

Methodological Advances in Diatom Research for Aquatic Biomonitoring and Water Quality Evaluation

Sneha Sindhwal¹, Rinku¹, Abhishek Saxena¹, Mireya Navarro Márquez², Roberto Parra-Saldivar³, Archana Tiwari^{*1}

¹Diatom Research Laboratory, Amity Institute of Biotechnology, Amity University, Noida, Uttar Pradesh, 201313, India

²Universidad Autónoma de Nuevo León, Facultad de Agronomía, Francisco Villa S/N Col. Ex Hacienda El Canadá 66415, General Escobedo, N.L., México.

³Magan Centre of Applied Mycology, Cranfield University, Cranfield MK43 0AL, UK

*Corresponding author: panarchana@gmail.com

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24 **Abstract**

25 Diatoms, a unique class of microalgae, are pivotal in global ecosystems due to their
26 photosynthetic abilities and adaptability to diverse aquatic environments. Composed of opaline
27 silica, they serve as sensitive indicators for water quality monitoring. This review addresses
28 various diatom research techniques, including sampling and isolation. It highlights methods for
29 different substrata, such as epiphyton, epipelon, and epipsammon, as well as traditional
30 isolation techniques, such as agar plate methods and serial dilution. Automation techniques,
31 such as flow cytometry (FACS), are also discussed. The review emphasizes the potential of
32 diatoms for pollutant removal, particularly heavy metals, and presents various diatom-based
33 indices for water quality assessment. Future research is encouraged to refine isolation
34 techniques, enhance the effectiveness of indices, and explore biochemical pathways that could
35 improve diatoms' bioremediation capabilities, thereby leveraging their potential for
36 environmental monitoring and remediation.

37 **Keywords:** Axenic Cultures, Bioremediation, Diatom-Based Indices, Isolation, Sample
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48 **1. Introduction**

49 Diatoms are unicellular eukaryotic microalgae that exhibit remarkable diversity in both
50 morphology and ecology, with over 200,000 estimated species thriving in marine, freshwater,
51 and brackish ecosystems (Rinku et al., 2024). These organisms possess frustules made of
52 opaline silica, which serve multiple functions: they provide mechanical protection and enable
53 regulated nutrient exchange. Furthermore, diatoms exhibit a rapid sensitivity to environmental
54 changes in pH, nutrient levels, temperature, and salinity, rendering them effective ecological
55 indicators (Saxena et al., 2021). Their unique characteristics make them significant in
56 understanding ecological dynamics and monitoring aquatic health.

57 Diatoms are crucial not only for primary productivity and carbon cycling but also for aquatic
58 bioassessment. Changes in species composition of diatoms serve as reliable indicators of
59 environmental stressors, which can be both short- and long-term, including eutrophication,
60 organic pollution, and toxic contamination (Costa & Schneck, 2022; Kiran Marella et al.,
61 2020). Consequently, diatom-based biotic indices such as the EPI-D and TDI have gained
62 widespread use in evaluating the ecological status of freshwater systems globally (Blanco,
63 2024; Saxena et al., 2021).

64 Despite the established significance of diatoms in biomonitoring, ongoing methodological
65 constraints affect the accuracy and repeatability of studies. The processes of isolating and
66 identifying diatom taxa remain notably labor-intensive and heavily reliant on the operator's
67 skill, mainly due to the morphological similarities among species and the intricate challenges
68 encountered during sample preparation (Mora et al., 2019). However, recent technological
69 advancements are making a substantial impact on the field. Innovations such as high-resolution
70 microscopy techniques, including Scanning Electron Microscopy (SEM), Transmission
71 Electron Microscopy (TEM), and confocal microscopy, are enhancing observation capabilities.

72 Additionally, the implementation of automated cell isolation methods, such as
73 micromanipulation and Fluorescence-Activated Cell Sorting (FACS), along with
74 advancements in metabarcoding and AI-driven taxonomic analysis, is streamlining research
75 processes and alleviating operational bottlenecks in diatom studies (Tapolczai et al., 2019;
76 Percopo et al., 2022; Yu et al., 2022).

77 This review offers a thorough evaluation of recent methodological advances in diatom research,
78 with a focus on key areas. It discusses improved sampling strategies that enhance the collection
79 of diatom samples and emphasizes the significance of physicochemical analyses in
80 understanding diatom environments. Additionally, the review highlights innovations in
81 isolation techniques and microscopic tools, which are essential for accurate diatom
82 identification and study. A key aspect of this review is the emerging integration of molecular
83 techniques and bioinformatics, which are revolutionizing the field. Together, these
84 methodological innovations are poised to advance diatom-based ecological biomonitoring,
85 setting the stage for more effective environmental assessments.

86 **2. Background and Ecological Relevance of Diatoms**

87 Diatom-based paleolimnology in Lake Michigan provides insights into historical ecological
88 conditions by analyzing diatom remains in sediment cores. This approach helps reconstruct
89 past environmental changes, contributing to a better understanding of freshwater ecosystems
90 over time (Reavie et al., 2025). In Tursujuq Park, Nunavik, Canada, the diatom biogeography
91 reveals diverse species distributions influenced by varying environmental parameters such as
92 water chemistry and climate (Alibert et al., 2025). Additionally, examining patterns and drivers
93 of diatom diversity and abundance in the global ocean highlights significant trends related to
94 anthropogenic impacts and natural variability, shedding light on broader ecological dynamics
95 within aquatic environments (Pierella Karlusich et al., 2024). Overall, these studies underscore

the importance of diatoms as key indicators of ecological health and change across diverse aquatic systems.

Diatoms contribute significantly to Earth's primary production, atmospheric oxygen, and carbon fixation, accounting for 20-25% of the global output. In oceans, they account for up to 40% of primary production, comparable to terrestrial rainforests. They support diverse animal populations, including fish, seabirds, and marine animals, by serving as a primary food source (Serôdio & Lavaud, 2022). As a species-rich biological group, diatoms contribute to biodiversity and genetic resources in fluvial ecosystems (Tréguer et al., 2018). They are responsive to organic contaminants, making them suitable for bioremediation (Kiran Marella et al., 2020). Benthic diatoms have become a prominent tool for ecological bioindication due to their sensitivity to changes in environmental conditions (Costa & Schneck, 2022; Kiran Marella et al., 2020).

3. Sampling and Preservation Strategies

3.1 Site selection and substratum types (epiphyton, epipelon, epilithon, epipsammon, artificial substrates)

Diatoms, a group of microalgae, inhabit a variety of marine environments, including soil, rock surfaces, and aquatic plants. They are commonly found in benthic and planktonic habitats, with benthic diatoms residing on submerged surfaces and planktonic varieties thriving in open waters (Agarwal, 2025; Lewandowicz et al., 2025). Unique habitats also include attachment to seabird feathers (Croll & Holmes, 1982). Effective sampling is crucial for the successful isolation of diatom species, making it imperative to collect diatom and water samples throughout different seasons to ensure representativity. Prompt collection and isolation of viable cells are essential, as dead or damaged cells may compromise sample quality (Saxena et al., 2021). Long-term water sample storage is discouraged due to potential environmental

changes that can lead to cell death, and meticulous site documentation, including location and physical characteristics, is essential (British Standard, 2003; Saxena et al., 2021). For sampling site selection, several scales are considered: broad (catchment area), intermediate (designated areas), and fine (specific locations). Physicochemical conditions significantly influence community structure and aquatic ecosystems, affecting diatom community composition and distribution, thereby necessitating careful consideration during site selection (Karthick & Taylor, 2010).

Diatoms thrive on various substrate types, and selecting the appropriate surface is vital as different substrate support specific diatom communities. Four primary diatom assemblages (Figure i) are recognized (i) Epiphyton: This group thrives on macrophytes such as *Nymphoides* sp. and *Typha* sp. Sampling involves cutting plants and scraping the stems to collect the brown diatom film, which should be stored in sterile containers with methanol for preservation (Karthick & Taylor, 2010), (ii) Epipsammon: For sampling this assemblage, surface sediment from lake edges is collected using small tubes. Unwanted materials are removed by shaking and allowing the sand to settle, effectively isolating diatoms attached to the substrate (Lewandowicz et al., 2025), (iii) Epipelon: Sampling epipelon can be challenging, requiring methods that distinguish between live and dead diatoms. A technique using a Perspex cylinder for shallow waters and mechanical corers for deeper waters is recommended (Videska, 2025), (iv) Epilithons: These include submerged rocks, pebbles, and cobbles, which are favorable for diatom sampling due to their extensive diatom communities. Sampling involves scrubbing the upper surface of the rocks while avoiding the lower surfaces to prevent contamination (Car & Kaleli, 2025; Lewandowicz et al., 2025). Lastly, when suitable natural substrates are lacking, artificial substrates with rough or smooth surfaces can be used. It is recommended that these be submerged for approximately 4 weeks before sampling to allow diatom communities to establish, with longer periods in shaded or cooler environments to ensure equilibration with

local conditions (British Standard, 2003; Taylor et al., 2007). Another critical factor, seasonal variations, significantly affects diatom communities, with various ecological factors such as temperature, rainfall, nutrient levels, etc., influencing species dominance at different times of year (Hamid et al., 2023). For instance, a study in China found that in the Danjiangkou Reservoir, *Discostella* was most abundant in summer and winter, accounting for 98.84% and 62.71% of the diatom abundance, respectively. At the same time, *Aulacoseira* was most abundant in winter and spring, comprising 60.90% and 60.62%, respectively (C. Zhang et al., 2022). Studies have observed that during winter, epilithic and epiphytic substratum types are the most abundant due to their high attachment and stable communities (Lu et al., 2007). While epipellic diatoms are also most abundant during the winter and monsoon seasons, these taxa disappear due to monsoon floods (Aykut et al., 2025). Having described sampling methods, the next step is proper preservation to maintain the integrity of diatom communities.

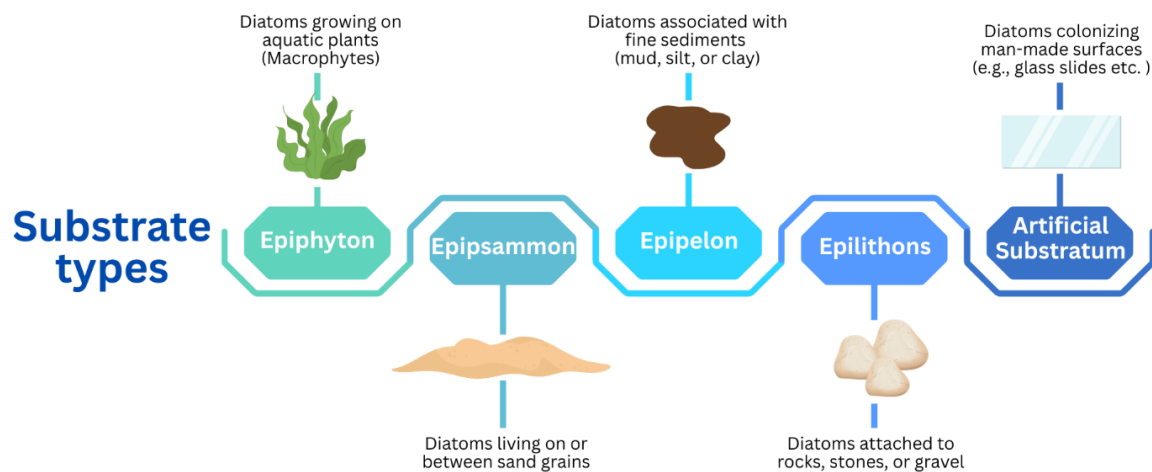


Figure i: Overview of various substrate categories for diatom sample collection and analysis

3.2 Sample collection, preservation, and transport techniques

If samples are processed within a few hours, no preservation is required, and they can be stored in a cool, dark place (Karthick & Taylor, 2010). Otherwise, allow the sample to settle for 24 hours or centrifuge it (British Standard, 2003). For short-term preservation, Lugol's iodine solution is recommended, while 20% ethanol (by volume) is suitable for longer durations (Karthick & Taylor, 2010). Alternatively, 4% formaldehyde (formalin) can also be used for preserving diatom samples (Table i) (El Amin & Prihantini, 2023).

Table i. Comprehensive techniques employed for effective long-term preservation and integrity of biological samples for microscopic and molecular analysis

Preservation solutions	Recommended Concentration	Advantages	Disadvantages	References
Lugol's Iodine	1%	Increases the organism's weight which reduces the time of the sedimentation and has instant effect on the samples	Cause discoloration, which makes the identification hard.	(El Amin & Prihantini, 2023)
Ethanol	20%	After preserving the samples in the ethanol, it can be used for molecular analysis	Cannot be used for Samples which are in situ sediment trap	(Sano et al., 2020)
Formalin (Formaldehyde)	4%	Good preserving and fixing agents make cell characteristics more visible,	Unsuitable for molecular analysis after preservation	(Anderson & Karlson, 2017; Sano et al., 2020)

		Good for long time preservation		
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4. Processing and Microscopic Characterization

4.1 Cleaning and oxidation procedures (peroxide, acid, incineration)

The processing and preparation of diatom samples is crucial for accurate identification and analysis. Upon laboratory return, an initial microscopic examination of collected samples is recommended to ascertain the presence of live cells; samples comprising primarily dead cells, characterized by empty frustules devoid of chloroplasts, should be discarded (Karthick & Taylor, 2010). Diatom identification requires cleaning cells to ensure clear morphology for observation under a microscope (Table ii) (Trobajo & Mann, 2019). One of the prominent cleaning techniques is the hydrogen peroxide method, particularly advantageous for samples laden with organic matter that necessitate potent oxidizing agents (British Standard, 2003). This method, favored for scanning electron microscopy (SEM) studies, offers a gentler alternative compared to more corrosive reagents. It can be executed through two techniques: the hot method and the cold method. In the hot method, 20 ml of H₂O₂ is added to 5-10 ml of the diatom sample, heated to 90°C for 1-3 hours, followed by the addition of hydrochloric acid (HCl) and subsequent washing with hot HCl. Conversely, the cold method involves directly adding H₂O₂ to the sample and allowing it to stand for four days (Karthick & Taylor, 2010). Ensuring proper ventilation is crucial during these procedures (Taylor et al., 2007).

Acid Oxidation (HCl + KMnO₄, Nitric + Sulfuric Acid) method incorporates adding 10 ml of potassium permanganate (KMnO₄) to 5-10 ml of diatom suspension for 24 hours, then introducing concentrated hydrochloric acid (32%) under fume hood conditions. The mixture is subsequently heated to 90°C for 1-2 hours, with oxidation monitored by the addition of hydrogen peroxide. The final steps include cooling, rinsing with distilled water, and

centrifugation at 2500 rpm for 25 minutes, repeating the washing process four times. Alternatively, the Nitric + Sulfuric Acid method can be used post-decalcification by heating a strong acid mixture (2:1 nitric and sulfuric acid) with the sample. Decalcification is typically performed before nitric or sulfuric acid treatment to prevent insoluble calcium precipitates (Taylor et al., 2007). To perform decalcification, hydrochloric acid is incrementally added to the sample in a fume hood until effervescence indicates carbon dioxide release. Once effervescence ceases, the sample is centrifuged with distilled water at 2500 rpm for 10 minutes, and this washing step is repeated four times to eliminate residual acid (Karthick & Taylor, 2010). The incineration method is suitable for delicate or weakly silicified diatoms, reducing the risk of damage from other cleaning techniques. It involves placing 2-3 drops of diatom suspension on a cover slip, covering it with distilled water, and heating it on a hot plate until the moisture evaporates. The sample is then heated to 350°C, transforming from brown (organic burn-off) to black (carbon formation) and eventually to white (remaining material). Post-cooling, the cover slip is rinsed to remove precipitates and reheated to eliminate moisture (Taylor et al., 2007). Once the diatom frustules are prepared, the following procedure is the slide preparation for microscopy imaging

Table ii. Methods for cleaning diatom frustules: Techniques to remove biological residues for accurate microscopic examination

S.No	Technique	Advantages	Disadvantages	References
1	Hydrogen Peroxide Method	<ul style="list-style-type: none"> - Suitable for SEM - Less corrosive than other methods 	<ul style="list-style-type: none"> - Time-consuming (especially cold method) - Forms bubbles which can affect the diatom frustules 	(Karthick & Taylor, 2010)

2	Hot HCl and KMnO₄ Method	- Effective for organic matter removal	- Requires fume hood - Involves strong acids which can over clean the samples and affect diatom frustules	(Karthick & Taylor, 2010; Taylor et al., 2007)
3	Decalcification Method	- Removes calcium carbonate - Prevents unwanted precipitations in later steps	- Only needed if carbonates are present - Acid exposure affects the diatom frustules	(Karthick & Taylor, 2010)
4	Nitric and Sulphuric Acid Method	- Strong oxidizing mix - Very effective for high organic content	- Highly corrosive and can dissolve the frustules - Requires careful handling and ventilation	(Taylor et al., 2007)
5	Incineration Method	- Ideal for delicate or lightly silicified diatoms - Avoids chemical exposure	- Heat may affect the diatom morphology - Needs precision in temperature control	(Taylor et al., 2007)

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210 **4.2 Slide preparation for light and electron microscopy**

211 When preparing diatom slides, a high-refractive-index mounting medium is used to enhance
212 visibility. Common media include Pleurax (RI 1.73), Naphrax (RI 1.69), Hyrax (RI 1.71), and
213 ZRAX (RI 1.7) (Taylor et al., 2007). To prepare a slide, place 0.2 ml of diatom sample on a
214 cover slip, evaporate the water at room temperature, and mount with a high refractive index
215 medium like Naphrax. Heat the slide to 130°C for 15 minutes, then reheat if the cover slip

moves after cooling. Check the slide under a microscope, aiming for 3-4 diatoms per field of view. Finally, store the slides in a glass container with methanol. For SEM analysis, the diatom sample suspension is dried onto a specimen stub with a coverslip attached using resin. Silver paint is applied to the stub's edge for good electrical conductivity, and the sample is then coated with gold. For TEM analysis, formvar-coated grids are used, onto which the sample suspension is dried before observation (Battarbee et al., 1990).

4.3 Advanced imaging tools for diatom characterization (SEM, TEM, CLSM, AFM, DHM)

Diatoms are microscopic organisms which, are analyzed using various microscopy and imaging techniques that enhance taxonomic studies and diversity assessments. The identification of diatoms heavily relies on their morphological features, specifically the frustule shape and structure (Fig. ii) (Burfeid-Castellanos et al., 2022; Kutako et al., 2023; Salido et al., 2020). Light Microscopy is a traditional method for diatom observation, utilizing immersion oil to enhance contrast and resolution, and typically achieving 100X magnification. While it effectively reveals characteristics such as striae and shape, it suffers from limited resolution due to diffraction, limiting the observation of intricate details (Kaur et al., 2024; Piper, 2011). Transmission Electron Microscopy (TEM) offers high-resolution two-dimensional imaging of diatom frustules and intracellular organelles. This technique involves transmitting electrons through a sample, which requires a vacuum to prevent interference from air particles. The capability of TEM to produce detailed images is marked by its importance in diatom research (Kaur et al., 2024; Tang & Yang, 2017). Scanning Electron Microscopy (SEM) provides high-resolution three-dimensional images of diatom structures by directing an electron beam onto the sample surface. This results in detailed imagery that reveals complex patterns that light microscopy or TEM cannot. Nevertheless, SEM faces challenges such as sample contamination, vacuum requirements, the inability to analyze live cells, and higher operational costs (Jiang et al., 2015; Kaur et al., 2024). Fluorescence Microscopy utilizes the unique

fluorescence emitted by diatoms' pigments (e.g., chlorophyll a, c, and fucoxanthin) when exposed to electromagnetic radiation. This method helps identify diatoms by the specific colors and wavelengths emitted as the pigments' electrons return to the ground state (Kaur et al., 2024; Millie et al., 2002). Confocal Laser Scanning Microscopy (CLSM) facilitates non-destructive imaging of living specimens by enabling optical sectioning to produce in-focus images. It is particularly beneficial for observing organisms enveloped in biofilms without disturbance and can differentiate between living and non-living samples. CLSM's ability to capture 3D visualizations enhances the understanding of biofilm structures (Norton et al., 1998). Atomic Force Microscopy (AFM) provides not only high-resolution three-dimensional images but also measures molecular interactions within diatoms. AFM is instrumental in exploring diatom structure, morphology, and biomineralization, as well as in taxonomical classification. Proper sample preparation is crucial, involving diatom cleaning and substrate attachment for practical imaging (Chakraborty et al., 2022; Mišić Radić et al., 2023). Digital Holographic Microscopy (DHM) emerges as an efficient means of monitoring living diatom cells, capturing phase and light-intensity information. This method enables quantitative phase imaging and unique identifiers for diatom analysis while also assessing lipid content through specific holographic sequencing. Innovative sample preparation strategies involve transferring treated diatom frustules to designated environments for imaging (Saito et al., 2022; Yourassowsky et al., 2024; Zetsche et al., 2016).

Light Microscopy, lacks the resolution which leads to taxonomic implications such as misidentification, overestimated distribution etc. Hence, to overcome these implication and better resolution advanced techniques like SEM, TEM, DHM etc have been introduced which not only enhanced the resolution of the images but also helps in better identification (Table iii) (Morales et al., 2009). Methodological errors can be occurred while sample processing and

microscopic characterization which can affect the diatom identification. Errors like operator variation which include sample focusing, recognition of morphological features etc, which collectively affect the taxonomy identification (Taylor et al., 2007). Other errors can be occurred during preservation such as damage to frustules, morphological distortion etc which can hinder the sample identification (El Amin & Prihantini, 2023). Also, during slide preparation various error can be occur such as bubble formation, unevenly spread of sample etc which affect microscopic imaging. Hence, it is necessary to address these errors for effective diatom analysis (Clason et al., 2017). After processing the sample and identifying the species, the next step is to isolate the species, cultivate them, and assess their biomass.

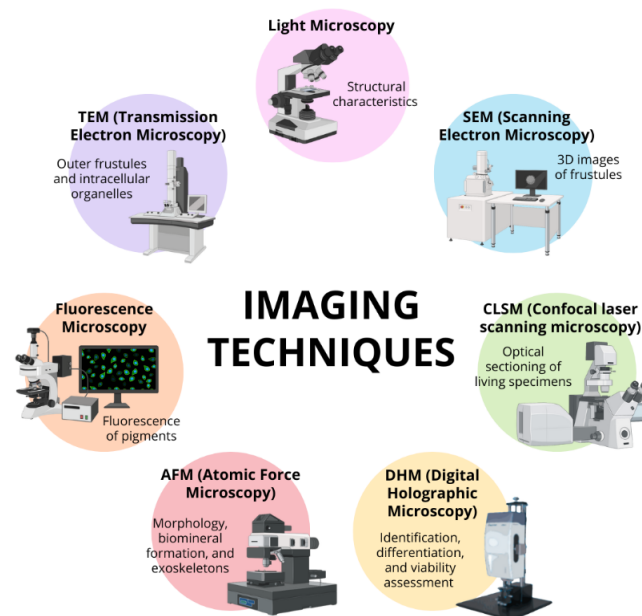


Figure ii. Advanced imaging techniques used for morphological observation and diversity of diatoms

Table iii. Comparative assessment of advanced imaging techniques

Techniques	Resolution	Advantages	Limitations	References
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Light Microscopy	<ul style="list-style-type: none"> - Low 	<ul style="list-style-type: none"> - Resolution upto 100x - Characteristics features are visible 	<ul style="list-style-type: none"> - Limited resolution compared to advanced microscopy - Due to diffraction fine details of diatoms are not visible 	(Kaur et al., 2024)
Scanning Electron Microscopy	<ul style="list-style-type: none"> - Very High resolution (nm) 	<ul style="list-style-type: none"> - Visualizes 3D structure of sample - Fine details are visible 	<ul style="list-style-type: none"> - If organic or inorganic materials are present it will hinder the image 	(Jiang et al., 2015)
Transmission Electron Microscopy	<ul style="list-style-type: none"> - Very High resolution 	<ul style="list-style-type: none"> - Provides 2D image of diatom outer shell and cell organelles 	<ul style="list-style-type: none"> - Fine details are not visible 	(Kaur et al., 2024)
Confocal laser scanning Microscopy	<ul style="list-style-type: none"> - Moderate resolution 	<ul style="list-style-type: none"> - Allows 250 nm Z-axis scans - Shows 3D structure at tissue and cellular level 	<ul style="list-style-type: none"> - Expensive - Time consuming 	(Kus, 2015)
Atomic Force Microscopy	<ul style="list-style-type: none"> - High resolution 	<ul style="list-style-type: none"> - Used in studying topography of diatom frustules - Allows high image resolution 	<ul style="list-style-type: none"> - Probe can affect the sample - Time consuming 	(Gebeshuber et al., 2003; Kaur et al., 2024)

Fluorescence Microscopy	- Moderate resolution	- Helps in visualizing the pigments - Live cells visualization	- Morphological details are limited	(Kaur et al., 2024)
Digital Holographic Microscopy	- Moderate resolution	- Current spatial resolution of DHM is approximately 100 nm - 3D structure can easily visualize without pre treatment	- No sample preparation for biological sample has been established yet	(Saito et al., 2022)

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281 5 Isolation, Cultivation, and Biomass Assessment

282 5.1 Isolation and purification methods (micropipetting, dilution, FACS, 283 micromanipulation)

284 The isolation and purification of diatoms are critical for obtaining specific species from
285 samples. Key methods include: Micropipetting, known as the Zumstein single-cell method,
286 improved by Pringsheim in 1946, which uses a heated Pasteur pipette to create a thin tube,
287 allowing effective cell separation (Vu et al., 2018). Centrifugation utilizes gravitational forces
288 to separate organisms by size, with smaller cells remaining in the supernatant and larger ones
289 settling at the bottom. Filtration is a pre-isolation technique that classifies particles by size and
290 shape. Filtration can involve organic fibers such as cellulose acetate or uniform-diameter
291 circular holes in polycarbonate. The streaking and Pour Plate Method uses solid agar plates;
292 this traditional method involves streaking samples with a bacterial loop or mixing them with
293 agar before incubation, thereby allowing direct cultivation of diatoms. The Atomized Cell

Spray Technique inoculates agar plates with a fine mist of liquid suspension generated by forced sterile air, emphasizing the need for a clean, sterile environment (Andersen, 2005). Serial dilution is effective for isolating specific species from contaminated samples. This technique involves multiple dilution steps until no cells are left for transfer (Saxena et al., 2021). FACS (Fluorescence-Activated Cell Sorting) is an advanced automated method that sorts cells based on their light scattering properties and fluorescent signals, capable of sorting thousands of cells within seconds (Vu et al., 2018). The micromanipulator method enhances isolation precision by using a micromanipulator attached to an inverted microscope, allowing careful selection of single cells and reducing contamination risks (Saxena et al., 2021; Snelling et al., 2013). Phototaxis-based isolation utilizes the natural movement of organisms towards light. This method uses an apparatus that separates cells based on their phototactic responses, effectively isolating targeted diatoms (Andersen, 2005; Yim & Lee, 2005). These techniques (Figure iii) illustrate the diverse approaches available for diatom isolation and purification, each tailored to specific challenges in obtaining pure cultures (Table iv).

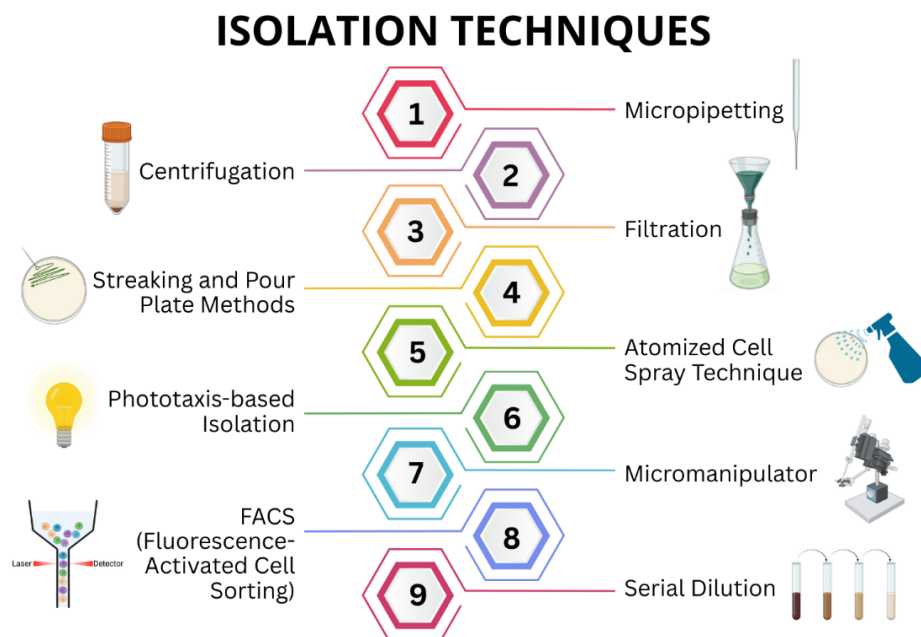


Figure iii. Isolation strategies employed for culturing and purifying diatom

310 **Table iv. Advantages and disadvantages of isolation techniques** (Andersen, 2005; Vu et al.,
311 2018)

Techniques	Advantages	Disadvantages	Techniques most appropriate for
Micropipetting	Highly specific	Time consuming	Ideal for single cell isolation
Centrifugation	Concentrate large cells	Difficult to obtain single species	Ideal for segregating smaller and larger organisms
Filtration	Rapid and convenient technique	Cannot isolate single species	Ideal for size-based separation
Streaking and pour plate method	Easy and simple method	Many species do not grow on agar	Suitable for rapid growing culture and for obtaining axenic culture
Atomized Cell Spray	Samples are evenly spread on agar plates	Risk of Contamination from air	Ideal for isolating cultures with agar
Phototaxis based isolation	Non-invasive process and segregates motile and non-motile sample	It's a steady process	Ideal for sample which exhibits phototaxis
Micromanipulator	Highly precise and low contamination	Expensive and low throughput	Ideal for precise isolation of single species
FACS	Rapid and provide pure cultures	Highly expensive and may damage cells	Ideal for fluorescence or cell size-based cell sorting
Serial Dilution	Cost-effective and simple process	Slow process and sometime diluting may eliminate target species	Ideal for single species isolation from mixed population by diluting

5.2 Establishment of axenic cultures

Axenic cultures are pure cultures containing a single species or strain, free of contamination. To achieve axenicity, various techniques can be employed, including antimicrobial agents like potassium tellurite and antibiotics. Antibiotics work either by inhibiting bacterial growth (bacteriostatic) or killing bacteria by damaging cell walls or contents. However, antibiotics may hinder the diatom growth and can trigger mutations posing risk to culture integrity and promoting antibiotic resistance. Alternative techniques like lysozyme treatment, sodium hypochlorite, sulfonamides, and UV irradiation helps in achieving the axenic cultures. Maintaining axenic cultures of diatoms can be challenging due to the possibility of contamination especially robust bacteria (Parikh et al., 2025). Methods such as serial transfer, cryopreservation, and specific treatments (e.g., 0.1% I2 solution for *Chaetoceros*) help maintain and purify axenic cultures (Andersen, 2005; Vu et al., 2018).

5.3 Growth and biomass estimation: Cell count, dry weight, metabolite profiling

Culture media are critical for the growth and metabolism of microorganisms, particularly diatoms, as they provide essential nutrients under controlled in-vivo conditions (Gautam et al., 2013). These media are categorized into three types: solid, liquid, and semi-solid. Solid media, exemplified by agar, support diatom attachment and colony formation, while liquid media, such as nutrient-enriched broths, allow for cell suspension and nutrient absorption. Semi-solid media utilize lower agar concentrations for specific applications, including culture transfers (Saxena et al., 2021).

Diatom cultivation necessitates specialized media tailored to their physiological needs, such as WC, PM, Chu, and F/2-Si media. For instance, PM medium is designed for diatoms in acidic environments, whereas WC medium is intended for diatoms in alkaline environments. F/2-Si medium is a versatile option, enhanced with silicate to promote frustule biosynthesis. The

biochemical components of these media—including nitrogen, phosphorus, silicon, iron, and trace elements—play pivotal roles in processes such as amino acid synthesis, protein synthesis, ATP synthesis, and nucleic acid synthesis. Diatoms exhibit diverse trophic strategies—photoautotrophy, heterotrophy, and mixotrophy — that enable them to adapt to varying environmental conditions. The careful selection of culture media and growth methodologies is essential for maximizing diatom biomass growth, yield, lipid accumulation, and metabolic diversity, thereby enhancing their utility in sectors such as biofuel production and aquaculture (Yang et al., 2014; Yodsuwan et al., 2017).

To assess diatom growth, metrics such as cell counts and biomass measurements can be used. Cell counting, often conducted using hemocytometers, is typically performed in triplicate to minimize errors, with immobilization techniques used for motile cells and proper homogenization to avoid clumping (Reza Moheimani et al., 2013; Bhattacharjya et al., 2020). Biomass and ash-free dry weight are standard methods for quantifying biomass. Dry weight is calculated post-filtering and drying biomass on a pre-weighed filter, whereas ash-free dry weight calculations involve adjusting for weight loss after combustion at high temperatures. Biomass productivity is expressed in grams per liter per day ($\text{gL}^{-1}\text{d}^{-1}$), while lipid productivity combines specific growth rates with lipid yields to measure metabolic output (Indrayani et al., 2020; Reza Moheimani et al., 2013; Wang et al., 2014). Finally, diatoms produce both primary and secondary metabolites: primary metabolites comprise proteins, lipids, sterols, and carbohydrates; secondary metabolites include polyphenols and carotenoids (Table v). Accurate estimation of these products is key for evaluating the potential commercial applications of diatom cultures (Nieri et al., 2023).

Table v. Overview of various diatom-derived metabolites and their estimation techniques

Metabolites	Estimation Method	Analysis Technique	References
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Primary Metabolites			
Lipids	Bligh and Dyer Method	NMR spectroscopy provides structure of lipids with quantitative and qualitative analysis; while MS is used for identification and quantifying of lipids, it includes GC-MS, LC-MS etc.	(Bligh & Dyer, 1959; Thien Do NGUYEN et al., 2017; Gerhardtova et al., 2024)
Fatty Acids			
Triacylglycerols			
Polar Lipids			
Oxylipins			
Carbohydrates			
Chrysolaminarin		GC-MS is used for identification	

Exopolysaccharides (EPS)	Anthrone Method	and quantification of monosaccharides and EPS analysis;NMR spectroscopy is used in the studies of chrysolaminarin and polysaccharides	(Koehler, 1952; Templeton et al., 2012; Alothman et al., 2024)
Proteins	Bradford Method, Lowry Method	UV-Spectroscopy helps in quantification of protein at specific wavelength (600 nm for Bradford and 500 to 750 nm for Lowry method)	(Barbarino & Lourenço, 2005)
Sterols	Saponification Method	GC-MS used for identification and quantification of sterols.	(Fagundes et al., 2021)
Amino Acids	Ninhydrin Assay	HPLC widely used for amino acids profiling, GC-MS used for quantification of amino acids	(Zhang et al., 2016)

Secondary Metabolites			
Carotenoids	Spectrophotometric Assay	UV-Vis spectroscopy measures absorbance of pigments at specific wavelengths; HPLC separates and quantify chlorophyll and carotenoids	(Sudhakar M P et al., 2013; Kuczynska et al., 2015)
Chlorophyll a & c			
Fucoxanthin			
β-Carotene			
Diadinoxanthin, Zeaxanthin, Antheraxanthin, Diatoxanthin, Violaxanthin			
Polyphenols		UV-Spectroscopy estimates polyphenols, HPLC quantifies and separates polyphenols	(Hajimahmoodi et al., 2010)
Flavonoids	Aluminum Chloride Method	UV-Spectroscopy screens flavonoids, HPLC separates and quantifies flavonoids	(Ghasemzadeh et al., 2015)

Phenolic Acids	Folin-Ciocalteu Method	HPLC quantifies and separates phenolic acids, GC-MS measures volatile phenolic acids	(Singleton et al., 1999)
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6. Diatom-Based Indices and Statistical Approaches

Diatoms serve as biological indicators for assessing aquatic habitat conditions and are widely used as Biological Quality Elements (BQEs) for water biomonitoring. Diatom-based indices, such as the Specific Pollution Sensitivity Index (SPI), Watanabe's Index (WAT), and Biological Diatom Index (BDI), provide a steady approach to monitoring water quality and detecting ecological pollution. These indices are based on principles like saprobic systems (Table vi), autecology, and community structure analysis, allowing researchers to understand ecological conditions and responses to environmental stress (Table vii) (Blanco, 2024; Méndez-Zambrano et al., 2024; Musa & Greenfield, 2018).

The development of diatom-based indices for water quality assessment has expanded considerably, particularly across the temperate regions of the Northern Hemisphere (Birk et al., 2012). Commonly used European indices include the Trophic Diatom Index (TDI), the Pampean Diatom Index (IDP), the Biological Diatom Index (IBD), and the Pollution Tolerant Index (Besse-Lototskaya et al., 2011), while in North America, diatom indices such as the Indices of Biotic Integrity and the Eastern Canadian Diatom Index (IDEC) are commonly used (Potapova & Charles, 2007). Because these indices were developed under temperate Northern Hemisphere conditions and the research has suggested that their applicability often declines when used in rivers from different climatic and biogeographical regions (Bere, 2016). Various studies have suggested that regional or catchment-specific indices provide more accurate assessments than nationally or internationally derived indices. Although diatoms were traditionally viewed as cosmopolitan with consistent ecological tolerances, recent research indicates that many species exhibit regionally variable responses shaped by climate, soils, geographical morphology, vegetation, and catchment characteristics. As a result, there is a growing need to recalibrate existing indices to local conditions or to develop region-specific

indices that more accurately reflect taxa–environment relationships, thereby improving monitoring sensitivity and reliability (Oeding & Taffs, 2017).

Table vi. Various classes based on diatom index score

Class	Diatom Index Score	Trophic State	References
Bad quality	Less than 9	Eutrophy	(Eloranta & Soininen, 2002; Venkatachalapathy & Karthikeyan, 2015)
Poor quality	Between 9 to 12	Meso-eutrophy	
Moderate quality	Between 12 to 15	Mesotrophy	
Good quality	Between 15 to 17	Oligo-mesotrophy	
High quality	More than 17	oligotrophy	

Diatom indices are calculated by the formula developed by Marvan and Zelinka in 1961, and calculated by a software called OMNIDIA (Karthick & Taylor, 2010; Musa & Greenfield, 2018):

$$Index = \frac{\sum_j^n = 1a_j s_j v_j}{\sum_j^n = 1a_j v_j}$$

Where,

a_j = species (j) abundance in sample

v_j = indicator value as per the species

s_j = species pollution sensitivity

Table vii. Various Diatom-based indices

Diatom Based indices	Origin	Taxonomy	Interpretation	Significance	Limitations	References
Watanabe's Index (WAT)	Japan	Species	Organic Pollution level	Signifies higher levels of nutrient enrichment of the species	Limited to specific species	(SUMITA & WATANABE, 1983)
Biological Diatom Index 2006	France	Species	Water Quality	Signifies sensitivities to different pollution	Might not cover all types of pollution	(Coste et al., 2009)

(BDI-2006)						
Trophic Diatom Index (TDI)	England	Species	Inorganic Pollution	Signifies tolerance to organic pollution of the species	Limited to specific trophic levels	(Musa & Greenfield, 2018; Norian et al., 2022)
Specific Pollution Index (SPI)	France	Species	Organic Pollution	Signifies species tolerance to organic pollution	May not account for all types of organic pollution	(Musa & Greenfield, 2018)
Generic Diatom Index (GDI)	France	Genera	Inorganic Nutrient Contamination	Signifies genera tolerance to inorganic nutrient contamination	Might fail to account for the sensitivity of particular species to specific contaminants	(Musa & Greenfield, 2018)
Sladeczek Saprobic Index (SLA)	Czechia	Taxon level	Organic Pollution	Signifies the effect of organic pollution	May get influenced by other environmental factors	(Dalkıran & Zünbülğil-Ünsal, 2024)
Diatom Pollution Tolerance Index (PTI)	Central Europe	Taxon level	Water Quality	Signifies the assessment of the water quality	Not ideal for assessing all aquatic ecosystem	(Méndez-Zambrano et al., 2024)
Pampean Diatom Index (IDP)	United States of	Species	Organic Pollution	It represents organic contamination as I, II, III and IV	Might not cover all ecological conditions	(Méndez-Zambrano et al., 2024)

	America					
Descy's pollution metric (DESCY)	France	Species	Pollution level	It signifies the sensitivity of diatom species towards the nutrient enrichment	May vary in non-european regions	(Descy, 1979)
Eutrophication/ diatom-based pollution index (EPI- D)	Italy	Species	Organic Nutrients	It signifies the sensitivity of species towards the organic nutrients	Gets influenced by regional environmental conditions	(Torrise & Dell'Uomo, 2006)
Steinberg and Schiefele trophic metric (SHE)	Germany	Species	Eutrophication level	It classifies the aquatic habitat into three sites: heavily polluted sites, poor nutrient sites and unpolluted sites	It does not specify other pollution types such as organic pollution, inorganic pollution etc.	(Steinberg & Schiefele, 1988)
DAIpo index	Japan	No Taxon level	Organic Nutrients	It signifies by the values in which higher the level, greater the amount of saprophytic species	Might not account for various environmental conditions affecting saprophytic species	(Méndez-Zambrano et al., 2024)

Principal Component Analysis (PCA) and Canonical Correspondence Analysis (CCA) are statistical tools used in biomonitoring to understand how ecological factors influence diatom communities. PCA simplifies large datasets by identifying patterns, while CCA directly correlates species data with environmental variables. Studies of rivers such as the Chambal River in India and South African rivers have used these methods to link diatom communities to variables such as phosphate, pH, and conductivity. These analyses support the development of diatom-based indices that calculate optimal environmental conditions for each species using weighted-average regression (Costa & Schneck, 2022; Solomon et al., 2025; Srivastava et al., 2017).

$$u_k = \frac{\sum y_{ik} x_i}{\sum y_{ik}}$$

Where, u_k is the species' optimum, y_{ik} is its abundance in sample i , and x_i is the environmental value in that sample.

Diatom indices, such as the Trophic Diatom Index (TDI) and Specific Pollution Sensitivity Index (IPS), use species optima and pollution sensitivity to produce a single score reflecting water quality. These indices have been effective in tracking human impacts on water quality in various ecosystems, including tropical reservoirs. By combining PCA and CCA with diatom indices, researchers can identify key ecological factors and assess diatom responses, providing a powerful tool for evaluating and monitoring water quality in rivers, lakes, and reservoirs (Blanco, 2024; da Silva-Lehmkuhl et al., 2022; Yu et al., 2021).

6.1 Integration with physicochemical parameters

Physicochemical parameters of water are critical for evaluating its quality and health, encompassing factors such as color, temperature, and various contaminants, including microorganisms and chemicals. According to Sharma et al., (2020), these contaminants can be

422 characterized using specific measurement parameters including pH, temperature, electrical
423 conductivity (EC), turbidity, total alkalinity, total dissolved solids (TDS), total hardness, total
424 suspended solids (TSS), biochemical oxygen demand (BOD), dissolved oxygen (DO), and
425 chemical oxygen demand (COD) . The choice of these parameters depends on the water's
426 intended use—drinking, domestic, agricultural, or industrial.

427 Physical parameters such as pH, temperature, color, turbidity, odor, and TDS are measured
428 using various instruments. For example, pH levels can be determined with a pH meter or strip,
429 temperature with a thermometer, TDS with a TDS meter, and turbidity with nephelometers
430 (Bhat et al., 2018). Each parameter plays a unique role: pH indicates hydrogen ion
431 concentration, while temperature influences biological activity and the solubility of gases such
432 as oxygen. Electrical conductivity reflects water's electrical conductivity, and turbidity reveals
433 water clarity based on suspended particles. Total alkalinity measures acid-neutralizing capacity,
434 and total hardness—linked to calcium and magnesium salts—affects scaling in pipes and
435 industrial systems (Kumar et al., 2020; Rout et al., 2021; Upadhyay et al., 2011)(Kumar et al.,
436 2020; Rout et al., 2021; Upadhyay et al., 2011).

437 (APHA, 1998; Bhavan et al., 2012; CCME, 2001; CPCB, 1993; WHO, 2017)Heavy metal
438 assessments are crucial for determining water quality due to the harmful effects these metals—
439 such as cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), iron (Fe), manganese (Mn),
440 cobalt (Co), nickel (Ni), zinc (Zn), and mercury (Hg)—have on human health and aquatic
441 ecosystems. These metals typically originate from industrial discharge, agricultural runoff, and
442 mining operations (Sehgal et al., 2012). Advanced analytical techniques such as atomic
443 absorption spectroscopy (AAS) and inductively coupled plasma mass spectrometry (ICP-MS)
444 are used to detect these metals. Nitrogen levels are measured via spectroscopic analysis using
445 Nessler's reagent, while sulfate concentrations are assessed using the nephelometric method.
446 Magnesium is quantified by EDTA titration in buffered conditions, whereas calcium is

determined by complexometric titration with specific indicators at high pH. Additionally, silicates and phosphates are estimated spectroscopically with molybdate ions under acidic conditions. Sodium and potassium concentrations are analyzed by flame photometry, with dilution factors applied to samples exceeding standard limits (Tomar & Upadhyay, 2018). Overall, a comprehensive assessment of physicochemical water parameters is vital for safety, regulatory compliance, ecological balance, and the health of aquatic systems. This necessitates the use of advanced analytical instruments and proficient expertise to detect and mitigate potential water quality risks, underscoring the importance of water quality assessments in environmental management and public health (Suthar et al., 2010).

Recent studies underscore the potential of diatoms in phycoremediation, particularly their ability to bio-accumulate heavy metals from wastewater (Marella et al., 2020). . For instance, the diatom species *Navicula pelliculosa* has been experimentally shown to effectively uptake and detoxify cadmium (Cd), lead (Pb), and zinc (Zn) from aquatic environments, suggesting its viability for heavy-metal remediation applications (Irving et al., 2009). Furthermore, in marine ecosystems, diatoms are implicated in hydrocarbon bioremediation. Research indicates that specific diatom species promote the degradation of aliphatic and aromatic hydrocarbons found in oil-contaminated seawater by secreting exopolysaccharides and engaging with associated microbial communities (Paniagua-Michel et al., 2024). Notably, cultivating the model diatom *Phaeodactylum tricornutum* in wastewater produced from oil fields has led to a significant reduction of both inorganic and organic pollutants while also facilitating substantial biomass production. This highlights the dual advantages of employing diatoms not only for bioremediation but also for biomass valorization, demonstrating their multifaceted role in environmental cleanup initiatives (Gillard et al., 2021). These findings suggest that diatom-based strategies can extend beyond simple bioindication to remove pollutants and enhance ecosystem health actively.

7. Applications and Future Perspectives

7.1 Diatom as bioindicator for aquatic health

Diatoms are vital components of aquatic ecosystems, playing significant roles in water quality monitoring and assessment, as well as in reconstructing paleoenvironments (Xin, 2011). Their morphological diversity, particularly the silicified cell walls of various diatom species, contributes to the biotic characterization of environmental samples (Carvalho et al., 2011). Diatom indices obtained through microscopic methods are widely accepted and can effectively correlate with eutrophication levels and pollution conditions (Yogeshwaran et al., 2025). As bioindicators, diatoms are sensitive to various ecological conditions and can reveal changes in water quality in streams and rivers (Glevitzky et al., 2025). For instance, studies in the Locumba River in Peru indicated that a decrease in diatom diversity, coupled with increased contaminant levels, occurred downstream, demonstrating their utility for water quality assessment (Calizaya-Anco et al., 2013). Similarly, in Ecuador's Jimbitono River, epilithic diatoms were used to assess water quality, revealing varying contamination levels across monitoring stations (Heredia et al., 2024). Diatoms also serve as early indicators of changes in relatively undisturbed lakes. Furthermore, beyond their monitoring capabilities, diatoms offer a sustainable solution for municipal wastewater treatment, adept at removing nutrients, heavy metals, and emerging pollutants (Rahman et al., 2024; Ramstack Hobbs et al., 2022). Diatoms can adsorb, oxidize, decompose, and precipitate toxic substances, including heavy metals. Their presence thus reduces ecological stress in aquatic systems and maintains. Because diatoms respond sensitively to changes in nutrient and chemical composition, they are also widely recognized as bioindicators of water quality in marine, brackish, and freshwater environments (Pandey et al., 2018). Moreover, they contribute to water quality improvement through their fast nutrient uptake ability, utilizing excess nitrogen, phosphorus, and trace metals present in wastewater effluents. Their unique cellular structure, particularly the siliceous

frustule and large central vacuole, supports their capacity to absorb and detoxify pollutants (Marella et al., 2020)

7.2 Molecular and bioinformatics integration

Molecular-based diatomic indices are set to supersede traditional indices due to their improved sensitivity (Carvalho et al., 2011). Recent advances in omics technologies, along with advanced bioinformatics tools for analyzing complex omics data, facilitate the use of diatoms as key organisms for water quality evaluation via molecular biomarkers. Fully sequenced diatom species are currently utilized in toxicological studies to investigate the effects of pollutants. The use of molecular studies with diatoms is anticipated to become fundamental for ecological assessment of environmental samples (Carvalho et al., 2011; Villar et al., 2025).

The drive to incorporate molecular methods into bioindicator research is fueled by the potential to enhance understanding and accuracy (Livingstone, 1993). Molecular biomarkers, including mRNA probes, are likely to promote the broad adoption of these tools (Hoshi et al., 2025). Significant advancements in bioinformatics have occurred across various fields, particularly in molecular biology, proteomics, genomics, metabolomics, biotechnology, and agriculture (Hodgman, 2006; Villar et al., 2025). Furthermore, assessing microbial presence and abundance associated with disease can support monitoring of marine environmental health, with bioinformatics contributing to the assessment of potential interferences. Increasing research is being conducted to compare molecular and morphological techniques for examining communities, such as nematode communities (Chen et al., 2010; Domingues et al., 2025)(Chen et al., 2010; Domingues et al., 2025).

In recent years, significant methodological advancements have revolutionized the analysis of diatom communities, moving beyond traditional molecular biomarker techniques. High-throughput metabarcoding using markers such as 18S rRNA, rbcL, and ITS has emerged as an

effective means to assess diatom community composition directly from environmental samples, eliminating the need for culturing. This approach enables the detection of both abundant and rare taxa, thereby providing a comprehensive overview of ecological status (Apothéloz-Perret-Gentil et al., 2021; Vlaović et al., 2025; Vasselon et al., 2025). In conjunction with metabarcoding, environmental DNA (eDNA) methodologies are gaining traction for water-quality monitoring. These non-invasive and expedient techniques enable rapid assessments of diatom biodiversity and hold promise for early-warning systems across aquatic ecosystems, including lakes, rivers, and coastal areas (Wang et al., 2024). Furthermore, advancements in automated image analysis and deep-learning-based taxonomy represent a transformative shift in the field. Machine-learning tools, notably convolutional neural networks (CNNs), exhibit proficiency in recognizing frustule morphology with accuracy that rivals that of seasoned taxonomists. This technology significantly minimizes the time and subjectivity associated with microscopic identification, positioning it to become essential within diatom-based biomonitoring frameworks (Keck et al., 2023). The synthesis of bioinformatics workflows that amalgamate omics data, eDNA metabarcoding, and AI-driven image analysis is paving the way for the creation of next-generation diatom indices. These indices promise improved reproducibility, scalability, and suitability for high-frequency monitoring, aligning well with the tenets of circular and digital environmental management.

To quantitatively assess the relative effectiveness of molecular and bioinformatics approaches in diatom-based biomonitoring, it is essential to understand the performance of various metrics reported across multiple studies. These metrics include the number of taxa detected, sensitivity, specificity, throughput, and congruence with classical microscopy. Metabarcoding and eDNA approaches generally detected 15–35% more taxa than traditional microscopy, particularly capturing rare or cryptic species (Apothéloz-Perret-Gentil et al., 2021; Vlaović et al., 2025). Automated image analysis and machine-learning-assisted taxonomy achieved classification

accuracies of 85–95% compared with expert taxonomists, while significantly reducing operator time (Keck et al., 2023). Despite these promising developments, several technical constraints remain. Primer bias, incomplete reference databases, and discrepancies between morphological and molecular identifications can limit the accuracy of metabarcoding and eDNA approaches (Apothéloz-Perret-Gentil et al., 2021; Vasselon et al., 2025). Similarly, machine-learning-based automated taxonomy requires high-quality training datasets and computational resources, which may restrict routine application in some contexts. Acknowledging these limitations is crucial for developing robust, reproducible, and globally comparable diatom-based bioassessment frameworks.

7.3 Future directions for bioindicator research

Future research in bioindicators, particularly diatoms, is expected to expand with a focus on the correlation between bioindicator monitoring and its impact on communities, interspecies comparisons for identifying effective bioindicators, and assessing community proficiency in using these organisms (Fahmi et al., 2025). This review is essential for advancing bioindicator research and applications in environmental monitoring. Concurrently, ecotoxicology research aims to enhance the utility of bioindicators and biomarkers. There is also a growing emphasis on innovative adsorbents for pollutant removal, such as nanocellulose, metal-organic frameworks (MOFs), graphene-based composites, and biochar (Fahmi et al., 2025; Feodorov et al., 2024). Future efforts will focus on developing scalable production methods, improving material stability, and establishing sustainable regeneration techniques. Moreover, integrating bioinformatics with green technologies is seen as a promising avenue for bolstering environmental protection initiatives (Akhtar et al., 2024; Kruthi et al., 2024).

Diatom-based bioindicator research will necessitate global standardization of bioassessment indices, including consistent protocols for sampling, slide preparation, sequencing workflows,

and data reporting. The creation of inter-laboratory calibration initiatives and shared reference libraries, encompassing both morphological traits and DNA barcodes, will facilitate uniform interpretation of ecological status across different geographic regions, as highlighted in relevant studies (Vasselon et al., 2025). A key priority is integrating morphological and molecular methodologies, moving beyond a competitive perspective toward a complementary approach. The combination of classical microscopy with techniques such as metabarcoding, environmental DNA (eDNA) assays, automated image analysis, and machine-learning-assisted taxonomy is expected to enable the development of hybrid diatom indices. These indices will harness both ecological accuracy and taxonomic sensitivity, showcasing findings from multiple studies that eDNA-based diatom indices reliably correlate with environmental quality in contrast to traditional methods. Additionally, machine-learning strategies applied to metabarcoding data can significantly enhance predictive power for bioassessment tasks (Kerk et al., 2023). Future research should prioritize the application of molecular datasets to regulatory frameworks, emphasizing the validation of threshold values and ecological status classifications derived from high-throughput sequencing methods. Recent studies also indicate the practicality of employing eDNA-based diatom monitoring in freshwater quality assessment programs, underscoring its potential for environmental monitoring and regulatory applications (Kutty et al., 2022).

8. Conclusion

Diatoms have recently garnered global interest for their multifaceted applications across fields such as pharmaceuticals, nutraceuticals, and nanotechnology. Additionally, they are recognized for producing valuable compounds, including lipids, fucoxanthin, and polysaccharides. Moreover, diatoms are utilized as effective tools for water biomonitoring, offering insights into the quality of various aquatic ecosystems. Although various diatom-based indices have been developed to assess water quality, these tools are often limited to specific geographic regions,

necessitating the creation and validation of new indices to broaden their applicability. Furthermore, isolating diatoms is crucial for establishing axenic cultures, which are essential for understanding their diversity. Ongoing research is imperative to sustain and enhance axenic culture maintenance. Therefore, a comprehensive study of diatoms is essential to harness their full potential in scientific and practical applications.

References:

- Agarwal, Nilesh. (2025). *Diatoms Basic and Applied Research*. Educohack Press.
- Akhtar, M. S., Ali, S., & Zaman, W. (2024). Innovative Adsorbents for Pollutant Removal: Exploring the Latest Research and Applications. *Molecules* 2024, Vol. 29, Page 4317, 29(18), 4317. <https://doi.org/10.3390/MOLECULES29184317>
- Alibert, M., Hamilton, P. B., Pienitz, R., Dorion, H., & Antoniadis, D. (2025). Diatom biogeography of Tursujuq Park, Nunavik, Canada. *Hydrobiologia*, 852(16), 4187–4209. <https://doi.org/10.1007/S10750-025-05859-5>
- Allothman, A., Emwas, A. H., Singh, U., Jaremko, M., & Agusti, S. (2024). Metabolomics-based analysis of the diatom *Cheatocecos tenuissimus* combining NMR and GC-MS techniques. *MethodsX*, 12, 102695. <https://doi.org/10.1016/j.mex.2024.102695>
- Andersen, R. A. (2005). *Algal Culturing Techniques*. Elsevier Academic Press.
- Anderson, D. M., & Karlson, B. (2017). Appendix 4. Preservatives and methods for algal cell enumeration. <https://doi.org/10.25607/OBP-365>
- APHA. (1998). *Standard methods for the examination of water and waste water* (American Public Health Association, Ed.; 19th ed.). American Water Works Association, Water Environment Federation..
- Apothéloz-Perret-Gentil L, Bouchez A, Cordier T, Cordonier A, Guéguen J, Rimet F, Vasselon V, Pawlowski J. Monitoring the ecological status of rivers with diatom eDNA metabarcoding: A comparison of taxonomic markers and analytical approaches for the inference of a molecular diatom index. *Molecular Ecology*. 2021 Jul;30(13):2959-68.
- Aykut, T. O., Balkis-özdelice, N., Durmuş, T., & Solak, C. N. (2025). Seasonal Variation of Epipelagic Diatom Assemblages and Their Relationships with Abiotic Variables in the Utility

624 and Drinking Water Source of İstanbul: Büyükçekmece Reservoir. *Turkish Journal of*
625 *Fisheries and Aquatic Sciences*, 25(6). <https://doi.org/10.4194/TRJFAS26745>

626 Barbarino, E., & Lourenço, S. O. (2005). An evaluation of methods for extraction and
627 quantification of protein from marine macro-and microalgae. *Journal of Applied Phycology*,
628 17, 447–460. <https://doi.org/10.1007/s10811-005-1641-4>

629 Barua, P. (2025). Diatoms as Microbial Architects of the Silica Cycle: Ecological Significance,
630 Biogenic Bioactives, and Emerging Biotechnological Applications. *Microbial Bioactives*,
631 8(1), 1–12. <https://doi.org/10.25163/MICROBBIOACTS.8110298>

632 Battarbee, R. W., Charles, D. F., Dixit, S. S., & Renberg, I. (1990). Diatoms as indicators of
633 surface water acidity. In E. F. Stoermer & J. P. Smol (Eds.), *The Diatoms* (pp. 85–127).
634 Cambridge University Press. <https://doi.org/10.1017/CBO9780511613005.006>

635 Bere, T. (2016). Challenges of diatom-based biological monitoring and assessment of streams in
636 developing countries. *Environmental Science and Pollution Research*, 23(6), 5477–5486.
637 <https://doi.org/10.1007/S11356-015-5790-Y>

638 Besse-Lototskaya, A., Verdonschot, P. F. M., Coste, M., & Van De Vijver, B. (2011). Evaluation of
639 European diatom trophic indices. *Ecological Indicators*, 11(2), 456–467.
640 <https://doi.org/10.1016/j.ecolind.2010.06.017>

641 Bhat, B. N., Parveen, S., & Hassan, T. (2018). Seasonal assessment of physicochemical parameters
642 and evaluation of water quality of river Yamuna, India. *Advances in Environmental*
643 *Technology*, 4(1), 41–49. <https://doi.org/10.22104/aet.2018.2415.1121>

644 Bhavan, M., Shah, B., & Marg, Z. (2012). *Indian standard specification for drinking water*.
645 https://cpcb.nic.in/wqm/BIS_Drinking_Water_Specification.pdf

646 Birk, S., Bonne, W., Borja, A., Brucet, S., Courrat, A., Poikane, S., Solimini, A., Van De Bund, W.,
647 Zampoukas, N., & Hering, D. (2012). Three hundred ways to assess Europe's surface waters:
648 An almost complete overview of biological methods to implement the Water Framework
649 Directive. *Ecological Indicators*, 18, 31–41. <https://doi.org/10.1016/j.ecolind.2011.10.009>

650 Blanco, S. (2024). What do diatom indices indicate? Modeling the specific pollution sensitivity
651 index. *Environmental Science and Pollution Research*, 31(20), 29449–29459.
652 <https://doi.org/10.1007/s11356-024-33115-1>

Bligh, E. G., & Dyer, W. J. (1959). A RAPID METHOD OF TOTAL LIPID EXTRACTION AND
 PURIFICATION. *Canadian Journal of Biochemistry and Physiology*, 37(8), 911–917.
<https://doi.org/10.1139/o59-099>

British Standard, E. U. (2003). *Water quality — Guidance standard for the routine sampling and
 pretreatment of benthic diatoms from rivers*.

Burfeid-Castellanos, A. M., Kloster, M., Beszteri, S., Postel, U., Spyra, M., Zurowietz, M.,
 Nattkemper, T. W., & Beszteri, B. (2022). A Digital Light Microscopic Method for Diatom
 Surveys Using Embedded Acid-Cleaned Samples. *Water (Switzerland)*, 14(20).
<https://doi.org/10.3390/w14203332>

Calizaya-Anco, J., Avendaño-Cáceres, M., & Delgado-Vargas, I. (2013). [Evaluation of the quality
 of river water with diatoms (Bacillariophyceae): an experience in Tacna, Peru]. *Revista
 Peruana de Medicina Experimental y Salud Publica*, 30(1), 58–63.
<https://doi.org/10.1590/S1726-46342013000100012>

Car, A., & Kaleli, A. (2025). Marine benthic diatoms from the Adriatic Sea (NE Mediterranean):
 review of investigations and checklist with updated nomenclature. *Acta Botanica Croatica*,
 84(2), 116–140. <https://doi.org/10.37427/BOTCRO-2025-024>

Carvalho, R. N., António, D. C., & Lettieri, T. (2011). Diatoms for Omics Applications in Water
 Monitoring. *Hydrol Current Res*, 1, 1. <https://doi.org/10.4172/2157-7587.S1-001>

CCME. (2001). *Canadian Council of Ministers of Environment: Water Quality Index User's
 Manual*. <https://www.scirp.org/reference/referencespapers?referenceid=3757384>

Chakraborty, I., Chakrabarti, S., Managuli, V., & Mazumder, N. (2022). Atomic Force Microscopy
 Study of Diatoms. *Diatom Microscopy*, 81–109. <https://doi.org/10.1002/9781119711568.CH4>

Chen, X. Y., Daniell, T. J., Neilson, R., O'Flaherty, V., & Griffiths, B. S. (2010). A comparison of
 molecular methods for monitoring soil nematodes and their use as biological indicators.
European Journal of Soil Biology, 46(5), 319–324.
<https://doi.org/10.1016/J.EJSOBI.2010.05.002>

Clason, T., Acker, F., Morales, E., & Marr, L. (2017). IDENTIFICATION AND ENUMERATION
 OF DIATOMS ON MICROSCOPE SLIDES.

Costa, A. P. T., & Schneck, F. (2022). Diatoms as indicators in running waters: trends of studies on
 biological assessment and monitoring. In *Environmental Monitoring and Assessment* (Vol.

194, Issue 10). Springer Science and Business Media Deutschland GmbH.
<https://doi.org/10.1007/s10661-022-10383-3>

Coste, M., Boutry, S., Tison-Rosebery, J., & Delmas, F. (2009). Improvements of the Biological Diatom Index (BDI): Description and efficiency of the new version (BDI-2006). *Ecological Indicators*, 9(4), 621–650. <https://doi.org/10.1016/j.ecolind.2008.06.003>

PCB. (1993). *General standards for discharge of environmental pollutants part-a: effluents*; .

Roll, D. A., & Holmes, R. W. (1982). October 1982] *Short Communications* 765 *A Note on the Occurrence of Diatoms on the Feathers of Diving Seabirds*.
<https://academic.oup.com/auk/article/99/4/765/5185729>

da Silva-Lehmkuhl, A. M., Blanco, S., & Bicudo, D. C. (2022). A new Trophic Diatom Index for Tropical Reservoirs (TDIR). *Hydrobiologia*, 849(21), 4651–4666.
<https://doi.org/10.1007/s10750-022-05003-7>

Dalkıran, N., & Zünbülğil-Ünsal, B. (2024). Trophic and saprobic diatom-based indices are not indicating the same ecological status in a shallow lake, Türkiye. *Community Ecology*, 25(2), 249–265. <https://doi.org/10.1007/S42974-024-00199-1/TABLES/7>

Descy, J.-P. (1979). *A new approach to water quality estimation using diatoms*. *Beih. Nova Hedw.*, 64, 305–323. <https://researchportal.unamur.be/en/publications/a-new-approach-to-water-quality-estimation-using-diatoms-beih-nov>

Domingues, V. da S. P., Seldin, L., & Jurelevicius, D. (2025). Understanding the implicit effects of 16S rRNA gene databases on microbial bioindicator studies. *Aquatic Toxicology*, 283.
<https://doi.org/10.1016/j.aquatox.2025.107351>

El Amin, M. H., & Prihantini, N. B. (2023). Characteristics of Diatom in Different Preservation Methods: A Comparison Study. *Jurnal Penelitian Pendidikan IPA*, 9(3), 1327–1331.
<https://doi.org/10.29303/jppipa.v9i3.2775>

Eloranta, P., & Soininen, J. (2002). Ecological status of some Finnish rivers evaluated using benthic diatom communities. *Journal of Applied Phycology*, 14(1), 1–7.
<https://doi.org/10.1023/A:1015275723489>

Agundes, M. B., Alvarez-Rivera, G., Vendruscolo, R. G., Voss, M., da Silva, P. A., Barin, J. S., Jacob-Lopes, E., Zepka, L. Q., & Wagner, R. (2021). Green microsaponification-based

method for gas chromatography determination of sterol and squalene in cyanobacterial
biomass. *Talanta*, 224, 121793. <https://doi.org/10.1016/J.TALANTA.2020.121793>

Fahmi, M. I. N., Mahanal, S., Zubaidah, S., & Ibrohim, I. (2025). Bioindicator Trends in Studying
Environmental Pollution: A Systematic Review. *Jurnal Penelitian Dan Pengkajian Ilmu
Pendidikan: E-Saintika*, 9(2), 107–126. <https://doi.org/10.36312/E-SAINTIKA.V9I2.1704>

Feodorov, L., Nita-Lazar, M., & Cornea, C. P. (2024). Exploring current frontiers of environmental
challenges by bioindicators and biomarkers. *Scientific Bulletin. Series F. Biotechnologies*,
Vol. XXVIII.

Gautam, S., Arya, A., & Vinayak, V. (2013). Protocol to Establish Axenic Cultures for Diatoms of
Fresh Water. *International Journal of Science and Research (IJSR) ISSN*.
<https://doi.org/10.21275/ART20162689>

Gebeshuber, I. C., Kindt, J. H., Thompson, J. B., Del Amo, Y., Stachelberger, H., Brzezinski, M.
A., Stucky, G. D., Morse, D. E., & Hansma, P. K. (2003). Atomic force microscopy study of
living diatoms in ambient conditions. *Journal of Microscopy*, 212(3), 292–299.
<https://doi.org/10.1111/J.1365-2818.2003.01275.X>

Gerhardtova, I., Jankech, T., Majerova, P., Piestansky, J., Olesova, D., Kovac, A., & Jampilek, J.
(2024). Recent Analytical Methodologies in Lipid Analysis. *International journal of
molecular sciences*, 25(4), 2249. <https://doi.org/10.3390/ijms25042249>

Ghasemzadeh, A., Jaafar, H. Z. E., & Rahmat, A. (2015). Phytochemical constituents and
biological activities of different extracts of *Strobilanthes crispus* (L.) Bremek leaves grown in
different locations of Malaysia. *BMC Complementary and Alternative Medicine*, 15(1), 1–10.
<https://doi.org/10.1186/S12906-015-0873-3/FIGURES/3>

Gillard JT, Hernandez AL, Contreras JA, Francis IM, Cabrales L. Potential for biomass production
and remediation by cultivation of the marine model diatom *Phaeodactylum tricornutum* in oil
field produced wastewater media. *Water*. 2021 Sep 29;13(19):2700.

Glevitzky, M., Corcheș, M. T., & Popa, D. M. (2025). Assessing Pollution and Diatom-Based
Bioindicators in the Arieș River, Romania. *Environments*, 12(11), 398.
<https://doi.org/10.3390/environments12110398>

Hajimahmoodi, M., Faramarzi, M. A., Mohammadi, N., Soltani, N., Oveisi, M. R., & Nafissi-
Varcheh, N. (2010). Evaluation of antioxidant properties and total phenolic contents of some

742 strains of microalgae. *Journal of Applied Phycology*, 22(1), 43–50.

743 <https://doi.org/10.1007/S10811-009-9424-Y>/METRICS

744 Hamid, T., Shoaib, N., & Siddiqui, P. J. A. (2023). Seasonal variation of diatom biodiversity with
745 relation to environmental factors in Northern Arabian Sea. *Indian Journal of Geo-Marine*
746 *Sciences (IJMS)*, 52(02), 101–113. <https://doi.org/10.56042/IJMS.V52I02.6926>

747 Heredia, B., Méndez, P., & Osorio, M. (2024). Epilithic Diatoms as Bioindicators of Water Quality
748 in the Jimbitono River, Morona Santiago, Ecuador. *ESPOCH Congresses: The Ecuadorian*
749 *Journal of S.T.E.A.M.*, 3(2), 21–38. <https://doi.org/10.18502/ESPOCH.V4I1.15797>

750 Hodgman, C. , U.-C. Y. , T. G. , & D. I. (2006). The implications for Bioinformatics of integration
751 across physical scales. *Journal of Integrative Bioinformatics*. .

752 Hoshi, K., Kuwata, A., & Sawada, K. (2025). Newly detected polar lipids from cultured diatom
753 strains of north pacific ocean: possible new diatom biomarker? *European Association of*
754 *Geoscientists & Engineers.*, 2025.

755 Indrayani, I., Haslianti, H., Asmariyani, A., Muskita, W. H., & Balubi, M. (2020). Growth, biomass
756 and lipid productivity of a newly isolated tropical marine diatom, *skeletonema* sp. Uho29,
757 under different light intensities. *Biodiversitas*, 21(4), 1498–1503.
758 <https://doi.org/10.13057/biodiv/d210430>

759 Irving EC, Baird DJ, Culp JM. Cadmium toxicity and uptake by mats of the freshwater diatom:
760 *Navicula pelliculosa* (Bréb) Hilse. *Archives of environmental contamination and toxicology*.
761 2009 Oct;57(3):524-30.

762 Jiang, W., Pan, H., Wang, F., Jiang, M., Deng, X., & Li, J. (2015). A rapid sample processing
763 method to observe diatoms via scanning electron microscopy. *Journal of Applied Phycology*,
764 27(1), 243–248. <https://doi.org/10.1007/S10811-014-0324-4>

765 Karthick, B., & Taylor, J. C. (2010). *Protocols for Collection, Preservation and Enumeration of*
766 *Diatoms from Aquatic Habitats for Water Quality Monitoring in India*.

767 <https://www.researchgate.net/publication/249341664>Kaur, G., Kaur Mohal, A., Pandey, P.,
768 Chaudhary, P., Kaur, M., Das, P., Kaur, A., & Professor, A. (2024). FORENSIC
769 EXTRACTION AND MICROSCOPY TECHNIQUES FOR DIATOM ANALYSIS-
770 REVIEW. *Bulletin of Pure and Applied Sciences Zoology (Animal Science)*, 43(2S), 1442–
771 1456. www.bpasjournals.com

772 Keck F, Brantschen J, Altermatt F. A combination of machine-learning and eDNA reveals the
773 genetic signature of environmental change at the landscape levels. *Molecular Ecology*. 2023
774 Sep;32(17):4791-800.

775 Kiran Marella, T., Saxena, A., & Tiwari, A. (2020). Diatom mediated heavy metal remediation: A
776 review. *Bioresource Technology*, 305, 123068.
777 <https://doi.org/10.1016/J.BIORTECH.2020.123068>

778 Koehler, L. H. (1952). Differentiation of Carbohydrates by Anthrone Reaction Rate and Color
779 Intensity. *Analytical Chemistry*, 24(10), 1576–1579. <https://doi.org/10.1021/ac60070a014>

780 Kruthi, M. N., Panja, S., Kumar, A., Sengupta, A., & Narad, P. (2024). Application of
781 bioinformatics: Integrated computational tools in green technologies. *Microbial Approaches
782 for Sustainable Green Technologies*, 112–135. [https://doi.org/10.1201/9781003407683-
783 6/APPLICATION-BIOINFORMATICS-NITHYA-KRUTHI-SEBIKA-PANJA-ABHISHEK-
784 KUMAR-ABHISHEK-SENGUPTA-PRIYANKA-NARAD](https://doi.org/10.1201/9781003407683-6/APPLICATION-BIOINFORMATICS-NITHYA-KRUTHI-SEBIKA-PANJA-ABHISHEK-KUMAR-ABHISHEK-SENGUPTA-PRIYANKA-NARAD)

785 Kuczynska, P., Jemiola-Rzeminska, M., & Strzalka, K. (2015). Photosynthetic pigments in
786 diatoms. In *Marine Drugs* (Vol. 13, Issue 9, pp. 5847–5881). MDPI AG.
787 <https://doi.org/10.3390/md13095847>

788 Kumar, V., Sharma, A., Kumar, R., Bhardwaj, R., Kumar Thukral, A., & Rodrigo-Comino, J.
789 (2020). Assessment of heavy-metal pollution in three different Indian water bodies by
790 combination of multivariate analysis and water pollution indices. In *Human and Ecological
791 Risk Assessment* (Vol. 26, Issue 1, pp. 1–16). Taylor and Francis Inc.
792 <https://doi.org/10.1080/10807039.2018.1497946>

793 Kus, J. (2015). Application of confocal laser-scanning microscopy (CLSM) to autofluorescent
794 organic and mineral matter in peat, coals and siliciclastic sedimentary rocks — A qualitative
795 approach. *International Journal of Coal Geology*, 137, 1–18.
796 <https://doi.org/10.1016/J.COAL.2014.10.014>

797 Kutako, M., Hiransuchalert, R., Sarayut Watchasit, Kaewduang, M., Hanchana, O.,
798 Setthamongkol, P., Chindudsadeegul, P., Gunbua, V., & Jaritkhuan, S. (2023). Morphological
799 and molecular comparison as a useful tool for identification of the three centric marine
800 diatoms (Bacillariophyceae: Chaetoceros). *Archives of Microbiology*, 205(5).
801 <https://doi.org/10.1007/s00203-023-03525-9>

80 Kutty SN, Loh RK, Bannister W, Taylor D. Evaluation of a diatom eDNA-based technique for
803 assessing water quality variations in tropical lakes and reservoirs. *Ecological Indicators*. 2022
804 Aug 1;141:109108.

805 Lewandowicz, W., Grabowska, M., Wojtal, A. Z., Puczko, K., & Więcko, A. (2025). Taxonomic
806 Diversity: Importance, Threats, and Status of Diatoms from Lowland Urban Springs
807 (Northeast Poland). *Water* 2025, Vol. 17, Page 2293, 17(15), 2293.
808 <https://doi.org/10.3390/W17152293>

809 Livingstone, D. R. (1993). Biotechnology and pollution monitoring: Use of molecular biomarkers
810 in the aquatic environment. *Journal of Chemical Technology & Biotechnology*, 57(3), 195–
811 211.
812 <https://doi.org/10.1002/JCTB.280570302>;JOURNAL:JOURNAL:10974660;WGROU:STRI
813 NG:PUBLICATION

814 Lu, F. L., Neyransoylu, E., & Ondokuzmayisuniversity, G. (2007). *Seasonal Variations and*
815 *Occurrence of Epiphytic Diatom Assemblages on Mats of Cladophora glomerata in Lake*
816 *Ladik, Samsun, Turkey*. 28(4), 373–384.

817 Marella, T. K., López-Pacheco, I. Y., Parra-Saldivar, R., Dixit, S., & Tiwari, A. (2020). Wealth
818 from waste: Diatoms as tools for phycoremediation of wastewater and for obtaining value
819 from the biomass. *Science of the Total Environment*, 724.
820 <https://doi.org/10.1016/j.scitotenv.2020.137960>

821 Marella TK, Saxena A, Tiwari A. Diatom mediated heavy metal remediation: A review.
822 *Bioresource Technology*. 2020 Jun 1;305:123068

823 Méndez-Zambrano, P., Ureta Valdez, R., Tierra Pérez, L., & Flores Orozco, Á. (2024).
824 *Biomonitoring of Benthic Diatoms as Indicators of Water Quality, Assessing the Present and*
825 *Projecting the Future: A Review*. 4, 1020. <https://doi.org/10.56294/saludcyt20241020>

826 Millie, D. F., Schofield, O. M. E., Kirkpatrick, G. J., Johnsen, G., & Evens, T. J. (2002). Using
827 absorbance and fluorescence spectra to discriminate microalgae. *European Journal of*
828 *Phycology*, 37(3), 313–322. <https://doi.org/10.1017/S0967026202003700>

829 Mišić Radić, T., Vukosav, P., Čačković, A., & Dulebo, A. (2023). Insights into the Morphology
830 and Surface Properties of Microalgae at the Nanoscale by Atomic Force Microscopy (AFM):

831 A Review. In *Water (Switzerland)* (Vol. 15, Issue 11). MDPI.
832 <https://doi.org/10.3390/w15111983>

833 Mora D, Abarca N, Proft S, Grau JH, Enke N, Carmona J, Skibbe O, Jahn R, Zimmermann J.
834 Morphology and metabarcoding: a test with stream diatoms from Mexico highlights the
835 complementarity of identification methods. *Freshwater Science*. 2019 Sep 1;38(3):448-64.

836 Morales, Eduardo & Siver, Peter & Trainor, Francis. (2009). Identification of diatoms
837 (Bacillariophyceae) during ecological assessments: Comparison between Light Microscopy
838 and Scanning Electron Microscopy techniques. *Proceedings of the Academy of Natural*
839 *Sciences of Philadelphia*. 151. 95-103. 10.1635/0097-
840 3157(2001)151[0095:IOBDE]2.0.CO;2.

841 Musa, R., & Greenfield, R. (2018). Use of diatom indices to categorise impacts on and recovery of
842 a floodplain system in South Africa. *African Journal of Aquatic Science*, 43(1), 59–69.
843 <https://doi.org/10.2989/16085914.2018.1443907>

844 Nieri, P., Carpi, S., Esposito, R., Costantini, M., & Zupo, V. (2023). Bioactive Molecules from
845 Marine Diatoms and Their Value for the Nutraceutical Industry. In *Nutrients* (Vol. 15, Issue
846 2). MDPI. <https://doi.org/10.3390/nu15020464>

847 Norian, A., Amini, F., Sakhaei, N., Archangi, B., & Mokhtarpour, A. (2022). Evaluation of
848 biodiversity of phytoplankton and determination of biological health quality of Arvand River
849 (south west of Iran) using Trophic Diatom Index (TDI). *Iranian Journal of Fisheries*
850 *Sciences*, 21(4), 1047–1063. <https://doi.org/10.22092/ijfs.2022.127619>

851 Norton, T. A., Thompson, R. C., Pope, J., Veltkamp, C. J., Banks, B., Howard, C. V., & Hawkins,
852 S. J. (1998). Using confocal laser scanning microscopy, scanning electron microscopy and
853 phase contrast light microscopy to examine marine biofilms. *Aquatic Microbial Ecology*,
854 16(2), 199–204. <https://doi.org/10.3354/AME016199>

855 Oeding, S., & Taffs, K. H. (2017). Developing a regional diatom index for assessment and
856 monitoring of freshwater streams in sub-tropical Australia. *Ecological Indicators*, 80, 135–
857 146. <https://doi.org/10.1016/J.ECOLIND.2017.05.009>

858 Pandey, L. K., Sharma, Y. C., Park, J., Choi, S., Lee, H., Lyu, J., & Han, T. (2018). Evaluating
859 features of periphytic diatom communities as biomonitoring tools in fresh, brackish and

860 marine waters. *Aquatic Toxicology*, 194, 67–77.
861 <https://doi.org/10.1016/J.AQUATOX.2017.11.003>

862 Paniagua-Michel J, Banat IM. Unravelling diatoms’ potential for the bioremediation of oil
863 hydrocarbons in marine environments. *Clean Technologies*. 2024 Jan 17;6(1):93-115.

864 Parikh, H. S., Dave, G., Singh, P. K., & Tiwari, A. (2025). Exploring the significance of xenic and
865 axenic cultures for diatoms: methodological insights, scope, and biotechnological
866 implications. *Systems Microbiology and Biomanufacturing*, 5(3), 969–989.
867 <https://doi.org/10.1007/S43393-025-00363-2>

868 Percopo I, Ruggiero MV, Sarno D, Longobardi L, Rossi R, Piredda R, Zingone A. Phenological
869 segregation suggests speciation by time in the planktonic diatom *Pseudo-nitzschia allochroa*
870 sp. nov. *Ecology and Evolution*. 2022 Aug;12(8):e9155.

871 Pierella Karlusich, J. J., Cosnier, K., Zinger, L., Henry, N., Nef, C., Bernard, G., Scalco, E.,
872 Dvorak, E., Jimenez Vieira, F. R., Delage, E., Chaffron, S., Ovchinnikov, S., Zingone, A., &
873 Bowler, C. (2024). *Patterns and drivers of diatom diversity and abundance in the global ocean*.
874 <https://doi.org/10.1101/2024.06.08.598090>

875 Piper, J. (2011). A review of high-grade imaging of diatoms and radiolarians in light microscopy
876 optical- and software-based techniques. *Diatom Research*, 26(1), 57–72.
877 <https://doi.org/10.1080/0269249X.2011.587377>

878 Potapova, M., & Charles, D. F. (2007). Diatom metrics for monitoring eutrophication in rivers of
879 the United States. *Ecological Indicators*, 7(1), 48–70.
880 <https://doi.org/10.1016/j.ecolind.2005.10.001>

881 Rahman, T. T., Jiang, T., Zhang, C., Rao, Y., Sims, R. C., Hou, L., Rahman, T. T., Jiang, T., Zhang,
882 C., Rao, Y., Sims, R. C., & Hou, L. (2024). Diatoms in wastewater treatment: Potentials,
883 applications, and values of biomass. *CREST*, 54(7), 557–580.
884 <https://doi.org/10.1080/10643389.2023.2259278>

885 Ramstack Hobbs, J. M., Heathcote, A. J., VanderMeulen, D. D., & Edlund, M. B. (2022).
886 Integrating water quality monitoring and diatom community trends to determine landscape-
887 level change in protected lakes. *Ecosphere*, 13(8), e4199.
888 <https://doi.org/10.1002/ECS2.4199>;PAGEGROUP:STRING:PUBLICATION

88Reavie, E. D., Bramburger, A. J., & Cai, M. (2025). The recent diatom-based paleolimnology of
890 Lake Michigan. *Journal of Paleolimnology* 2025 73:4, 73(4), 347–360.
891 <https://doi.org/10.1007/S10933-025-00363-1>

89Reza Moheimani, N., Borowitzka, M. A., Isdepsky, A., & Fon Sing, S. (2013). Standard methods
893 for measuring growth of algae and their composition. In *Algae for Biofuels and Energy* (pp.
894 265–284). Springer Netherlands. https://doi.org/10.1007/978-94-007-5479-9_16

89Rinku, Saxena, A., Singh, P. K., Manoylov, K., & Tiwari, A. (2024). Isolation and identification of
896 marine diatoms based on frustules morphology using scanning electron microscopy: Isolation
897 and identification of marine diatoms using frustules morphology. *Indian Journal of*
898 *Experimental Biology (IJEB)*, 62(07), 584–592. <https://doi.org/10.56042/IJEB.V62I07.12070>

89Rout, P. R., Shahid, M. K., Dash, R. R., Bhunia, P., Liu, D., Varjani, S., Zhang, T. C., &
900 Surampalli, R. Y. (2021). Nutrient removal from domestic wastewater: A comprehensive
901 review on conventional and advanced technologies. *Journal of Environmental Management*,
902 296, 113246. <https://doi.org/10.1016/J.JENVMAN.2021.113246>

90Saito, M., Kitamura, M., Ide, Y., Nguyen, M. H., Le, B. D., Mai, A. T., Miyashiro, D., Mayama, S.,
904 & Umemura, K. (2022). An Efficient Method of Observing Diatom Frustules via Digital
905 Holographic Microscopy. *Microscopy and Microanalysis*, 28(6), 2172–2176.
906 <https://doi.org/10.1017/S1431927622012508>

90Salido, J., Sánchez, C., Ruiz-Santaquiteria, J., Cristóbal, G., Blanco, S., & Bueno, G. (2020). A
908 low-cost automated digital microscopy platform for automatic identification of diatoms.
909 *Applied Sciences (Switzerland)*, 10(17). <https://doi.org/10.3390/app10176033>

91Sano, M., Makabe, R., Kurosawa, N., Moteki, M., & Odate, T. (2020). Effects of Lugol's iodine
911 on long-term preservation of marine plankton samples for molecular and stable carbon and
912 nitrogen isotope analyses. *Limnology and Oceanography: Methods*, 18(11), 635–643.
913 <https://doi.org/10.1002/LOM3.10390>

91Saxena, A., Tiwari, A., Kaushik, R., Iqbal, H. M. N., & Parra-Saldívar, R. (2021). Diatoms
915 recovery from wastewater: Overview from an ecological and economic perspective. *Journal*
916 *of Water Process Engineering*, 39. <https://doi.org/10.1016/j.jwpe.2020.101705>

917ehgal, M., Garg, A., Suresh, R., & Dagar, P. (2012). Heavy metal contamination in the Delhi
 918 segment of Yamuna basin. *Environmental Monitoring and Assessment*, 184(2), 1181–1196.
 919 <https://doi.org/10.1007/S10661-011-2031-9>

920eródio, J., & Lavaud, J. (2022). Diatoms and Their Ecological Importance. In *Life Below Water*
 921 (pp. 304–312). Springer, Cham. https://doi.org/10.1007/978-3-319-98536-7_12

922harma, R., Kumar, R., Satapathy, S. C., Al-Ansari, N., Singh, K. K., Mahapatra, R. P., Agarwal,
 923 A. K., Le, H. Van, & Pham, B. T. (2020). Analysis of Water Pollution Using Different
 924 Physicochemical Parameters: A Study of Yamuna River. *Frontiers in Environmental Science*,
 925 8, 581591. <https://doi.org/10.3389/FENVS.2020.581591/BIBTEX>

926ingleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and
 927 other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in*
 928 *Enzymology*, 299, 152–178. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)

929nelling, A. M., Swann, G. E. A., Leng, M. J., & Pike, J. (2013). A Micro-manipulation Technique
 930 for the Purification of Diatoms for Isotope and Geochemical Analysis. *Silicon*, 5(1), 13–17.
 931 <https://doi.org/10.1007/S12633-012-9115-1/FIGURES/1>

932olomon, K. J., Stevenson, R. J., Surratt, D., Whelan, K. R. T., Tobias, F. A. C., Johnson, K. M., &
 933 Gaiser, E. E. (2025). Robust species-optima estimates from non-uniformly sampled
 934 environmental gradients. *Journal of Paleolimnology*, 73(3), 179–194.
 935 <https://doi.org/10.1007/S10933-025-00354-2/FIGURES/5>

936rivastava, P., Grover, S., Verma, J., & Khan, A. S. (2017). Applicability and efficacy of diatom
 937 indices in water quality evaluation of the Chambal River in Central India. *Environmental*
 938 *Science and Pollution Research*, 24(33), 25955–25976. [https://doi.org/10.1007/s11356-017-](https://doi.org/10.1007/s11356-017-0166-0)
 939 0166-0

940teinberg, C., & Schiefele, S. (1988). Biological indication of trophy and pollution of running
 941 waters. *Z. Wasser - Abwasser-Forsch*, 21, 227–234.
 942 [https://www.researchgate.net/publication/235347103_Indication_of_trophy_and_pollution_of](https://www.researchgate.net/publication/235347103_Indication_of_trophy_and_pollution_of_running_waters)
 943 [_running_waters](https://www.researchgate.net/publication/235347103_Indication_of_trophy_and_pollution_of_running_waters)

944udhakar M P, Ananthalakshmi J S, & Beena B Nair. (2013). Extraction, purification and study on
 945 antioxidant properties of fucoxanthin from brown seaweeds. *Available Online*

946 *Www.Jocpr.Com Journal of Chemical and Pharmaceutical Research*, 5, 169–175.
 947 www.jocpr.com

948 uthar, S., Sharma, J., Chabukdhara, M., & Nema, A. K. (2010). Water quality assessment of river
 949 Hindon at Ghaziabad, India: impact of industrial and urban wastewater. *Environmental*
 950 *Monitoring and Assessment*, 165(1–4), 103–112. <https://doi.org/10.1007/s10661-009-0930-9>

951 Tang, C. Y., & Yang, Z. (2017). Transmission Electron Microscopy (TEM). *Membrane*
 952 *Characterization*, 145–159. <https://doi.org/10.1016/B978-0-444-63776-5.00008-5>

953 Tapolczai K, Keck F, Bouchez A, Rimet F, Kahlert M, Vasselon V. Diatom DNA metabarcoding
 954 for biomonitoring: strategies to avoid major taxonomical and bioinformatical biases limiting
 955 molecular indices capacities. *Frontiers in Ecology and Evolution*. 2019 Oct 29;7:409.

956 Taylor, J. C., Harding, W. R., Archibald, C. G. M., & South Africa. Water Research Commission.
 957 (2007). *A methods manual for the collection, preparation and analysis of diatom samples*,
 958 *version 1.0*. Water Research Commission.

959 Templeton, D. W., Quinn, M., Van Wychen, S., Hyman, D., & Laurens, L. M. L. (2012).
 960 Separation and quantification of microalgal carbohydrates. *Journal of Chromatography A*,
 961 1270, 225–234. <https://doi.org/10.1016/J.CHROMA.2012.10.034>

962 Thien Do NGUYEN, H., Ramli, A., & Man KEE, L. (2017). A Review on Methods Used in
 963 Analysis of Microalgae Lipid Composition. *J. Jpn. Inst. Energy*, 96(12).

964 Tomar, D., & Upadhyay, R. (2018). Heavy metals in Yamuna river: A Review. *Int. J. Adv. Sci*, 540.

965 Torrisi, M., & Dell’Uomo, A. (2006). BIOLOGICAL MONITORING OF SOME APENNINE
 966 RIVERS (CENTRAL ITALY) USING THE DIATOM-BASED EUTROPHICATION /
 967 POLLUTION INDEX (EPI-D) COMPARED TO OTHER EUROPEAN DIATOM INDICES.
 968 *Diatom Research*, 21(1), 159–174. <https://doi.org/10.1080/0269249X.2006.9705657>

969 Tréguer, P., Bowler, C., Moriceau, B., Dutkiewicz, S., Gehlen, M., Aumont, O., Bittner, L.,
 970 Dugdale, R. C., & Finkel, Z. (2018). Influence of diatom diversity on the ocean biological
 971 carbon pump. *Nature Geoscience*, 11(1), 27–37. <https://doi.org/10.1038/s41561-017-0028-xi>

972 Trobajo, R., & Mann, D. G. (2019). A rapid cleaning method for diatoms. *Diatom Research*, 34(2),
 973 115–124. <https://doi.org/10.1080/0269249X.2019.1637785>

- 974 Upadhyay, R., Dasgupta, N., Hasan, A., & Upadhyay, S. K. (2011). Managing water quality of
975 River Yamuna in NCR Delhi. *Physics and Chemistry of the Earth, Parts A/B/C*, 36(9–11),
976 372–378. <https://doi.org/10.1016/J.PCE.2010.03.018>
- 977 Vasselon V, Rivera S, Ács É, Almeida SB, Andree K, Apothéloz-Perret-Gentil L, Bailet B,
978 Baričević A, Beentjes K, Bettig J, Bouchez A. Proficiency testing and cross-laboratory
979 method comparison to support standardisation of diatom DNA metabarcoding for freshwater
980 biomonitoring. *Metabarcoding and metagenomics*. 2025 Jan 10;9:e133264.
- 981 Venkatachalapathy, R., & Karthikeyan, P. (2015). *Application of Diatom-Based Indices for*
982 *Monitoring Environmental Quality of Riverine Ecosystems: A Review* (pp. 593–619).
983 https://doi.org/10.1007/978-3-319-13425-3_28
- 984 Videska, A. (2025). *Structure of planktonic and benthic diatom communities in the Zelenci spring*
985 [Univerza v Ljubljani]. <https://repozitorij.uni-lj.si/IzpisGradiva.php?id=173375>
- 986 Villar, E., Zweig, N., Vincens, P., Cruz de Carvalho, H., Duchene, C., Liu, S., Monteil, R., Dorrell,
987 R. G., Fabris, M., Vandepoele, K., Bowler, C., & Falciatore, A. (2025). DiatOmicBase: a
988 versatile gene-centered platform for mining functional omics data in diatom research. *The*
989 *Plant Journal*, 121(6), e70061. <https://doi.org/10.1111/TPJ.70061>
- 990 Vlaović L, Lemonnier C, Alric B, Rimet F, Ciric M, Miletić S, Milovanović Ž, Petrović V,
991 Vidaković D. Diatom metabarcoding vs. morphology in monitoring freshwater and saline
992 lakes in Serbia. *Frontiers in Ecology and Evolution*. 2025 Sep 29;13:1677773.
- 993 Vu, C. H. T., Lee, H. G., Chang, Y. K., & Oh, H. M. (2018). Axenic cultures for microalgal
994 biotechnology: Establishment, assessment, maintenance, and applications. In *Biotechnology*
995 *Advances* (Vol. 36, Issue 2, pp. 380–396). Elsevier Inc.
996 <https://doi.org/10.1016/j.biotechadv.2017.12.018>
- 997 Wang B, Li R, Lan X, Kong D, Liu X, Xie S. Benthic diatom eDNA metabarcoding for ecological
998 assessment of an urban river: A comparison with morphological method. *Ecological*
999 *Indicators*. 2024 Sep 1;166:112302.
- 1000 Wang, X. W., Liang, J. R., Luo, C. S., Chen, C. P., & Gao, Y. H. (2014). Biomass, total lipid
1001 production, and fatty acid composition of the marine diatom *Chaetoceros muelleri* in
1002 response to different CO₂ levels. *Bioresource Technology*, 161, 124–130.
1003 <https://doi.org/10.1016/j.biortech.2014.03.012>

1004 WHO. (2017). Guidelines for drinking-water quality, 4th edition, incorporating the 1st addendum.
 1005 Geneva: World Health Organization. Geneva, Switzerland. *World Health Organization*.
 1006 <https://www.who.int/publications/i/item/9789241549950>

1007 Xin, X. (2011). Application of Diatoms in Water Quality Monitoring and Reconstruction of
 1008 Palaeoenvironment. *Journal of Anhui Agricultural Sciences*.

1009 Yang, M., Zhao, W., & Xie, X. (2014). Effects of nitrogen, phosphorus, iron and silicon on growth
 1010 of five species of marine benthic diatoms. *Acta Ecologica Sinica*, 34(6), 311–319.
 1011 <https://doi.org/10.1016/j.chnaes.2014.10.003>

1012 Yim, J. H., & Lee, H. K. (2005). Axenic culture of Gyrodinium impudicum strain KG03, a marine
 1013 red-tide microalga that produces exopolysaccharide. In *Article in The Journal of*
 1014 *Microbiology*. <https://www.researchgate.net/publication/8080083>

1015 Yodsuwan, N., Sawayama, S., & Sirisansaneeyakul, S. (2017). Effect of nitrogen concentration on
 1016 growth, lipid production and fatty acid profiles of the marine diatom Phaeodactylum
 1017 tricornutum. *Agriculture and Natural Resources*, 51(3), 190–197.
 1018 <https://doi.org/10.1016/j.anres.2017.02.004>

1019 Yogeshwaran, M., Thacker, M., Wadmare, N., Vigneshwaran, A., Radhakrishnan, C., Pardhi, S., &
 1020 Karthick, B. (2025). Soil diatoms and their applications as an indicator of environmental
 1021 changes. *Applied Soil Ecology*, 213, 106277. <https://doi.org/10.1016/J.APSOIL.2025.106277>

1022 Yourassowsky, C., Theunissen, R., Dohet-Eraly, J., & Dubois, F. (2024). Lipid quantification in
 1023 living microalgal cultures with digital holographic microscopy. *Frontiers in Photonics*, 4.
 1024 <https://doi.org/10.3389/fphot.2023.1301708>

1025 Yu, P., You, Q., Pang, W., Cao, Y., Bi, Y., & Wang, Q. (2021). Development of a Periphytic
 1026 Diatom-Based Comprehensive Diatom Index for Assessing the Trophic Status of Lakes in the
 1027 Lower Reaches of the Yangtze River, China. *Water*, 13(24), 3570.
 1028 <https://doi.org/10.3390/w13243570>

1029 Yu W, Xiang Q, Hu Y, Du Y, Kang X, Zheng D, Shi H, Xu Q, Li Z, Niu Y. An improved automated
 1030 diatom detection method based on YOLOv5 framework and its preliminary study for
 1031 taxonomy recognition in the forensic diatom test. *Front Microbiol*. 2022; 13: 963059. DOI:
 1032 <https://doi.org/10.3389/fmicb.2022>.

1033 Zetsche, E. M., El Mallahi, A., & Meysman, F. J. R. (2016). Digital holographic microscopy: A
1034 novel tool to study the morphology, physiology and ecology of diatoms. *Diatom Research*,
1035 31(1), 1–16. <https://doi.org/10.1080/0269249X.2016.1140679>

1036 Zhang, C., He, Y., Li, W., Guo, X., Xiao, C., & Zhao, T. (2022). High-Throughput Sequencing of
1037 Diatom Community, Its Spatial and Temporal Variation and Interrelationships with
1038 Physicochemical Factors in Danjiangkou Reservoir, China. *Water (Switzerland)*, 14(10).
1039 <https://doi.org/10.3390/W14101609>

1040 Zhang, G., Li, J., Zhu, T., Gu, Q., & Li, D. (2016). Advanced tools in marine natural drug
1041 discovery. *Current Opinion in Biotechnology*, 42, 13–23.
1042 <https://doi.org/10.1016/J.COPBIO.2016.02.021>

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Sindhwal, Sneha; Rinku; Saxena, Abhishek; Navarro-Márquez, Mireya; Parra-Saldívar, Roberto ...

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