methods.

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Chapter 10
Diatoms in Forensics: Adding New Dimension to the Growing Relevance of Diatoms in Improving Lives

Shalini Dhyani and Kavita Bramhanwade

Abstract Forensic science is the application of science which provides relevant evidence against crime. It is a broad field which comprises a diverse array of disciplines that includes DNA analysis, fingerprint analysis, etc. to solving a crime. The diagnosis of drowning is one of the most difficult tasks in forensic practices, and consequently an enormous number of tests have been carried out to allow a confirmation. However, still, it is difficult to distinguish a drowning from other deaths. Diatom testing is recently an important supporting method to determine the death by drowning. Diatoms are most common type of phytoplankton, helpful in relating suspects and fatalities to crime sight in and around water. This chapter provides brief information about the use of diatoms in solving drowning cases and some case studies related to this, forensic limnology, and the opportunities and importance of diatom test. This also discusses the challenges and constraints in diatom-supported forensic investigations.

Keywords Diatoms · Forensics · Drowning · Diatom test

10.1 Introduction

To resolve legal issues, forensic science employs biological, physical, and social sciences. Commerce, anthropology, dentistry, pathology, pharmacology, entomology, psychiatry, economics, engineering, and computer forensics have all been associated with the term forensics. To make sense of an occurrence and give investigative leads, forensic evidence is obtained, reviewed, appraised, interpreted, and presented (Maras and Miranda 2014). Forensic science is used in modern lawsuits at all phases of criminal, civil, and governmental cases. It provides law enforcement agencies with technical assistance and professional services to help

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P. Srivastava et al. (eds.), *Insights into the World of Diatoms: From Essentials to Applications*, Plant Life and Environment Dynamics,
https://doi.org/10.1007/978-981-19-5920-2_10

them determine case evidence, establish liability provenance, and apply the law properly, as well as relevant evidence using science and justice (He and Li 2022).

Drowning is defined by the World Health Organization (WHO) as "the process of experiencing respiratory disruption as a result of submersion/immersion in liquid." Drowning is one of the most challenging diagnoses in the realm of human forensic pathology to prove (Piegari et al. 2019).

It is frequently considered a "diagnostic of exclusion." Generally, the postmortem assessment of drowning is based on combining both the alterations induced by the kind of suffocation and those caused by thanatological changes caused by the length of time the corpse was submerged in water into one diagnosis. Due to advancements in forensic science technology, this approach has become obsolete (Marella et al. 2019). Records, including bodies, can be swept away; gathering forensics is difficult, and, because drowning is a typical occurrence, investigators may mistakenly believe that deaths are the result of an accident. They appear to be a simple instance of drowning at first glance, but an autopsy shows a different story. These types of crimes are on the rise in every corner of our country (Biswas et al. 2015). After ruling out all other probable possibilities for why the victim ended up in the water, such as drug overdoses and heart attacks, medical experts establish drowning as the cause of death. Prosecutors must then establish that the drowning was deliberate, which sometimes necessitates relying on circumstantial evidence. Because there is little research on the subject and local police statistics aren't usually well documented, it's difficult to tell how many killings involving drowning there are.[1] Environmental forensic evidence is frequently encountered during investigation process and is recognized for its ability to provide useful circumstantial information about the setting of a specific criminal incident. Although traditional research has focused on the investigation of terrestrial soil and sediment traces, the forensic evaluation of aquatic crime scenes, particularly those originating in freshwater environments, is growing rapidly (Scott et al. 2016). Regardless of their social standing, all such victims are entitled to justice (Biswas et al. 2015). Because of the crucial contributions of diverse forensic sciences to the consistency of legal outcomes, a thorough grasp of these applied fields and their potential dangers is required before using them. To reduce the potential of inaccuracy, forensic science departments use standard methodologies and protocols to ensure that forensic outputs are comparable, consistent, and traceable (He and Li 2022).

Diatoms not only contribute 20% to oxygen synthesis, but they also play a key role in solving drowning-related cases. Diatoms are particularly sensitive to changes in their surroundings, such as temperature and nutrition levels. As a result, the number of diatom species differs from one water source to the next. Diatoms can be found in a wide range of aquatic settings. Diatoms have a range of features, including wide occurrence in water, a great diversity of species, habitat specificity, and a high potential for preservation, making them suitable in forensic geosciences

[1] https://www.nbcnews.com/news/us-news/drowning-one-hardest-homicides-prove-these-investigators-want-change-n1011911.

(Rana and Manhas 2018). Diatoms have a number of characteristics that make them an excellent topic for forensic research. Because of their small size, they can easily be transferred from the crime scene by people or objects, and the offenders are unlikely to be aware of their presence. The tenacity of the silica-based cell wall permits them to survive in the human body even after decomposition has progressed to the point where death by pathological methods may be more difficult. Diatoms have distinct morphology that allows them to be differentiated from each other, and their frequency and variability result in highly varied assemblages of diatoms in bodies of water. While diatoms have typically been employed to diagnose death by drowning, new research is revealing their enormous potential for use as trace evidence in a variety of forensic cases.

Diatoms are photosynthetic, unicellular eukaryotic microorganisms that are often categorized as algae. They're mostly stationary. Around 15,000 species of diatoms have been identified, but many more are yet to be discovered. The siliceous coating of the cell, which is enclosed in a pair of silica valves, is what makes them unique. Because silica is practically inert and indestructible, the silica portions of the body survive death. The silica components aid in the identification of these various organisms (Levkov et al. 2017). Diatoms (*Bacillariophyceae*) are unicellular, photosynthetic, autotrophic creatures with a unique structure called frustules, which consists of two thecas or silica cell walls. Each frustule is divided into two pieces, known as valves, and one of those is significantly smaller than the other and fits inside the other. The diatoms are split into two primary orders based on the morphology of the frustule: Centrales and Pennales. The Centrales are radially symmetric, while the Pennales are symmetric in both directions. There are more over 200,000 species of diatoms. Diatoms are constructed of siliceous cell walls that can be extensively structured with a range of pores, ribs, minute spines, marginal ridges, and elevations that aid in the identification of genera and species.[2] Freshwater, marine water, and soil are the most common habitats for these tiny organisms. However, due to cleaning agents like chlorine, they can't thrive in home pools. They have a gelatinous covering that can be simple or branched. A frustule, which is made up of two valves connected at the girdle by a connective, surrounds all the diatoms.

Considering the relevance of the diatom in forensics, the present chapter focusses on this aspect of diatoms and discusses the applications of limnology in forensic science and attempts to provide an overview to the subject that is of significant interest for solving crimes in a smaller duration.

[2] https://www.forensicevents.com/blog-details/Diatoms-in-Forensic-Science/72.

10.2 Forensic Limnology

The applicability of biology to law enforcement is known as forensic biology. Forensic anthropology, forensic botany, forensic entomology, limnology, forensic serology, forensic odontology, wildlife forensics, and DNA forensics are all sub-disciplines of forensic anthropology. Forensic biology is being used to establish that an accused was present at a scene of the crime, to recognize illegal products derived from endangered species, to solve the case by correlating crime scene proof to accused persons, to examine airplane bird strikes, and to explore bird collisions with wind turbines.[3]

There's even a forensic profession dedicated to defining the legal significance of diatoms in forensic investigations. It's known as forensic limnology.[4]

> The exploration of freshwater ecology for the occurrence of diatoms (especially) to solve forensic and medical situations is known as forensic limnology.

The study of freshwater ecology in a legal context, particularly the study of diatoms, is referred to as forensic limnology. Diatoms are minute algae creatures that can be found in a variety of aquatic habitats, including both moving and stagnant water. Because of the richness and diversity of diatoms, their research can be quite useful in some legal cases.[5] In forensic pathology, establishing death by drowning is a complex endeavor. Due to submersion, they are microscopic enough to be taken up further into circulation via the lungs. Diatoms can be found in the lungs, kidneys, and bone marrow.[6] To confirm the cause of such deaths, a number of tests have been devised, with the diatom test emerging among the most significant. The test involves determining whether or not there are diatoms in the body getting examined.[7] Given the capacity to retrieve freshwater traces over long time scales, the retention of diatoms sticking to garments following lengthy exposure to fire suggests that research to gather any damaged evidence are valuable (Scott et al. 2016).

10.3 Diatom Test as a Cardinal Key to Solving Drowning Cases

For many years, the judicial system has used scientific techniques in its many proceedings. Some of these, such as the use of diatoms in forensic research, are naturally far less common, although diatom taxonomy and ecology play a vital role

[3] https://gifsa.ac.in/forensic-biology/.

[4] https://forensicreader.com/significance-of-diatoms-in-forensic-science/.

[5] https://aboutforensics.co.uk/forensic-limnology/.

[6] https://forensicyard.com/importance-of-diatoms-in-forensic-investigations/.

[7] https://indianexpress.com/article/explained/mansukh-hiran-death-case-diatom-test-explained-7234656/.

in some types of investigations. A diatomist may be able to supply evidence to investigators that will help the court reach a decision, and this evidence can be used by either the prosecutor or the defenses (Peabody and Cameron 2010). In a criminal investigation, forensic geoscience is concerned with the analysis of geological materials to compare and eliminate environmental samples from a known source, or to identify an unknown provenance. Diatom analysis is a tool in the forensic geoscience approach that has the ability to give an impartial ecological evaluation of uncovered evidence (Scott et al. 2014). The most common use of diatoms in forensic science is in the diagnosis of drowning deaths. Drowning is a relatively common form of death by accident, with thousands of people dying each year. The majority of these people die in noncontentious situations, such as when there are witnesses or strong evidence of self-harm, such as a note. When a body is still warm, a pathologist may have minimal trouble concluding that the victim drowned. The histological symptoms of drowning, on the other hand, are frequently ephemeral and obscured by the filthier effects of decomposition. When bodies were recovered from water bodies, investigating agencies have difficulty determining the specific reason of death in cases where the body has been putrefied/decomposed/skeletonized or has been moved to the location by floating along the tides of water (Vinayak 2010). It is simple for criminals to throw the dead body in water, and make it appear as if it drowned. In such instances, the only test that can be used to generate evidence of death from drowning or other causes is the diatom test. Furthermore, in circumstances where a subject has been seriously injured prior to being submerged in water, it is critical to ascertain whether death is the result of these injuries or drowning (Peabody and Cameron 2010). When a person drowns, diatoms and water enter the bloodstream and travel to distant organs such as the bone marrow. The biological samples besides the water sample from the location where the dead person was discovered are then transported to the laboratory, where diatom presence is investigated using phase contrast microscopy after acid digestion, centrifugation, and microscopy (Vinayak 2010). The basic principle of the diatom test in drowning is based on the assumption that diatoms are present in the environment where drowning occurred and that inhaled water enters the alveolar spaces of the lungs and transfer from the alveoli into the blood stream, transporting microscopic unicellular algae known as diatoms to different parts of the body (Fig. 10.1). The diatoms discovered within the body of a drowned person could provide additional or even decisive evidence to back up the death diagnosis. It is possible to determine if the drowning occurred antemortem or postmortem (Kaushik et al. 2017).

In clothing that has been in touch with soil and water, three diatom extraction procedures can be tested: washing in water (RW), washing in ethanol (RE), and immersion in H_2O_2 solution (H). To determine the extent of diatom retention on processed clothing samples, scanning electron microscopy (SEM) analysis is performed. To evaluate the efficacy of each approach in gathering a meaningful sample for analysis, the complete diatom yield and species richness statistics from each experimental sample are collected. Correspondence analysis is used to investigate similarity (Scott et al. 2014).

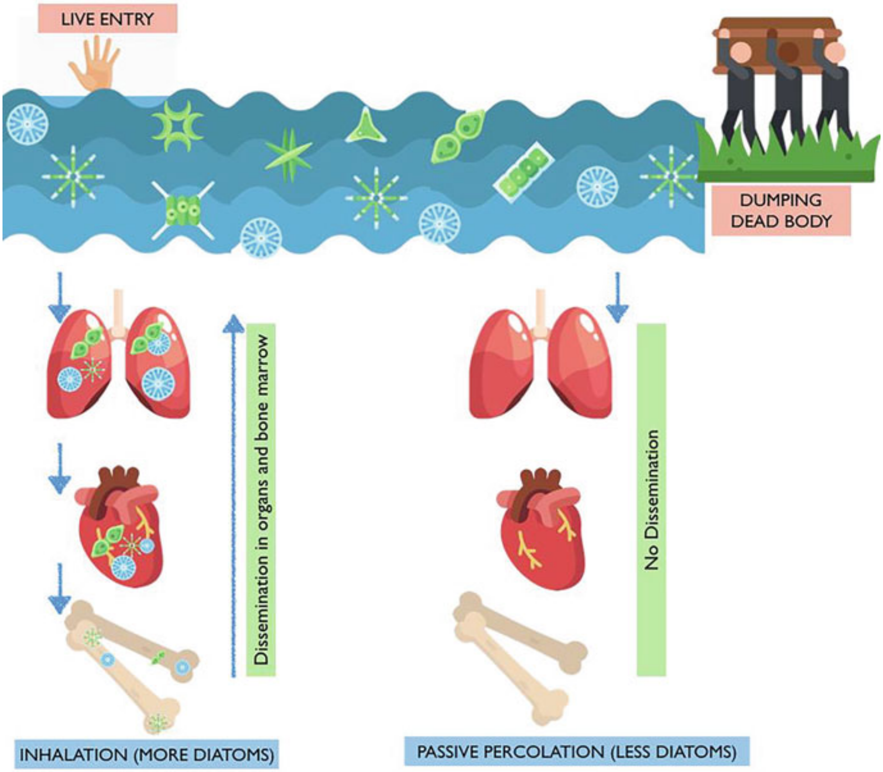

Fig. 10.1 Showing relevance of diatom test as a cardinal key to solving drowning cases

10.4 Opportunities and Importance of Diatom Test

Some of the relevant diatom applications in forensics for solving crimes and to reach appropriate decisions are as follows:

1. **Connecticut Case, 1991, USA:** While fishing at a suburban Connecticut pond in July 1991, two boys were savagely attacked by multiple teenage thugs. Sediment-encrusted sneakers were confiscated and examined for aquatic microbes in an attempt to link the suspects to the crime site. Diatoms and scaled chrysophytes (planktonic algae) were found in abundance. The algae populations on the sneakers were very similar, indicating exposure to a same freshwater ecosystem, most likely the crime scene pond. Additional analysis revealed that *Mallomonas caudata* was the dominating scaled chrysophyte species in each sample and that the ratios of three species of the diatom *Eunotia* did not differ significantly across all samples. These findings further reinforced the theory that all of the samples came from the same location and helped solving the case (Siver et al. 1994).

2. **Mansukh Hirani Case, 2021, Mumbai, India:** The Maharashtra (ATS) used a forensic test known called diatom testing to find leads in the suspected murder investigation of Mansukh Hirani in July 2021. Hirani's body was discovered with a fabric mask around his mouth and pieces of cloth shoved into his mouth, despite the fact that he could swim. While doctors at the Chhatrapati Shivaji hospital in Kalwa, who performed the post-mortem examination, declined to speculate on the cause of death, they did say the body had no visible injuries. At JJ Hospital's forensic laboratory, a diatom test was performed, but the results were inconclusive. Hirani was conscious when he was tossed in the creek, or he leapt on his own, according to ATS officials.
3. **Cold Case of DB Cooper's Money (1973–2019), USA**: The DB Cooper hijacking is the sole unsolved hijacking case of the USA. Diatoms can be found in any water body. Some diatom species, such as *Asterionella formosa*, have a wide seasonal variation in abundance; hence, diatoms can help to define the period of year when an object was immersed in water. Money in three bundles was discovered on the Columbia River in Portland 9 years after the crime. This burial place was some 30 km away from his claimed location; thus, there was no obvious reason for the money to have ended up there. Diatoms were discovered on a recovered bill, indicating that the money had been immersed before burial. The species mix on the bills was compared to a control group. The bills' species mix was matched to a test bill found immersed in the Columbia River in November, the month of the crime. The Cooper bill was found to also include diatoms from summer bloom species, indicating that the money was not buried dry and that the immersion took place months after the kidnapping in late November. This finding dismisses the majority of current interpretations about the crime and indicates that diatoms could be used to narrow seasonal dates in forensics (Kaye and Meltzer 2020).
4. **Death Due to Drowning in Water Tank, Haryana, India:** A 19-year-old boy's body was discovered drowned in a water tank. An autopsy revealed no signs of damage. Three types of diatom species were discovered in nitric acid extracts of internal organs (rib cage, collarbone, femur, and lungs) (*Navicula lanceolata*, *Navicula oblonga*, and *Gomphonema gracile*). The water sample from which the body was recovered contained a variety of diatom species. As a result, drowning was determined to be the cause of death (Malik et al. 2013).
5. **38 Human Drowning Cases, Haryana, India**: In the Forensic Science Laboratory at Karnal, 38 human drowning case studies of Haryana (India) were investigated and resolved via the "diatom test." Crime exhibits, as well as water samples from which the deceased individuals were found, were transferred to the laboratory after a medicolegal assessment. Exhibits were acid digested, centrifuged, and then examined under a microscope for the existence of diatoms, if any existed. The crime disclose (sternum, clavicle, liver, lungs, spleen, kidney, intestine, heart, and putrefied viscera) the identical diatom kinds as were present in water samples of the drowning sites of the corresponding instances, making 34 cases positive for the diatom test (Verma 2016).

6. **17 Drowning Cases, Himachal Pradesh, India:** The current research was carried out at the State Forensic Science Laboratory's Biology and Serology Division in Shimla Hills Junga, Himachal Pradesh, India. After a postmortem examination of the deceased individual, a water sample, in addition to hard bones (sternum, clavicle, femur), soft tissue (spleen, liver, kidney), and peritoneal/pleural cavity fluid, was sent to the research laboratory for diatom detection. Cases were filed and reviewed in accordance with established procedures. In this investigation, 17 drowning cases were investigated for the presence of diatoms, where 15 males and 2 females aged 11 to 63 years old were among the 17 cases. Diatoms were identified in 12 cases (drowning deaths), while 5 cases were discovered to be clear (death other than drowning) (Kaushik et al. 2017).

10.5 Challenges and Constraints in Diatom-Supported Forensics

Although it is a time-consuming as well as an arduous operation, diatom testing is now a key supporting tool for determining drowning deaths and drowning sites (Zhou et al. 2020), and that is clear from different case studies mentioned in the chapter. Although the discovery of diatoms in the organs is regarded as a significant "biological marker" for the identification of drowning in clinical pathophysiology, false-positive results are still a possibility. The principal critique of the diatom test for drowning identification is based on the possibility of ante- and postmortem diatom penetration, as well as the discovery of diatoms in non-drowned human bodies. However, qualitative and quantitative studies on diatoms in non-drowned tissues have produced paradoxical and confusing results (Lunetta et al. 2013). Original research from the 1970s, 1980s, and 1990s yielded mixed results when it came to the accuracy and consistency of diatom analysis. Modern research methodologies utilized as component of the diatom analytical technique have been capable of reducing false-positive results, improve the capacity to discern among true and false positives, and alleviate many of the flaws identified in previous studies. Piegari et al. (2019) supported with evidence that the diatom test is a useful tool in human as well as veterinary forensic pathology for confirming drowning diagnoses. The histological and anatomical pathology findings in drowning cases are less specific because they can be seen in a variety of causes other than drowning, but diatom density and location differed among drowned and non-drowned victims. Although having some weaknesses, such as diatom introduction into bodies prior to death and some details surrounding false-positive results, remain significant concerns, diatom analysis is a useful forensic tool, whose consistency has been reinforced by modern research and can be reliant upon to ascertain a confirmed diagnosis of death by drowning when coupled with current strengths, such as climatic variables and environmental precision (Lunetta et al. 2013).

10.6 Conclusion and Way Forward

The principal task of forensic investigation in drowning cases is to confirm whether the individual died due to drowning or not. Diatoms have been used in various types of investigations from several years but for forensic investigations far less common. Nowadays, the most common use of diatoms in forensic science is the diagnosis of drowning deaths. Using diatom test, it is possible to determine whether drowning occurred antemortem or postmortem.

Although some of the case studies reported successful use of diatom test in drowning investigations, it is a time-consuming as well as a laborious operation. During investigation, sometimes false-positive result is also possible.

However, a modern research methodology in diatom testing is capable of reducing false-positive results. It was proved very imperative by providing the actual cause of death and useful tool in human as well as veterinary forensic pathology for confirming drowning diagnosis. In future, addition of insightful knowledge will provide smarter results than present.

Acknowledgments Authors acknowledge Knowledge Resource Centre (KRC), CSIR-NEERI, Nagpur, India, for plagiarism check using licensed version of i-thenticate under the number.

Author Declaration Authors declare no conflict of interest in drafting or publishing this chapter.

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Chapter 11
Diatom Silica a Potential Tool as Biosensors and for Biomedical Field

Raunak Dhanker, Parul Singh, Drishti Sharma, Priyanka Tyagi, Mithlesh Kumar, Richa Singh, and Suraj Prakash

Abstract Brown algae, diatoms, have been the subject of extensive investigation in recent decades because they include active chemicals with a wide range of biological activities, including antibacterial, anticancer, antioxidant, anti-inflammatory, antidiabetic and antiparasitic capabilities. As of late, diatoms have many applications in biotechnology and are appropriate to deliver recombinant proteins/peptides, such as monoclonal antibodies, antibodies and presently for the generation of biosensors as well as sedate delivery specialist. Because of their biodegradability, ease of functionalisation, and moo-fetched and uncomplicated features compared to synthetics, diatom-based nanoparticles are used as drug delivery vehicles. Additionally, diatom-based nanoparticles are a viable option for delivering anticancer medications while also reducing cancer chemotherapy side effects. In this chapter, we attempted to compile the published data related to brown algae as a biosensor, medicate conveyance operator, focused on medicate conveyance utilising hereditarily built diatom biosilica.

Keywords Diatoms · Silicate cell wall · Biosensor · Drug delivery vehicle · Cancer therapy · Genetically engineered biosilica

11.1 Introduction

Diatoms are a big and diversified family of golden-brown algae that includes anything from minute filamentous forms to large, complex seaweeds (Phaeophyceae; Dhanker et al. 2022). Phaeophytes, like other Heterokontophyta members (Ochrophyta, Stramenopiles), have plastids with a girdle lamella, three-stacked thylakoids and chloroplast ER (endoplasmic reticulum). All have a heterokont motile stage (unequal flagella) and photosynthetic pigments such as chlorophylls a, c_1 and c_2, carotene, diatoxanthin and fucoxanthin (Andersen 2004).

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P. Srivastava et al. (eds.), *Insights into the World of Diatoms: From Essentials to Applications*, Plant Life and Environment Dynamics,
https://doi.org/10.1007/978-981-19-5920-2_11

Fucoxanthin, a xanthophyll pigment, generally conceals the other pigments in phaeophytes, giving them their distinctive brown colour. Phaeophytes differ from most other heterokont groups in that they have (1) cellulose, alginic acid and various polysaccharides in their cell walls; (2) physodes, which are cellular inclusions of polyphenolic polymers; (3) chloroplasts with thylakoids in stacks of three, enclosed by a girdle lamella; and (4) laminarin, α-1,3-glucan as their main storage product (Pueschel and Stein 1983).

As storage reserves, several species create mannitol, sucrose, glycerol or oils. The nuclear envelope is separated by a peripheral endoplasmic reticulum. All Phaeophyceae members are multicellular in the vegetative phase, unlike other members of the phylum; none are unicellular in the vegetative phase, which is the prevalent morphology in other golden-brown groups. In most organisms, haploid and diploid generations alternate, which might be isomorphic or heteromorphic. Although there are many simpler filamentous forms, many are macroscopic seaweeds with sophisticated tissues and reproductive mechanisms (Guiry and Guiry 2014). Fewer than 1% of the class's estimated 1836 species in 285 genera have been identified in watery environments (Wilce 1966). Some freshwater organisms have adapted to life in brackish water. There are three to seven genera of freshwater brown algae and up to 13 species worldwide, according to various authors. In most organisms, haploid and diploid generations alternate, which might be isomorphic or heteromorphic. Although there are many simpler filamentous forms, many are macroscopic seaweeds with sophisticated tissues and reproductive mechanisms.

About two decades ago, the first characterisations of the biochemical processes and components involved in diatom silicification were made (Hildebrand et al. 2006; Kröger et al. 1999, 2000, 2002). More recent research has shed light on how higher-order silica structure construction might take place (Tesson and Hildebrand 2010, 2013; Scheffel et al. 2011). Recent publications have detailed the discovery of novel components engaged in higher-order processes, real-time imaging of dynamics and genetic modification of silica structure (Kotzsch et al. 2017; Tesson et al. 2017). These recent researches have offered strategies for not only identifying but also clarifying the connections and spatiotemporal dynamics of the components involved in silica cell wall formation. This type of holistic understanding will be required to have a better understanding of cell wall formation (Hildebrand et al. 2018).

The ability to selectively remove malignant cell populations while leaving healthy cells alone is a critical goal in anticancer therapy. Although the benefits of using nanoporous silica-based materials as drug delivery vehicles have recently been established, their production requires the employment of expensive and toxic chemicals. Nanoporous biosilica is generated from diatom microalgae to deliver chemotherapy medicines to cancer cells (Delalat et al. 2015). This chapter concentrates on the evolutionary history of brown algae, overview of brown algae's silicate cell, as well as the role of brown algae as a biosensor and its biomedical applications.

11.2 Evolutionary History of Brown Algae

Brown algae (Phaeophyceae) are complex photosynthetic entities with an evolutionary history that is substantially distinct from that of green plants, to which they are only distantly related. These seaweeds are the most common species in rocky coastal ecosystems, and they have a variety of remarkable adaptations to their typically harsh surroundings. Brown algae are also one of the few eukaryotic lineages with sophisticated multicellularity (Cock et al. 2010; Dhanker and Tiwari 2021).

The old, twentieth-century understanding of brown algal categorisation, which was based on a combination of life cycle organisation, thallus architecture and gametic features, was invalidated by molecular phylogenies. For example, phylogenetic evidence clearly refuted the long-held theory that the physically more complex orders were diverged from filamentous Ectocarpales early in the brown algae's development. The Ectocarpales, on the other hand, were near cousins of the Laminariales, one of the most morphologically complex groups of brown algae. Based on molecular phylogenies, ancestral state reconstructions show that parenchymatous growth has returned to filamentous growth several times (Trevor et al. 2020). Similarly, life history features and gametic differentiation show complex evolutionary patterns, with transitions from isogamy to anisogamy to oogamy occurring multiple times independently, with the genetic basis just recently identified. Because of this versatility, molecular data has been crucial in accurately characterising brown algal relationships.

The genome of Ectocarpus has revealed indications of its ancient evolutionary background as well as more recent events related to the origin of the brown algal lineage. The former includes the various origins of the genes that make up the genome, many of which were acquired through endosymbiotic events, whereas the latter includes the recent emergence of new gene families and the evolution of an unusual genome architecture, both in terms of gene structure and organisation (Cock et al. 2010). It's likely that events on both periods influenced the genesis of sophisticated multicellularity in brown algae. The long-term preservation of completeness and diversity within essential gene families, such as the brown algal receptor kinase family, appears to have been just as significant as the more recent development of novel proteins (Cock et al. 2010).

Early in the evolutionary history of brown algae, the orders Discosporangiales and Ishigeales diverged from the other brown algal lineages at the beginning of the Mesozoic Era (~250 Ma). These two orders have only 11 species, yet they are very different from other brown algae.

The SSDO clade diverged from the lineage that gave origin to the remaining existing brown algae orders during the Mid-Mesozoic (approximate timeframe for the Jurassic period, 200–145 Ma) and diversified into what are now four orders: Sphacelariales, Syringodermatales, Dictyotales and Onslowiales. The most prominent of these lineages is Dictyotales, which today comprises a large number of brown algal species (Trevor et al. 2020).

The life cycle is a fundamental biological element that drives the evolution of many properties such as reproductive systems and dispersal modes, and it must be considered in order to properly comprehend a species' biology. Brown macroalgae have a diverse range of life cycles, sexual systems and reproduction strategies (Trevor et al. 2020). Their life cycles range from isomorphic haplodiplontic life cycles (e.g. *Dictyota dichotoma*), in which both the gametophyte and sporophyte display identical levels of multicellular development, to diplontic life cycles, in which only the diploid generation is multicellular (e.g. *Fucus* spp.) The cycle is called heteromorphic when the gametophytes and sporophytes have distinct morphologies. Except in a few taxa, such as *Scytosiphon*, where the haploid phase is a big, upright thallus and the diploid phase is a prostrate crust, the diploid sporophyte is generally dominant (i.e. larger) than the haploid gametophyte (Heesch et al. 2021; Trevor et al. 2020).

The primordial form of brown algal sexual reproduction was haplodiplontic, with identical haploid and diploid phases, according to phylogenies based on morphological and molecular features (i.e. isomorphic; Heesch et al. 2021; Trevor et al. 2020). Several lineages have experienced changes to this isomorphic life cycle, including a reduction in the size of the gametophyte generation (transition to a heteromorphic cycle, e.g. Syringodermatales, prior to the ancestor of the BACR) or the loss of this haploid generation (transition to a diplontic life cycle, e.g. Ascoseirales, Fucales, genus *Tilopteris* in Tilopteridales). There have been no transitions back to a haplodiplontic life cycle, implying that conversions to diplontic cycles were irreversible. Multiple transitions from heteromorphic to isomorphic life cycles, on the other hand, have happened (Trevor et al. 2020). The study of the evolutionary processes that drive these transformations is still a fruitful field of study for brown algae.

11.3 Emerging Application of Microalgae

11.3.1 Brown Algae as Biosensor

11.3.1.1 Biosensors

Biosensors or organic sensors are tools made from a transducer and bioreceptor that can pick out analytes and flip that data right into a measurable sign.

There are elements of biosensors such as bioreceptor and transducer (Brayner et al. 2011):

1. Bioreceptor: Bioreceptor can be created from any enzyme, antibody, nucleic acid, complete cell, tissue and microorganism.
2. Transducer: Transducer is the opposite of a biosensor and having optical, electrochemical, thermal, mass-based, ion—susceptible and resistant (Mona et al.

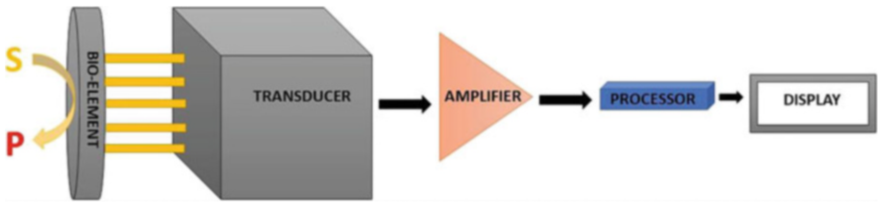

Fig. 11.1 Schematic diagram of biosensor

2020). Selectivity and specificity rely upon an organic popularity gadget related to appropriate transducers (Fig. 11.1).

Basic precept of biosensor is especially concerned with three elements:

- Biological popularity detail
- Transducers discover and transduce signs from organic goal
- Then transduction of indicators from organic to electric powered indicators (Mona et al. 2020)

11.3.1.2 Need of Algal Biosensor

This technique quickly and accurately detects low concentrations of pollutants in fluids, water and air. Environmental safety issues require fast, accurate and efficient technology. Bio-algae biosensors are based on microalgae and cyanobacteria (turquoise algae). Algae biosensors detect herbicides, volatile organic compounds (VOCs), heavy metals and more. Algae biosensors measure various metabolic activities of an organism. Toxic and dangerous substances have a great influence on the metabolic activity of cells, and this effect is transmitted in the form of signals. The main purpose of these sensors is to detect pesticides, herbicides and fungicides. Algae are used in bioassays for aquatic risk management and environmental monitoring (Kashem et al. 2019; Dhanker et al. 2021, 2022; Mathew et al. 2021). Algae are so sensitive and reproducible that they are used to eliminate toxicity (Durrieu et al. 2004; Dhanker et al. 2022; Mathew et al. 2021). However, biosensors have been developed to assess aquatic toxicity. Photosynthetic activity is inhibited by electrochemical oxygen reduction and chlorophyll fluorescence. The alternative activity of algae protoplasts and the gravity or phototaxis of algae are electrochemically monitored (Tatsuma et al. 2009). The main advantage of biosensors is that they are highly selective for each type of contaminant. Heavy metals inhibit enzyme synthesis as an inhibitor of alkaline phosphatases and esterases and inhibit pesticide attack on PSII as chlorophyll fluorescence released from photosynthetic activity (Durrieu et al. 2004).

11.3.1.3 Types of Biosensors

Primarily there are two sorts of biosensor:

1. **Natural:** These algal strains happen in common conditions. These organisms generally work in living beings (*Chlorella vulgaris*). Characteristic algal biosensors are for the most part working on the photosynthetic action of green growth. In these sorts of biosensors, the movement of photosynthesis in living cells is affected due to the nearness of different poisons. A few biosensors are based on the fluorescence of chlorophyll put away in chloroplasts. Algal biosensors right now utilise the chlorophyll fluorescence as the quantifiable flag. Chlorophyll fluorescence is utilised to measure the herbicides that influence the photosynthesis at PSII for illustrating triazines and atrazine (Durrieu et al. 2004).
2. **Hereditarily altered:** They are hereditarily altered quality of any microorganism. Manufactured biosensors have so numerous focal points. Natural biosensors have certain limitations, but to overcome these problems, biotechnological modified strains of biosensors are used, work with high efficiency and are easily detectable. According to Shao et al. (2022) a freshwater Cyanobacteria, *Synechocystis* sp. strain PCC6803, was genetically modified with the gene *Lucia luciferase* of firefly (a novel bioluminescent alga) which is sensitive to a wide range of compounds like herbicides and other pollutants. Important application of these biosensors is important for quick screening of water samples or determining toxicity of pollutants to harm the environment (Guleri et al. 2020; Shao et al. 2022). The biosensors could also help to indicate the type of pollutant and potential of pollutant to harm.

11.3.1.4 Working of Algal Biosensors

Biosensor consists of a bioreceptor that senses a biological element and transducer which determines the signals (biochemical) and converts it into an optical or electrical signal. Biosensors facilitate fast, accurate, rapid and low concentration screening of a number of compounds. Depending on the type of biological element used, the response/signal varies. The signal is then amplified and filtered using a signal processing unit (SPU), and the outcome of the SPU is an analog signal which is equal to the biological quantity measured. However, specifically in algal biosensors, the fluorescence emitted by the photosynthetic activities of algae is used to facilitate the detection of toxic substances. The identical signals are obtained with optical and conducto-metric transducers. This device is planned to watch different synchronous metabolic exercises of immobilised green growth (Durrieu et al. 2006).

11.3.2 Diatoms-Based Sensors

11.3.2.1 Hierarchical Porous Structure

Diatom frustules feature a remarkable permeability structure, with pores dispersed at different scales from nano to micro. They can be thought of as prefabricated 3D nanodevices. More than 10^5 unique diatom species have been depicted. They are easily processed, yielding large quantities of hereditarily regulated silica frustules. These three-dimensional silica shells could thus serve as the foundation for novel electronic devices, such as gas sensors that can detect contamination faster and more effectively than conventional devices (Livage and Sicard 2011). The subject of optical microsensors for unstable compounds will be a fascinating application of diatom frustules. An expansive surface range is a critical feature for an optical transducer that must be sensitive to vapors and glasses in order to provide a genuinely successful interaction with a few adsorbates. Because the diatom pores' measurements are accurate within a nanometer range, a wide range of unstable chemicals (solvents, hydrocarbons, etc.) and even pure gases can penetrate and condense within them. Diatoms' varied levels of porosity enable hint mixing between the explanatory gas test and the locator, allowing for appealing biomolecular intuitive observation. Silica is known to have photoluminescent capabilities within the visible range, around 2.2 eV, due to Si–O organisation abandons (Livage and Sicard 2011).

For silica diatom frustules, similar photoluminescence (PL) emanation within the yellow region is also monitored. Surface-oxygen stoichiometric surrenders are linked to this glow activity. It can thus be impacted by even minor changes in the surrounding gas environment. Within the thickness of luminous states, gas particles are adsorbed on these surrenders, leading them to an altar. As a result, the presence of gases can either quench or boost photoluminescence (PL) emission. Gas detection at low concentrations is very sensitive, and for diatom frustules with the highest specific surface area, a detection limit of 50 ppb was achieved. Recent research using the silica skeleton of the marine diatom *Thalassiosira rotula* has revealed that photoluminescence (PL) is greatly influenced by the environment. In the visible area, silica frustules have a broad emission band centred at roughly 2.26 eV, with a full width at half maximum of 600 meV. The strength of the PL signal decreases when exposed to NO_2. The PL flag has been deactivated because NO_2's electrophilic capabilities can attract electrons from the silica substrate. *Thalassiosira rotula* frustules have been observed with a notable variation of PL concentrated at low (sub-ppm) NO_2 concentrations (shown as $[NO_2]$), and immersion of the extinguishing impact occurs at NO_2 concentrations of the order of 10 ppm (Lettieri et al. 2008; De Stefano et al. 2005). Gases and natural vapors affect both the optical escalated and crest placements. A few compounds, such as acetone or ethanol, extinguish the glow, while others, such as pyridine, successfully improve it, depending on their electronegativity and polarising capability. These miracles allow for the separation of various chemicals and were utilised to create the first diatom-based photoluminescence gas-sensing devices (Steraro 2007). As a result, these genuine living beings are excellent candidates for optical detecting materials for dangerous gas detection or contamination testing. By providing distant components inside the culture media, the photoluminescent characteristics of silica frustules can be changed.

For example, germanium can be metabolically implanted in *Pinnularia* sp. frustule biosilica. Within the blue range, between 450 and 480 nm, a few Si atoms are replaced by tetravalent Ge, and germanium-doped frustules display both photoluminescent and electroluminescent features (Jeffryes et al. 2008; Qin et al. 2008). Titanium has also been metabolically implanted into silica frustules using a two-stage cell-cultivation process (Jeffryes et al. 2008), resulting in the formation of a semiconducting TiO_2 coating.

In this situation, conductivity estimates can be used to limit the total amount of distant gases like NO_2. The modified frustule functions as a microelectrode. The hydrated silica $SiO_2 \cdot nH_2O$ in diatom frustules is unknown. Si–OH receptive hydroxyl bunches enable chemical surface modification and subsequent silica shell functionalisation. Later research has shown that the frustule surface of *Coscinodiscus wailesii*, a diatom with spiral symmetric valves, may be chemically changed and covalently attached to several types of bioprobes, acting as a helpful support in the development of fluorescence biosensors. Antibodies have been linked to the frustules of *Coscinodiscus concinnus*, a marine diatom. According to fluorescence estimates, these antibodies can still recognise their antigens when attached to the hazy silica surface of diatom microshells. Changes in the photoluminescence emission of diatom frustules reflect the specific antibody–antigen recognition (De Stefano et al. 2008; Gale et al. 2009). Diatom frustules appear to be used as layouts for the production of nanostructured materials as well.

Silica shells can be chemically transformed into different oxide materials while retaining their three-dimensional nanostructure. Through a shape-preserving gas–silica uprooting response, silica has been transformed into a contemporary composition in this preparation, currently known as fundamental 'bioclastic and shape-preserving inorganic transformation'.

The silica shell can be converted to MgO by heating it in magnesium vapour at 900 °C for 4 h (Sandhage et al. 2002). As a result, a variety of additional nanostructured oxide materials (TiO_2, ZrO_2, $BaTiO_3$) have been created (Bao et al. 2007). Silica can be reduced to permeable silicon, resulting in previously unimagined microelectronic consequences. A synergistic combination of natural nanostructures and synthetic chemical functionalisation could result in a large variety of 3D micro/nanostructures with chemistry and characteristics that can be designed for detection applications (Guleri et al. 2020; Livage and Sicard 2011).

11.3.2.2 Photonic Crystals Made of Diatom Frustules

A few diatoms continue to function as 'living opals', with glowing qualities arising from their unusual porous structure. They then occasionally transport moo (gaps)

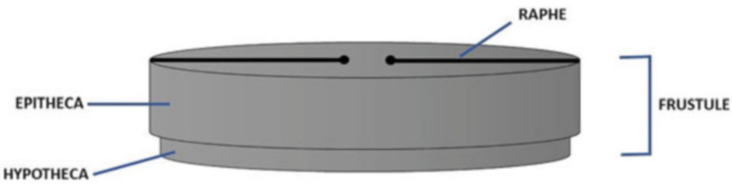

Fig. 11.2 Schematic diagram of diatom

and tall (silica) dielectric stable materials with grid measurements close to the wavelength of visible light. In *Coscinodiscus granii*, a hexagonal cluster of pores with a large grid within the valve and a square cluster with a small cross section within the support (Fuhrmann et al. 2004) have been discovered. As a result, diatom frustules might be depicted as 'living photonic crystals'. Between light and matter, something solid may happen intuitively. In 'photonic crystals', light behaves similarly to electrons in semiconducting materials. As a result, diatom frustules take on the appearance of opals (Fig. 11.2).

Silica frustules typically hold light in the blue region, a feature that protects diatoms from excessive illumination and improves their photosynthetic behaviour (Yamanaka et al. 2008). This is due to a specific assimilation resulting from the occasional conveyance of pores inside the silica frustule, which continues to function as a photonic precious stone piece waveguide. These diatomic nanostructures can be used to locate unstable chemicals in photonic microsensors. The capillary condensation of natural vapors inside the pores causes a change in the normal refractive file, which can be detected using a few optical techniques (De Stefano et al. 2009). Lin et al. (2010) have described diatom-based sensors for fast label-free electrochemical localisation of cardiovascular biomarkers. A cluster of gold nanoelectrodes on a silicon chip make up the biosensor. Each sensor is covered with a diatom frustule, resulting in a thick layer of nanowells. Their permeable structure facilitates the spread of biospecies and allows for true management of 'molecular flow'. At low pg/mL levels, fiery indicators in the human blood have been found, with affectability sufficient to identify patients at risk of cardiovascular infection (Livage and Sicard 2011).

11.4 Brown Algae as Drug Delivery Agent

Lipids, carbohydrates, peptides and carotenoids, which are derived from brown algae and have anticancer and antibacterial properties, are used in biomedicine and pharmaceutical biotechnology. Photosynthetic marine microorganisms have biotech uses and are also suitable hosts for the production of recombinant peptides such as monoclonal antibodies and vaccines. Diatom is a brown algae that is eukaryotic and has a distinctive cell wall called frustule. The silica frustule structure possesses unique advantages for drug administration, including well-organised three-dimensional pores, microchannels, chemical inertness and a homogeneous nanopore structure. Frustules could be easily designed, shielded and functionalised for medication delivery and loading in the future (Khavari et al. 2021). Because of their easy functionalisation, biodegradability, low cost and simple features compared to manufactured silica nanoparticles, diatom-based nanoparticles are exploited as drug delivery vehicles (Vona et al. 2015). As a result, diatom-based nanoparticles can be used to deliver anticancer medications while also reducing the negative effects of cancer chemotherapy. Diatom-based nanoparticles have been employed in drug delivery systems in recent years. Biodegradability, wide surface area and low toxicity are the key advantages of these nanoparticles over other carriers. Diatoms are utilised in the production of growth factors, antibodies, vaccines, hormones and immunological regulators in medical and pharmaceutical biotechnology (Yan et al. 2016; Aw et al. 2012).

11.4.1 Diatom-Based Nanocarriers in Drug Delivery

Drug delivery systems are created to deliver medications or genes to specific cells, such as cancer cells. Patients with genetic diseases frequently have a missing or damaged genome. In this context, silicon nanoparticles (NPs) have been found to be a successful technique (Dolatabadi and de la Guardia 2011). As they transport medications to specific tissues in the body, sophisticated drug delivery systems can now overcome the constraints of conventional pharmaceuticals (e.g. poor solubility/stability and high toxicity). Micelles, liposomes and silicon oxide NPs are some of the most utilised drug carriers, each with its own set of advantages and disadvantages (Dhanker et al. 2021).

Silica-based NPs like SBA-15 and MCM-41 offer chemical and physical qualities include tailorable pore size, thermal ability, a large surface area and high loading capacity. The limitations of NPs have been noted as being time-consuming and costly, as well as requiring toxic ingredients and a lot of energy (Aw et al. 2012; Maher et al. 2018). The use of marine resources in biomedicine is becoming increasingly popular (Chao et al. 2014). Diatom biotechnology has received a lot of interest in recent years as a major field for research and manufacturing of high-value molecules with therapeutic uses (Gordon et al. 2009). As previously stated, microalgae are a major source of different polysaccharides such as carrageenan, laminarin, fucoidan and alginate, which can be transformed into NPs and interact with biomolecules via hydrophilic groups on the surface (Shankar et al. 2016).

The production of diatoms (brown algae) with an amorphous silica shell is both expensive and simple. Porous silica (SiO_2) NPs can be found in their fossils [frustules/diatomaceous earth (DE)]. DE and living diatoms have also been found to be sources of silica NPs, with DE having more frustules (Maher et al. 2018; Sasirekha et al. 2019). Amino group functionalisation preserves plasmid DNA while also delivering it to the nucleus. Park et al. (2008) found that receptor-mediated endocytosis reduced cytotoxicity and improved transfection effectiveness.

Furthermore, Aw et al. (2011) examined the oral delivery of a hydrophilic drug (gentamicin) and a hydrophobic drug (indomethacin) through porous silica diatoms and found that indomethacin loading (smaller than gentamicin) was higher and had a stronger interaction with DE, while both drugs' drug release behaviour was similar (Aw et al. 2011). Bariana et al. (2013) modified diatoms with phosphonic acid and organosilanes for co-delivery of hydrophilic (gentamicin) and hydrophobic medicines (indomethacin). They discovered that hydrophilic modifications like 16-PHA (16-phosphono-hexadecanoic acid) and OTS (7-octadecyltrichlorosilane) improved drug loading and controlled release of gentamicin, whereas hydrophilic modifications like APTES (3-aminopropyltriethoxysilane) and 2-CEPA (2-carboxyethyl–phosphonic acid) increased drug loading and controlled release of indomethacin. Modifications to diatomaceous earth (diatomite, DE) could change and improve its properties; for example, oligo(ethylene glycol) methacrylate copolymers on diatom microcapsules make these agents stimulus-responsive carriers for the delivery of levofloxacin, an antibiotic used against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Vasani et al. 2015).

Furthermore, diatom frustules treated with DOPA/Fe_3O_4 (dopamine-terminated Fe_3O_4 NPs) used their magnetic characteristics to control an external magnetic field (Losic et al. 2010). Sasirekha et al. (2019) used chitosan to functionalise *Amphora subtropica* for doxorubicin (DOX) release and loading in their study. Furthermore, Janićijević et al. (2015) reported that diatomite treated with aluminium sulphate is an efficient carrier for diclofenac sodium, resulting in higher adsorbent loading and longer release than the control.

A disulfide bond was used to attach cargo molecules in biomimetic silica carriers (e.g. bioactive peptides/proteins, medicines) with R5 silaffin peptide in another work. After that, R5-cargo conjugates were entrapped in silica particles, and it was discovered that reductive and acidic conditions were beneficial for cargo release from this complex (Lechner and Becker 2013). Natural silica NPs produced from *Coscinodiscus concinnus* (diatom) for the administration of streptomycin (hydrophilic drug), comparing the drug release efficiency time of the treated diatoms to the untreated diatoms. Due to surface adsorption, streptomycin was also absorbed inside the pores and into the hallow diatom structure (Gnanamoorthy et al. 2014). According to Vona et al. (2015), increasing silanol on diatom surfaces improved drug encapsulation of ophiobolin A, a fungi-derived anticancer chemical. Surface functionalisation was found to be an effective technique for drug loading on diatoms in a previous study, and ophiobolin A release was reported to be prolonged (Vona et al. 2015; Delasoie and Zobi 2019), as shown in Table 11.1. In a work by Cicco et al. nanoporous silica-based particles were functionalised with cyclic nitric oxide 2,6,6-tetramethylpiperidine-*N*-oxyl and loaded with antioxidant, antibiotic (ciprofloxacin) and antibacterial effects on fibroblast and osteoblast-like cell development (Cicco et al. 2015). The combined effects of diatom surface functionalisation and shape on drug loading and release qualities were studied by Bariana et al. (2013). Diclofenac sodium (DS) is a nonsteroidal anti-inflammatory medication (NSAID) used to treat inflammation and pain (Table 11.1).

Table 11.1 Some pharmaceutical products and drug candidates from brown algae

Compound (product)	Preparation technology	Biological activity and pharmaceutical application
Magnesium alginate/$NaHCO_3$ (Gastrotuss® baby syrup)	Pharmaceutical excipient	Children and infants from the first days of life reflux treatment
Sodium alginate/$KHCO_3$ (Algicid® suspension/tablets)	Pharmaceutical excipient	Adult reflux treatment
Sodium alginate/$NaHCO_3$/$CaCO_3$ (Gaviscon Double Action Liquid®)	Pharmaceutical excipient	
Sodium alginate oral suspension sachet	Pharmaceutical excipient	
Alginate-based reflux suppressant	Pharmaceutical excipient	Heartburn in pregnancy
Polyethylene glycol matrix/hydrated alginate (Flaminal Forte® gel)	Functional modification: hydrogel	Leg and diabetic ulcers, pressure sores, complex grazes, burns, oncology and wound dermatosurgery
Propylene glycol sodium/calcium alginate (Saf-Gel® gel)	Functional modification: hydrogel	Dry and sloughy necrotic wounds, pressure and venous ulcers, second-degree burns, cuts, abrasions and skin tear, noninfected diabetic foot ulcers
Ester of hyaluronic acid/sodium alginate (Hyalogran® dressing)	Pharmaceutical excipient	Variety of exuding wounds including leg ulcers, pressure sores, ischaemic and diabetic wounds, particularly those which are covered with slough and necrotic tissue or areas that are difficult to dress
Calcium alginate (SeaSorb® dressing)	Functional modification: hydrogel	Heavily exuding wounds including leg and pressure ulcers, diabetic ulcers and second-degree burns, cavity wounds
Calcium/sodium alginate (Kaltostat® dressing)	Functional modification: hydrogel	Moderately to highly exuding chronic and acute wounds and for wounds with minor bleeding
Sodium alginate/poloxamer (Guardix-SG® adhesion barrier)	Pharmaceutical excipient	In spine and thyroid surgeries to reduction of the incidence postoperative adhesions
Sodium alginate (Natalsid® suppositories)	Pharmaceutical excipient	Chronic haemorrhoids, proctosigmoiditis and chronic anal fissures after surgical interventions in the rectum
Propylene glycol alginate/enamel matrix derivative (Emdogain® gel)	Esterification: gel	1-, 2- and 3-wall intrabony defects, class II mandibular furcation defects with minimal interproximal bone loss, recession defects
Alginate oligosaccharide (OligoG®)	Chemical depolymerisation	Cystic fibrosis, treatment of chronic obstructive pulmonary disease (COPD), improvement of antibacterial and antifungal therapy, antifungal activity
Propylene glycol alginate sodium sulfate oligosaccharides (PSS)	Chemical depolymerisation and sulfation	Anticoagulant and antithrombotic activity, blood viscosity reduction

(continued)

Table 11.1 (continued)

Compound (product)	Preparation technology	Biological activity and pharmaceutical application
Propylene glycol mannuronate sulfate (PGMS)	Chemical depolymerisation and sulfation	Hyperlipidemia and ischaemic cardiovascular and cerebrovascular diseases
Oligomannuronates (GV-971)	Chemical depolymerisation	Alzheimer's disease and neurodegenerative diseases
Polymannuroguluronate sulfate (HS911)	Chemical depolymerisation and sulfation	HIV/AIDS, hepatitis B virus
Oligomannurarate sulfate (JG3)	Chemical depolymerisation and sulfation	Inhibition of tumour angiogenesis, metastasis and tumour growth
Polyguluronate sulfate sodium (PGS)	Chemical depolymerisation and sulfation	Kidney stones and bladder stones; anti-inflammatory

11.4.1.1 Cancer Therapy with Diatom-Based Nanocarriers

DSNs (diatomite silica nanoparticles) are suitable for cancer therapy. Anticancer chemicals are efficiently transported into the cytoplasm of human epidermoid carcinoma cells by DSNs (Ruggiero et al. 2014). Liposomes and polymeric nanoparticles have recently been employed in chemotherapy, and dual-drug delivery may be used with two or more medicines (Nouri et al. 2017). When the concentration of DOX is higher than PTX, for example, paclitaxel (PTX) and DOX have shown synergistic interactions in the treatment of solid tumours. Because of their self-assembly and ease of production, DE particles are ideal for the regulated release of these medicines (Kabir et al. 2020).

DNPs functionalised with ATPES (3-Aminopropyl) triethoxysilane and polyethylene glycol (PEG) had greater cellular absorption and a longer drug release profile (Terracciano et al. 2015). Curcumin is an anti-inflammatory, antioxidant and cancer-fighting compound. Curcumin's medicinal use is currently limited by issues such as low bioavailability, fast dimerisation and poor absorption. In cancer therapy, natural diatoms treated with polydopamine are effective carriers for curcumin administration (Uthappa et al. 2019). Furthermore, diatomites activated by oxidising acids have been used as an ophiobolin A carrier (a fungal anticancer molecule) to extend the release of this agent (Vona et al. 2015).

Todd et al. (2014) found that diatoms loaded with iron oxide NPs might be employed as smart carriers for the delivery of small molecules and pharmaceuticals by manipulating an external magnetic field using their magnetic characteristics (Todd et al. 2014). This compound binds to HCT-116 colorectal cancer cells at the pH of the GI (Gastro Intestinal) tract, and the anticancer drug is slowly released under light irradiation, resulting in a twofold increase in cytotoxicity against HCT-116 (Delasoie et al. 2020). Free diatoms, on the other hand, have exhibited extremely low cytotoxicity against Caco-2, HT-29 and HT-116 (colon cancer cells), resulting in increased prednisone and mesalamine release and penetration in the GI tract (Zhang et al. 2013). Two medications have been delivered using diatom silica microparticles in the treatment of GI disorders. In GI diseases, prednisone and mesalamine have a longer half-life. Furthermore, drug permeability across Caco-2/HI-29 cells has been reported to improve, indicating that these particles are viable options for colon cancer treatment (Zhang et al. 2013). Diatomite NPs are similarly effective at transporting siRNA to suppress gene expression inside human epidermoid cancer cells (H1355).

11.5 Targeted Medication Delivery Using Genetically Modified Diatom Biosilica

The capacity to selectively target malignant cell populations while leaving healthy cells untouched is a key goal in anticancer therapy. Although the use of nanoporous silica-based materials as drug delivery vehicles has proven to be beneficial, their manufacture needs harmful and expensive chemicals. Chemotherapeutic medicines are delivered to cancer cells using nanoporous biosilica produced from diatom microalgae. *Thalassiosira pseudonana* has been genetically modified to display an IgG-binding domain of protein G on the surface of biosilica, allowing cell-targeting antibodies to be attached. Biosilica showing specific antibodies sorbed with drug-loaded nanoparticles specifically targets and kills B-lymphoma and neuroblastoma cells. In a subcutaneous mouse xenograft model of neuroblastoma, treatment with the same biosilica causes tumour growth regression. These findings suggested that genetically modified biosilica frustules could be employed as adaptable 'backpacks' for delivering poorly water-soluble anticancer medicines to tumour locations (Delalat et al. 2015).

Because of their longer drug release profiles and great efficacy in delivering hydrophobic medicines, research on porous silica-based particles has verified their applicability for drug-delivery applications.

1. The utilisation of nanoporous silica materials for drug delivery is a cornerstone of the rapidly growing field of nanomedicine.
2. Oxidised porous silicon and mesoporous silica have been the most extensively studied silica materials for drug delivery.
3. Both have thermal stability, a large surface area, configurable pore size, great biocompatibility, chemical inertness and biodegradability (at least in the case of porous silicon).
4. One significant disadvantage of using these materials is that their synthesis requires toxic chemicals (namely, silanes and hydrofluoric acid) and is both expensive and time-consuming.

The goal of this research was to provide a general approach for attaching antibodies and hydrophobic medicinal molecules to diatom biosilica without using covalent cross-linking or chemical solvents. This method's strategy entails (a) genetic engineering of antibody-binding protein domains into diatom biosilica and (b) the insertion of medicinal molecules into silica-binding carriers (Delalat et al. 2015).

To achieve (a), the currently developed method known as live diatom silica immobilisation (LiDSI) (Poulsen et al. 2007; Sheppard et al. 2012) is used to incorporate GB1, an immunoglobulin G (IgG)-binding domain of protein G19, into the biosilica of the diatom *T. pseudonana* in vivo and investigate IgG antibody attachment to the genetically engineered biosilica.

To achieve (b), hydrophobic drug molecules are encapsulated in cationic micelles and liposomes (Tanaka et al. 2010; Blanco et al. 2013) then their biosilica-binding characteristics are investigated, and the drug molecules are released. Because loading the diatom frustules with a hydrophobic chemical from an organic solvent would denature the antibody, this two-step technique is critical. Once the biosilica has adhered to tumour cells, drug-loaded nanoscale vehicles can be deployed (Delalat et al. 2015).

11.6 Conclusion and Future Perspectives

Brown algae have been broadly investigated for giving inexhaustible and profoundly esteemed items for more than a century, and the brown algal polysaccharides have an extraordinary potential for pharmaceutical applications. Brown algae as biosensors are exceedingly delicate and reproducible. This strategy is more solid and regularly usable as compared to other explanatory methods. Diatom shells, in addition, contain unique 3D shapes and are used to make nanoparticles for medical and biomolecule delivery. Medicate stacking and discharge from DENPs appear to be improved by unique shape and functionalisation. Several studies have looked into modified DENPs for drug delivery (DOX, camptothecin, paclitaxel) in the treatment of colon and breast cancer, with promising results. Hereditarily adjusted biosensors are demonstrated to be more straightforward, dependable, quick and exact. Biosensors are successful but have a few impediments, since within the field condition, diverse variables create numerous impacts on their action. Encouragement and research work is required to make a viable biosensor.

Acknowledgement The authors expressed their gratitude to GD Goenka University for the support and infrastructure provided to the authors.

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Chapter 12
Diatoms in Biomedicines and Nanomedicines

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Abstract Diatoms are unicellular photosynthetic microalga typically found in an aquatic environment, featuring a 3D nanopatterned silica enclosure called 'frustules'. The deposition of silica by diatoms allows the formation of a porous micro- or nanoscale 3D structure shells with several significant properties like thermal stability, high chemical and mechanical resistance, high specific surface area, tailorable surface chemistry with simple chemical functionalisation/modification procedure, optical/photonic characteristics, biocompatibility and eco-friendly. These characteristics are advantageous in fulfilling a wide range of environmental, agricultural, industrial, biotechnological and biomedical applications. Moreover, high renewability, ease of cultivation through an artificially induced environment, abundant availability and mineable fossilised mineral deposits (diatomaceous earth or diatomite) are economically favourable. This chapter will cover the recent advancements in the application of diatoms in the biomedical field like nanoparticle synthesis, drug delivery, bioimaging/biosensing and tissue engineering.

Keywords Diatoms · Drug delivery · Diatom nanoparticles · Tissue engineering · Biosensors/bioimaging

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P. Srivastava et al. (eds.), *Insights into the World of Diatoms: From Essentials to Applications*, Plant Life and Environment Dynamics,
https://doi.org/10.1007/978-981-19-5920-2_12

12.1 Introduction

Diatoms are photosynthetic unicellular microalgaes commonly found in soil and water (Dugdale and Wilkerson 1998). There are more than 110,000 known species across 200 genera recognised with silica-based shells, and these shells have a wide range of diverse shapes and structures containing pores with a diameter ranging from nano to micrometres (Parkinson and Gordon 1999). Through CO_2 fixation, it plays a vital role for photosynthetic producers within the food chain, globally contributing significantly to carbon stabilisation and immobilising a large percentage of the ocean's CO_2 (Kurkuri et al. 2011). The diatom cell wall is known as frustules which are well-organised porous silica micro shells (silicon dioxide hydrate, $SiO_2 \cdot nH_2O$) (Gordon et al. 2009). The frustule, on the other hand, is bilaterally symmetrical and consists of two valves known as thecae, one of which is slightly larger (epitheca) and overlaps the other (hypotheca) (Lopez et al. 2005). The frustules are organised through stacks of several inner layers known as areolae, cribrum and cribellum. Areolae are a honeycomb-like porous chamber roofed with cribrum having tiny and highly ordered pore patterns; cribellum is the thin siliceous membranes with several tiny pores, as shown in Fig. 12.1. The synthesis of frustules occurs with the help of a specialised compartment known as silica by intracellular polymerisation of silicic acid monomers (Chao et al. 2014). Silaffins transport proteins that appear to facilitate the formation of silica deposition in diatom frustules. The frustules can vary in size, morphology and function depending on the environment for their growth and species of diatom (Bradbury 2004).

In recent years, drug delivery application of mesoporous material of silica has been extensively studied to address the traditional challenges related to drug

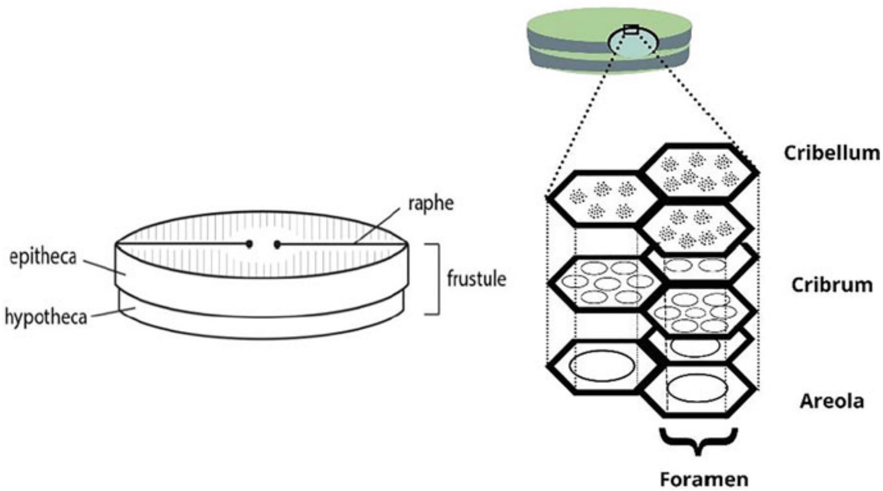

Fig. 12.1 Schematic structure of centric diatom frustule

delivery, such as low solubility and bioavailability, unappaling properties of pharmacokinetics and short activity (Vallet-Regí et al. 2007). They provide numerous benefits in the drug development process when utilised as a drug carrier in a drug delivery system because of their unique qualities, such as large surface area, adjustable structure, chemical resistivity, mechanical rigidity, increased drug loading capability, etc. (Bariana et al. 2013). Apart from their massive success in the drug delivery system, mesoporous material also has drawbacks, such as complicated synthesis processes, time-intensive, costly, high energy, unsafe and generating waste. MCM-41 and SBA-15, being the most popular series, also couldn't escape such drawbacks (Slowing et al. 2007; Vallet-Regí et al. 2007). As a consequence, there was a need for an alternative that could fit in place of mesoporous silica, yet again nature has offered an alternative that has the potential to replace the mesoporous silica. Diatoms are closely similar to mesoporous silica and are renewable, abundantly present, cost-effective, non-toxic and compatible with the drug delivery system (Bariana et al. 2013; Rabiee et al. 2021). Diatoms have been extensively studied for their potential application in a variety, and several researchers have independently proven them, such as molecular separation, drug delivery, immunoprecipitation, photonics, biosensing, nanofabrication, etc. (Fuhrmann et al. 2004; Lopez et al. 2005; Gordon et al. 2009; Aw et al. 2013; Bariana et al. 2013; Rea et al. 2016). The diatom's surface can be easily modified with simple chemical processes. The silicon dioxide building blocks provide scope for tailoring the surface properties in various engineered biomaterials for fulfilling desired biomedical applications. The hydroxyl groups on the diatomaceous earth (DE) surface are exploited for modification through well-established chemical processes. The surface functionalisation processes resemble previously discovered processes for modifying synthetic silica particles, including various applications such as organic monolayers and inorganic layers of oxide, proteins, polymers and coating with metal (Howarter and Youngblood 2006). Most of the processes include the modification through silanol (SiOH) groups present on the DE surfaces, which are reactive moieties. Several other reactive species, e.g. $-NH_2$, -COOH, -SH and -CHO, are employed for the surface modification exploiting silanol groups, which provide coupling points for several chemical and biological particles like drugs, DNA, proteins, antibodies, sensing probes, etc., hence, helping in the immobilisation of these particles (De Stefano et al. 2013; Terracciano et al. 2013). It is worth mentioning that the silanisation process occurs through the covalent bond of Si-O-Si, which provide a stable attachment for various active moieties on the surface of the diatom (Losic et al. 2010). Figure 12.2 illustrates the distinctive surface functionalisation of diatom microcapsules using organosilanes through the self-assembled layer (Rea et al. 2016).

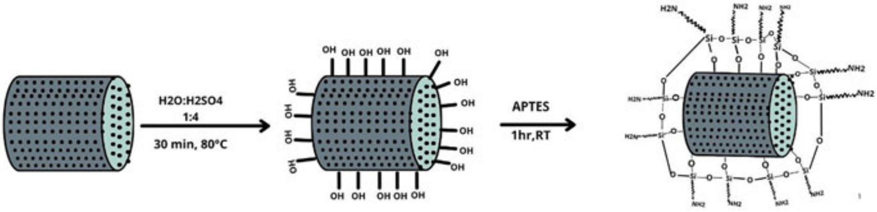

Fig. 12.2 Schematic represention of surface functionalisation of diatoms

12.2 Application of Diatoms

12.2.1 Diatoms for the Biosynthesis of Nanoparticles (NPs)

The characteristics of gold nanoparticles were widely anticipated for a catalytic property, photonic bandgap behaviour, localised surface plasmon resonance (LSPR) response and surface-enhanced Raman spectroscopy (SERS) (Link and El-Sayed 2003; Meldrum and Cölfen 2008). When reducing 4-nitrophenol to 4-aminophenol ($NaBH_4$) in the presence of reductants, the catalytic characteristics were significantly verified (Yu et al. 2010). Similarly, gold nanoparticles were fabricated by depositing gold onto the silica of diatomaceous earth (DE) via photo deposition. The molecules in the solution get captured in the pore structure, and the formed gold nanoparticle amplifies spectroscopy signals by many folds through the SERS effect (Onesto et al. 2018).

The photosynthetic pigments released by the diatoms (*Amphora* sp.) were noticed to have a reducing property implicated successfully in the synthesis of polycrystalline spherical silver nanoparticles via bioreduction of the silver ions into silver nanoparticles. The formulated silver NPs displayed high antimicrobial effects, especially gram-positive or gram-negative microorganisms (Jena et al. 2015). Similarly, another silver nanoparticle was synthesised on the diatom's surface by exploiting fucoxanthin. Reducing silver ions by the current carotenoid with a hydroxyl group and proteins averted the possible future aggregation and/or sedimentation of NPs with a carboxyl group (Chetia et al. 2017). Remarkably, under a given physiological setting, diatom frustules derived from peptides demonstrate excellent conditions for synthesising silver NPs. The kinetic reaction of silver NP formation was a highly sensitive photoreduction process—the reaction rate was hugely dependent on the peptide-diatom interaction (Gupta et al. 2018). Therefore, silver NPs are a highly antimicrobial compound and are effective against problematic pathogens such as *Streptococcus pneumonia*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Aeromonas* sp., etc. Hence, it resulted in the increased popularity of silver NPs.

Consequently, several attempts were made with different diatom species to prepare silver NPs (Mishra et al. 2020). Similarly, several attempts have been undertaken using various techniques to create NPs, such as *Nitzschia* diatoms for the biosynthesis of silicon-germanium oxide nanocomposite (Mansuy-Aubert et al. 2013). Another study has indicated the plausible preparation for gold-silica nanocomposites with the feasible structural organisation (spherical, hexagonal and triangular) using *Amphora copulate* diatoms (Roychoudhury et al. 2016). The mechanism for the biosynthesis of silver NPs via bioreduction of silver ions into NPs within the diatoms were implicated with the role of photosynthetic pigments, although silanol (with hydroxyl group) was identified with a crucial role in forming silver NPs (Mishra et al. 2020). The synthesised NPs were tested for a few other properties, and in a study, it was undoubtedly noticeable that the affinity of biosynthesised NPs bond with DNA without any further modification requisites—which was confirmed using agarose gel electrophoresis—"Y"-shaped chainlike and coiled structures were observed for the interacted particles and DNA molecules. Such properties could be highly beneficial in the field of biomedical applications. However, the biosynthesis of nanocomposites is possible only on the surface of the live diatoms, whereas dead diatoms remain unaffected (Roychoudhury et al. 2016).

12.2.2 Diatoms in Drug Delivery

The ideal drug delivery system should target only specific diseased cells with an adequate concentration of drug discharge and minimum adverse effect on healthy tissues (Wagner et al. 2006; Maher et al. 2018). Furthermore, most of the new drug targets and currently popular drug molecules are contained in the Biopharmaceutics Classification System, which shows inadequate physicochemical properties such as class II or class IV, poor solubility, poor uptake into cells, restricted biodistribution, efficacy, etc. Therefore, over the past, scientists have focused on developing new enhanced techniques for drug delivery, which led to the concept of drug carrier systems. These carriers are designed to protect drugs from degradation/rapid clearance, lift physiochemical properties and elevate cellular uptake. As a result, scientists have practically developed several drug carrier systems with enhanced drug distribution and efficient immobilisation of drugs in the tissues of interest with few negative consequences (El-Aneed 2004; Taylor and Triggle 2007; Van der Meel et al. 2013). Evidently, the physicochemical properties of synthetic silica are much fruitful for this purpose. However, the major drawbacks were the high cost of manufacturing, time-consuming and high cost of manufacturing. In addition, the usage of harmful solvents for the manufacturing process also poses a threat to the final product due to trace residue (Tran et al. 2009; Maher et al. 2018). Although these drawbacks could be overcome through biosilica exploitation with the propensity towards diatoms as bioresource—diatoms have high regeneration capacity, and a huge amount of DE silica already exists. Therefore, biosilica has huge potential for producing futuristic drug carriers with minimal negative impact all-embracing (Anderson et al. 2000; Dolatabadi and de la Guardia 2011).

In 1999, The first-ever idea of exploiting diatoms to construct productive material was mentioned by Morse. He showed the current incapability of humans to produce a synthetic replica of the architectural construct of silica as produced by diatoms (Morse 1999). The release of gold nanoparticles from DNA-functionalised diatom surfaces has been successfully controlled and scalable in medication delivery (Rosi et al. 2004). However, few studies also demonstrated (in vitro) the practical application of DE silica as a drug carrier through the encapsulation of therapeutic drugs (Aw et al. 2011, 2012). Indomethacin was loaded onto the DE silica with a drug loading capacity of 22 wt% drug loading capacity and a 2-week continuous release of the drug, which depicts the efficacy of the DE silica for the application as a drug carrier. The drug release ensued in two phases, and the first phase exhibited a rapid release for incessant 6 h due to surface detachment of the adsorbed drug. The other phase lasted 2 weeks and had zero-order kinetics due to the slow release of drugs from diatoms' interior pores. Likewise, prednisone and mesalamine were also put onto DE silica for oral administration. The results validated the drug's prolonged release, and toxicity testing revealed that diatom frustules had no deleterious effects even at 1000 g/mL when tested in various cells (Caco-2, HT-29 and HCT-116) individually and Caco-2/HT-29-cocultured cells (Zhang et al. 2013).

The hydroxyl-enriched DE silica surface provides huge scope for the modification to enhance the surface properties to achieve improved drug loading and release profile (Aw et al. 2013). Several strategies have been developed in recent years to modify silica surfaces with metal and inorganic oxide layer coating, polymer, proteins and organic monolayer; these strategies are frequently used for developing synthetic silica (Rabiee et al. 2021). The most popular method is the application of the silanol group to functionalised it with several reactive species (e.g. -NH_2, -COOH, SH and CHO), generating a vigorous coupling point for biological and chemical moieties such as drugs, proteins, antibodies, aptamers, DNA, sensing probes, etc. (De Stefano et al. 2013; Terracciano et al. 2013). Mesoporous silica-based NPs are the finest, with a pore size of ~2–50 nm for the drug delivery aptitudes (Terracciano et al. 2018). Remarkable characteristics of diatoms such as thermal stability, modification thru the simple chemical procedure, enormous surface area (up to 200 m^2/g), biocompatibility, mechanical resistance, eco-friendly, optical/photonic characteristics and ease of genetic manipulation make it an excellent choice for drug/gene delivery (Rabiee et al. 2021). Some of the examples of drug delivery employing diatoms are mentioned in Table 12.1. Fabrication of DE silica for the drug delivery system via the silanisation process is quite a popular procedure due to its chemical stability due to Si-O-Si covalent bonds (Mohammadinejad et al. 2015; Schröfel et al. 2011). Figure 12.2 provides a pictorial representation of the chemically induced modification of the surface of diatom microcapsules via the formation of a self-assembled layer using organosilanes (Pytlik et al. 2017). In a study, various modifications were tested by imparting hydrophilic (2-carboxyethyl-phosphonic acid), APTES (3-aminopropyl triethoxysilane), and AEAPTMS (3-aminopropyl trimethoxysilane) and hydrophobic (16-phosphono-hexadecanoic acid) properties to microcapsule (Fig. 12.3). Observation shows contrasting behaviour; the hydrophilic modification showed enhanced drug loading having 15–24 wt% and prolonged drug release (6–15 days), whereas hydrophobic modification showed lower drug loading and rapid drug release (Maher et al. 2018). A thorough analysis

Table 12.1 Some examples of drug delivery systems employing diatoms

Drugs	Surface functionalisation	Loading process/loading capacity	Release duration	Remarks	References
Mesalamine	–	Immersion; 9.9–11.5%		• Functions as permeation enhancer for oral ingestion.	Zhang et al. (2013)
Prednisone				• Minimises toxicity of diatoms	
Indomethacin	–	Wetted and sonicated; 22%	14 days (6 h—burst release)	• For implant and oral ingestion	Aw et al. (2012)
Indomethacin	Graphene oxide-APTES	Immersion; 30%		• Controlled drug delivery	Kumeria et al. (2013)
Indomethacin	mPEG-silane	Simple incipient wetness; 14–24%	13–26 days	• Improving drug loading and release profile	Bariana et al. (2013)
Gentamicin	7-OTS				
	3-GPTMS				
	2-CEPA				
	16-PHA				
	APTES				
Indomethacin Gentamicin	Dopamine (DOPA) functionalised iron oxide NPs	Immersion; 28%	8 h	• Enhanced targeted drug delivery	Medarević et al. (2016)
Sorafenib	APTES	Silanisation; 10.4 ± 1.1%	4–24 h	• Dual biofunctionalisation	Terracciano et al. (2015)
	APTES-PEG (polyethyline glycol)	DNPs-PEGylation; 22 ± 2%		• Enhanced stability, biocompatibility, cellular uptake for anti-cancer drug	
	APTES-PEG-CPP (cell-penetrating peptide)	DNPs-PEGylation and peptide bioconjugation; 17 ± 2%		• Increased solubility	

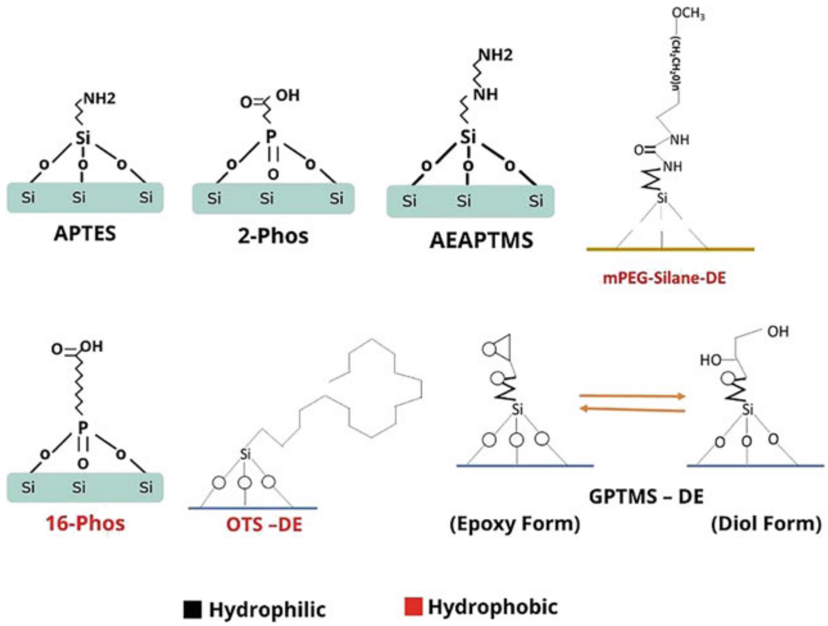

Fig. 12.3 Structure of DE microparticle structure as well as surface functionalisation with organosilanes (APTES, GPTMS, OTS and mPEG-Silane) and phosphonic acid (2-CEPA and 16-PHA) to make DE surfaces hydrophilic or hydrophobic

of the different hydrophobic and hydrophilic DE surface modifications for the hydrophobic indomethacin and the hydrophilic gentamicin (hydrophilic). Similarly, Bariana et al. (2013) have successfully analysed the grafting of several functional groups, and their interfacial properties were confirmed through Fourier transform infrared (FTIR) analysis. Their findings are shown in Table 12.2; water-soluble drugs (gentamicin) and water-insoluble drugs (indomethacin) were tested against hydrophilic and hydrophobic modifications. Because of a polar carboxyl, amine or hydrolyzed epoxy group in hydrophilic changes, indomethacin release was prolonged, but hydrophobic modifications with a long-chain hydrocarbon resulted in gentamicin release being prolonged (Kim et al. 2009; Bariana et al. 2013).

12.2.3 Diatoms in Biosensing and Bioimaging

Biosilica can be improved by incorporating antibodies, enzymes, drugs and DNA aptamers as detection components to obtain a series of promising biosensors. In one study, in order to detect breast cancer cells from normal cells using iron oxide NPs, researchers chemically modified biosilica structures from *Chaetoceros* sp. diatoms to create a new functionalised system containing trastuzumab antibodies. In vitro investigations using these nanosystems in applying a magnetic field show that

Table 12.2 Comparative overview of loading capacity for the hydrophobic and hydrophilic modification with hydrophobic and hydrophilic drug. 3-Aminopropyltriethoxysilane (APTES); 7-octadecyltrichlorosilane (OTS); 3-(glycidyloxypropyl) trimethoxysilane (GPTMS); 2-carboxyethylphosphonic acid (2 CEPA); 16-phosphono-hexadecanoic acid (16 PHA); methoxy-poly-(ethylene-glycol)-silane (mPEG-silane)

	Indoemthacin (water-insoluble drug)	Gentamicin (water-soluble drug)
Hydrophobic functionalisation	**(Wt.% loading)**	**(Wt.% loading)**
OTS-DE	14 ± 5	–
16-PHA-DE	14 ± 5	22 ± 5
mPEG-silane-DE	17 ± 5	–
Hydrophilic functionalisation	**(Wt.% loading)**	**(Wt.% loading)**
APTES-DE	22 ± 5	15 ± 5
GPTMS-DE	19 ± 5	–
2-CEPA-DE	24 ± 5	16 ± 5

SKBR3 cells may be selectively trapped and separated (Esfandyari et al. 2020). APTES was employed to detect bovine serum albumin, and frustules from *Amphora* sp. diatoms were functionalised. A significant reduction in photoluminescence intensity of bovine serum albumin was obtained at 445 nm as a result of interaction with the amine-functionalised diatom bovine serum albumin protein complex, with a detection limit (LOD) of 3105 M (Viji et al. 2014). Subsequently, cardiovascular protein biomarkers (myeloperoxidase and C-reactive proteins) use nanoporous biosilica materials such as silicon chips with an array of gold electrodes and instantaneous label-free electrochemical properties. It can be determined from human serum samples. For devices with the potential for point-of-care protein biomarker detection, they can be used as a biosensor platform with significant sensitivity (1 pg/mL) and selectivity (Lin et al. 2010).

It has been discovered that the biosilica frusta from the diatom *Coscinodiscus concinnus* can be used as an optical biosensor with high sensitivity and low-level detection (LOD) of 100 nM. These new systems could be beneficial for laboratory particle applications. Gold nanoparticles were integrated into a biosilica-based ultrasensitive surface-enhanced Raman spectroscopy (SERS) immunoassay to detect interleukin 8 (IL8) in the human blood (De Stefano et al. 2009; Kamińska et al. 2017). Diatom biosilica was used to make ultrasensitive immunoassay biosensors with increased fluorescence spectroscopy and imaging, which are valuable attributes for biosensing applications with advantages (Squire et al. 2018). In another study, diatomaceous earth biosilica was used to fabricate nanoplasmonic sensors using in situ growth of silver NPs (based on SERS). These sensors can be used for various purposes, including medical and biomedical research (biomolecule identification), food detection and quality monitoring of air and water (Kong et al. 2016). Optical imaging and magnetic resonance imaging can benefit from porous silica-based NPs (Calfon et al. 2011). Herr et al. examined fluorescence intensities of dye-doped silica-based NPs and diaptamers only and found that aptamers manipulated with dye-doped silica-based NPs exhibited superior brightness and stability compared to diaptamers alone (Herr et al. 2006). The main advantage of these nanoparticles was that the silica matrix prevented dye fading, enabled long-term imaging of tumour cells and was suitable as a highly sensitive biosensor (Santra et al. 2001). It has been demonstrated that diatomite NPs paired with a nontargeting siRNA may be localised in H1355 lung malignant cells for more than 72 h using Raman imaging (Managò et al. 2018). Due to their low systemic toxicity, chemical/thermal stability and cost-effectiveness, these NPs can be used as potential anti-cancer therapeutics and nanovectors. Aptamer-bound NPs were prepared for tumour cell extraction and fluorescence imaging (Medley et al. 2011). Researchers used fluorophore-doped silica-coated magnetic and silicon-based NPs to detect and isolate malignant cells (Wu et al. 2015). The hormone N-terminal pro-B-type natriuretic peptide (NT-proBNP), a well-known cardiovascular disease biomarker, was detected using diatom frustules as fluorescent imaging immunoassay platforms. The glass slides inhabited by diatoms were aminated with glutaraldehyde (GA) for surface functionalisation and APTES for amination. The reaction of aldehyde functional groups with amine groups occurred with anti-NTproBNP, antibodies and bovine serum albumin (BSA). BSA inhibited the remaining active aldehydes and restricted non-specific binding. The antibody binds to the antigen and creates a sandwich structure for the antiNT-ProBNP (FITC) fluorescent tag. You can then evaluate the fluorescence and report the detected event (Squire et al. 2019).

12.2.4 Diatoms in Tissue Engineering

Biosilica materials are used for in vivo and on bone healing (Saos-2 cells) because it is highly stable and biocompatible because of their unique properties, such as being morphogenetically active and assisting in the mineralisation of osteoblast-like cells (Granito et al. 2017; Venkatesan et al. 2015; Wang et al. 2012). The procedure for preparing microcapsules of β-tricalcium phosphate (β-TCP) encapsulated in a polymer (D, lactide coglycolide) alone or combined with silicate-in or silica is depicted in the image (Wang et al. 2014a, b). In comparison to those containing simply β-TCP, silica-containing microspheres increased Saos-2 cell adhesion. Following that, as a source of biosilica, biocomposite and chitosan were created for bone tissue engineering utilising the lyophilisation approach resulting in chitosan-biosilica composite scaffolds which were highly porous (Tamburaci and Tihminlioglu 2018). The wet chitosan-diatomite composite scaffolds' compression moduli were comparable to that of chitosan alone. Biosilica and polyphosphate have previously been used because they affect osteoblasts in morphogenesis. Their effects have been assessed in differentiating mesenchymal stem cells and human pluripotent stromal cells (hMSCs) encapsulated in biocompatible plant polymer alginate beads (Wang et al. 2014a, b). Induction of both biosilica and polyphosphate into osteogenic cells resulted in increased mineralisation, morphogenetic protein 2 expressions of bone

(BMP-2) and increased expression of alkaline phosphatase. The type I and type II expression of collagen differed depending on whether they were exposed to polyphosphate or biosilica. Osteogenic cells express a lot of collagen type I. In chondrogenic cells, the level of collagen II transcript was found to be higher than the osteogenic cells. When combined with morphogenetically active inert alginate polymers, polyphosphate and biosilica can be used to transfer in 3D printing of human mesenchymal stem cells and fractures (Wang et al. 2014a, b). As we know, Chitosan-coated diatoms are biocompatible and can absorb fluid and hemostasis influence, which help prevent bleeding (Feng et al. 2016). Chitosan/dopamine/diatom biosilica composite beads have desirable biocompatible hemostasis potential. Dopamine was used as a bioglue to combine chitosan and diatom biosilica. The system is built on a porous structure that can quickly absorb large amounts of water and stop the bleeding immediately (Wang et al. 2018). A unique and ecologically friendly calcium-doped biosilica system for water absorption has been established to integrate calcium into the diatom frustules of *Coscinodiscus* sp. with excellent competence and biocompatibility. In vivo, it can also consolidate blood clotting pathways, accelerate blood clotting and manage haemorrhages more quickly (Li et al. 2018).

12.3 Conclusion

In recent years, diatoms have emerged and become popular for various applications in several fields such as drug delivery, nanotechnology, biosensors, bioimaging, photodynamic therapy, biophotonics and molecular filtrations. Diatoms have 3D nanostructures with unique hierarchical porous structures with large surface area, tailorable surface modification, excellent biocompatibility, chemical stability and distinctive optical and photoluminescence capabilities that are difficult to obtain with synthetic silica. Besides, the generation of diatoms took place in genetically controlled cellular processes, hence, providing scope for synthesising various new-fangled biomaterials via biotechnological aids. Porous and mesoporous silica-based nanoparticles are most famous for drug/gene delivery due to their advantageous properties such as high biocompatibility, adjustable surface chemistry, rate of drug loading, solubility and release profile and, foremost, their cost-effectiveness. However, future studies should target their renewability, enhancing bioavailability, biodegradability analysis and occurrence of plausible toxicity.

Furthermore, they have been highlighted for their probable application in biotechnology, ecological monitoring, phytoremediation of heavy metals and hazardous pollutants, biofuel production and CO_2 fixation. With such broad applicability, it has a high chance of industrial level cultivation. However, for the industrial level requirement, it should be studied meticulously to improve and eliminate any undesired characteristics.

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Chapter 13
Recent Advances in Biomedicine: Diatomaceous Applications

Vivek Narkhedkar and Kavita Bramhanwade

Abstract At global scale, biomedical industry being one of the major drivers of economic and social development requires continues advancements to cope up with the changing needs of the society. Consequently, the future demands that the growth and development in present time must not compromise with the needs of future generation. In this context, the applications of microbes in the medicine sector become inevitable. The recent advances suggest the prospective sustainable utilization of diatoms in drug delivery, hemorrhage control as biosensors, anticancer agents, antimicrobial, etc. The uniqueness of diatoms by the virtue of its silica-based nanostructure has offered a great opportunity for efficient applications in medicines. Moreover, diatomaceous properties viz. nano size, chemical inertness, thermal stability, porous nature, and modifiable surface makes diatoms promisable tool for interdisciplinary applications. Another significant aspect of diatom utilization is its eco-friendly nature. Being the natural cosmopolitan microbe, diatoms have overcome the environment contamination problems posed by the use of chemicals. In this chapter, an attempt has been made to present the significant improvement in the utilization of diatoms in biomedical applications.

Keywords Diatoms · Surface modification · Biomedical application · Drug delivery · Biosensor

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P. Srivastava et al. (eds.), *Insights into the World of Diatoms: From Essentials to Applications*, Plant Life and Environment Dynamics,
https://doi.org/10.1007/978-981-19-5920-2_13

13.1 Introduction

The social and economic trends of the Anthropocene are now diverted towards sustainable development to cope with the changing health and climatic scenario. Various interdisciplinary approaches are now being employed to address this vital issue for attaining sustainable growth.

For example, a significant research input in biotechnology, in terms of capital and time, has helped humans to explore the microbial potential for consumption. Moreover, industrial microbes' uses and the improvised techniques had supported excellent gain on capital input. Evidently, the published scientific literature in this century reflects diverse applications of microbes, such as yeast, bacteria, and microalgae in research (Sharma et al. 2021).

Certainly, owing to its silica-based shell walls, many scientists have gained attention towards the fine architecture of microalgae, such as diatoms. The advances in microscopy have revealed the amazing characters of these microorganisms. Currently, material research is focusing on the utilization of their potential as nanomaterials (Ragni et al. 2017). Unlike artificial nanomaterials, biological nanostructures are a worthy choice due to their lower impact on the environment, scalability, greater structural reproducibility, and low cost of production (Ragni et al. 2018). In the living world, from microbes to vertebrates, many organisms produce minerals which in turn contribute to the development of features such as teeth, shells, exoskeleton, and bones. This phenomenon of biomineralization involves more than 62 biominerals, chiefly phosphates and calcium carbonates, ferric hydroxides/oxides, and silicates (Kumari et al. 2020). In contrast to synthetics, at physiological conditions, biomineralization allows obtaining nanostructured materials showing superior properties without the requirement of high pressure and high temperature for its synthesis (Livage 2018).

The silica micro-/nanoparticles offer numerous advantages compared to other nanomaterials by the virtue of their properties like chemical inertness, particle size, ease of surface modification, greater surface porosity, biocompatibility, and thermal stability (Albert et al. 2017; Chao et al. 2014; Maher et al. 2018; Rea et al. 2017; Simovic et al. 2011; Terracciano et al. 2018). The artificial synthesis of mesoporous silica utilizes toxic chemicals, needs advanced skill, generates nonrecyclable by-products, etc. (Maher et al. 2018; Delasoie and Zobi 2019). As a solution to these issues, Morse (1999) proposed the production of mesoporous silica by employing natural microbe, diatoms.

The biomedical uses of diatoms and research in pertaining areas have gained a lot of research interest since the last decade (Panwar and Dutta 2019). The very differentiating character of diatom is the frustule, a distinctive three-dimensional structure of its shell (Tramontano et al. 2020). Once the frustules are obtained from living algae or fossil remains, called diatomaceous earth (DE), it offers a unique possibility for drug delivery, theranostics, micro-/nano-devices, and many other medical applications (Delasoie and Zobi 2019). The traditional drug delivery system is limited by many aspects such as uncertainty in solubility, poor targeting, high

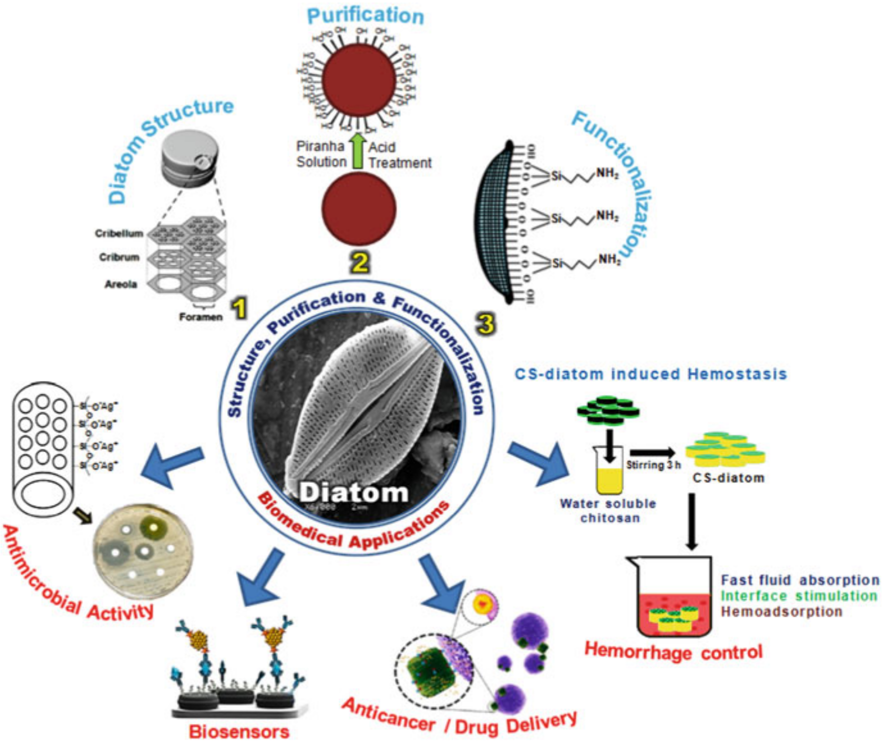

Fig. 13.1 Overview of diatom structure and its biomedical applications. (Modified after Rabiee et al. 2021; Ragni et al. 2018)

toxicity, and less stability. In this scenario, the natural biomineralization process is considered as a role model for the elaboration of artificial materials and competent drug carriers (Maher et al. 2018).

In this chapter, we attempt to enlighten the recent advances in biomedicine by emphasizing the potential application of the diatom biosilica-based system. This chapter is purely based on the recently published scientific literature. This approach deals with the structure and modification of diatom silica, the function of diatom-based nanoparticles (NPs) in drug delivery, and the role of biosilica in advanced medical applications (Fig. 13.1).

13.2 Diatom or Diatomite Silica: Structure, Purification, and Surface Modification

13.2.1 Diatom Structure

Diatoms are unicellular eukaryotic algae, which are cosmopolitan in aquatic environments having considerable biological, ecological, and geochemical effects on the development and sustenance of life on earth. Diatoms are microscopic nanofabrication industries that employ simple self-assembly procedures to produce complex porous 3D silica shells called a frustule. The frustules exhibit various shapes such as triangular box, drumlike, circular box, and many more. Although frustules show different kinds of shapes, there are also some common characters like the void pill-box structure formed by two overlying valves closed together by girdle bands. The primary role of silicaceous porous shell is physical protection nevertheless; external characters of frustules exhibit multiple properties like nutrient sieve, antibacterial, optimized energy, and light-harvesting functions and movements (Maher et al. 2018).

Diatom frustules have numerous distinctive structural, mechanical, optical, excellent biocompatibility, photonics properties, and high surface area with micro-/nanoscale porosity that ensures its utility for a wide range of applications. Diatom frustule's hollow porous microcapsular structure makes them perfect for the improvement of micro-/nanodrug carriers for a range of medical treatments like microrobotics and theranostics (Rea et al. 2017; Maher et al. 2017). This biomaterial can be procured in two possible ways: firstly through cultivation, harvest, and isolation of frustules from living diatoms and secondly, via mining DE or diatomite (Jiang et al. 2014). DE is a significant tool for diverse applications in fields like drug delivery, nanofabrication, molecular separation, biosensing, energy storage, chromatography, water purifications, etc. (Maher et al. 2018).

13.2.2 Purification

The raw DE minerals possess impurities like alkaline metals, alkaline earth, organic materials, and iron that could obstruct the properties of frustules essential for a specific purpose. In the industries, DE is processed by crushing, purification, and separation based on the size. In crushing, milling equipment is used to get finely crushed DE with sub-micrometer to several micrometer particle size frustules and broken pieces. Later, the purification step is done to eliminate the unwanted impurities. It involves both chemical and physical purification methods.

The physical purification procedure involves burning the impurities, i.e., calcination of the raw DE at high temperatures (greater than 600 °C). But, such calcination of raw DE consists of toxicity and is thus not suitable for commercial uses. While the chemical methods are preferable as they utilize the acid treatment of a raw

DE in a hot medium, herein, it is noteworthy to mention that the time required for acid leaching treatment is greatly reliant on the source of raw DE (that includes a type of impurity) (San et al. 2009). Such treatment makes the purified frustules appropriate and secure for biomedical uses (Terracciano et al. 2018). The purification process developed by Rea et al. (2017) is based on crushing, sonication, and filtration. These steps, in turn, help to get NPs for drug delivery uses. Later, for purification, a piranha solution (10% hydrogen peroxide, 2 M sulfuric acid for 30 min at 80 °C) was employed on the nanopowder along with 5 M hydrochloric acid (overnight treatment at 80 °C). The techniques such as dynamic light scattering (DLS) along with SEM and TEM confirmed the porous nature and nanometric size (300 nm) of the powder. Furthermore, after the purification treatment, EDXS analysis, FTIR spectroscopy, and photoluminescence confirmed the enhancement of the silica nanopowder (Aw et al. 2011).

13.2.3 Surface Functionalization

Intrinsically, the purified DE surface presents the potential of the DE surface for the generation of novel bioengineered materials for applications in biomedicine. The hydroxyl group constituted by the silica over the DE surface could be used to gain functionalized chemical modification strategies. The most familiar method of surface modification is based on functionalizing the reactive silanol (SiOH) groups on diatom surface with reactive species (e.g., -SH, -CHO, -COOH, and -NH_2). These modifications built concrete bonding sites for the arrest of chemical or biological entities such as sensing probes, aptamers, enzymes, antibodies, proteins, DNA, drugs, etc. Along with that silanization is another popular method which forms the covalent Si-O-Si bonds for stable binding of various active entities on the diatom surface. After that surface modification with the required terminal chemical group arrests various biological moieties like nucleotides, antibodies, etc. This enables improved drug loading for biosensing and drug delivery. The commonly used methods for arresting active biomolecules on the surface of chemically modified diatomaceous earth include both covalent and non-covalent interactions. The key issue while employing the non-covalent interaction is dependence on the solution, such as a change in ionic strength or pH may change the bond strength. Thus, it may lower or strengthen the non-covalent bond's stability. Therefore, covalent binding is more preferable in terms of reproducibility and stability (Maher et al. 2018; Tramontano et al. 2020).

13.3 Biomedical Applications of Diatom Silica

13.3.1 Drug Delivery Systems

The need of the hour for the pharmaceutical industry is the delivery of specific drug at a defined concentration to target in the human body, wherein causing minimal or no side effects on the healthy tissues/part of the body (Ferrari 2005; Wagner et al. 2006). The choice of the perfect drug is limited due to some undesirable limitations such as uncertain solubility, less stability, high toxicity, poor targeting, and, thus, side effects. Consequently, recent years have witnessed a rise in research and developmental activities pertaining to advanced drug delivery systems (aDDS). Unlike traditional drugs, the enhancement in the physicochemical properties of advances achieved in the aDDS system has overcome the different barriers (Yan and Chen 2014).

The potential of diatom's biological structure for the construction of raw material was realized by Morse (1999). He suggests that the precision in biological silica synthesis is genetically controlled and far from the competence of human engineering. The first attempt at deliberate use of diatoms as a delivery system was shown by Rosi et al. (2004). The report demonstrates the controlled loading and release of gold nanoparticles from the DNA-functionalized surface of the diatom. The available reports also suggest diatomaceous microcapsules as efficient carriers in both implant and oral applications (Ragni et al. 2017).

In recent drug delivery reports, antihyperalgesic effects of ibuprofen were demonstrated (Janicijevic et al. 2018). The delivery system used aluminum salt-modified diatomite for carrying the drug ibuprofen. The modified system showed greater drug loading ability and increased drug release in in vitro conditions. Unlike pure ibuprofen, in vivo experiments on rats confirmed the enhanced efficiency of the ibuprofen-diatomite complex for the same doses. In another report, natural silica obtained from diatom *Amphora subtropica* was used for doxorubicin drug delivery (Sasirekha et al. 2019). Results revealed that, unlike artificial nanomaterials, *Amphora* frustules are the superlative alternative for drug delivery. Furthermore, studies on lung cancer cell line (A549) showed low toxicity and persistent drug delivery.

It is established that drug quantity and the approach of encapsulation govern the efficacy of the drug delivery system. A recently published report suggests the enhanced efficacy of porous carriers in drug encapsulation. Wherein biogenic silica from frustules of *Cyclotella* sp. was employed for encapsulating Isorhamnetin with the help of a silicon microfluidic device (Mancera-Andrade et al. 2019). The frustules showed 48% drug release in the first hour and the residual drug release in the later 3 h. In 2019, curcumin drug delivery was achieved by diatom modified with polydopamine (Uthappa et al. 2019). It was proved to be an efficient catalyst for dye degeneration and systematic drug release. The results reported that the diatom-curcumin-polydopamine-ligand folic acid complex could serve as an excellent system for drug delivery in tumor therapy. Additionally, the diatom-polydopamine complex was modified with silver NPs for anionic (Congo red) and cationic (methylene blue) dye degradation.

Recently, an inventive approach for drug delivery over cancer tissues expressing transcobalamin (II) receptor present in the colon was published (Delasoie et al. 2020). The delivery system utilized in this approach was based on the vitamin B12-modified diatom frustules. Moreover, microparticles could be photoactivated to produce free radicals or carbon monoxide, which in turn stimulated apoptosis in the tumor cell.

In cancer treatments, Kabir et al. (2020) reported to devise self-assembled micro-/NPs from diatomaceous earth to overcome the failure of the combined chemotherapy and multidrug resistance. They employed dual delivery of chemotherapeutic drugs and supersede the antagonistic effect by taking different molar ratios of drugs. Saxena et al. (2021) loaded the curcumin drug onto the *Thalassiosira weissflogii* frustules. The study indicates that unlike acidic conditions, the rate of drug discharge was quicker at physiological conditions. During cell viability testing, they observed no lethal effects of curcumin-loaded biosilica, while toxicity was observed against human renal adenocarcinoma cell lines.

The applications of diatom silica structures for nucleic acid delivery in gene therapy are also astonishing. Thus, consequently, small interfering RNA (siRNA) delivery is now recognized as a successful approach for cancer treatment. The pioneering attempt in using diatoms for delivery of siRNA constitutes a binding of poly-D-arginine peptide/siRNA complex to diatom with APTES (3-aminopropyltriethoxysilane) surface-modified (Rea et al. 2014). The delivered siRNA acts to suppress the expression of a cancerous gene, known as gene silencing. Eventually, this leads to cell death (Maher et al. 2018; Tramontano et al. 2020). Similarly, Martucci et al. (2016) reported amino-silanization-modified diatomite nanoparticle for siRNA delivery to lymphoma cells. The kinetics of internalization of diatomite nanoparticles deduced via Raman imaging explained the cytosolic location of nanoparticles bound to RNA interference (Manago et al. 2018). It was supposed that internalization occurred through endosmosis, and NPs co-existed in lipidic vesicles.

13.3.2 Biosensors

Recent reports suggest the probable exploitation of biosilica for getting a variety of biosensors (Rabiee et al. 2021). The low cost of making and efficiency in filtration are suitable choices for biosensor designs. Such perceptive devices possess a biomolecular detection element associated with a transducer, capable of inducing a signal with respect to the altering concentration of the target molecule to be sensed. As the porous nature of frustules could be optimized, it has the ability to integrate into frustule-specific sensing chambers of biosensors. This would help to achieve a selective transport of the molecules. By virtue of its enormous refractive property, frustules magnify signals and therefore could be utilized as fluorescent probes

Table 13.1 Some recent studies on diatom-based biosensor applications in medicine

Sr. No.	Diatom species	Methods used	Biomedical application	References
1.	*Chaetoceros* sp.	Iron oxide NPs	Differentiate breast cancerous and normal cells from each other	Esfandyari et al. (2020)
2.	*Amphora* sp.	APTES (3-aminopropyltriethoxysilane)	Detection of bovine serum albumin	Viji et al. (2014)
3.	Diatom biosilica	Photonic crystal-enhanced fluorescence imaging immunoassay	Clinically relevant detection of N-terminal pro-B-type natriuretic peptide (NT-proBNP) and the facile screening of heart failure	Squire et al. (2019)
4.	Biosilica	Gold NPs integrated into biosilica-based ultrasensitive surface enhanced Raman spectroscopy (SERS) immunoassay	Interleukin 8 detection in human blood	Kaminska et al. (2017)
5.	DE biosilica	Silver NPs	Biomedical studies for molecular detection	Kong et al. (2016)
6.	*Amphora* sp.	Covalent immobilization of *Salmonella typhi* antibody onto the crosslinked diatom substrates via glutaraldehyde	Diagnosis of typhoid	Selvaraj et al. (2018)

(Mishra et al. 2017). Here, Table 13.1 exemplifies different examples of biosensors in medicinal use.

13.3.3 Tissue Engineering with Biosilica

Silicon plays a very significant function in osteogenesis and bone maintenance. It stimulates mineralization and enhances osteoblast cell functions. Abnormal long bone and bone deformation is often linked to silicon/silica deficit (Le et al. 2016).

In nature, diatomite serves as a rich and cheap source of natural silica that possesses applications in regenerative medicines (Maher et al. 2018). By the virtue of biocompatibility and greater stability, biosilica is utilized in on-site and in vivo bone repair (Rabiee et al. 2021), where silica shells of the diatom *Thalassiosira weissflogii* were utilized to augment propagation and linking of human osteosarcoma cell line MG63 and L murine fibroblasts. Two drugs, first antibiotic ciprofloxacin and second antioxidant cyclic nitroxide 2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO), were used to modify the diatom silica surface. The antibiotic was supposed to heal allied bacterial contamination, while the antioxidant avoids inflammation by dealing with the reactive oxygen species. After the incubation period as compared to the control, results confirmed enhanced cell viability for both cells (Cicco et al. 2015). Moreover, for bone tissue engineering, the production of diatomite and chitosan biocomposite as the origin of biosilica was achieved by the lyophilization technique (Tamburaci and Tihminlioglu 2018).

13.3.4 Hemorrhage Control Using Biosilica

In hypovolemic shock, unrestrained hemorrhage if not cured early could result in death. Currently available hemostatic drugs have many limitations, such as the drug QuikClot zeolite may consequently cause tissue burning because of high heat that may reach 95 °C. Alternatively, silica diatom agents are nonimmunogenic, noncytotoxic, and cheap hemostatic drugs that are reported to surmount limitations (Feng et al. 2016). Unlike zeolite, no heat generation was observed in silica diatoms due to greater plasma absorptivity.

Feng et al. (2016) coated two purified frustules, commercially available diatomite and lab-cultured diatoms, with chitosan in various concentrations. Later, modified frustules were tested for in vivo blood clotting and in vitro hemolysis. Results revealed that in contrast to uncoated diatom, chitosan-coated frustules showed insignificant hemolysis. Conversely, unlike QuikClot zeolite and gauze in rat-tail amputation, much-reduced blood clotting time was attained for chitosan-coated frustules. Furthermore, the chitosan-dopamine-diatom biosilica complex showed appropriate biocompatibility for hemostasis, where dopamine acts to fasten diatom biosilica with chitosan and developed porous structure leads to quick hemostasis due to adsorption of a high amount of water (Wang et al. 2018). In another case, *Coscinodiscus* sp. frustule-derived calcium-doped biosilica having many hemostatic properties was obtained (Li et al. 2018). In a rat-tail amputation experiment, it was reported that modified frustules of Ca-biosilica have strengthened the blood coagulation process. The quicker hemorrhage potential of modified frustules was attributed to the rich calcium and silanol group interface. The main advantages of Ca-biosilica were simple eco-friendly preparation process, efficacy, and superior biocompatibility.

13.3.5 Anticancer Effects

The treatment of cancer is continuously evolving. Diatom-based drug delivery of therapeutic agents is showing much potential for applications. Moreover, some of the modified diatom-based agents reported having effective anticancerous activity, such as marennine, oxylipins, polysaccharides, monoacylglycerides, haslene lipid, chrysolaminarin fucoxanthin, fatty alcohol esters, stigmasterol, and adenosine (Hussein and Abdullah 2020).

In diatom *Skeletonema marinoi*, isolated monoacylglycerides showed cytotoxic activities by activating 3/7 apoptotic pathways in hematological and colon cancer cell lines (Miceli et al. 2019). Lauritano et al. (2016) cultured different diatoms in variable conditions. Later, the extracts were checked for any potential activities. Eventually, antibiofilm activity against *Staphylococcus epidermidis*, anti-inflammatory activity, and anticancer activity was found in six diatom species. It was noteworthy that experiments on normal human cells showed a nontoxic nature against all the six evaluated diatoms.

13.3.6 Antimicrobial Effects

The natural diatomaceous earth is an excellent compound material against microbes. It is believed to be effective for water treatment (Sherief et al. 2021). The diatom extracts showed antibacterial activities against Gram-positive as well as Gram-negative bacteria (Lauritano et al. 2016). Moreover, NPs synthesized from diatom *Skeletonema* sp., *Chaetoceros* sp., and *Thalassiosira* sp. were reported to have antibacterial properties against *Aeromonas* sp., *Streptococcus pneumonia*, *Staphylococcus aureus*, *Bacillus subtilis,* and *Escherichia coli* (Mishra et al. 2020). In the conducted study, silver NPs were supplemented over a diatom surface to prepare the silver-diatom NPs complex. Such modified complex was reported to possess more antimicrobial potential against *Aspergillus Niger* and *Staphylococcus aureus* than diatomaceous earth (Sherief et al. 2021).

13.4 Conclusions and Future Prospect

In recent years, diatoms were proven to be a cost-effective natural source of nanostructured silica. Diatom's porous frustules having properties like greater surface area and easy chemistry of the surface proved its value as a cheap substitute for the improvement of the multifunctional silica system. The current research trend on diatoms concludes that chemically modified and biofunctionalized frustules have potential future aspects in biosensing, drug delivery, cell proliferation, and adhesion. Moreover, the benefits of modified frustule's silica surface also include biomedical applications such as increased drug solubility, enhanced biocompatibility, and more drug encapsulation at the specific target site. However, prospects of diatom research should include biodegradability and long-term studies for possible toxicity, although current studies found no or negligible toxicity. More research should be undertaken to explore other areas of research by virtue of its cheap and eco-friendly nature.

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Chapter 14
Perspectives on the Ecosystem Services and Need for Conservation of Diatomite and Diatomaceous Earth Landscapes for India

Harini Santhanam

Abstract Diatoms, diatomaceous earth, diatom-rich sediments and sedimentary rocks, diatomaceous ooze, as well as diatomites form crucial parts of global landscapes providing significant mined diatomite resources for industrial and commercial applications. While the presence of huge reserves at present and low quantities used globally deem this an "inexhaustible" resource, excessive and/or persistent mining will give rise to many socioecological complications of the regions where they are located and need futuristic assessment, monitoring mechanisms and policy frameworks to ensure sustained usages avoiding land degradation. Sustainable resource management of diatom-based resources also have the potential to contribute to conjunctive water use operations through landscape management and the maintenance of natural infrastructure promoting eco-resilience. The present works provide a comprehensive discussion of the above points related to the conservation and usage of diatomite resources, with special emphasis on the Indian scenario. Field-based, technology-based, and people-based interventions are described in detail as means for achieving national targets of Sustainable Development Goals (SDGs) 6, 14, and 15.

Keywords Diatom · Diatomite · Diatomaceous earth · SDG 6 · SDG 14 · SDG 15 · MGNREGS

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P. Srivastava et al. (eds.), *Insights into the World of Diatoms: From Essentials to Applications*, Plant Life and Environment Dynamics,
https://doi.org/10.1007/978-981-19-5920-2_14

14.1 Introduction

Diatoms are important parts of the coastal and marine biogeochemical cycles, which contribute to the aquatic productivity being benthic drivers of stoichiometric changes especially in the shallow water ecosystems. Diatoms have been critically linked to ecological endpoints and human beneficiaries through biological processes of production and consumption via the ecological production function (e.g. Rhodes et al. 2017). By the virtue of their siliceous shells, live diatoms provide the various ecosystem services (ES) during the lifetimes. For example, diatoms are the producers in aquatic ecosystems; they function as prey to higher organisms, facilitating trophic interactions under nutrient-abundant and/or limited conditions. Marine and coastal diatoms such as *Biddulphia* sp. provide special regulating services in the form of buffering of the CO_2 to prevent ocean acidification and simplification of coastal food chains (e.g. Harvey et al. 2019), enhancing the coastal eco-resilience to natural habitat changes (e.g. Santhanam et al. 2018).

Both natural and anthropogenic processes impact the ES of diatoms, which are present in living states in the ecosystem (Vidal-Abarca et al. 2016). For example, changes in the riparian condition negatively impacts the provisioning and regulating ES of diatoms in aquatic ecosystems. Anthropogenic pollution above critical loads impacts their abundances and existences critically, even when they exhibit pollution tolerance (e.g. Mutinova et al. 2020). The ES delivery of diatoms is also intricately linked to that of the other biotic organisms such as the fishes and higher crustaceans (e.g. Brown et al. 2021). Apart from their provisioning ES, diatoms can also contribute directly to special ES as well-recognized indicators of habitat changes as well as for the movement of water masses (Lebour 1930; Nair 1960) in transitional water environments. For example, the *Biddulphia* sp., known for its mesohaline preference (Madondkar et al. 2007), could also be an appropriate 'proxy' for the salinity shift during high-impact events (especially in a bloom condition; Santhanam et al. 2018). Under this perspective, the window of natural salinity shifts in diatom-dominated tropical lagoons is important from two standpoints: one, that concerns the in situ biota, and the other, in relation to the overall trophic quality of the environment. It is important to remember that the nature of salinity changes can be quantitative, i.e. dilution of the amount of salt in the water column or, say, a reversible increase. The diatoms that comfortably thrive through these active phases of salinity shifts can well be the "indicators" of such quantitative changes undergone by the ecosystem. However, the cumulative effects of such shifts over a period of time can only be inferred from the changes in the populations of those species which translates, in ecological terms, to lesser standing stock, lesser primary production and substantiated oligotrophicity that portend a decrease in the environmental quality. Recently, the regulating ES of diatoms has also been highlighted through their contributions to the microphytobenthos biofilms in the sediments, which is a thin photosynthesising layer of the intertidal flats. These diatom-rich biofilms provide various critical ES such as nourishment of the food web and enhancement of sediment stability (Andriana et al. 2021). On a different note, Galović et al. (2017) reported the high suitability of Croatian diatomites as inert insecticides, reflecting the wide range of ES that diatoms and diatomite contribute toward.

After their death, diatoms settle down at the bottom of the aquatic ecosystems and are known as “diatomaceous earth” (DE), which transformed into diatomite “rocks” and are mined for variable uses as filters in industrial and domestic processes. Being natural materials, DE is also considered as a safe or neutral substance for food-grade or allied usages. Thus, they contribute to both provisioning and regulating ES of both the aquatic and land ecosystems where they are present in either live or dead statuses. These single-celled diatoms occur abundantly at both the present-day benthic environment and were common in many ancient environments as well. The skeletons of the diatoms are made up of a delicate lattice of silica (SiO_2), and each species has a distinctive skeleton, settling to the bottom of the water body (Wallace et al. 2006), forming the diatomic environment and landscapes. The ubiquitous nature of the diatomites originating in both freshwater and marine environments provides a source of the “rocks” mined for industrial uses. However, the relevance of the formation of the DE landscapes needs to be understood in detail.

DE landscapes and the presence of biogenic sediments form a critical link between the ancient waterscapes and the uniquely transformed terrestrial landscapes of the present times. The processes of conversion of a diatom-rich water body into diatomite-rich landscapes, formed over periods of thousands to millions of years, are particularly transformed over the geological periods of the Miocene, the Plio-Pleistocene, and during the early to middle Holocene (Lindqvist and Lee 2009; Owen et al. 2009; Flower 2013). Many global examples document the conversion of a diatom-abundant ecosystem into the DE landscape. A typical example pertains to a report on the freshwater diatomites found in the Kožuf Mountain area, where DE is reported to have been formed over several million years during the changeover from the mild and wet early Pliocene climate in the region to drier in the late Pliocene (Boev et al. 2019). Volcanic activity in the Kožuf Mountain area was reported to be closely related to the distribution of rare earth elements in the diatomite and tridymite. The formation of DE landscapes is quite unique considering the present example, where the Tikveš Pliocene lake had formed on the periphery of the large Kožuf Mountain volcanic activity zone. Precipitation is collected in the natural depressions that originated due to geological and geomorphological changes in the aftermath of the volcanic activity. The availability of nutrients such as phosphorus and extensive pyroclastic materials which flowed into the lake provided a nutrient-rich landscape for the establishment of a diatom-abundant water ecosystem. These further transform into variable landscapes in ecological successions, producing a thick layer that eventually formed diatomite found in the Vitačevo sedimentary lake sequences (Reka et al. 2018). The diatomite that results from such transformations is reported to be very fine-grained sedimentary rocks with a chalklike appearance, rich in silica, finely porous, and with a low density and high porosity. Greater saturation of silicate microfossils, siliceous substances, sediments, biofilms, and other clastic substances and further compression and lithification over several years give rise to the diatomite rocks (Inglethorpe 1993). The origin and distribution of the diatomite resources is explained in Zahajská et al. (2020) with several examples.

14.2 Diatomite-Rich Sediments and Landscapes Impart Natural Eco-Resilience

The fact that DE are known to be the natural filters, being good absorbents and sequestrating media for the several substances, providing ligand sites for cationic and anionic exchanges, makes its source rocks as natural landscape facilitating the filtration and preservation of freshwater resources. As shown in Fig. 14.1, there are many similarities in the biofiltration characteristics of modern constructed lake ecosystems with the diatomaceous landscapes. The design of the former system fitted with slow sand filters, diatomaceous earth fittings as porous, permeable layer in the bottom of the lake along with planted bed filtration mechanism which are used as blue-green infrastructures draws a huge parallel with the natural filtering capacities of the wet or dry diatomaceous landscapes, where the intercalated layers of diatom-rich sedimentary rocks, gravel, sand, and layers with diatomaceous ooze provide a natural mechanism for filtration, which is perhaps the largest-scale of operation possible for capture, harvest, and storage of the natural precipitation in the form of rain or snowmelt.

In general, it is known that the hydrological cycle is uniquely linked to the geochemical and geomorphological changes in a landscape. Studies have established this link through a variety of depositional versus evaporation cycles indicated closely by the presence or absence of diatoms in the ecosystems investigated. For example, Bellanca et al. (1989) provide a detailed description of the minerology and isotopic geochemistry of the formations of Miocene-age outcroppings in southeastern Spain where beds of economically-significant quantities of diatomite reserves were found to be alternating irregularly with carbonate bed rocks. The results indicated the impacts of the seasonal changes on the biostratigraphy of the diatomites in variable depositional conditions, which had resulted in the overall deepening of a lake (Bellanca et al. 1989). More recently, Sánchez-González et al. (2021) highlighted that the ecosystem-level response to rise in aridity in geological periods resulted in the substitution of planktonic species in the water with benthic species dominated by diatoms as an eco-resilience measure for natural preservation of the lacustrine ES. Such studies prove that the presence of diatomite landscapes plays a critical role in water conservation, preserving the hydrological services of a region as well as in regulating the biogeochemical cycling of nutrients.

Globally, much attention is being provided toward promoting conjunctive water management policies, which not only help in the local as well as regional level water conservation and sustainable management but also in preventing the degradation of the quality of the land and aquatic ecosystems by regulating hydrological cycles. The UNESCO (2020) definition of Conjunctive Water Management (CWM) is as

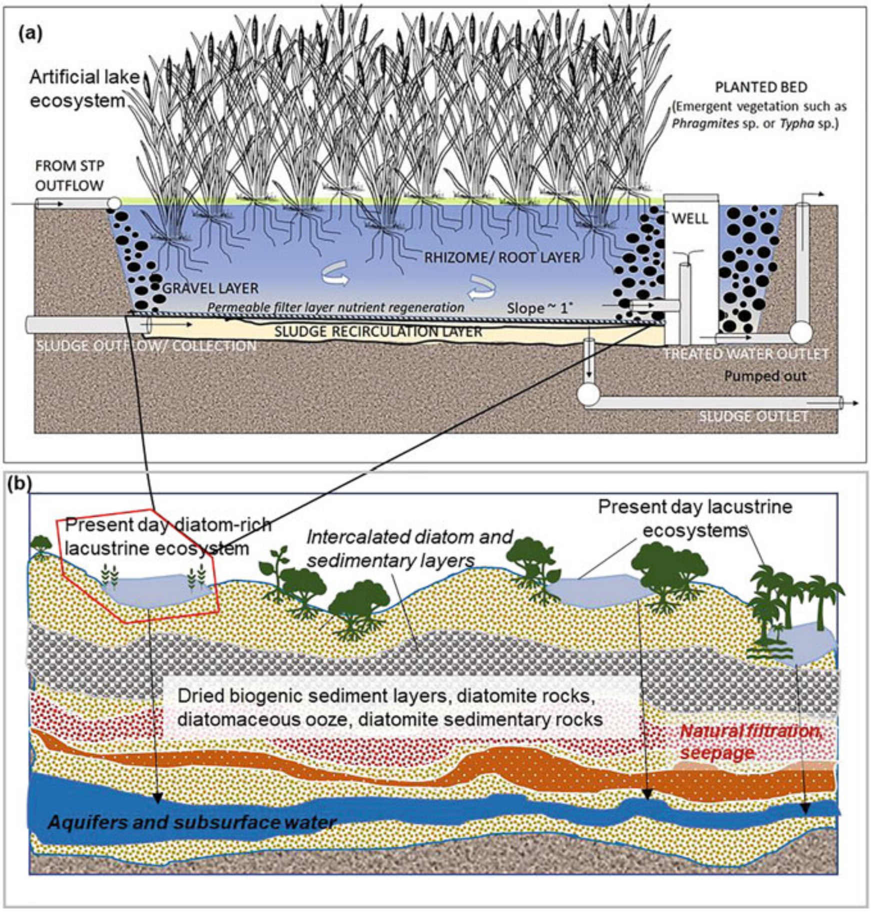

Fig. 14.1 Illustration of the natural filtration and water conservation services of (**a**) a constructed planted bed ecosystem for treatment of wastewater and (**b**) a whole diatomite landscapes with diatom-rich sedimentary layers and diatomite rocks as nature-based blue-green infrastructures

follows: "*Conjunctive Water Management is an approach to water resources management in which surface water, groundwater and other components of the water cycle are considered as one single resource, and therefore are managed in closest possible coordination, in order to maximize overall benefits from water at the short and at the long term*" (Van der Gun 2020). Further, it has been stated that "*Any approach to water resources management that takes the linkages within the water cycle systematically into account may be called Conjunctive Water Management*".

Thus, CWM can be practiced by two phases of action: (1) maintaining the hydraulic connectivity in the natural water cycle through direct linkages with the ecosystems and (2) regulating the human water use chain indirectly by attaching sociocultural values to the landscapes and waterscapes. The above discussions highlight the unique roles of DE landscapes in not only preserving and facilitating the hydraulic connectivity (regulating ES) but also as an important socioecological landscapes providing nature-based solutions (NbS) for large-scale harvesting and storages of rainwater (provisioning ES), preventing soil erosion by improving stability (Special ES), preventing shallowness of lacustrine environments, preventing eutrophication by sequestrating excess nutrients (especially N and P; regulating ES), and providing socioeconomic benefits for dependant human communities either as diatomites (provisioning ES) as cultural microfossil environments for research (cultural ES). Through all these functions and processes, the DE landscapes are crucial to promote the water recharge contributing positively to SDG 6 targets, preserving/enhancing storage capabilities of marshlands and/or inlands as a part of the CWM plans (SDG 6 and 14), as well as promoting the eco-resilience of the region to land degradation and/or desertification (SDG15), where these landscapes are prevalent.

14.3 Perspectives Related to Diatomite Mining in Global and Indian Scenarios

It has been reported that globally, the United States of America (USA) is the world's leading producer and exporter of diatomite, where more than 800,000 metric tons are mined annually, and approximately 100,000 metric tons were reported to be exported by 2011 (Crangle 2018; Source: https://www.earthmagazine.org/article/mineral-resource-month-diatomite/). About ten exclusive mining areas in California, Nevada, Oregon, and Washington provide the diatomite resources for seven companies to produce the products for industrial applications in USA. In the same country, the filtration markets were reported to be the major consumers (~67%) of all domestically mined diatomite in 2011 (Crangle 2018).

With respect to India, in the mainland region, the major diatomite reserves are distributed in Gujarat, Rajasthan, Tamil Nadu, and Andhra Pradesh. In the Andaman and Nicobar archipelago, the islands of Camorta and Trincat are known to possess diatomite resources. The official estimates of the resources are available as of April 2015 have been reported to be about 2.89 million tonnes, largely distributed in Rajasthan (72%) and Gujarat (28%) in India (IBM 2019). However, these DE landscapes have not been mined for diatomites except for siliceous earth without biogenic sediments or microfossils (since 1991–1992 as per the data reported in IBM 2019). These regions are also not marked for any futuristic sustainable mining operations, providing a huge scope for India to assess and enhance diatomite mining activities without the loss of these natural landscapes.

Recently, Zahajská et al. (2020) highlighted the lack of understanding and classification of diatomite-related resources, mentioned by different names in the scientific and industrial literature as diatomite, diatomaceous earth (DE), diatomite-rich sediments (DRS), diatomite sedimentary rock, and diatomaceous ooze. The differences in the percentages of the diatomaceous silica seem to be reported differently in different scientific and nonscientific contexts related to sedimentology, quaternary science, industrial applications, and mining. The respective definitions for the diatomite resources also rely on the percentages (usually varying between 50% and 95% or 99%) to refer to the respective diatomite-based resources. The apparent confusion in such related terminologies and definitions makes it difficult to associate the estimates of the resources or plan the appropriate sustainable resource management perspectives. These issues are compiled and highlighted here for the first time in Fig. 14.2.

Global reports have highlighted the fact that exploitation of minerals like diatomite can result in serious consequences such as accelerated sedimentation and siltation, vulnerability to eutrophication, over-enrichment of iron, and loss of the lacustrine ES (e.g. Jørgensen et al. 2005). In the 1970s, the mining for diatomite in Lake Myvatn (Iceland) was linked to the increase in the rate of deposition of iron by a factor of 32% by the end of as reported by Jónasson (1979). The diatomite excavation also has increased the phosphorus loading rates into the lake by 20%. More seriously, the nitrogen loading was found to have increased by more than 90%. The eutrophication caused high incidence of *Anabaena* sp. blooms, resulting in the complete loss of the lake ES. Recognizing the dangers of rampant mining for biogenic sediments and diatomite, a Lake Conservation Act, 1974 for the Myvatn area had been promulgated to check the loss of the ES. Thus, it is important to recognize the values of the diatomite landscapes and to promote natural resource management options for utilizing these resources sustainably. As a first step, the need is to produce a futuristic sustainable natural resource management plan for diatomite landscapes, whereby conservation of both the present-day diatom-rich sediments of the benthic environments and the dried diatomite landscapes with rock deposits is provided with adequate attention.

As shown in Fig. 14.2, the environmental risk assessment for DE landscapes and DRS is quite essential to understand the resource usage statuses and the index of sustainability. For example, the study of the status of land degradation corresponding to the Sustainable Development Goal 15 (Life on Land) is an essential part of understanding the appropriate usage criteria for DE landscapes (Fig. 14.3). While in previous sections, the impacts of anthropogenic mining processes leading to degradation of the DRS/DE landscapes have been described, it is quite tedious to perceive their indirect links as causes for land degradation, unless the hydrogeological processes that are manifested due to the excessive mining activities can be established as in the case reported by Jónasson (1979) for Lake Myvatn. Such studies linking the significance of DE landscapes to fulfilment of national and global targets of SDG 15 (preventing desertification) are quite important to address the interlinked socioecological issues underlying mining of DRS and DE. Thus, environmental risk assessments become critical components to assess and quantify the ES values of DE landscapes.

Further, from a policy perspective, the DE and DRS are presently considered as 'infinite' resources (IBM 2019), which is not practical since the exploitation of the DE resources will tend to lead towards their depletion. Hence, value assessment in

Current Perception (D)	Environmental risks (R)	Data and Policy intervention needed (P)
Diatomaceous 'rocks' are the sources for Diatomaceous earth (DE)	Disconnect in sourcing DE and DRS as per the ecosystem which bear them, their corresponding land degradation, hydrogeological statuses, link to the overland flooding and prevalent ecological processes	Reliable data synthesis on the DE resources from the Natural Resources Management (NRM) perspective Need to place eco-restorative options for heavily mined regionsin future (e.g. Rajasthan)
Diatomaceous earth sources (rocks and ores) are found on land	Most originate on dried wetlands or marshlands with no or less resemblance to their original landscapes	Understand the wetland/ marshland characteristics of DE landscapes in the perspective of land-use before mining
DE resources are infinite (e.g. IMY 2018)	In the absence of research/data, it may not be correct to assume the resources are infinite and may lead to unsustainable mining especially in futuristic scenarios	Need present and futuristic policies to regulate the sustainable mining options with mandatory Nature-based Solution (NbS) assessment and valuation

Fig. 14.2 Definitions, current perceptions, environmental risks associated with diatomite resources, and the need for targeted policies

terms of their NbS values to CWM, as well as on preserving sustainable land-use practices, must be considered while planning NRM activities for DE landscape conservation. Policy interventions, which will provide plans for sustainable

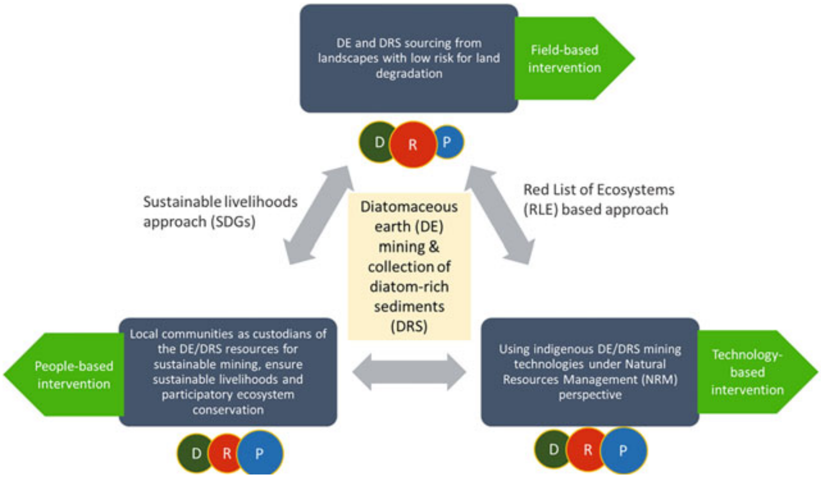

Fig. 14.3 Some perspectives and stages for sustainable management of diatomite landscapes

extraction of DE or DRS, are present lacking and are required to be formulated for futuristic scenarios. While DE remains to be inexpensive options for industrial filtration needs, as well as in clarification of oils, etc., their end usages are not yet part of the mainstream discussions as the presence of absorbed substances, including toxins and heavy metals, on industrially used DE needs to be disposed of securely. These aspects provide large concerns in the proper utilization of the DE and DRS materials.

From the perspectives of intervention for sustainable management of DE landscapes, three types of interventions become possible: (1) field-based intervention, (2) technology-based intervention, and (3) people-based intervention. Under field-based intervention, the policies must be targeted toward allowing the mining of DE/DRS landscapes, where such activities can cause low environmental risks. For example, extensive mining in desert landscapes (Rajasthan, for example) may contribute to higher incidences of soil erosion which need to be addressed through estimating the optimum extraction rates for the next 100 years. Such targeted policies with potentials for conservation of the DE landscapes will not only contribute to the preservation of the ES of diatomites but also improve the field-based awareness about DRS from wetlands and marshlands. Field-based interventions and monitoring would be crucial to assess the conditions of the ecosystems in the landscapes as per the Red List of Ecosystems protocol provided by the IUCN. These inputs would be useful to prevent the land degradation in advance (Keith et al. 2013, 2015; Rodríguez et al. 2015).

From the technological perspective, the intervention must focus on the use of indigenous and traditional DRS/DE mining methodologies which ensure sustainable extraction rates. The communities benefitting from the commercial use of these resources must also associate with the indigenous methodologies at local scales, despite the availability of industrial-scale mining methodologies. This will deter the overexploitation of the diatomite resources and at the same time improve the regulatory and cultural ES values associated with them. Policy perspectives in this regard need to be explored in detail.

In the third method of intervention, the public participatory framework must be established through Sustainable Livelihoods Approaches (SLA), which provide opportunities for the local communities to take ownership of the DE resources, to manage their sustainable utilization, by "allowing" sustainable extraction for industrial usages. Policies for enhancing the awareness about the DE landscapes among the resident human communities must be made mandatory to provide a channel for their participation in the sustainable development at local scales as custodians of these landscapes. Further, policy implementation can be promoted through incentivizing the sustainable resources' usage through social schemes for natural resource management. One such scheme is the Mahatma Gandhi National Rural Employment Guarantee Scheme (MGNREGS) in India, which promotes unskilled laborers to contribute toward the creation of water assets through activities such as pond rejuvenation (e.g. Dhyani et al. 2021). These social schemes provided assets of high conservation values in the past decades for rural communities in exchange for employability and social security. Conservation of DE landscapes can be practically implemented through co-management with local communities, citizen scientists, and traditional researchers.

14.4 Conclusions

The present work highlights the significance of the diatoms, diatomite landscapes, and diatomaceous resources in providing multiple ecosystem services for human communities. Exploring their significance in the context of sustainable land development activities as well as water conservation and in promoting eco-resilience, the present work provides insights on the undervaluation as well as the issues surrounding the perception of these resources from mining perspectives. Although currently termed inexhaustible, preservation and conservation of the diatom-based natural resources is critical from several socioecological points of view discussed in the present work. Thus, the insights from the study provide the scope for the development of futuristic targeted end-policy intervention mechanisms to promote sustainable use of the natural resources. Finally, the significance of DE landscape conservation presented in this study provides the impetus for the diatom-based scientific investigations to explore and adopt resilience building of coastal ecosystems in India.

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Chapter 15
Biofuels from Diatoms: Potential and Challenges

Jyoti Verma, Akriti, Hemlata Pant, and Ambrina Sardar Khan

Abstract Biofuel is the hope of this planet to ensure safe and sustainable use amid increasing rate of pollution and global warming. The term biofuel may be misleading for some that it is only substitute of fossil fuels, which is not true. Biofuel is a broad term including bio-oil, biodiesel, bioethanol, biogas, etc. finding its use in transportation, cosmetics, cooking, nutrient supplements, etc. The generation of biofuel from diatoms is third-generation biofuel production. Either the lipid from diatoms is extracted as bio-oil or the whole biomass of diatoms is used as biocrude. Apart from the mainstream uses of biofuel, the diatom culture produces many by-products which find their use in multiple fields. The public authority of India reported Biofuels Policy in 2008 to advance its production and utilization. The main obstacle on the way is economic production of biofuel from diatoms, to make it worth choosing over other options. Diatoms grow fast but they produce lipids slow, and the process of extraction is even more tedious. However, with the use of recent technologies, proper management and planning, this method proves to be the most efficient and environment friendly way of biofuel production. Diatoms don't have a large number of cells in the body to support, they use carbon dioxide and other nutrients from waste, or eutrophied water bodies produce biofuel and clean their nearby environment in return. It is high time we develop this method to make it feasible for greater good.

Keywords Biofuels · Diatoms · Microalgae · Hydrocracking · Sustainable energy

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P. Srivastava et al. (eds.), *Insights into the World of Diatoms: From Essentials to Applications*, Plant Life and Environment Dynamics,
https://doi.org/10.1007/978-981-19-5920-2_15

15.1 Introduction

Biofuel is a kind of fuel (liquid or gaseous), produced over a short time span from the biotic component of our environment rather than very slow natural processes involved in the formation of fossil fuels. It could be derived from plant, algae material or animal waste. These materials could be replenished readily, making biofuel a source of renewable energy. The word fuel associated with biofuel could lead to assumptions, that it could be used only for transportation purposes. Biofuel's advantage is not limited to one field only but also extended to energy generation, heat production, charging electronics, clean oil spills and grease, lubrication, removal of paint and adhesive, as cooking oil and there are many yet to be discovered to utilize their full potential.

15.1.1 Diatoms

Diatoms, a significant component of phytoplankton, are microalgae. They contain silica-based tiny shell walls. The lineage is traditionally divided into two orders: radially symmetrical centric diatoms, or centrales, and bilaterally symmetrical pennate diatoms, or pennales. The first order is further classified into polar and non-polar centrics, whilst the latter order comprises the classes Bacillariophyceae and Fragilariophyceae based on the presence or absence of a raphe; each group emerged and developed progressively over the Mesozoic era when CO_2 levels decreased. Diatoms displaced a substantial amount of other algae (mostly cynobacteria and few green algae) in the ocean throughout the Mesozoic era, according to the fossil record (Hildebrand et al. 2014). They evolved around the Jurassic Period. They have the ability to biosynthesize a variety of commercially valuable chemicals.

Each year, living diatoms generate 20% to 50% of the oxygen produced on the Earth, absorb about 6.7 billion metric tons of silicon from the waters in which they dwell and account for nearly half of the organic material present in the seas (Treguer 1995). Diatoms are natural nanotechnology factories that have been discovered in the fossil record for over 100 million years. Diatoms are less sensitive to turbulence in the ocean than any other phytoplankton (Wyatt 2014).

Diatoms also produce other useful items such as semiconductors, health foods (glucosamine) and chitin fibres. These incredible microscopic algae will be able to absorb some of the cheapest, most abundant elements on Earth—like silicon and nitrates—and make a steady stream of affordable products with nothing more than sunlight, practically any sort of water and carbon dioxide (Rorrer 2012).

15.1.2 Biofuel from Diatoms

During the vegetative period of growth, diatoms create oil drops that are kept intracellularly as a reserve material, with percentages ranging from less than 23% to greater than 45% of dry cell weight. Membrane-bound polar lipids, triglycerides and free fatty acids are a variety of lipids found in diatoms. Other substances discovered include sterols, waxes and acyl lipids. The concentration varies by species, depending on the availability of nutrients and other growth requirements. Lipid fractions can range from 70% to 85% in some species, whilst 15% to 25% is more common. Diatoms are particularly promising for biofuel production because of (a) their widespread prevalence and competitive advantage over other microalgae, (b) their rapid growth, doubling their biomass every few hours, (c) their growth could be controlled by availability of silicates and (d) almost all of their biomass could find profitable use (Wang and Seibert 2017).

Bio-oil is a type of oil derived from diatom lipids that can be enhanced by transesterification and other methods. **Biocrude** is natural crude, such as oil, that has been thermochemically transformed from diatom biomass (Wang and Seibert 2017) (Fig. 15.1).

Diatoms are a good contender for a 'drop-in' fuel replacement. There are two ways to obtain biofuels from diatoms or any other microalga: (1) direct lipid extraction followed by biofuel processing (which is a common practice) and (2) thermochemical conversion of the entire biomass portion into a biocrude (this is not straightforward but is advantageous over the first one) (Wang and Seibert 2017).

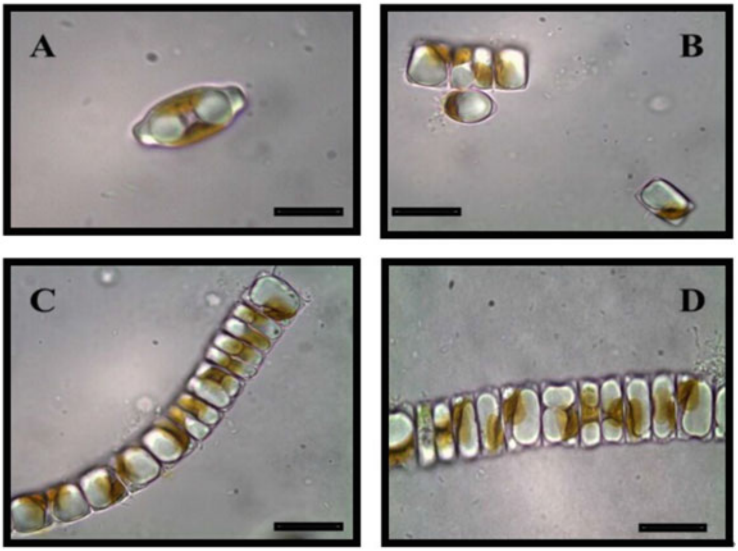

Fig. 15.1 *Diadesmis confervaceae* in solitary and chain forms observed under 100× (oil immersion in III). (Source: Mar. Drugs 2015, 13, 2629–2665; doi: 10.3390/md13052629)

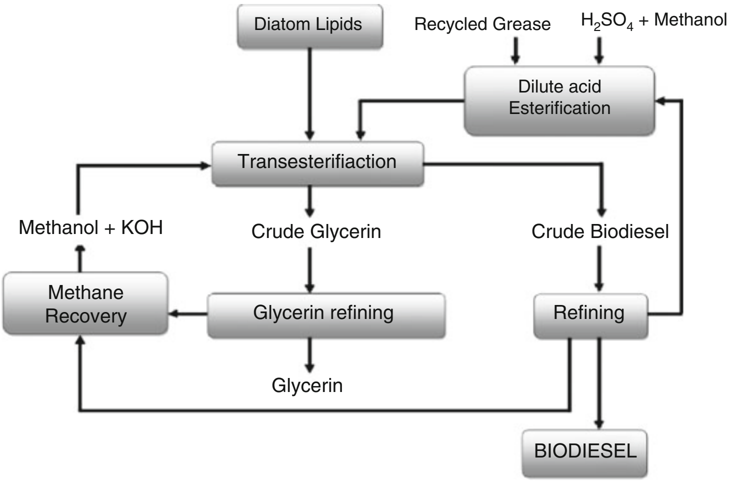

Fig. 15.2 Flow chart showing different fate of diatom lipid. (Source: Ramachandra et al. (2009) 3: 764 ; doi: 10.1021/IE900044J)

Hydrothermal liquefaction (HTL) can utilize all the biomass as a feedstock, regardless of lipid content, and can process wet feedstock directly (without the need for a time-consuming drying procedure). Carbon in various types of biomasses, including carbohydrates and proteins, can be used by HTL. The goal of HTL techniques is to convert carbon to oil rather than extracting existing oil from microalgae (Wang and Seibert 2017) (Fig. 15.2).

15.2 History

Until recently, it was widely assumed that the cost of creating algal biomass was higher than that of producing biodiesel crops. For research purposes, *Phaeodactylum tricornutum* is designated as a model diatom (Graham et al. 2012). Its lipid content is stated to be between 21% and 26% of dry weight, and its doubling time (Td) is 25 h. Cerón-García et al. (2013) evaluated *P. tricornutum's* mixotrophic growth on several carbon sources. Although all diatoms synthesize and store lipids as food reserves, marine diatom species have been examined for lipid content in relation to biofuels (Fig. 15.3).

Weyer et al. (2010) of the Jawkai Bioengineering R & D Center in Shenzhen, China, has achieved sustainable diatom yields of over 120 MT dry weight per hectare per year. The HTL process can convert one-third of the dry weight of diatoms to biocrude which is more than 36,000 L of biocrude per hectare.

A version published by New Zealand's prominent biochemist Chisti (2007) presents algae as an oil producer that is 130 times more productive than soybeans.

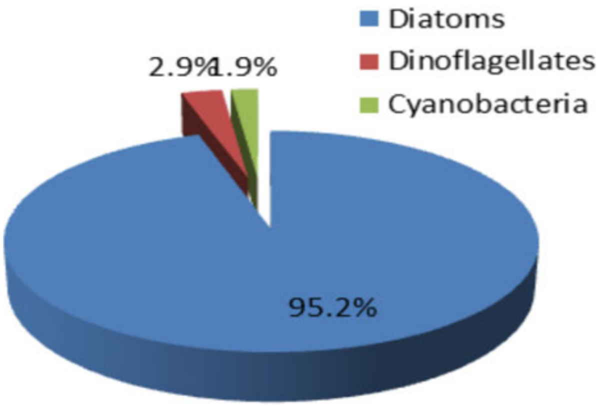

Fig. 15.3 Pie chart showing contribution of different microalgae in biofuel production. (Source: Beetul et al./Biofuel Research Journal 2 (2014) 58–64)

15.3 Methodology

15.3.1 Harvesting

Traditional methods such as **centrifugation**, **filtration** and **flocculation** are very popular as the energy concentrated, not easy to perform, and involve high chemical induction for harvesting process. Natural existing surfactants (as in the case of *Chaetoceros*) produced by the microalgae themselves may deliver a partial result. Similar surfactants will allow the use of froth separation to concentrate diatoms. Csordas and Wang (2004) have shown that froth separation can remove up to 90 of *Chaetoceros* from its culture medium (after diatom discarding, the medium can be reused with applicable treatment).

15.3.2 Breaking the Cell Wall

The approach of producing lipids from diatoms and converting them to bio-oil involves: (a) harvesting cells after the growth period and application of stress conditions to amplify lipid production, (b) destroying cells to extract lipids and then (c) altering lipids configuration to biodiesel by transesterification. Rossignol et al. (1999) stated that the cell breakage is due to the sudden and a rapid transmission of diatoms from a state of extreme elevated pressure (30–270 MPa) to one of low pressure (0.1 MPa). This sudden change is very damaging for diatoms. Their experiments showed significant splintering of *Haslea ostrearia* cells occurred at 30 MPa. Kelemen and Sharpe (1979) in their studies have defined and stated that there is a certain threshold of a pressure that triggers the destruction of 50% of the various microbial cell populations. They have shown that cells do not rupture at a defined pressure; instead, a critical pressure must first be applied before the cells begin to rupture. **High-pressure rapid release (HPQR)** cell destruction methods have been shown to be effective in recovering intracellular metabolites (nucleic acids, enzymes, proteins, pigments, etc.) from diatoms. This technology complements or competes with traditional laboratory-scale technologies such as **sonication or shear-based system** previously used for the extraction of **marennine**, a blue pigmentation that is exhibited by the marine diatom *Haslea ostrearia Simonsen*. Additional methodology of destruction is **to use the application of one or more powerful but very quick and rapid electric field shock to diatoms** suspended in a moderate aqueous solution for facilitating electrical conductivity. If the field is strong enough, many temporary aqueous pores will be created. If there are high concentrations of dissolved ions and molecules in the internal space of diatoms, an osmotic pressure difference will occur. Water from external media would enter the cells and increase the pressure in the diatom's lipid bilayer, the membrane-protected area. The pressure difference must be large enough to destroy the diatoms. The average lifespan of pores is controversial, probably more than a second, so multiple pulses may be required. The temperature rise is generally small (Weaver 2010).

15.3.3 Hydrothermal Liquefaction of Diatoms

This methodology eliminates the necessity for the breaking of cell unit and is considered to be more economical and viable approach for the reduction in the production cost of a biocrude yield process. The dry weight of wet diatom is mostly 80%–90% water because of their large water content, and relatively with low heat capacity, before using in heat, power generation or other biofuel production operations, it should be pretreated. Hydrothermal liquefaction (HTL) equipment functions under subcritical water conditions and is thus well suited to transform wet biomass into liquid energy (Zhou 2015). The innovation is like the regular geographical cycles that prompted the development of unrefined petroleum; however, this happens in minutes rather than a long period of time. HTL involves water as the transporter under sub-basic circumstances to deteriorate the biomass and structure of more important and more limited chain atoms. The immediate treatment of wet biomass by HTL maintains a strategic distance from the need for drying, which ought to fundamentally work on the proficiency of the overall thermal process. Goudriaan et al. (2000) asserted that the biomass thermal processing effectiveness during hydrothermal treatment (HTU) in a 10-kg (dry weight) *h*—1 pilot workshop establishment can be as high as 75. Biocrude is the main produce of the procedure, counting for about 45 of the feedstocks on an ash-free, dry weight base. Biocrude had an advanced heating value of 30–35 MJ/kg, which might be additionally upgraded if desired. Bohlmann et al. (1999) elaborated a similar procedure applying a novel high-pressure microwave oven reactor to reduce the energy consumption of algal biofuel yield. Brown et al. (2010) drew the transfiguration of the oceanic microalga, *Nannochloropsis* sp . into biocrude plus a gaseous produce harnessing hydrothermal processing from 200 °C to 500 °C and a set holding time of 60 min. Similar study by Minowa et al. (1995) recounted a 37% oil production from

Dunaliella tertiolecta (moisture content, 78.4 wt% and carbon content), applying direct HTL at around 300 °C and 10 MPa. To have more understanding of these techniques' efficacy, Aresta et al. (2005) evaluated and compared these various transformation procedures, namely, pyrolysis, supercritical CO_2, hydrothermal and organic solvent extraction, for diatom biodiesel production. HTL was found to be a better and efficient technology than any other known technologies for harnessing biofuel out of diatoms.

The studies reported that by the conversion of fatty acids in biocrude into alkanes, the property and quality of the fuel enhances due to the reduction of the oxygen content of the bioproducts. Previous records stated that the biocrude obtained with direct conversion without utilizing the catalyst was not of good quality and have low commercial values as its properties completely changes. Levine et al. (2011) in an inert setting experimented that all their sampled liquefaction catalysts delivered higher productions of biocrude in *Nannochloropsis* sp .; still, the heating value of the biocrude (ca. 38 MJ/kg) and its essential composition weren't reactive to the catalyst applied, for most of the duration. Nevertheless, in the case of a backed Ni catalyst, the biocrude obtained had untraceable sulfur content. The desulfurization process was, however, exceptional in the case of Ni catalyst. Reports stated that the increased protein and lipid contents in algal dry weight increased the production of biocrude. However, the nitrogen content is completely dependent on the feedstock utilized to extract biocrude. Zhang et al. (2010) found that diatoms, which are N-containing hetero-aromatic compounds and are used for biocrude, managed to increase in intensity with rising resulting temperature and are characterized with 10% of the total known peak area. These substances may be considered as unsuitable outputs as they are structurally stable and are dangerous for the biocrude production at large scale.

15.4 Indian Scenario

The public authority of India reported Biofuels Policy in the year 2008 to advance the creation and the utilization of biodiesel to accomplish an objective of mixing 20% biodiesel with diesel by 2017. Biodiesel can be delivered from palatable, non-eatable oils and fats. The biodiesel creation from eatable oils is unrealistic because of its utilization for consumable purposes and 80% of palatable oils are imported. Non-palatable sources are Jatropha, Pongamia, Neem, Sal and so forth. Microalgae can possibly deliver 1,36,900 L, whilst Jatropha can create 1892 L of oil for every section of land. Microalgae have the most elevated biofuel creation potential, i.e. 15 to multiple times more than earthbound oil seed crops on an area premise (Alam et al., 2012) (Fig. 15.4).

Important factors to be thought about for choosing appropriate microalgae species are cell biomass, macromolecule content, macromolecule quality, rate of growth, response to conditions like lightweight, temperature and nutrient input, and growth medium. Production of microalgal strain and improvement of its growth is the most

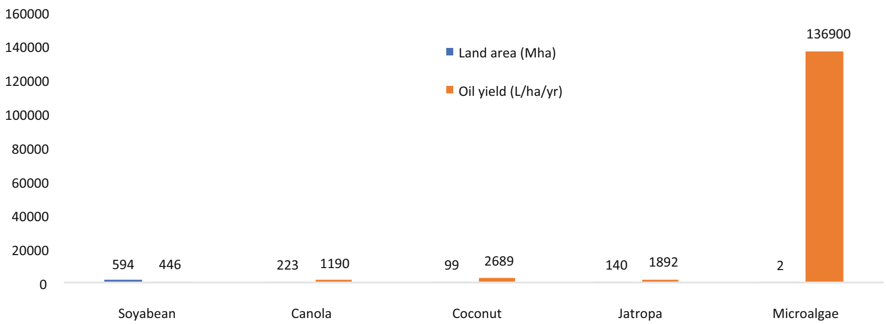

Fig. 15.4 Land required and oil productivities from different feedstocks. (Source: J Integr Sci Technol, 2014, 2(2), 72–75)

significant in determining the political economy of biofuel production. Sudhakar et al. (2012) reported that if microalgae are farmed on 0.06% of the overall expanse, 75 g/m ^{2}/day of protoctist biomass and 35 mL/m ^{2}/day of microalgal oil will be made. Bajpai et al. (2014) also reported that if microalgae are cultivated in <2%–3% of the total expanse, it will fulfil the nation's liquid fuel demand. Robles-Medina et al. (2009) urged that the Sundarbans delta ground of 100 islands (Bay of Bengal) will be used for protoctist cultivation and extraction of biodiesel. Compared to edible and non-edible oil-based biodiesel, the soundness of biodiesel from microalgae is the intercalary advantage, which suggests that the biodiesel remains unchanged in fuel quality for an extended amount of time.

In India, broad work has been done on the use of microalgae for food and drug applications; however, very little work has been done on development, reaping, oil creation, biodiesel change and its use as motor fills. Lately, microalgae have been found to deliver 19,000–57,000 L contrasted with 2000–2500 L of biodiesel per section of land from eatable and non-consumable oils (Kumar and Sharma 2014). Saranya et al. (2018) researched on diatom consortia across diverse lentic and lotic habitats of the Aghanashini estuary with varied levels of nutrients primarily influenced by distribution of flora and fauna to understand the role of environmental parameters and nutrient levels in species composition and community structure. This effort was an essential prelude to phyco-prospecting potential candidates for third-generation biofuel production. Hierarchical cluster revealed highly productive clusters that are capable of accumulating higher lipids under certain environmental conditions over other species. **Regression modeling** was performed to understand the probable lipid productivity potential by integrating physicochemical and nutrient parameter which provided an empirical equation relating lipid and other critical factors affecting lipid content of diatoms. This empirical modeling is capable of providing lipid content details right at the sampling stage without cultivating diatoms under laboratory conditions. Rajaram et al. (2018) considered exploring the impacts of CO_2 on biomass, unsaturated fats, carbon-hydrogen and biochemical gathering of the marine diatom *Amphora coffeaeformis* RR03. The outcome showed that *A. coffeaeformis* RR03 contained high biomass efficiency and biochemical creation in various development conditions. *A. coffeaeformis* RR03 showed the most extreme development of 5.2 × 106/mL on the 21st day of development under CO_2 supply. The bio-unrefined petroleum creation from *A. coffeaeformis* RR03was 36.19 megajoule (MJ). Marella et al. (2017) concentrated on metropolitan wastewater from eutrophic Hussain Sagar Lake to develop a diatom cultivating consortium, and the impacts of silica and the following metal advancement on development, supplement evacuation and lipid creation were assessed; results exhibit the possibility to deliver feedstock for inexhaustible biodiesel creation.

15.5 Potential

Diatoms can ingest CO_2 (alongside certain contaminations all the whilst) and can be exceptionally helpful in advantageous power age/diatom creation, oil refining/diatom creation, or brewery/diatom creation connections. They can eliminate natural NOx from burning gases. At the point when utilized in fluid, fuel creation will leave almost no waste material (toxins) behind. The leftover development medium can be reused. Proteins created (with non-warm handling) are good for animal feed (Fig. 15.5).

The frustules from diatoms (which are covered with nanosized openings), which endure the HTL interaction, are possibly great material for the adsorption of major contaminants from wastewaters coming from mills, industries, textiles, etc., particularly heavy metal; up to 99.9% of the copper can be eliminated from wastewater by

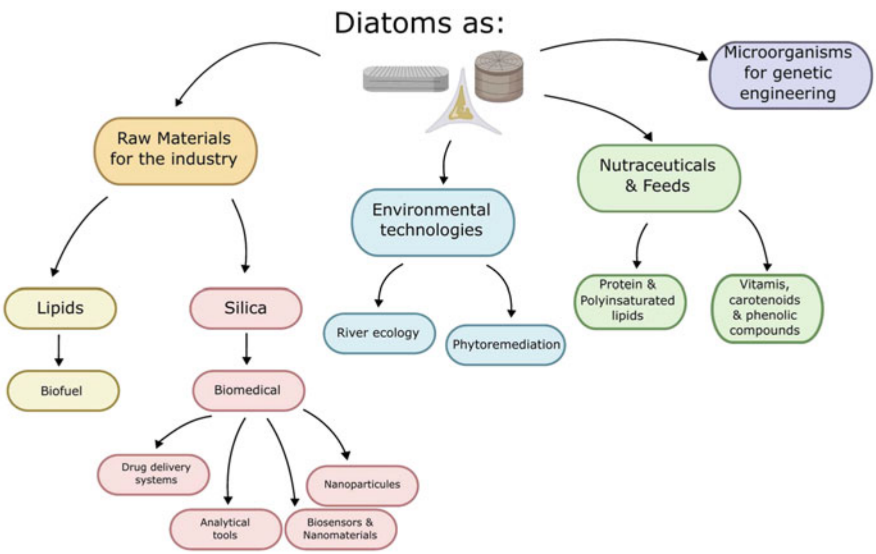

Fig. 15.5 Potential of diatoms in different fields other than biofuel. (Source: Article in Frontiers in Marine Science. February 2021. doi: 10.3389/fmars.2021.636613)

separating the water through diatom frustules according to the study reported by Wang (2015).

Assuming we are to utilize untreated or even treated wastewater to substitute composts, we can save the expense of the manure, and by cleaning up of the wastewater, we ought to likewise acquire another income stream to counterbalance the expense of the biocrude. The capacity to eliminate supplements can likewise be utilized to advantage in the treatment of wastewater. On the off chance that creation productivity, for example, yield per unit region per unit time, is anything but a genuine concern, and if we can match the constituent or the composition of the supplements in the treatment lakes or ponds to that expected by the diatoms, we can diminish the supplement load in the wastewater to near nothing (Wang and Seibert 2017).

This may be used as environmentally friendly fillers for polymers, thus widely employed in our everyday activities, or may be even as materials for composites or metal additive producing. Several potential coproducts embody specific organics like food-grade carotene, prescription drugs and pigments, also as compounds like polysaccharides, carbohydrates, surfactants and different polymers. The alga *Phaeodactylum tricornutum* is cultivated in out-of-door photobioreactors for the assembly of long-chain unsaturated fatty acids that can be used as food supplements (Graham et al. 2012).

Oil (lipids) from diatoms can likewise be utilized to deliver cooking oil in applications where HTL isn't utilized. The top notch of the lipids created will bring about good quality oil for human utilization, and expansive future accessibility ought to assist with the lack of cooking oil, which is a crisis and a serious issue in the coming time. The utilization of food crops for biodiesel creation prompts contest between the utilization of farming area for food creation and its utilization for fuel creation with a subsequent expansion in food expenses and possible environment and biodiversity misfortunes. Diatoms can deliver as much as multiple times the volume of oil per unit of land region, contrasted with business oilseed crops. There are other energy fuel sources; yet sadly, there is not a viable alternative for cooking oils from natural sources (Wang and Seibert 2017).

Diatomaceous earth is a mineral material consisting chiefly of the siliceous fragments of various species of fossilized remains of diatoms. In cosmetics and personal care products, diatomaceous earth may be used in the formulation of bath products, soaps, detergents, cleansing products, face powders, foundations and skin care preparations (https://www.cosmeticsinfo.org/ingredients/diatomaceous-earth).

15.6 Challenges

The extraction of lipids from diatoms is a very tedious process and requires a large amount of labor and attention; apart from this it also requires surplus amount of financial aid (Fig. 15.6).

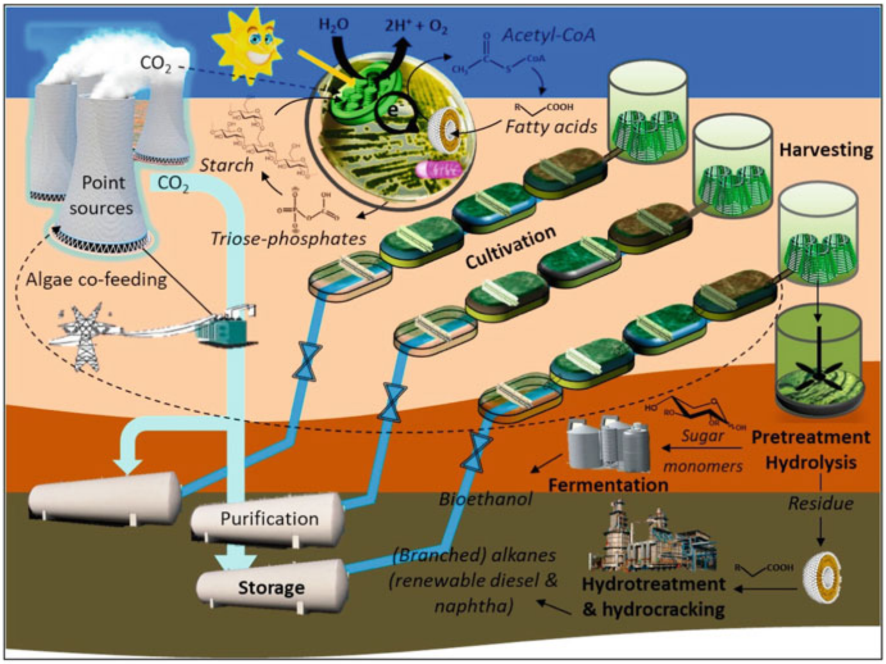

Fig. 15.6 Complex process of biofuel production from algae (diatom). (Source: A review on sustainable microalgae based biofuel and bioenergy production: Recent developments; https://doi.org/10.1016/j.jclepro.2018.01.125)

Even after its numerous benefits over other biofuel substitutes, the economic cultivation and production of biofuel from diatoms have not gained popularity because of the following reasons:

(a) The asexual division in diatoms make them smaller in size.
(b) Grow quick, however, production of oil is slow in them.
(c) Wild alga quickly displaces the alga being cultivated.
(d) Initial capital prices, the necessity to get rid of heat and the critical issue and high price of maintenance activities.
(e) The method of getting careful lipid profiles for microalgae is slow and exhausting.
(f) The major prices of alga production, not reckoning facility investments, square measure fertilizers (including silicate), dioxide and electricity make the method terribly pricey and nearly not possible for industrial production.

15.7 Solutions

The surplus water in the lake will give it a time to reduce the organic load by itself. The excessive water enhances the decomposition of organic content by aerobic decomposition. Further, the city water shall be treated utilizing the diatoms as it enters the aquatic bodies via city wastewater plants. Hence, eliminating supplements and contaminants from the wastewater at the source ought to be a viable and a suitable methodology. This is important since even an efficiently designed wastewater treatment plant eliminates minimal inorganic supplements from city wastewater. It for the most conversion happens from organic into inorganic ones, so cleaning the water would become a significant specialty for diatoms (Wang and Seibert 2017) (Figs. 15.7 and 15.8).

(a) Limited but however continuous replacement of the native algae species underneath culture with the leading economical diatoms from the wild guarantees constant economical production.
(b) When we economically harness algae for biocrude, we are really keen on fixing carbon content and not to harness oil essentially. The entire notion is ought to be intended to produce natural carbon from inorganic carbon. Whether the natural carbon is as oil or sugar or whatever else, HTL can generate the products that we need.

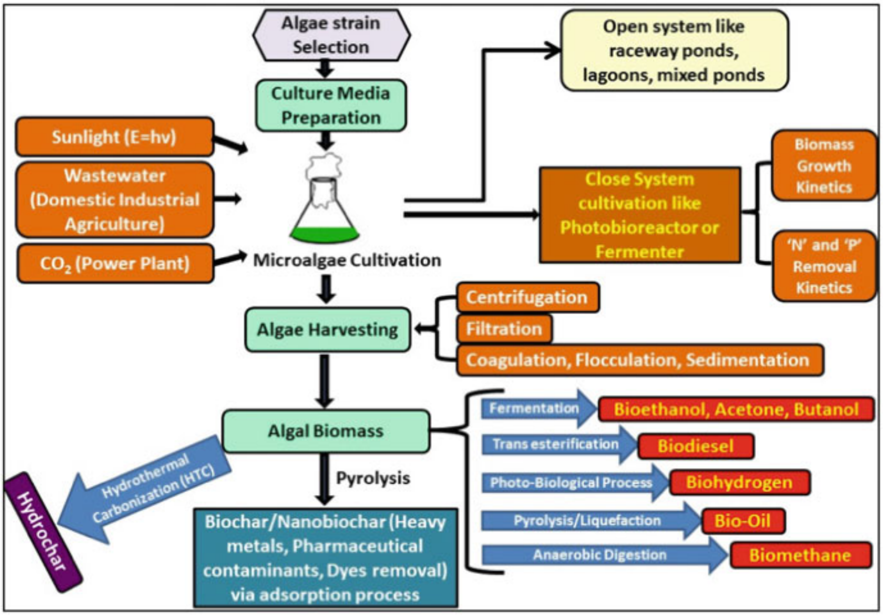

Fig. 15.7 Process and products of an algal (diatom) culture. (Source: Ajay Kumar (2021);249–275 (2021). https://doi.org/10.1007/s42250-020-00221-9)

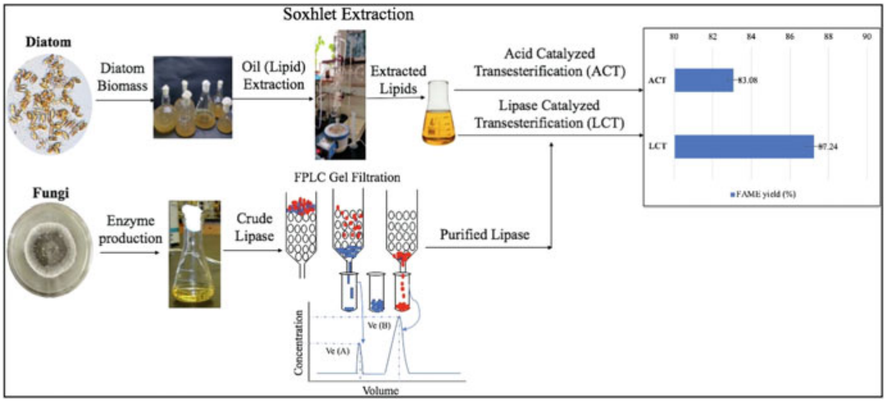

Fig. 15.8 Catalytic upgrade of lipids extracted from diatom. (Source: https://doi.org/10.1016/j.renene.2020.02.053)

(c) Using domestically isolated strains, we tend to additionally avoid the issues that may come back from introducing foreign (invasive) species into native waters.
(d) If the diatom can be maintained in an open system, growing at log phase by limiting, among various contents like the concentration of salt, nutrients, silica, etc., then it will outgrow non-diatom species and thereby retain its dominant position within the production system (*Chaetoceros* sp . a marine phytoplankton has been unendingly cultivated with success in an exceedingly industrial, open-production system in Hawaii, exploitation using the similar technique). The management of aquatic animals that kill diatoms has conjointly been achieved during this system by repeatedly applying stresses (e.g. pH stress), and also the impact of predators will be reduced.
(e) A new technique was reported for the direct, quantitative determination of lipid profiles on living microalgal cells by single-cell laser-trapping Raman spectroscopy. Lipid profiles were reported for single living cells of *Botryococcus braunii, Chlamydomonas reinhardtii* and *Neochloris oleoabundans*, with the time to obtain spectra for fatty acids less than 10 s. **Raman spectroscopy** should make rapid monitoring of mass cultures of microalgae possible for the determination of optimal harvest time (Wu, 2014).
(f) Denitrogenation by catalytic upgrading will help in matching the level of algal biocrude transportation fuel.
(g) To lessen the expense of power (one of the expense issues referenced above), environmentally friendly power, for example, sun based, or wind power could be utilized where fitting.
(h) The modification of a collective or joined heat and power (CHP) plant or an internal heat exchanger network would assist in executing the immediate handling of wet biomass.

15.8 Discussion

Recent engineering life cycle comparisons, however, indicate that linking algal production to wastewater cultivation has the potential to offset many of the environmental burdens of algae biomass production for biofuel feedstocks and outperform terrestrial crops. Wastewater-linked algae cultivation also offers the prospect of effluent bioremediation, with the sale of algal feedstock potentially offsetting increased costs associated with new N and P reduction mandates. The creation of diatoms under subtropical circumstances is significantly more impacted by variation in accessible sun-powered illumination than the changes in temperature, as long as the temperature stays under 36 °C or more 25 °C. Subsequently, they can be delivered in desert regions, where saline water is accessible, and in the mild zone at low temperatures. Diatoms, coincidentally, have been found in the desert and high-altitude lakes. Even in cold temperate climates, algal biomass production can outperform soybean-based biodiesel production if the algal production is carried out in a photobioreactor within a greenhouse warmed by waste heat from an adjacent powerplant (Wang and Seibert 2017).

They are adapted to the high nutrient conditions of hypereutrophic lakes, for wastewater-linked biofuel feedstock production. If diatoms or other algae are to be grown in wastewater effluents as a potential source of biomass feedstock for biofuels and as a cost-effective means of reducing nutrient pollution to natural waters, then the diatoms must be grown to the maximum biomass permitted by the levels of phosphorus and nitrogen in the wastewater effluent. In that event, the diatoms will reduce phosphorus and nitrogen in the effluent before discharge to levels that would not cause eutrophication. A discharge level of less than 50 μg P/l of effluent would be in the mesotrophic range for natural waters and one of $<$10–15 μg P/l would be in the oligotrophic range for natural freshwaters. The cultures grown at the lower silicon levels showed a decline in lipid content as the time in stationary phase increased. It would be useful to determine the optimal time in stationary phase to produce the maximum lipid. In addition to these growth parameters, diatoms sink out of culture readily in the absence of stirring. All non-motile algal cells tend to sink in freshwater in the absence of mixing because their cytoplasm tends to be slightly denser than water. Diatoms sink more rapidly than many other groups of algae because their silicon impregnated cell walls are extremely dense (2600 kg/m ^{3}). The capacity of diatoms to sediment rapidly should be useful in harvesting their biomass for lipids. In diatom lipid production, shape matters, and it may be possible to use diatom cell shape as an indication of the potential to accumulate significant amounts of lipids for commercial application. Ways to deal with creating diatoms in the sea would have various benefits, including defeating the heat intensity challenge and a significant issue in close production frameworks. A close creation framework drifting on the water can scatter heat at almost no expense. As of late, NASA has examined the specialized plausibility of an interesting floating algal growth development and culture system for the coming time. (http://www.nasa.gov/centers/ames/research/OMEGA/). The study has exhibited that their OMEGA framework is

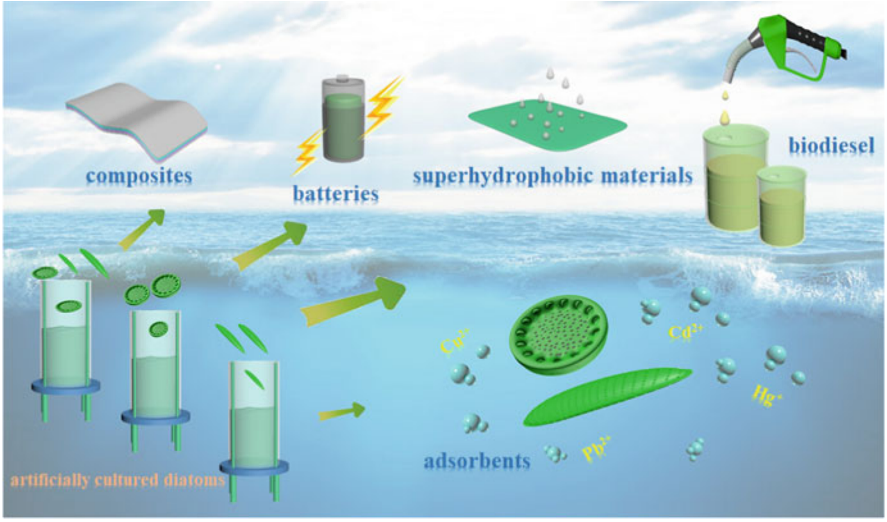

Fig. 15.9 Applications of artificially cultured diatoms. (Source: ES Energy & Environment 2020, 8, 3–4, https://doi.org/10.30919/esee8c486)

powerful in developing microalgae and treating wastewater on a limited scale; however, the monetary plausibility of this plan still needs to be shown (Wang and Seibert 2017).

To be successful, the microalgal growth model should be more like agriculture. Some of the suggestions for higher yield of diatoms are as follows: (a) semicontinuous harvesting (once a day), leading to highest quality light absorption at some stage in the year; (b) most useful strains must be grown during the two (the altering dominant strain have to be acquired, which need to be consistently seed to examine bioreactors; thus, one will be capable to use the most prolific resident strain for the local environments at all instances throughout the time); (c) substantial rheostat of invasive species or strain; (d) acceptable management of aquatic species that forage on diatoms; (e) optimum control or regulated temperature at all times; (f) optimum dietary supplementation have to be there throughout; and (g) diatoms normally in the log segment of their life cycle over prolonged durations of time (Wang and Seibert 2017) (Fig. 15.9).

15.9 Conclusion

The capacity to deliver biocrude, with the consumption of carbon dioxide and utilizing just wastewater or water sources that are undesirable for human utilization and watering system, as well as utilizing barren and waste land, offers nations genuine resource and expectations to produce biocrude in the future for

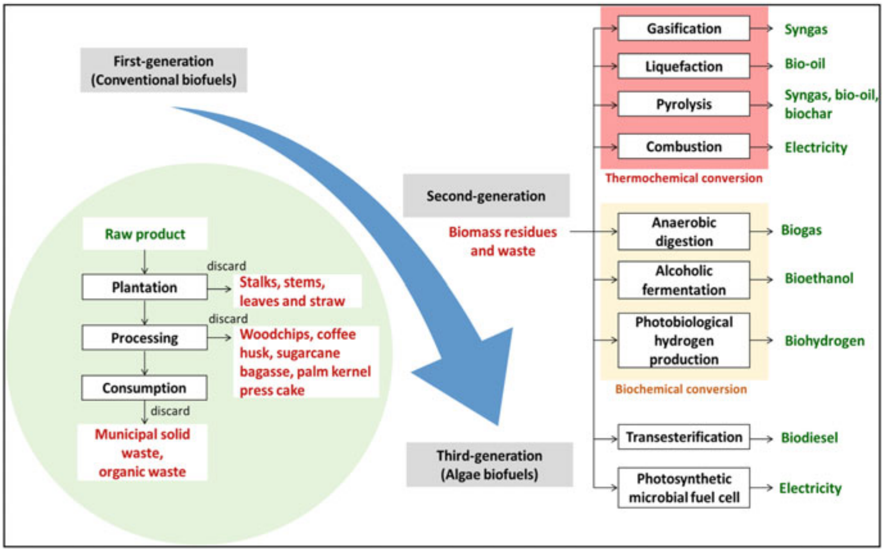

Fig. 15.10 Generation of biofuel using waste as resources. (Source: https://pubs.rsc.org/en/journals/journal/ra)

accomplishing, to some extent, an independence in maintainable fluid biofuel creation (Fig. 15.10).

We should know that the actual significance is of discovering the candy spot between maximizing organic carbon manufacturing and minimizing the conversion costs. This potential that booms price enhancements and organic carbon ought to outweigh oil content in the choice of a diatom species, assuming profitable HTL processing for biocrude production. This has genuine ramifications for strain determination and genetic designing work in all microalgae and bio-oil creation control. Diatoms don't have to help a variety of non-photosynthetic cells as in plants. Besides, on the grounds that they fill in a fluid climate, they are intrinsically more proficient at getting to water, utilizing CO_2 (utilizing a carbon-concentrating component), and breaking down dissolved nutrients. Utilizing CO_2 from substance or power plants, exploiting wind- and sun-oriented power and co-creating a variety of important substances and biofuels, we really want to contemplate on the business creation of diatoms for biofuel age. It is currently time to look again at these well-established issues and attempt back to foster new innovation that can be utilized at a suitable chance to increase diatom industry with authentic probability of success and progression (Wang and Seibert 2017).

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Chapter 16
Potential Industrial Application of Diatoms for a Greener Future

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Abstract The advantage of the complicated, microscopic, and industrially imperative diatoms is not a secret now and has recently astounded the scientific society with their miscellaneous potentiality. It attracts considerable attention due to their success in diverse environmental conditions. Diatoms are highly attractive for industrial applications due to their richness in natural lipids and carotenoids, especially in the field of biofuel, metabolites, and nutraceutical production. The possibility to utilize a diatom cell for industrial application has increased considerably accompanied with the advanced knowledge of microscopy, metabolic pathways, and genetic tools. Commercially it is feasible to perform the harvesting, primary culturing, and further downstream processing of diatom culture. Diatoms with their unique frustule structure, micro- to nanoscale properties, good thermal steadiness, proper surface area, surface functionalization procedures, and eco-friendliness have obtained a huge attention for their application in diverse topics of biotechnology and nanotechnology. In this chapter, an effort has been made to assemble the important development of diatoms in various industrial applications such as metabolite, feed, nutraceuticals, biofuel, pharmaceutical products, and nanostructure production.

Keywords Diatoms · Industrial application · Biofuel · Nanostructure · Metabolite

16.1 Introduction

The biggest challenge of today's world has paced up the scientific community's attention toward the arising issues related to resource limitations. The prime challenges of the world include, but are not limited to, clean water, energy, access to

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P. Srivastava et al. (eds.), *Insights into the World of Diatoms: From Essentials to Applications*, Plant Life and Environment Dynamics,
https://doi.org/10.1007/978-981-19-5920-2_16

affordable medicines, and healthy food. Recently, the International Conference on Key Engineering Materials 2020 researchers addressed the likely challenges in context to material science before the scientists from different areas of research. It was pointed out that innovations are required that rely on biomimetic to find a sustainable way for the production of structural and functional materials. The arguments pertaining to the economic meltdown, global health issues, and current environmental scenario favored the utilization of eco-friendly, renewable, and local resources. Optimistically, the forum promoted the idea that to counter the changing scenario of disrupted global demand and supply chain, such use of resources is inevitable (Kalyaev et al. 2020).

In this context, diatoms by the virtue of their worldwide distribution, a significant role in silicon and carbon recycling, a major share in the ocean's photosynthetic productivity is presumed to be a suitable alternative for today's crisis (Huang and Daboussi 2017; Yi et al. 2017). But conversely, diatoms are the least explored organisms for their utility, although average annual diatomaceous resource availability accounts for 132 MT dry diatoms/ha within approximately 5 years (Wang and Seibert 2017). Alongside, it is noteworthy that diatoms' share in the annual production of oxygen and organic carbon is approximately 20% and 40%, respectively (Treguer et al. 1995; Falkowski et al. 1998; Afgan et al. 2016). Diatoms constitute the rich diversity and dominating phytoplankton community possessing silica-built cell walls called frustules. Since the nineteenth century, this inherent capacity of silicon acquisition marked diatoms as an appealing microbial community (Sharma et al. 2021).

The electron microscopy and advanced genetic tools facilitated the research on frustule structure and confirmed the biochemical processes that constitute acquisition, transfer, and polymerization of silicon (Knight et al. 2016; Zulu et al. 2018). Moreover, this knowledge advancement in metabolic processes and elucidation of frustule structure could be a sustainable key to fabricating a vast range of commercial products, like biofuels, nutrient supplements, ecological tools, optoelectronics, etc. (Marella et al. 2020). Also, diatoms are capable to capture carbon and nitrogen released from different sources. This property could be utilized in waste management and the biofuel industries to generate fuels without carbon (Singh et al. 2017).

In the nutraceutical and pharma industries, diatoms can be explored to produce plant-based proteins, omega, and other important fatty acids (Wen and Chen 2001a, b). The industrial dependence for omega oils on the fishery sector could be reduced by replacing omega fatty acid diatoms. This will also decrease the issue pertaining to the biochemical composition of fish oil, which was arising due to the oceanic contamination and seasonal changes (Martins et al. 2013). The diatoms could also ease the commercial production of fucoxanthin and some other carotenoids, strong antioxidant pigments (Xia et al. 2013). Likewise, this multifaceted applicability of diatoms allows a colossal opportunity for sustainable development that could help to achieve carbon neutrality.

The structure of diatom frustules has provided enormous potential to build up various techniques and tools in biomedical industries (Mishra et al. 2017). The nanomaterials developed from diatom biosilica have major applications in the

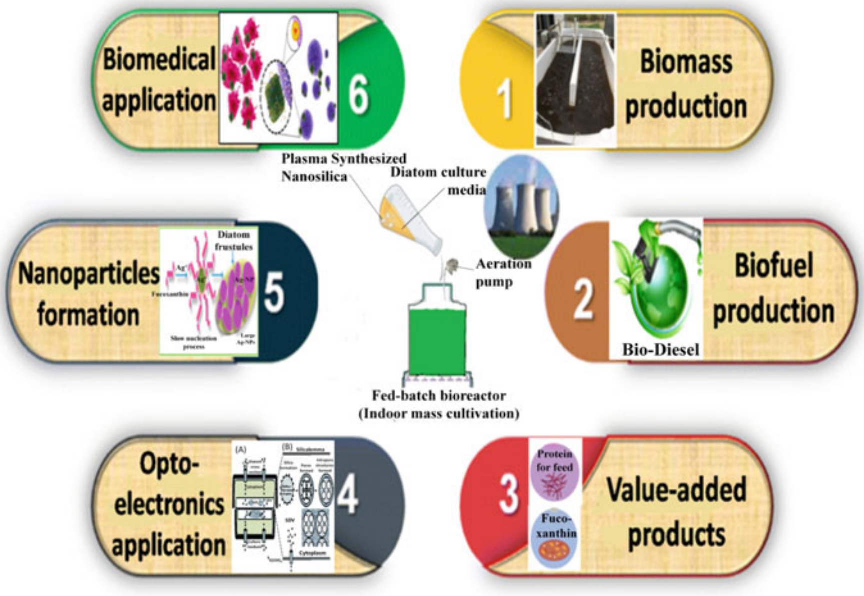

Fig. 16.1 Industrial applications of diatoms. (Modified after Hildebrand et al. 2012; Jayakumar et al. 2021; Popovich et al. 2020; Rabiee et al. 2021; Saxena et al. 2021)

recognition of highly sensitive compounds of biological origin by advanced optical and electronic techniques (Dolatabadi et al. 2011). In a new approach to diatom, metabarcoding has widened its applicability in exploring environmental issues such as algal blooms, acidification, and changing climate (Nanjappa et al. 2014).

In this context, this chapter intends to serve recent development in the potential use of diatoms in the industrial sector (Fig. 16.1). Herein, we attempted to produce inclusive knowledge on a diverse array of diatom applications for sustainable development. Indeed, the exploration of diatom's potential will significantly improve the steps progressing toward sustainable development. This will strengthen the economy along with decrease reliance on nonrenewable resources. Therefore, the need of the hour is to sustainably procure benefits and services from diatoms.

16.2 Industrial Applications

16.2.1 Biofuel Production

The advances in science and technology have created an era of industrialization and globalization. Although it has affected the environment largely, this is now an inevitable part of the human race. Industrialization is dependent on the transportation sector that runs approximately almost completely on nonrenewable energy sources (Rodrigue and Notteboom 2013). In this scenario, finding a sustainable carbon-neutral energy solution is the need of the hour. This will not only reduce the utilization of natural oils but will also shoulder the monitory development along with living in tune with nature. Consequently, in this context, biofuel generation via diatoms will serve as an optimistic solution due to its copious occurrence, cheap processing methods, and fast and handy growth (Wang and Seibert 2017). Diatoms in their vegetative growth phase produce oil food reserve till the arrival of a favorable condition. These oil contents of the oil glands produce more oil than many other oil seeds (Mishra et al. 2017). In comparison, oil yield in corn is 15 times less than that in diatoms; conversely, corn and maize occupy 66 times more land than diatom (Brocks et al. 2003). Also, it was reported that under stress condition diatom yields more oil (Ramachandra et al. 2009). They developed a modified diatom that secrete oil for daily oil extraction rather than oil storage and genetically modified diatom secretes gasoline without extra processing. Consecutively, substation of natural oil by diatom fuel may reduce greenhouse gases.

The cultures of diatom *Thalassiosira weissflogii* and *Cyclotella cryptic* under nitrogen starve condition enhance the lipid yield. Nitrogen deficiency works by affecting the de novo triacylglycerol synthesis and lipid remodeling. In *T. weissflogii* and *C. cryptic*, out of the total glycerolipids, approximately 82% and 88% are triacylglycerols, respectively. This accounts for suitability of diatoms for the large-scale production of biofuels (d'Ippolito et al. 2015), while diatom *Fragilaria capucina*'s ability to accumulate lipids to high level along with different temperature tolerance outstands its potential for biofuel production. Conversely, large industrial scale production is limited by the need for more optimized and improved protocols (Chaffin et al. 2012).

Additionally, high production of biodiesel could be achieved by transesterification of oil obtained from diatom *Nitzschia punctata*. This catalytic process is carried out by an enzyme lipase obtained from fungi *Cladosporium tenuissimum* (Saranya and Ramachandra 2020). In 2019, Popovich et al. reported the suitability of diatom *Navicula cincta* for biodiesel production along with the presence of value-addition compound, i.e., methyl palmitoleate formed by catalytic transesterification. The enhanced biodiesel yield of 81.47% from *Amphiprora* sp. was reported in the culture medium with 2% catalyst and methanol:oil::1.5:1 ratio at 65 °C for 3 h (Jayakumar et al. 2021). Table 16.1 mentioned few recent studies about different types of biofuel production using diatoms.

The study performed using cold-tolerant *Mayamaea* sp. JPCC CTDA0820 was reported to overcome the seasonal constraints for culture and growth (Matsumoto et al. 2017). This diatom was selected for culture in winter-like condition, i.e., at 10 °C. Consequently, the combined culture of winter diatom *Mayamaea* sp. along with *Fistulifera solaris*, diatom from another season, in outdoor reactor showed consistent whole-year production of biofuel. Although the advances in technology endorsed diatom-based biofuel production, available statistics revealed the different constraints of the biosynthetic process. Therefore, mixed form of fuel, i.e., biodiesel along with petrodiesel, would be a suitable alternative. The relative research data of

Table 16.1 shows recently reported studies of biofuel yield by a variety of diatom species

Sl. no.	Diatom species	Type of biofuel	Yield	References
1.	*Navicula cincta*	Biodiesel	97.6%	Popovich et al. (2019)
2.	*Amphiprora* sp.	Biodiesel	81.47%	Jayakumar et al. (2021)
3.	*Staurosirella pinnata* and *Phaeodactylum tricornutum*	Biomethane (CH_4)	79.2 ± 5.9 and 239.4 ± 6.7 mL CH_4/g organic fraction, respectively	Savio et al. (2020)
4.	*Phaeodactylum tricornutum*	Biodiesel, bioethanol and biomethane	1.72, 0.35 and 1361 m^3/year of biodiesel, bioethanol, and biomethane, respectively	Branco-Vieira et al. (2020)

pure petrodiesel and mixed fuel (petrodiesel, 80%; diatom fuel, 20%) revealed no significant performance differences. Moreover, blended biofuel showed reduced carbon monoxide emission, unburnt hydrocarbons, and less smoke (Soni et al. 2020).

Furthermore, the production of biofuels has started the utilization of genetically modified diatoms by the companies like Synthetic Genomics and Algenol Biofuels (Sharma et al. 2021).

16.2.2 Metabolite Production

The potential use of diatoms for the synthesis of bioactive chemicals and compounds had already gained attention of the industries (Vinayak et al. 2015). The intracellular metabolites, like amino acids, necessary lipids, and eicosapentaenoic acid (EPA), are reported to be produced from cultured diatoms for cosmetic and pharma industries (Lebeau and Robert 2003; Hemaiswarya et al. 2011). Also, nutritional contents such as vitamins, vegetarian protein, antioxidants, and animal feed could be produced using diatoms (Sharma et al. 2021).

In addition, considerable quantity of nutrients for animal feed as well as human diet was obtained from extracts of *Nitzschia inconspicua*, *N. laevis*, *N. saprophila*, and *Phaeodactylum tricornutum* (Kitano et al. 1997; Wen and Chen 2001a, b; Wah et al. 2015; Tocher et al. 2019). The living diatoms could also serve as larval feed, such as *Thalassiosira* and *Chaetoceros* (Spolaore et al. 2006), while feeding material for bivalve mollusks could be diatoms like *Skeletonema costatum, Tetrasel missuecica*, *Isochrysis galbana*, *Pavlova lutheri*, and *Thalassiosira pseudonana* (Hemaiswarya et al. 2011). While in France, diatom *Odontella aurita* had been marketed as food in 2002 (Pulz and Gross 2004; Buono et al. 2014). Also in rats, it had shown antioxidant effects (Haimeur et al. 2012) and the haslenes or polyunsaturated sesterpene oils are reported to have anticancer properties (Lebeau and Robert 2003; Hildebrand et al. 2012).

The EPA, a potent agent to prevent heart and blood-related illnesses, was successfully produced in various photobioreactors using cultivated *Nitzschia laevis* and *Phaeodactylum tricornutum* (Lebeau et al. 2002). Interestingly, diatom's self-defense mechanism consists of an array of chemicals that confers protection against pathogens. For example, high quantities of palmitoleic acid, an omega-7 monounsaturated fatty acid, along with many other bioactive agents, are produced by *Phaeodactylum tricornutum* against gram-positive bacteria (Desbois et al. 2009).

The culture conditions' modification influences the metabolite production. In *Amphora* sp., report suggests that change in nutritional supplements and culture temperature had elevated the synthesis of polyphenol and flavonoid (Chtourou et al. 2015). In marine diatoms, the increased contents of primary and secondary metabolite along with growth promotion was achieved by inductively coupled plasma (ICP) nanosilica as catalyst in *Chaetoceros* sp. and *Thalassiosira* sp. (Saxena et al. 2021). Gerin et al. (2020) revealed the freshwater diatom's industrial significance in photoautotrophic batch cultures. Therein, high biomass culture of *Nitzschia palea* and *Sellaphora minima* improved yield of EPA and fucoxanthin.

Moreover, from the established eco-friendly and cheap bioprocess at the pre-pilot scale, it can be concluded that biomass production and metabolite compositions of a diatom are not fixed. However, the production of primary and secondary metabolites is influenced by species or strain, light, growth phase stage, temperature, nutrient media, the extraction process, different stresses, etc. (Ingebrigtsen et al. 2016; Popovich et al. 2020).

Ingebrigtsen et al. (2016) reported the variability in the production and chemical constituents of both secondary metabolites and biomass. Such differences were attributed to the variations in temperature, light, species, phases of growth, nutrient media, sample processing, and several other aspects. The only species of diatom that was proven to be promising in industrial application in eicosapentaenoic acid production and aquaculture is *P. tricornutum* (Hamilton et al. 2015; Huang and Daboussi 2017).

16.2.3 Diatom-Based Nanofabrication

The synthesis of nanoparticles via the physicochemical process for commercial utilization necessitates more time and energy, needs increased pressures and temperature, and subsequently released hazardous chemicals into the environment (Farjadian et al. 2019). Therefore, a quick, cheap, and eco-friendly way of synthesizing nanostructures is in need of time (Kiani et al. 2020, 2021; Tavakolizadeh et al. 2021). Thus, the mass production of nanoparticles could be achieved by diatom-based synthesis for a variety of applications. This diatom-based biological nanofabrication prevails over the complex process and reduces the cost of both issues, i.e., miniaturization and production enhancement for all industrial technologies. Such many advantages are witnessed in silica frustules of diatomaceous algae over recent technology (Korsunsky et al. 2020). Likely, a study on diatomaceous earth-derived silicon nanostructure used as anode for Li-ion battery showed enhanced performance of battery (Wang et al. 2012; Campbell et al. 2016; Cui et al. 2019).

The smallest species of diatom *Nanofrustulum shiloi* was reported to be a potent producer of triangular gold nanoparticles with tricationic gold solution within 72 h. This gold-decorated nanosilica could be visualized in imaging without labels by the virtue of its self-fluorescent property (Roychoudhury et al. 2021a, b). In another case, the study performed to decrease the reflection of electromagnetic radiation showed the utility of silicon nanoflake coating as antireflective material (Aggrey et al. 2020). The hybrid material like biosilica coated with polydopamine was proposed to add the silver nanoparticles on the silica surface. This is supposed to be applicable to biomedicine, bioelectronics, and more (Vona et al. 2018).

Ragni et al. (2018) explored the probability of easy synthesis of photonic nanostructures having tailored fluorophores in the frustules of diatom *Thalassiosira weissflogii* by feeding the algae with altered photoactive materials. At room temperature, the silver nanoparticles were used as sensing material to observe water-dissolved ammonia was synthesized by diatom *Navicula* species (Chetia et al. 2017). Interestingly, it was hypothesized that by the virtue of peptide embedding, diatom frustules can grasp the metal nanoparticles that could prove as highly efficient energy devices (Gupta et al. 2018).

In 2017, Borase et al. reported the synthesis of gold nanoparticles from *Nitzschia* diatom species. It showed higher antibacterial properties of the mixture of antibiotics (streptomycin and penicillin) with gold nanoparticles than the isolated antibiotics and gold nanoparticles. It was suggested that biofabrication of silver into nanoparticle silver is due to the Chlorophyll-c and fucoxanthin, a photosynthetic pigment (Mishra et al. 2020). Moreover, nanoparticles derived from *Skeletonema* sp., *Chaetoceros* sp., and *Thalassiosira* sp. are employed for the antipathogenic activities against some of the bacteria (Mishra et al. 2020).

Some other notable reports on the utility of diatom nanofabrication include photodegradation of pollutants in the visible spectrum by frustule with titania-deposition (Chetia et al. 2018), acetaldehyde abatement by titania nanoparticles from the species *Thalassiosira pseudonana* (Ouwehand et al. 2018), Si-ZrO_2 nanoporous complex from *Phaeodactylum tricornutum* as an electrochemical sensor for the detection of methyl parathion (Gannavarapu et al. 2019), and multifaceted applications of silver-silica hybrid nanoparticles derived from *Gedaniella* species applicable in biosensing, electronic device designing, medical field, etc. (Roychoudhury et al. 2021a, b).

16.2.4 Biomedical Industry

The estimated monitory requirement for drug development and release to the market is 2.6 billion dollars (DiMasi et al. 2016). To overcome the current system of drug design and delivery (Aw et al. 2011a, b), it is much necessary to conduct detailed studies on the applications of diatomaceous frustules along with other biological alternatives for utilization in biomedicine (Sharma et al. 2021).

The optimistic characteristics of ideal drug delivery tools, such as thermostability, adjustable surface chemistry, specific surface area, etc., are the key benefits offered by the frustules of diatom. The porous structures of diatom frustules show multiple patterns from nano- to micrometer (Chandrasekaran et al. 2014; Cicco et al. 2015; Ragni et al. 2017). These properties of diatoms make it worthy of exploring silica-based applications in biomedicine (Mishra et al. 2017; Terracciano et al. 2018).

Exploring the idea of the suitability of diatoms in biomedicines, *Coscinodiscus concinnus* and *Thalassiosira weissflogii* are reported to be excellent drug carriers owing to their morphology and amorphous nature (Aw et al. 2011a; Gnanamoorthy et al. 2014). Moreover, microcapsules from diatoms could be implanted or administered orally as an efficient carrier for water-soluble and poorly soluble drugs (Aw et al. 2011a; Ragni et al. 2017). An interesting development was reported by Losic et al. (2010), wherein an altered diatom surface with iron oxide dopamine modification was designed for a drug carrier guidance system using magnetic properties. This allowed the sustained discharge of inadequately soluble drugs for about 2 weeks. Additionally, anticancer drug delivery to tumor sites was possible due to genetically altered biosilica (Delalat et al. 2015). In another case, diatom surface activated by dopamine-altered iron oxide nanoparticles was used in tumor healing drug release (Medarevic et al. 2016). The improvement to this system was reported due to the use of biosilica-based drug encapsulation with optimized delivery features (Kabir et al. 2020).

The surface modification of diatoms and alternation applications have an array of possibilities in the biomedicine industries, such as biosilica-modified surface of *Chaetoceros* sp. using iron-oxide nanoparticles comprised of trastuzumab antibody, was used for differentiating normal and breast cancer cells (Esfandyari et al. 2020), detection of interleukin 8 in human blood using integrated gold nanoparticles into biosilica (Kaminska et al. 2017), on site and in vivo bone repair using biosilica, that is stable and well-suited for biological system (Rabiee et al. 2021), and controlling hemorrhage with chitosan-coated diatoms (Feng et al. 2016).

16.3 Conclusion and Future Perspectives

The evident studies reported the surprising possible applications of diatoms. The cheap and eco-friendly aspects of diatom's industrial applications are steps to augment human use of renewable resources for dipping carbon emissions. Even though the industrial utilization of diatoms still demands upgradation, it is certainly a decisive research field for human welfare. Furthermore, recent progression in sequencing and processing greater biological data has made it feasible to store the biodiversity of diatoms.

The chief lacking point in diatoms' industrial use is the maximization of the various aspects of biofabrication. Nevertheless, it is expected to conquer such lacunas in the coming future by genetic engineering techniques. Finally, to enhance the diatom-based industrial sector, shift from the current policies by the government and decreasing the gap between industries and academicians is the call of the hour.

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Chapter 17
The Mechanism of Ecosystem Restoration and Resilience of Present-Day Coastal Lagoons by Coastal Diatoms and Their Implications for the Management of Successional Diatomite Landscapes

Harini Santhanam and Anjum Farooqui

Abstract Diatoms of coastal lagoons have been known to be excellent proxies of their environmental changes and are excellent proxies for eco-resilience given their capacities for achieving alternate stable states despite being impacted by extreme events such as floods. Thus, the coastal lagoon diatoms can be major agents of the restoration of the ecosystem statuses from a primary production perspective, slowly leading towards natural restoration of the ecosystem services. The present work illustrates this hypothesis through an example of natural restoration and resilience building of a coastal lagoon ecosystem, Pulicat lagoon in the aftermath of the 2015 South India. The factors contributing to the restorative processes and the mechanism of the building of resilience are illustrated. Further, a discussion based on the findings extends this understanding to the ecological successional events and transformation into diatomite landscapes with an abundance of diatom-rich sediments and diatomaceous earth as futuristic natural resources. The implications of the current study with respect to both the present-day diatom-rich lagoons and the possible futuristic diatomite landscapes are presented.

Keywords Coastal diatoms · *Biddulphia* sp. · Pulicat lagoon · Diatomite · Diatomaceous earth · Eco-resilience

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P. Srivastava et al. (eds.), *Insights into the World of Diatoms: From Essentials to Applications*, Plant Life and Environment Dynamics,
https://doi.org/10.1007/978-981-19-5920-2_17

17.1 Introduction

Ecosystem changes pertain to differential spatio-temporal scales and are more dynamic in ecotonal regions where the lateral and vertical changes in environmental characteristics are quite evident. The transitionary nature of the ecosystem changes at ecotones, such as coastal lagoons, makes the biotic indicators such as diatoms quite functional proxies of these changes from the environmental monitoring perspective. From the species assessment perspectives, diatoms also function as keystone species (e.g. *Skeletonema costatum*) influencing the biogeochemistry of the marine and coastal ecosystems with their broad as well as extensive links to silicate and phosphorus recycling within these ecosystems (e.g. Smayda 2011). Some characteristics of diatoms such as cell sizes, formation of the benthic resting states, the xanthophyll cycle pigments, etc. can be used to study the nature of changes undergone by the diatom cells in response to the changes in the ambient environment. Recently, these changes have been linked to stressors of anthropogenic origin and from the climate change perspective, which determine that the extent to which the biogeochemistry of these waters have also been altered resulting in the morphological or functional traits in the diatoms.

From the resilience perspectives, the presence of diatoms is also a unique indicator of the stable and alternate states. For example, the changes in the composition or the abundances of diatoms are indicators of the magnitude of influence of the forcing environmental factors operating within the ecosystem, which cause the changes in the stable states (Seddon et al. 2011). Natural events such as cyclonic storms and coastal flooding can also alter the stable ecosystem states which are well reflected by the diatom assemblages (e.g. Nodine and Gaiser 2014; Santhanam et al. 2018). Changes in ecological stoichiometry, for example, are reflected in the abundances of diatoms which can easily dominate the pelagic algae in the abundances of silicates—a stable proxy of environmental statuses over a long period of time. Santhanam et al. (2018) reported a sudden bloom of *Biddulphia* sp. in Pulicat, a coastal lagoon ecosystem which had been subjected to the effect of acute desalination in the aftermath of the 2015 cyclonic storm event in the Bay of Bengal (Santhanam and Natarajan 2018) which altered the ecosystem status of the lagoon.

Long-term responses of diatoms to changes in the stoichiometry have indicated the onset of nutrient disequilibrium and changes to the biomasses, which lead to the development of long-term stable states. For example, in the case of the blooms of the diatom, *Phaeocystis globosa*, which does not have any grazing pressure from zooplankters such as the copepods, the addition of nutrients to the coastal systems in North Sea translated into a very low transfer efficiency of the biomass (Gasparini et al. 2000). This in turn increased the abundances of the *P. globosa* leading to severe blooms above threshold statuses and probably resulting in low resilience. Figure 17.1 shows the ecological and biological factors related to diatoms which can be considered towards utilising them as robust proxies of eco-resilience in a coastal lagoon ecosystem.

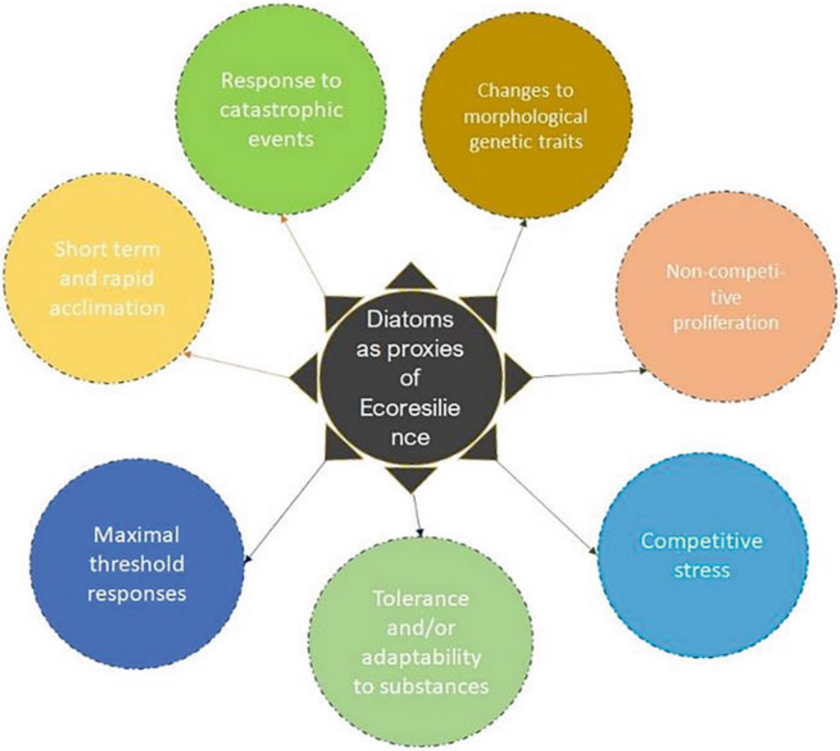

Fig. 17.1 Characteristics of diatoms which render them as suitable proxies of eco-resilience

17.2 Diatoms as Proxies of Eco-Resilience of Coastal Lagoons

The establishment of eco-resilience of coastal ecosystems depends on identifying and quantifying the thresholds and threshold responses to determine the changes in the alternate stable states. Diatoms are significant proxies which can help in identifying the threshold status; for example, Seddon et al. (2011) illustrated that the diatoms' responses to fast and slow processes in coastal systems can help to frame the policies to maximise resilience in coastal systems.

The complexity owing to the highly dynamical nature of the coastal ecosystems can be overcome to determine the changes in the stable states using the abundances and presence of diatoms. Virta et al. (2019) reported that using the diatom linkages to the changes in the sediment organic matter (OM), changes in the ecosystem productivity could be determined. Factors such as changes in the OM, precipitation and run-off quality impact the diatom communities, which directly affect the overall productivity; hence, diatoms can be useful indicators of the low and high production

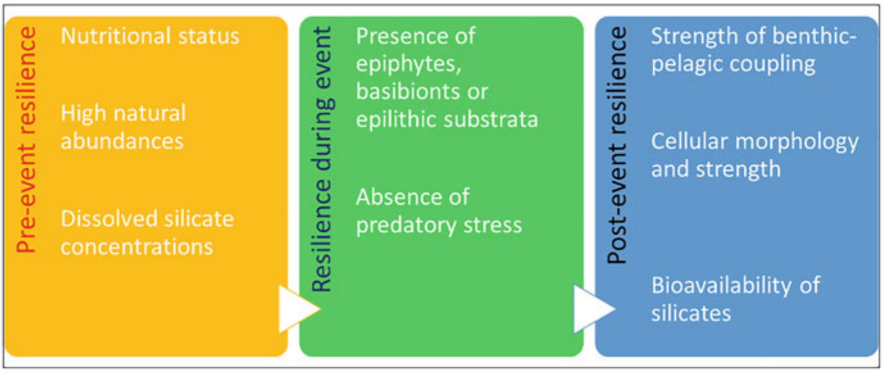

Fig. 17.2 Diatom-specific factors facilitating high resilience to natural hazard events such as floods in the pre-event, event and post-event phases in coastal lagoon systems

rates, which also signify the changes to the stable states. This observation is quite relevant to the relationships of the diatoms to the ecosystem services of coastal ecosystems. Figure 17.2 shows that the presence of dissolved silicates plays a major role in the increases in the diatom abundances or onset of blooms.

Xu et al. (2021) investigated the effects of acclimation and adaptation of coastal diatoms to environmental stressors, which influenced the ecosystem resilience with respect to arsenic. The study illustrated that short acclimation times and mid to high adaptations provided long-term resiliencies to three species of diatoms which are also globally distributed (*Phaeodactylum tricornutum*, *Thalassiosira pseudonana* and *Chaetoceros mulleri*). Coastal diatoms further possess the three criteria which make them robust functional proxies for resilience to climate change, namely, diversity, connectivity and adaptive capacity (Bernhardt and Leslie 2013).

17.3 An Illustrative Example of a Resilient Lagoon Ecosystem from India: Pulicat Lagoon

It is evident from the preceding discussion that factors such as the diatom cell morphologies, strength of the silicate shell, diatom type (whether centric or pennate), propensity for their epilithic and/or epiphytic existence and high nutrient concentrations (Fig. 17.2) provide them with high adaptative responses and the survival rates even under extreme conditions of local weather and/or the effect of high tidal circulation, water currents, etc. Thus, diatoms exhibit an inherent capacity for resilience and can be the drivers of the same within an ecosystem by promoting feedback mechanisms which kick-start the growth and proliferation of other phytoplankter groups, thereby leading the biotic-abiotic environmental responses which help to build the capacity for eco-resilience over the different trophic levels. In this way, diatoms can be considered the 'model' biota for investigating and developing nature-based, eco-resilience building measures which can be forged through human-mediated conservation practices for collapsing or collapsed coastal lagoon systems as per the Red List of Ecosystems (RLE) classified by the IUCN (Keith et al. 2013). This central idea is examined using a recent occurrence on the bloom of *Biddulphia* sp. provided in Santhanam et al. (2018) which is discussed in this section.

Biddulphia sp. are cosmopolitan oligohalobous to mesohalobous centric diatoms described as early as 1900 (Boyer 1900). The species of Biddulphia which have been recorded in the coastal waters of India are *Biddulphia mobiliensis*, *B. obtuse*, *B. pulchella*, *B. sinensis*, *B. tuomeyi* and *B. heteroceros* (Subrahmanyan 1946). Of these, *Biddulphia mobiliensis*, *B. obtuse*, *B. pulchella*, *B. sinensis*, *B. rhombus*, *B. longicrurus* and *B. heteroceros* are reported from the coastal waters of Bay of Bengal near Chennai (Subrahmanyan 1946). Redekar and Wagh (2000) reported the presence of two species from the western coast: *Biddulphia regia* and *B. sinensis*. The presence of a protective shell made of silica with concatenating or attached frustules in alternate angles and the presence of valves, well-developed spines and marked reticulations, granules or puncta characterise the diatom (Boyer 1900). The dominance of diatoms in the regions of 13°N latitudinal as well as the monsoon seasonal preference of the species of *Biddulphia heteroceros* and *B. mobiliensis* were observed. The characteristics and morphologies of *Biddulphia* sp. from the coast of India that are important in building its resilience and adaptive capacities are given in Table 17.1.

Pulicat lagoon, a restricted-type coastal lagoon ecosystem of about 450 km^2 situated in the south-east coast of India (13.3 and 13.6°N and 80.23 to 80.25°E), underwent extensive desalination during the recent 2015 South India flood event, owing to an influx of a huge volume of nutrient-laden freshwater from the rivers, which was reported in detail in Santhanam and Natarajan (2018). The salinity variation in the lagoon has been well documented (e.g. Santhanam and Amal Raj 2019). However, the desalination of the brackish water system was found to be phenomenal (~40%) which impacted the ecology and biotic distributions in short term (~ 3 months) before the natural seaward exchanges resumed. These changes were studied using diatoms in the surface sediments as proxies and in the post-flood scenarios; the development of the eco-resilience was largely reported coinciding with a bloom condition of the diatoms of *Biddulphia* sp.

Earlier studies had suggested a large presence of diatoms in sediment cores in general (Farooqui and Vaz 2000) and specifically *Biddulphia* sp. in Pulicat in the southern regions close to a river mixing zone (Araniar river zone). This area, which is within the limits of tidal influence, acts as the ideal niche for marine diatoms travelling into the lagoon from the sea. On the other hand, the northern portions of the lagoon, closer to another zone of river influx (the Kalangi river), were dominated by diatoms such as *Pleurosigma*, *Nitzschia*, *Gyrosigma*, *Navicula* and *Coscinodiscus*, while no dominance of *Biddulphia* sp. in the northern sectors had been reported prior to the 2015 flood event.

The presence of *B. sinensis* and *B. mobilinesis* from the central portions of the lagoon was observed alongside predominant macroalgal/weed vegetation (about 450 g/L and 230 g/L in the central and north-eastern sectors; Ramesh 2000);

Table 17.1 Species, specific characteristics and habitats of diatoms belonging to Biddulphiaceae observed at or near the south-east Indian coast (compiled from Subrahmanyan 1946; Venkataraman 1958; Kociolek et al. 1983; Redekar and Wagh 2000)

Species	Cell shape	Horns and processes	Valve spines	Valve surface characteristics	Aerolation	Length of apical axis	Habitat
Biddulphia heteroceros Grunov	Squarish or box-shaped	Presence of strong, polar horns	Presence of small spines	Higher between spines	9–10 areolae in 10 μ	45–48 μ	At the bottom mud of saline pools
Biddulphia mobiliensis Bailey	Linked in small chains	Horns unite the individual cells	Presence of two slender long spines	Surface flat between spines	Yes, sizes not available	26.6–76 μ	At the bottom mud of saline pools
Biddulphia pulchella Gray	Elliptical with undulating cell margin	Not described	Spines with long sutures	Middle part of the valve is raised	5–8 areolae in 10 μ	95–152 μ	Epiphytic on *Chaetomorpha* sp.
Biddulphia rhombus (Ehren)	Elliptical with rectangular girdle view	Presence of stout horns	Presence of two long spines	Siliceous valve with chromatophores, elliptic-lanceolate	On the valve: 9 in 10 μ; on the girdle: 12–14 in 10 μ	26.6–34.2 μ	Epilithic on pebbles immersed in seawater, epiphytic on Lyngbya and Polysiphonia; rarely pelagic
Biddulphia biddulphiana Boyer	Colonial cells united by their angles to form short chains	Swollen margin	Presence of strong coastae described	Elliptic with swollen margins, strongly sculptured, divided into 3 sections by the costae; girdle punctate in longitudinal lines	Yes, arranged in longitudinal and transverse rows, sizes not available	Not available	At the bottom as well as epiphytic
Biddulphia aurita (Lyngbye)	Box-shaped cells united by a thick mucilage pad over	Obtuse, marginal short processes	No central spine	Elliptical-lanceolate valves are present and the girdle zone is divided by a deep	Punctuated aerolae, 8–10 in 10 μ	24–30 μ	Littoral species is epiphytic on *Polysiphonia* sp.

	a zigzag chain that remains attached			groove with distinctive intercalary bands. The valve centres are almost flat but are sub-centrally depressed; strongly siliceous; numerous centrally-aggregated chromatophores			
Biddulphia dubia Cleve	Rhomboid, lanceolate and four sided	A stout horn-like process is observed from the side view of the valve at two angles, a short blunt process is noticed at another angle	Surface of valve may be perceived to either possess small spines or a raised wall of reticulations	Strongly sculptured, valve margins are striated; cell wall is quite strongly silicified and coarsely reticulated which may be irregular/quadrangular/heptagonal and unequal in size about 1.5–3.0 mesh per 10 μm; irregular to short cylindrical centrally aggregated chromatophores	Yes, irregular aerolation; sizes not available	30–55 μm	Littoral and benthic
Biddulphia laevis Ehr.	Zigzag filamentous colonies of short length containing up to 50 cells connected with mucilage	Stalked ocelli-like labiate processes bordered by a thin distal rim containing many porelli on external valve face	Peripheral spines present	Depressed ovoid centre with elevated circular area surrounding it	Yes, radial aerolae; 14–16 in 10 μ	40–60 μ	Epiphytic on *Cladophora glomerata*

however, their abundances are not available. Although short-lived mixed blooms of *Rhizosolenia*, *Chaetoceros*, *Biddulphia*, *Coscinodiscus*, *Thalassionema*, *Thalassiothrix* and *Bacteriastrum* and also species like *Skeletonema costatum* and Asterionella *japonica,* etc., had been reported from the southern sector (Kaliyamurthy 1975), the first record of a dominant *Biddulphia* sp. bloom at Pulicat in a post-flood scenario was evident after the 2015 event (Santhanam et al. 2018).

Qasim et al. (1973) reported that populations of *Biddulphia sinensis* dominated the Cochin backwaters under high nutrient concentrations of phosphorous and nitrate (singly or in combination) during monsoon months. *Biddulphia* sp., being large celled, possess a primary advantage by virtue of their sizes to attain greater half-life saturation states with respect to nutrient concentrations, outdoing even fast-growing but smaller-sized dinoflagellates such as *Ceratium furca (Ehrenberg)* (see Qasim et al. 1973). Studies by Castenholz (1964) and Humphrey (1979, 1983) suggested that *Biddulphia* sp. favoured underwater spectral illumination at lower wavelengths close to violet regime and shorter photoperiods (15 h) for greater growth and assimilation. Such studies illustrate the ability of *Biddulphia* sp. to grow abundantly rapidly even under low light, high turbidity as well as high flow conditions in the estuaries which are prevalent in the monsoon seasons.

Like most diatoms, *Biddulphia* sp. is known to lead an epiphytic and/or epilithic existence. *B. pulchella* have also been observed to be attached at the valve to another algae, *Lyngbya*, by mucilage and is found to be dominantly epiphytic on *Lyngbya* and *Polysiphonia.* Govindasamy and Anantharaj (2012) described the epiphytic existence of *Biddulphia pulchella* on the leaves of seagrass species *Enhalus acoroides* in Thondi coastal region (Southern Tamil Nadu coast)—a first record ever of such epiphytic existence of *Biddulphia pulchella* observed in January 2010. It is interesting to note that although another genus of seagrass, *Cymodacea serrulata*, is most predominant along the coast, epiphytic *B. pulchella* were observed from the leaves of *E. acoroides*. This interesting result indicates that the 'host' seaweed/seagrasses or macroalgae chosen for epiphytic existence of *Biddulphia* sp. could be quite selective under different environmental conditions.

Aziz (2005) reported planktonic and attached forms of *Biddulphia* sp. from the North-eastern Bay of Bengal along Bangladesh in bloom condition consisting of *B. aurita*, *B. dubia* and *B. pulchella*. While *B. aurita* (in blooms observed in July–September) was observed in a mixed bloom state alongside *Asterionella glacialis*, both *B. dubia* and *B. pulchella* were observed to be largely epiphytic forms: formerly on *Polysiphonia* sp. and the latter with both *Lyngbya* sp. and *Polysiphonia* sp.

Microzooplankton such as the copepods—*Calanus* sp. and *Eucalanus* sp.—are known to extensively feed on diatoms including *Biddulphia* sp. (Corner et al. 1972; Madhu et al. 2007). Other predators include the protozoans, *Tintinnopsis* sp., and exclusive filter feeding molluscan bivalves and oysters such as *Mytilus* sp., *Perna* sp. and *Crassostrea* sp. (e.g. Kasim and Mukai 2006). These predators including copepods and clams such as *Mytilus edulis* and *Perna viridis* are well distributed in Pulicat lagoon; *Crassostrea madrasensis* has been described to be the keystone species (Sanjeeva Raj 2006). However, the absence of these predatory influences as evidenced by the sedimentological analyses presented in Santhanam et al. (2018)

suggests that the diatoms, which were robust survivors of the flood conditions, benefitted by the absence of direct predators. It is interesting to note the low abundances of the resident pennate diatoms such as *Navicula* sp. which are usually quite unaffected by fluctuation in nutrient concentrations compared to centric diatoms as *Biddulphia* sp. (e.g. Mitbavkar and Anil 2002) were quite low in abundances compared to the latter in a post-flood scenario. The extensive reworking of the sediment observed at these sites combined with the habitat preference of pennate types towards the central and northern portions of the lagoon can explain this observation quite well. At site 1 close to the bar mouth, several specimens of tintinnids both separated and unseparated from their lorica were observed (Santhanam et al. 2018).

It is interesting to note the associative factor in the adaptative response to the flood which lead to the survival and resilience of *Biddulphia* sp. The diatoms of this species appear to have found a natural ally surprisingly not a competitor in the robust *Cladophora sp.* compared to other algae (e.g. pelagic diatom *Polysiphonia* or *Lyngbya*) and seagrasses (e.g. *Halophila* sp.). The latter seems to have been the source for its repository of nutrients (especially Si) as well as refuge to *Biddulphia sp*. Such close epiphytic association of *Biddulphia laevis* and *Cladophora glomerata* had been described in detail by Kociolek et al. (1983) in the Ohio river estuary, USA. The latter factor must have offered a comfortable substratum as well as protection from stronger currents (an obvious disadvantage for the pelagic diatoms prominent from their absences as observed during the microscopic survey (Santhanam et al. 2018). Another important consideration is the possible loss of seagrass beds (*H. ovalis*, etc.) observed in the lagoon with lowering of salinities (Lynda Keren and Moses Inbaraj 2013), making them unavailable to *Biddulphia* sp. for epiphytic association as was reported elsewhere (see Govindasamy and Anantharaj 2012).

With both organisms capable of surviving in lower salinities, the rapid growth and sustenance of both species must have been coincidental with the rapid increase in cell size and reproduction of *Biddulphia sp.* being greater than that of *Cladophora sp*. Further, given the fact that benthic diatoms can resuspend into the water column by the wind-stimulated turbulence provided both algal groups with a rich nutrient scape with just sufficient light condition for phototrophic fixation. The competitive stresses from microphytobenthos and phytoplankton could have been quite negligible as is observed during the monsoonal seasons (e.g. Sanilkumar et al. 2011). These factors working in favour of *Biddulphia* sp. has resulted in the formation of a 'bloom' condition, which also kick-started the recovery to brackish conditions.

It is also important to relate to the influence of concentrations of the silicic acid on the productivity of the diatoms in the lagoon. The northern regions (site 7 especially; Fig. 17.3) seem to have contributed more to the silicic acid concentrations in the lagoon (Santhanam et al. 2018). Silicate is highly soluble in alkaline conditions, and the higher concentrations of silicate ions in the hydroxide form are observed whenever the pH is found to be alkaline in the lagoon. The more alkaline northern regions thus show higher concentrations than the southern regions; furthermore, the riverine contribution of silicate to the system is higher from the Kalangi river rather than in the Araniar river. Although the availability of silicates is greater in the

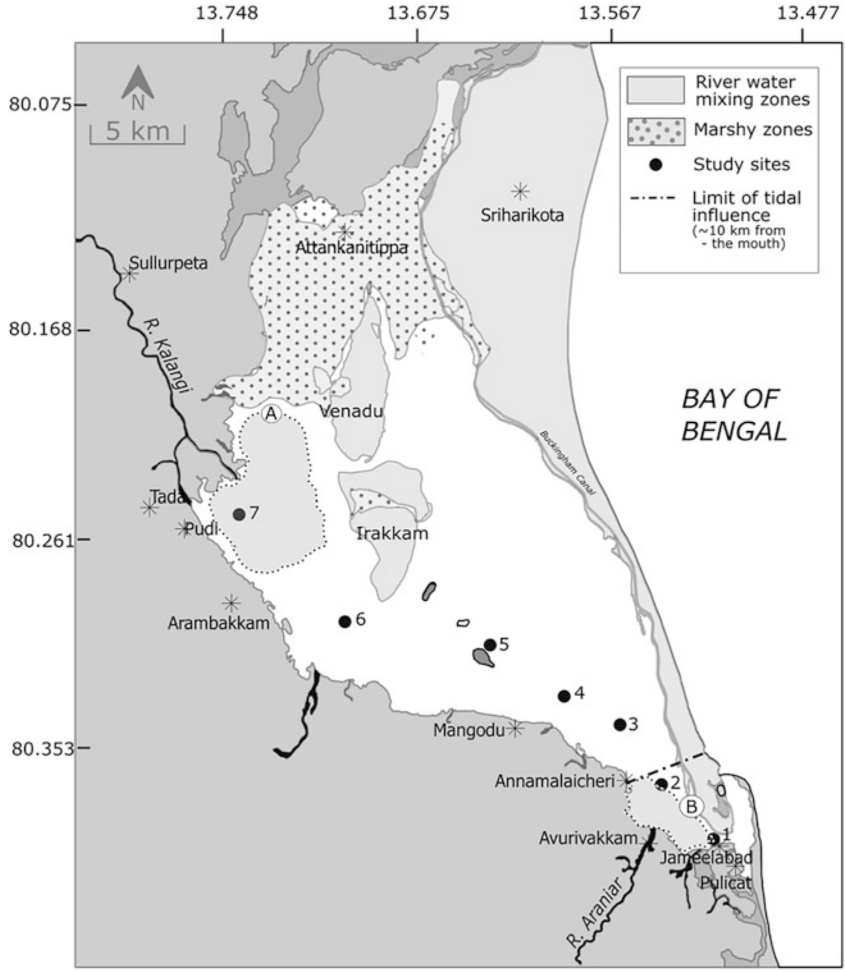

Fig. 17.3 Map of Pulicat lagoon ecosystem in South-east India showing the sampling locations (black dots). The regions under freshwater influence from the rivers Kalangi (NW) and Araniar (SW) are marked A and B with sampling sites 7 and 2, respectively, which are the low saline zones. The predominantly active southern mouth connects the lagoon with the adjacent sea, Bay of Bengal; the dot-dash line shows the limit of tidal influence within the lagoon (~10 km from the mouth; sources: Raman et al. 1977; Thangavelu 1983)

northern regions, its influence as a limiting factor for diatoms is well observed in the southern region which can reflect the trend during high-flood events as in the present case. The stagnation of the central region as illustrated from its salinity characteristics combined it the prevalent N and Si limitations in monsoon seasons renders it impossible for the onset of blooms here. However, the 2015 floods illustrated the alleviation of the generally Si-limited situation prevailing under ambient conditions in the southern zone which in combination with higher nitrates are responsible for sustaining bloom conditions.

17.4 Discussion and Conclusions

The concept of monitoring and assessing the eco-resilience of ecosystem was the central theme presented here. It is interesting to note that among the different diatoms, the strength of the siliceous skeletons of the large, centric diatoms like *Biddulphia sp.* seems to be advantageous to resist damage from impacts and help with sustenance during such high impact events such as cyclone-induced floods. With their established ability to rapidly utilise the available nutrients under a high-nutrient, high-turbid environment, *Biddulphia* sp. produced a bloom as observed during the study. This example illustrates the following implications for building eco-resilience at the ecosystem level even for collapsing ecosystem as Pulicat threatened by anthropogenic activities:

1. Diatoms such as *Biddulphia* sp. are important proxies for post-hazard monitoring of eco-resilience in coastal lagoons. They can hence indicate the levels of success of eco-restoration of the lagoons.
2. The case of Pulicat illustrates the important roles of robust diatoms in supporting primary production of lagoon ecosystems and hence can be explored for their applications as nature-based solutions (NbS) for ecosystem recovery, with special reference to post-flood scenarios in the absence of anthropogenic pressures.
3. The presence of diatoms from collapsed ecosystems which have transformed to dry landscape remains to be good indicators of the flood-bearing capacity of these regions which can be used as excellent markers for planners to build flood-resilient landscapes.

Over longer time periods of the order of thousands of years, the drying up of the lagoon beds with rich accumulation of such diatoms can produce diatom-rich substrata or sediment (DRS), which become diatomite ‘rocks’ (diatomaceous earth, DE; sedimentary layers rich in silicates) of the landscape. Thus, through successional conversion of the erstwhile wetland/lagoon systems into drier landscapes, the fossilised and sub-fossilised diatoms of older wetlands become ‘ores’ of the diatomite.

While diatomaceous earth remain critical substances for inexpensive, inert and durable filtration media over industrial processes and in manufacturing at present, there remain possibilities of their exploitation through excessive diatomite mining in the near futuristic scenarios. It needs to be recognised that though diatomite is deemed to be an extensive and ‘inexhaustible’ resource currently, with most resources located in Rajasthan and Gujarat, continual mining corresponding to demand for industrial use of DE can lead to their exploitation. Unregulated mining in futuristic scenarios may contribute to degradation of the land and the loss of the capacity of the dry diatomite landscapes to act as natural filters for the waters flowing downstream through the floodplains.

In conclusion, it is suggested that the observations presented herein need to be considered as part of the scientific efforts to build diatom-based natural resource management models focussing on the eco-resilience building initiatives of the present-day natural lagoon landscapes:

1. The adaptative capacities of the different diatoms must be researched to reconstruct the manner of natural recovery of lagoon ecosystems to floods and inundations. This will be useful to plan for successful wetland restoration and disaster risk reduction in the present day.
2. The links between diatoms, their epiphytes and epilithic associations are crucial to assess the extent to which successful natural restoration activities can be planned for lagoon systems in India.
3. Assessment of environmental risks associated with diatomite mining from ancient floodplains and coastal lacustrine environments must be extended to the studies on the ecosystem services assessments for present-day lagoon environments with diatom-rich benthic environments such as Pulicat and Chilika.
4. NbS-based assessment to capture the natural asset values of diatoms to wetlands and coastal lagoons must be facilitated in the present-day scenario to avoid any futuristic exploitation of these value resources.

Acknowledgements The author acknowledges the use of Turnitin software licensed to the National Institute of Advanced Studies, Bangalore, for plagiarism checking.

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