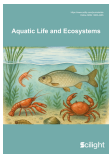
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Diversity and Seasonal Distribution of Bacillariophyta in Relation to Physicochemical Characteristics of the Dor River, Abbottabad, Pakistan

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Abstract: Bacillariophyta (diatoms) are important primary producers in freshwater ecosystems and are widely used as bioindicators of water quality and ecological conditions. This study investigated the taxonomic diversity, seasonal distribution, and relationships between Bacillariophyta assemblages and physicochemical characteristics of the Dor River, Abbottabad, Khyber Pakhtunkhwa, Pakistan, during 2021–2022. Monthly sampling was conducted at eight selected sites, and algal taxa were identified using standard morphological and taxonomic approaches. Physicochemical parameters were analyzed following standard protocols, while phytosociological indices and Principal Component Analysis (PCA) were used to characterize community structure and explore patterns in relation to environmental variables. A total of 46 Bacillariophyta taxa were recorded, representing two classes, ten orders, and nineteen families. Bacillariaceae, Gomphonemataceae, and Naviculaceae were among the most species-rich families, while Naviculales was the most species-rich order. Bacillariophyceae was the dominant class in the recorded assemblage. Seasonal patterns in diatom diversity and abundance varied throughout the study period, with higher values reported in December, February, and March. *Navicula laterostrata* had the highest reported density and Importance Value Index (IVI), whereas *Navicula cryptocephala* exhibited the highest frequency of occurrence. Diversity indices described variation in community structure across the sampling period. PCA revealed patterns of variation in diatom assemblages in relation to physicochemical parameters, suggesting potential relationships between environmental conditions and community composition without establishing direct causal effects. This study provides a baseline assessment of Bacillariophyta diversity in the Dor River and contributes information on the seasonal distribution of diatom assemblages and their relationships with measured water-quality parameters. Continued monitoring of diatom communities and physicochemical characteristics may support future assessments of river ecology and inform water-quality management and biodiversity conservation.

Keywords: bacillariophyta; freshwater diatoms; physicochemical parameters; seasonal distribution; water quality; bioindicators

1. Introduction

Freshwater ecosystems are among the most productive and biologically diverse environments on Earth, supporting a wide range of ecological processes and providing essential ecosystem services that sustain both biodiversity and human well-being. These ecosystems supply drinking water, support agriculture and fisheries,

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regulate hydrological and biogeochemical cycles, and contribute substantially to global carbon and nutrient dynamics. Despite occupying less than 1% of the Earth's surface, freshwater habitats harbor nearly 10% of all known species, highlighting their exceptional ecological importance. However, increasing anthropogenic pressures, including rapid urbanization, industrial development, agricultural intensification, excessive nutrient loading, habitat fragmentation, and climate change, have accelerated the degradation of freshwater ecosystems worldwide. These stressors alter physicochemical characteristics, modify habitat quality, disrupt biological communities, and ultimately threaten ecosystem functioning and biodiversity conservation [1].

Climate change has further intensified these challenges by altering precipitation regimes, increasing water temperatures, modifying river discharge patterns, and enhancing the frequency of extreme hydrological events. Such environmental changes directly influence aquatic biodiversity by affecting species distribution, community composition, primary productivity, and ecological resilience. Consequently, comprehensive monitoring of freshwater ecosystems has become increasingly important for understanding ecosystem responses to environmental change, identifying environmental variation at an early stage, and supporting evidence-based conservation and sustainable water resource management. Modern freshwater ecological assessments increasingly integrate biological indicators with physicochemical monitoring to provide a more comprehensive evaluation of ecosystem health than conventional chemical analyses alone [2].

Among the biological components of freshwater ecosystems, phytoplankton constitutes the foundation of aquatic food webs and play indispensable roles in primary productivity, nutrient cycling, carbon sequestration, and oxygen production. As primary producers, phytoplanktons transform solar energy into organic matter, supporting higher trophic levels and regulating ecosystem metabolism. Their short life cycles and rapid responses to environmental fluctuations make phytoplankton highly sensitive indicators of ecological change. Variations in nutrient availability, temperature, pH, conductivity, turbidity, and hydrological conditions rapidly influence phytoplankton community structure, allowing these organisms to reflect changing environmental conditions over relatively short temporal scales. Consequently, phytoplankton communities are widely used in freshwater ecological monitoring and environmental assessment programs throughout the world [3].

Among freshwater phytoplankton, diatoms (Division Bacillariophyta) represent one of the most diverse, abundant, and ecologically significant groups of micro Algae. Diatoms are unicellular or colonial photosynthetic organisms characterized by intricately ornamented siliceous cell walls (frustules), which exhibit remarkable morphological diversity and provide reliable taxonomic characteristics for species identification. Approximately 100,000 diatom species are estimated to occur globally, occupying nearly every aquatic habitat ranging from pristine mountain streams to estuaries and marine environments. Their ecological success results from exceptional physiological adaptability, rapid reproduction, efficient nutrient utilization, and broad environmental tolerance. Because of these characteristics, diatoms contribute nearly one-fifth of global primary production and play fundamental roles in silicon cycling, carbon fixation, and aquatic ecosystem functioning [4].

The ecological importance of diatoms extends far beyond their contribution to primary productivity. Numerous studies have demonstrated that diatom assemblages respond rapidly and predictably to changes in environmental conditions, making them among the most reliable biological indicators of freshwater ecological status. Species composition, abundance, and community diversity are influenced by nutrient enrichment, organic pollution, acidification, salinity, conductivity, temperature, hydrological alteration, and other physicochemical variables. Because individual species exhibit distinct ecological preferences and tolerance ranges, changes in diatom community composition provide valuable information regarding water quality and environmental disturbance. Unlike instantaneous physicochemical measurements that reflect conditions only at the time of sampling, diatom communities integrate environmental conditions over extended periods, thereby providing a more robust assessment of ecological integrity. Consequently, diatom-based biomonitoring has become an integral component of freshwater assessment programs worldwide and is increasingly incorporated into river health evaluation, ecological restoration, and environmental management strategies [5].

Furthermore, recent advances in freshwater ecology have considerably expanded the application of diatom research through the integration of traditional morphological taxonomy with ecological indices, multivariate statistical analyses, geographic information systems, remote sensing, environmental DNA (eDNA), and high-throughput sequencing technologies. These interdisciplinary approaches have improved species identification, enhanced understanding of community dynamics, and strengthened predictive models linking biological communities with environmental change. Although molecular techniques are increasingly used in ecological monitoring, morphological identification remains the international standard for freshwater bio monitoring because of its taxonomic reliability, historical continuity, and compatibility with long-term ecological datasets. Integrating classical taxonomy with modern ecological and statistical approaches therefore provides a comprehensive

framework for evaluating freshwater ecosystem health and predicting ecological responses under changing environmental conditions [6,7].

The objectives of this study were to:

(i) Document the taxonomic diversity of Bacillariophyta in the Dor River;
(ii) Investigate the seasonal and spatial distribution patterns of diatom communities;
(iii) Evaluate the physicochemical characteristics of river water and their seasonal variation;
(iv) Determine the relationships between Bacillariophyta assemblages and environmental variables using diversity indices and Principal Component Analysis; and
(v) Establish baseline ecological information for long-term biomonitoring, biodiversity conservation, and sustainable management of the Dor River ecosystem. The findings of this study provide valuable scientific evidence for understanding freshwater Algal ecology in northern Pakistan and contribute to the broader application of diatoms as potential biological indicator for river health assessment under increasing anthropogenic and climatic pressures.

2. Materials and Methods

2.1. Study Area

The present investigation was carried out in the Dor River, a perennial freshwater river located in the Hazara Division of Khyber Pakhtunkhwa (KPK), northern Pakistan. The river originates from the snow-fed mountains of Barengali near Nathia Gali and is primarily fed by melt water from the surrounding peaks, including Miranjani, Mushkpuri, and Thandiani as shown in Figure 1. Owing to its mountainous origin, the hydrological regime of the river is strongly influenced by seasonal snowmelt, precipitation, and climatic fluctuations. River discharge generally increases during spring and summer due to snowmelt and rainfall, whereas comparatively lower flow is observed during autumn and winter.

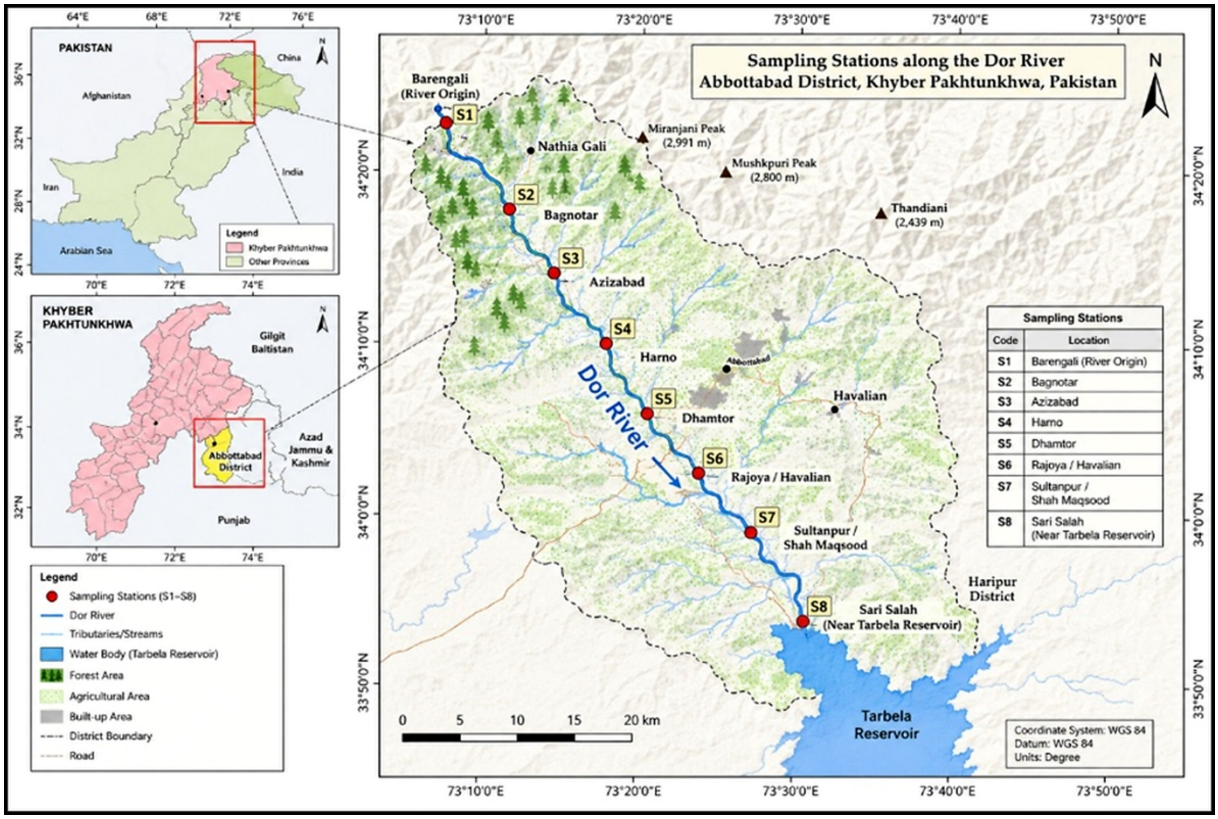


Figure 1. Map of the study area showing the eight sampling stations established along the Dor River, Abbottabad, Khyber Pakhtunkhwa, Pakistan.

The Dor River extends approximately 102 km before ultimately discharging into the Tarbela Reservoir in Haripur District. The upper reaches of the river (approximately 30 km) are characterized by relatively freshwater originating from forested catchments, whereas the remaining downstream section (approximately 72 km) is increasingly influenced by anthropogenic activities, including domestic wastewater discharge, agricultural runoff,

urban expansion, and small-scale commercial activities. These environmental gradients provide suitable conditions for evaluating the influence of physicochemical variables on freshwater Algal communities.

Geographically, the study area is located at approximately 34°08′37″ N latitude and 73°19′08″ E longitude within the western Himalayan region of Pakistan. The river flows through several populated areas, including Bagnotar, Azizabad, Harno, Dhamtor, Rajoya, Havalian, Sultanpur, Shah Maqsood, and Sari Salah before entering the Tarbela Reservoir shown in Figure 1. The catchment is characterized by a humid subtropical climate, with cold winters and moderately warm summers. Annual precipitation is mainly received during the monsoon season and winter rainfall associated with western disturbances, both of which considerably influence river flow and nutrient transport.

To investigate the diversity and seasonal dynamics of Bacillariophyta, eight representative sampling stations were established along the longitudinal profile of the river. The sampling sites were selected to represent upstream, midstream, and downstream reach exhibiting different ecological conditions, degrees of anthropogenic disturbance, water flow characteristics, and surrounding land-use patterns. This spatial arrangement enabled comprehensive evaluation of the relationship between environmental variables and diatom community structure. Monthly sampling was conducted continuously from January 2021 to December 2022, thereby covering all four climatic seasons and allowing assessment of both spatial and temporal variations in Bacillariophyta diversity and physicochemical characteristics. The geographical coordinates of each sampling station were recorded using a handheld Global Positioning System (Garmin eTrex 20x, Garmin International, Inc., Olathe, KS, USA), ensuring precise localization and reproducibility of sampling locations during the entire study period.

2.2. Sampling Design, Collection, Preservation, and Quantitative Enumeration of Algal Samples

A systematic monthly sampling programme was conducted from January 2021 to December 2022 at the eight sampling stations described in Section 2.1. Sampling was completed at all stations within two consecutive days during each monthly sampling occasion to maintain temporal consistency. Algal samples were collected separately from the available aquatic habitats, including planktonic water, submerged macrophytes, submerged rocks, sediments, and littoral habitats. Samples from different habitats were kept separate throughout collection and laboratory processing and were not pooled.

For quantitative sampling of planktonic Algae, 1 L of water was collected from the upper 0.5–1.0 m of the water column at each sampling unit using a sterile plastic scooper and, where appropriate, a phytoplankton net. Epiphytic Algae were collected from submerged macrophytes (*Chara*, *Nitella*, *Hydrilla*, and *Potamogeton*) by gently brushing the submerged plant surfaces with a soft brush, using approximately 10 g of plant material as the standardized sampling unit. Epilithic Algae were obtained by gently scraping a standardized area of approximately 25 cm^2 from the surface of submerged rocks using a sterile toothbrush. Sediment-associated Algae were collected from the surface sediment using a sterile spatula, with approximately 10 g of sediment used as the standardized sampling unit. Three independent replicate samples were collected for each available habitat at each station during each monthly sampling occasion. Sampling equipment was thoroughly cleaned between stations and habitat types to minimize cross-contamination.

Each sample was labelled with the sampling date, station code, habitat type, and replicate number. Samples were transported to the laboratory for processing, and samples intended for morphological examination were preserved in 4% formaldehyde. Before quantitative enumeration, each sample was thoroughly homogenized to distribute the Algal material as uniformly as possible. For planktonic and other liquid samples, a 1 mL well-mixed aliquot was transferred to a Sedgwick–Rafter counting chamber and allowed to settle before examination. Twenty randomly selected microscopic fields were examined under a compound light microscope, and the number of identifiable *Bacillariophyta* cells or valves was recorded for each taxon. Where valve morphology was used for identification, individual valves were used as the counting unit; where complete cells were distinguishable, individual cells were counted. The same counting procedure was applied consistently to replicate samples throughout the study period.

The raw microscopic counts were retained separately for station, month, habitat, replicate, and taxon. For planktonic samples, the observed count in the 1 mL examined aliquot was related to the original 1 L sample volume for volume-based abundance. Thus, the volume-based abundance was calculated as:

$$\text{Density (cells or valves } L^{-1}) = N \times V_t / Vc$$

where N represents the number of cells or valves counted in the examined aliquot, V_t represents the total sample volume, and Vc represents the volume examined. For epilithic samples, abundance was referenced to the standardized 25 cm^2 scraped surface area, while epiphytic and sediment-associated abundance was referenced to the standardized 10 g quantities of plant material and sediment, respectively. Consequently, counts obtained from

different habitat types were not treated as directly equivalent volume-based densities; each was interpreted according to its corresponding sampling unit.

For the community-level analyses, each taxon occurrence was recorded separately for every sampling unit. Therefore, when the same species occurred in different samples, its individuals or valves were counted again in each respective sample. The cumulative number of 846 individual cells/valves represents the sum of these repeated observations across the sampling stations, months, habitats, and samples. It therefore represents cumulative observed abundance and not the number of species. Species richness was determined independently from the number of distinct taxa identified during the study. The cumulative count was not itself considered a standardized density and was not assigned a unit of L^{-1}, cm^{-2}, or g^{-1}.

The resulting sample-level abundance and occurrence data were subsequently used for the phytosociological and diversity analyses described in Sections 2.5 and 2.6. This sampling and enumeration procedure provided a consistent quantitative basis for assessing the spatial and seasonal distribution of *Bacillariophyta* while maintaining the distinct quantitative basis of samples obtained from different aquatic habitats.

2.3. Morphological Identification and Microscopic Examination

Collected Algal samples were preserved in 4% formalin immediately after transportation to the laboratory to maintain their morphological characteristics. Permanent microscope slides were prepared following standard procedures for freshwater diatoms. Representative subsamples were mounted on clean glass slides and examined under a compound light microscope (Olympus, Japan) at magnifications ranging from 40× to 1000× using an oil immersion objective when required.

Species identification was based on detailed examination of valve morphology, including cell shape, size, symmetry, raphe structure, striae pattern, ornamentation, and other diagnostic characteristics. Measurements of valve length, width, and striae density were recorded using a calibrated ocular micrometer. Representative specimens were photographed using a digital microscope camera for taxonomic documentation as shown in Figure 2.

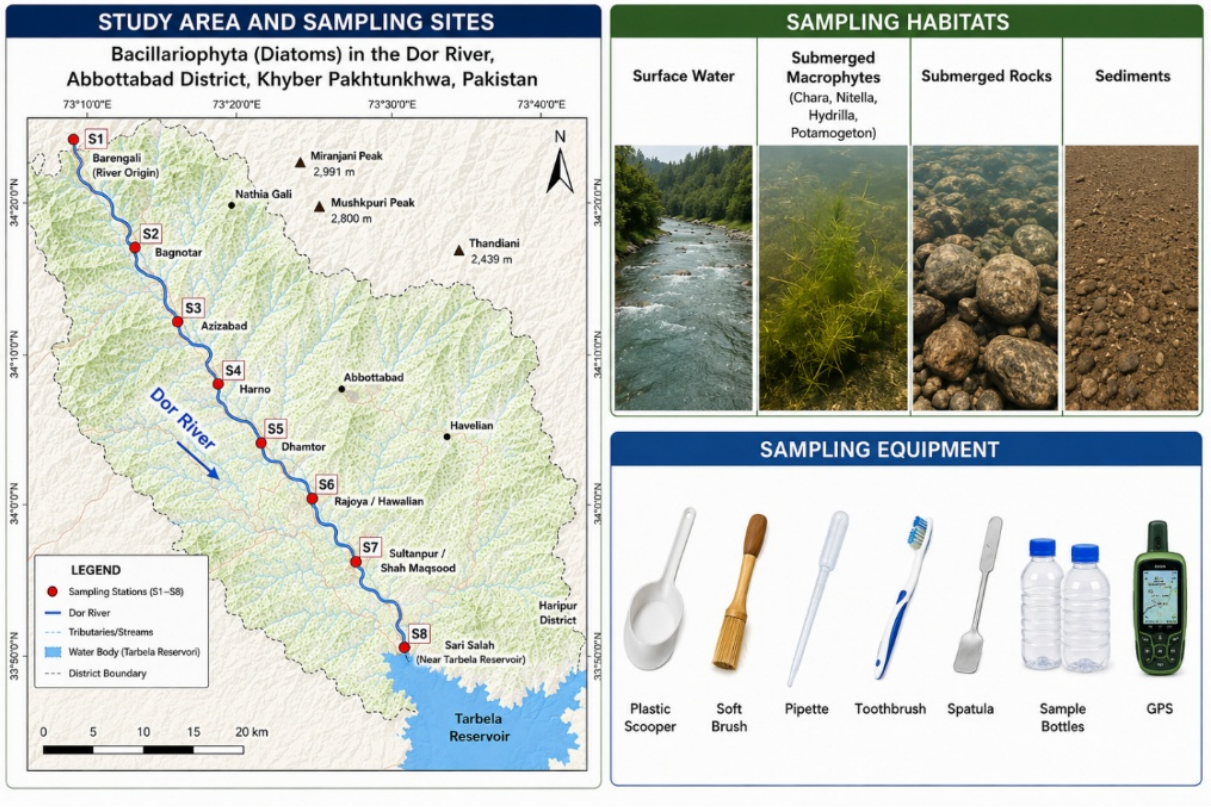


Figure 2. Schematic illustration of the study design adopted for the assessment of Bacillariophyta diversity and seasonal variation in relation to physicochemical characteristics of the Dor River, Abbottabad, Pakistan.

Taxonomic identification was performed using standard floras and identification keys, including Smith (1950), Prescott (1962), Patrick and Reimer (1966, 1975), Tiffany and Britton (1971), Krammer and Lange-Bertalot (1986–1991), and Wehr et al. (2015) [8–14]. The nomenclature and classification of all taxa were verified using AlgaeBase to ensure consistency with current taxonomic standards.

2.4. Physicochemical Analysis of Water Samples

Water samples were collected monthly from the eight sampling stations using pre-cleaned polyethylene bottles and transported to the laboratory in insulated ice boxes. All physicochemical analyses were completed within 24 h according to the standard procedures.

The analyzed parameters included water temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), turbidity, alkalinity, calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), potassium (K^{+}), bicarbonate (HCO_3^{-}), chloride (Cl^{-}), sulfate (SO_4^{2-}), nitrate (NO_3^{-}), and monthly precipitation. Water temperature, pH, EC, and TDS were measured using calibrated portable digital meters. Turbidity was determined using a nephelometric turbidity meter, while alkalinity, calcium, magnesium, chloride, bicarbonate, sulfate, sodium, potassium, and nitrate were analyzed following standard APHA (2017) laboratory methods.

Quality control was maintained by calibrating all instruments before analysis, using analytical-grade reagents, and analyzing samples in triplicate. The resulting physicochemical data were used to evaluate seasonal changes in water quality and their relationship with the diversity and distribution of Bacillariophyta in the Dor River.

2.5. Phytosociological Analysis

The phytosociological characteristics of the Bacillariophyta community were evaluated using standard ecological indices to determine species composition, abundance, and distribution within the Dor River. Species density, frequency, relative density (RD), relative frequency (RF), and Importance Value Index (IVI) were calculated following the methods described by Anjum [15]. These parameters were used to identify dominant species and assess their ecological significance within the diatom community. The calculated phytosociological indices provided quantitative information on the seasonal variation and community structure of Bacillariophyta across the eight sampling sites.

2.6. Statistical Analysis

The ecological and physicochemical datasets were analyzed using FAST (Version 4.03) and Microsoft Excel 2021. Species diversity was assessed using the Shannon–Wiener Diversity Index (H'), Simpson's Diversity Index (1–D), Margalef's Richness Index, Fisher's Alpha, Chao-1, Evenness, and Dominance Index. Principal Component Analysis (PCA) was performed to examine the multivariate relationships between *Bacillariophyta* community patterns and physicochemical variables and to identify environmental variables associated with variation in species distribution. Graphs and figures were prepared using Microsoft Excel. The results of PCA and ecological indices were interpreted in terms of patterns of association and ecological correspondence rather than statistical significance or causal effects.

2.7. Graphical Abstract

This study provides the first comprehensive assessment of the diversity, seasonal distribution, and ecological dynamics of Bacillariophyta (diatoms) in the Dor River, Khyber Pakhtunkhwa, Pakistan, during 2021–2022. Monthly sampling was conducted at eight representative sites to evaluate the relationships between diatom assemblages and physicochemical water characteristics. A total of 46 Bacillariophyta taxa belonging to 2 classes, 10 orders, and 19 families were identified, with *Navicula laterostrata* emerging as the dominant species. Seasonal peaks in species richness and diversity occurred during late winter and early spring, reflecting favorable environmental conditions. Multivariate analysis (Principal Component Analysis) and ecological diversity indices demonstrated that temporal variations in diatom communities were strongly associated with fluctuations in physicochemical parameters, indicating the combined influence of natural seasonal processes and anthropogenic disturbances as shown in Figure 3. The findings establish baseline ecological information for the Dor River and highlight the value of diatoms as sensitive bio indicators for freshwater ecosystem assessment, long-term ecological monitoring, and sustainable water resource management.

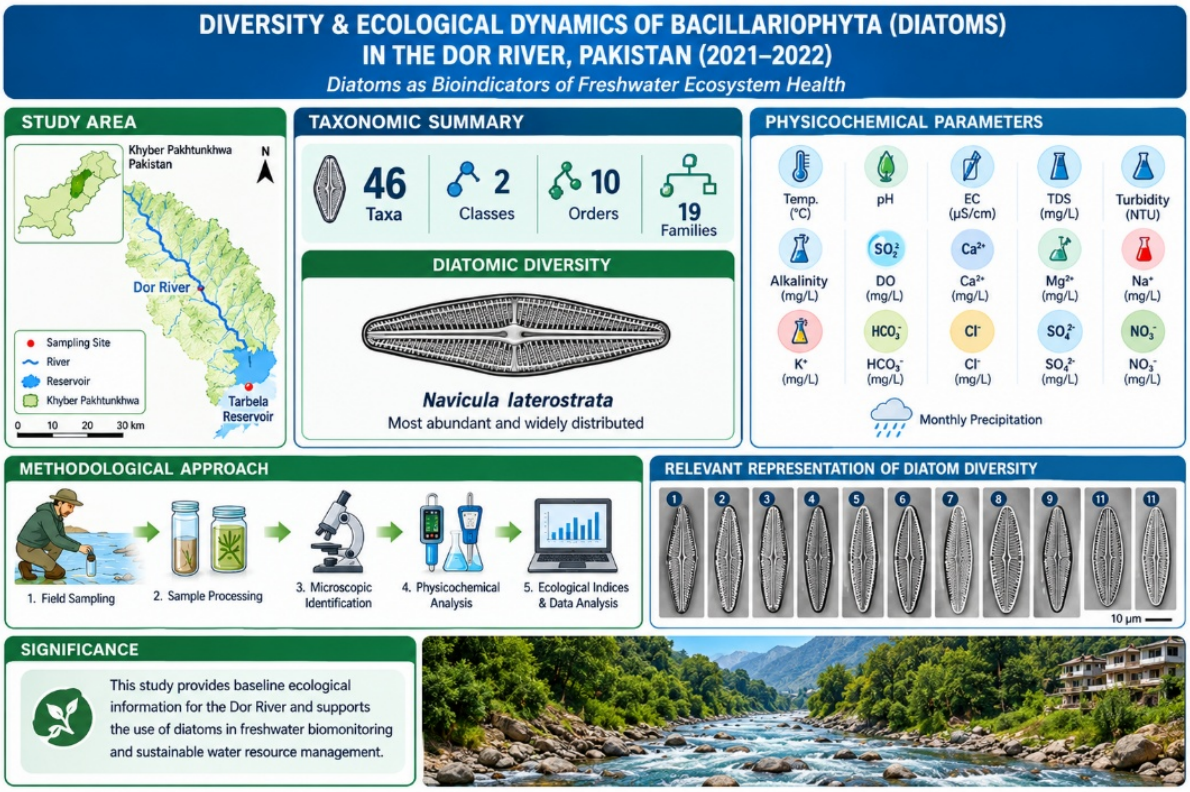


Figure 3. Overview of the study illustrating the assessment of Bacillariophyta diversity, seasonal distribution, and physicochemical characteristics of the Dor River, Abbottabad, Pakistan, through monthly field sampling, morphological identification, ecological analyses, and statistical evaluation.

3. Results

3.1. Taxonomic Diversity of Bacillariophyta

A comprehensive taxonomic survey conducted from January 2021 to December 2022 documented 46 taxa of Bacillariophyta in the Dor River, Abbottabad, Pakistan (Table 1). The recorded taxa represented a diverse assemblage of freshwater diatoms distributed across multiple orders and families, providing baseline information on diatom diversity and distribution in this river system in northern Pakistan (Figure 4).

At the family level, Bacillariaceae was the most species-rich family, comprising seven taxa, followed by Gomphonemataceae and Naviculaceae, each represented by six taxa. These families included commonly recorded genera such as *Nitzschia*, *Bacillaria*, *Gomphonema*, and *Navicula* (Figure 5). Their representation contributed substantially to the taxonomic diversity documented in the Dor River.

At the ordinal level, Naviculales was the most species-rich order, represented by taxa from several families, including Naviculaceae, Amphipleuraceae, Sellaphoraceae, Stauroneidaceae, Pinnulariaceae, Pleurosigmataceae, and Anomoeoneidaceae. Other recorded orders included Bacillariales, Cymbellales, Eunotiales, Rhopalodiales, Surirellales, Fragilariales, and Aulacoseirales. This distribution highlights the taxonomic breadth of the diatom assemblage recorded during the study period.

The final checklist comprised 46 taxa in total. The class-level assignments and corresponding numbers of orders and families should be reported according to the verified taxonomic classification in Table 1, ensuring that the class totals sum to 46 and that all taxa are consistently classified throughout the Abstract, Results, tables, and figures. This taxonomic inventory provides a baseline for future studies of diatom diversity and freshwater ecological monitoring in the Dor River.

The observed taxonomic composition demonstrates that the Dor River supports a rich and diverse diatom flora despite increasing anthropogenic pressures along downstream reaches. The presence of numerous environmentally sensitive taxa indicates that portions of the river still maintain favorable ecological conditions, while the occurrence of pollution-tolerant species suggests localized environmental disturbances associated with human activities.

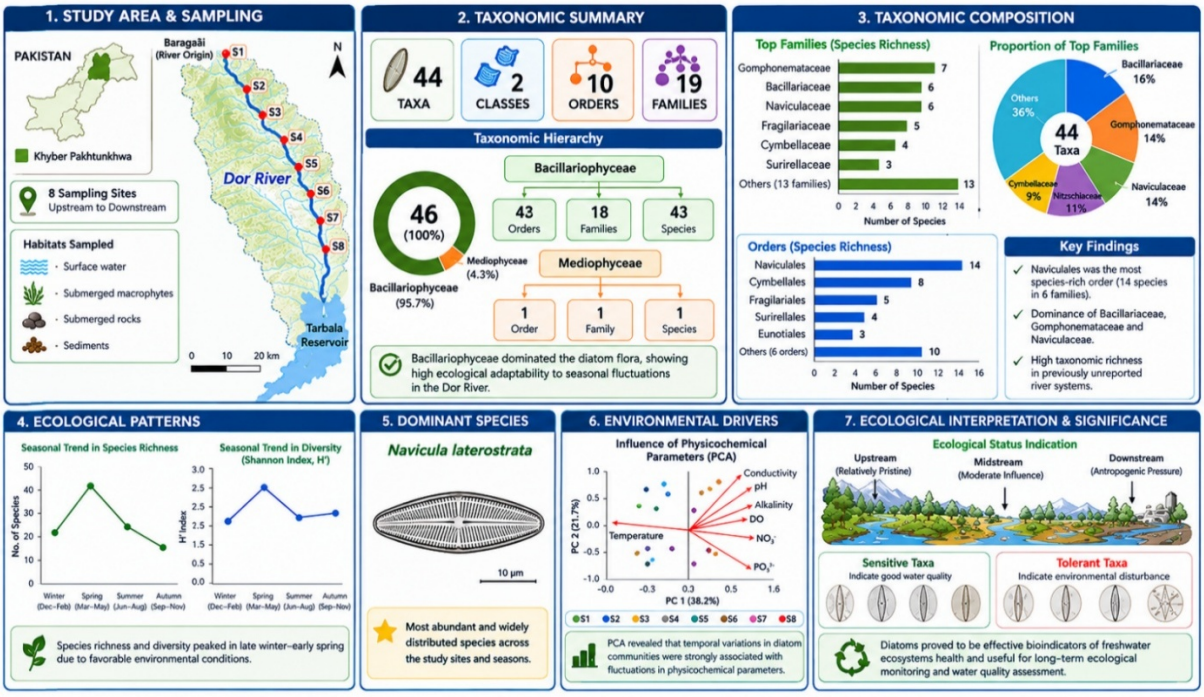


Figure 4. Taxonomic diversity of Bacillariophyta recorded from the Dor River, Abbottabad, Pakistan.

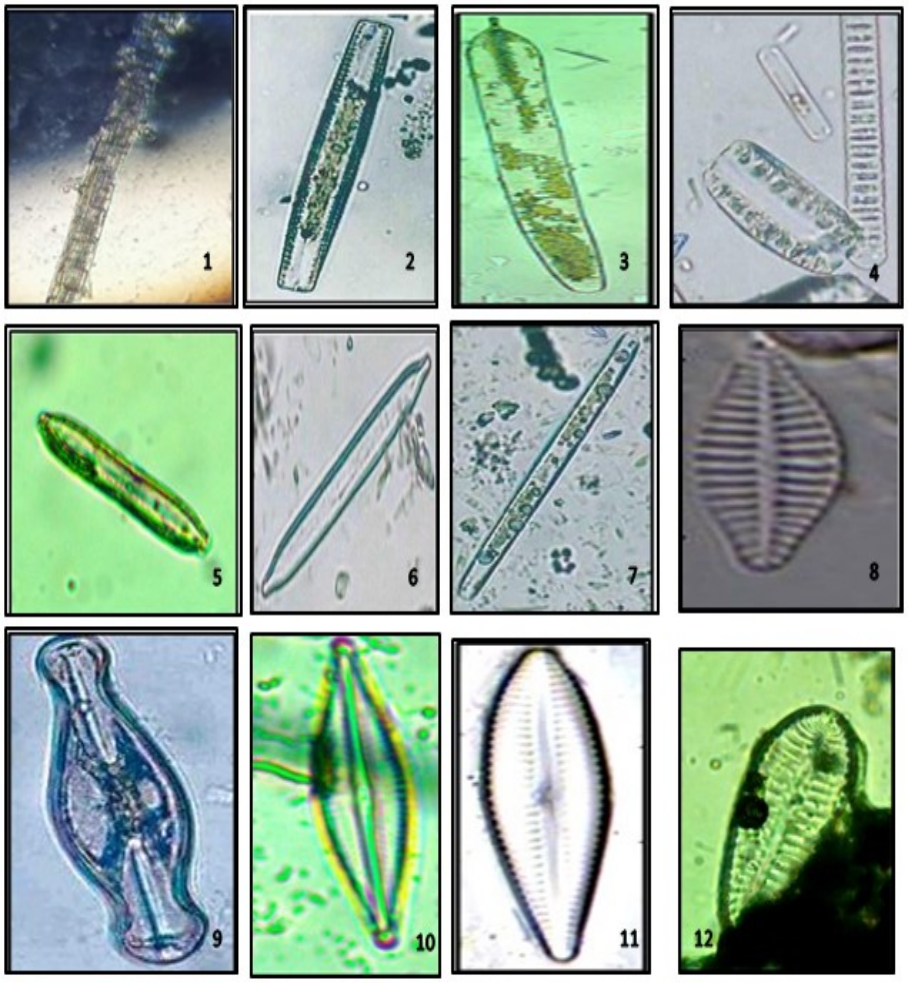


Figure 5. Light microscopic images of selected Bacillariophyta taxa recorded from the Dor River: (1) Bacillaria paradoxa; (2) Denticula elegans; (3) Nitzschia scalpelliformis; (4) Denticula thermalis; (5) Nitzschia constricta; (6) Nitzschia gandersheimiensis; (7) Nitzschia vermicularis; (8) Planothidium delicatulum; (9) Didymosphenia geminata; (10) Encyonema elginense; (11) Navicula radiosa and (12) Gomphonema affine.

Table 1. Checklist and taxonomic classification of Bacillariophyta (diatoms) recorded from the Dor River, Abbottabad, Khyber Pakhtunkhwa, Pakistan, during the study period (2021–2022).

Division	Class	Order	Family	No.	Species
Bacillariophyta	Bacillariophyceae	Bacillariales	Bacillariaceae	1	*Bacillaria paradoxa* Gmelin
				2	*Denticula elegans* Kützing
				3	*Nitzschia scalpelliformis* (Grunow) Grunow in Cleve & Grunow
				4	*Denticula thermalis* Kützing
				5	*Nitzschia constricta* (Kützing) Ralfs in Pritchard
				6	*Nitzschia gandersheimiensis* Krasske
				7	*Nitzschia vermicularis* (Kützing) Hantzsch
			Achnanthidiaceae	8	*Planothidium delicatulum* (Kützing) Round & Bukhtiyarova
		Cymbellales	Cymbellaceae	9	*Didymosphenia geminata* (Lyngbye) M. Schmidt
			Gomphonemataceae	10	*Encyonema elginense* (Krammer) D. G. Mann
				11	*Encyonema* sp.
				12	*Gomphonema affine* Kützing
				13	*Gomphonema truncatum* Ehrenberg
				14	*Gomphonema parvulum* var. *parvulum*
				15	*Gomphonema angustatum* (Kützing) Rabenhorst
			Rhoicospheniaceae	16	*Rhoicosphenia abbreviata* (C. Agardh) Lange-Bertalot
		Eunotiales	Eunotiaceae	17	*Eunotia monodon* Ehrenberg
			Diploneidaceae	18	*Diploneis puella* (Schumann) Cleve
		Naviculales	Naviculaceae	19	*Navicula* sp. 1
				20	*Navicula* sp. 2
				21	*Navicula cryptocephala* Kützing
				22	*Navicula laterostrata* Hustedt
				23	*Navicula radiosa* Kützing
				24	*Navicula viridula* (Kützing) Ehrenberg
			Amphipleuraceae	25	*Halamphora holsatica* (Hustedt) Levkov
				26	*Halamphora montana* (Krasske) Levkov
				27	*Gyrosigma eximium* (Thwaites) Boyer
			Sellaphoraceae	28	*Sellaphora pupula* (Kützing) Mereschkowsky
			Stauroneidaceae	29	*Stauroneis anceps*
			Pinnulariaceae	30	*Pinnularia microstauron* (Ehrenberg) Cleve
				31	*Pinnularia parva* Gregory ex Rabenhorst
			Pleurosigmataceae	32	*Pleurosigma salinarum* (Grunow) Grunow
		Rhopalodiales	Rhopalodiaceae	33	*Epithemia argus* (Ehrenberg) Kützing
				34	*Rhopalodia gibba* (Ehrenberg) Otto Müller
		Surirellales	Surirellaceae	35	*Cymatopleura solea* (Brébisson) W. Smith
				36	*Surirella elegans* Ehrenberg
				37	*Surirella ovalis* Brébisson
		Fragilariales	Fragilariaceae	38	*Fragilaria famelica* (Kützing) Lange-Bertalot
				39	*Staurosirella pinnata* (Ehrenberg) D. M. Williams
				40	*Staurosira construens* f. *venter* (Ehrenberg) P. B. Hamilton
				41	*Fragilaria tenera* (W. Smith) Lange-Bertalot
				42	*Fragilaria ulna* var. *ulna* (Nitzsch) Lange-Bertalot
		Aulacoseirales	Aulacoseiraceae	43	*Aulacoseira italica* (Ehrenberg) Simonsen
				44	*Aulacoseira granulata*
		Fragilariales	Fragilariaceae	45	*Diatoma vulgare* Bory
		Naviculales	Anomoeoneidaceae	46	*Anomoeoneis vitrea* (Grunow) R. Ross in Patrick & Reimer

3.2. Seasonal Distribution of Bacillariophyta

The seasonal dynamics of Bacillariophyta communities in the Dor River exhibited pronounced temporal variation throughout the study period (2021–2022), reflecting the influence of changing environmental conditions and hydrological regimes. Monthly monitoring revealed considerable fluctuations in species abundance, density, frequency, and community dominance, indicating that seasonal changes were associated with variation in the structure of the diatom assemblages.

A total density of 846 individuals was recorded during the investigation. The highest abundance occurred during March (131 individuals), followed by February (92 individuals) and December (85 individuals), whereas comparatively lower abundances were observed during May (50 individuals), October (56 individuals), and June (64 individuals). The increased abundance during late winter and early spring coincided with moderate water temperature, variation in nutrient concentrations, relatively stable hydrological conditions, and improved water transparency. These environmental conditions may have been associated with the observed increase in diatom abundance. Conversely, educed abundance during the summer months coincided with elevated water temperature, increased turbidity, and seasonal hydrological variation resulting from monsoonal rainfall, which can disturb benthic habitats and reduce light penetration.

Species-specific analysis revealed substantial variation in ecological dominance among the recorded taxa. *Navicula laterostrata* was identified as the most abundant species, with a density of 39 individuals, accounting for 4.60% of the total recorded density. *Fragilaria tenera* ranked second, contributing 33 individuals (3.90%), followed by several species of *Pinnularia*, *Navicula*, and *Gomphonema*, which exhibited moderate abundance throughout the sampling period. The dominance of *Navicula laterostrata* may indicate its ability to persist across the range of environmental conditions observed during the study period. Species belonging to the genus *Navicula* are well known for their ability to colonize diverse freshwater habitats and respond rapidly to variations in nutrient availability, pH, conductivity, and water flow.

Monthly species density also demonstrated distinct seasonal peaks. *Pinnularia microstauron* and *Navicula viridula* each attained their maximum density of nine individuals, particularly during the pre-monsoon period when environmental conditions were relatively stable. The occurrence of these taxa during cooler months coincided with moderate temperatures and lower suspended sediment loads, suggesting that these conditions may have provided suitable conditions for their persistence and growth.

Frequency analysis demonstrated that several taxa were widely distributed throughout the annual cycle. *Navicula cryptocephala* exhibited the highest frequency of occurrence, being recorded in nine of the sampling months, followed closely by *Navicula radiosa*, which was also present during nine sampling months. These species contributed 3.78% and 3.30% of the total relative frequency, respectively, indicating their persistent occurrence across diverse environmental conditions as shown in Figure 6. The high frequency of these taxa suggests broad ecological tolerance and their potential utility as indicator species for long-term biomonitoring of freshwater ecosystems.

The Importance Value Index (IVI) further highlighted differences in ecological dominance among species. *Navicula laterostrata* exhibited the highest IVI (6.818), followed by *Navicula cryptocephala* (6.621), indicating their relatively high ecological importance within the Bacillariophyta community of the Dor River as shown in Figure 6. The elevated IVI values indicate that these species were not only abundant but also widely distributed throughout the study period, reflecting their widespread occurrence and relative abundance under the environmental conditions observed during the study period.

Overall, the seasonal succession of Bacillariophyta observed in the present study demonstrates that diatom community composition is closely associated with seasonal environmental variability. The increased abundance recorded during late winter and early spring coincided with seasonal variation in temperature, nutrient concentrations, turbidity, and other measured water-quality parameters. In contrast, reduced abundance during the monsoon and early summer months coincided with increased turbidity, seasonal hydrological variation, and changes in the measured physicochemical characteristics. These conditions may have been associated with the observed reduction in diatom abundance.

3.3. Spatial Distribution of Bacillariophyta

The spatial distribution of *Bacillariophyta* along the Dor River showed variation in diatom occurrence and community composition across the river's longitudinal course. Sampling was conducted at eight stations: S1 (Barengali), S2 (Bagnotar), S3 (Azizabad), S4 (Harno), S5 (Dhamtor), S6 (Rajoya/Havelian), S7 (Sultanpur/Shah Maqsood), and S8 (Sari Salah) (Figure 1). Collectively, these stations represented different river sections and provided an overall view of the diatom flora throughout the study area.

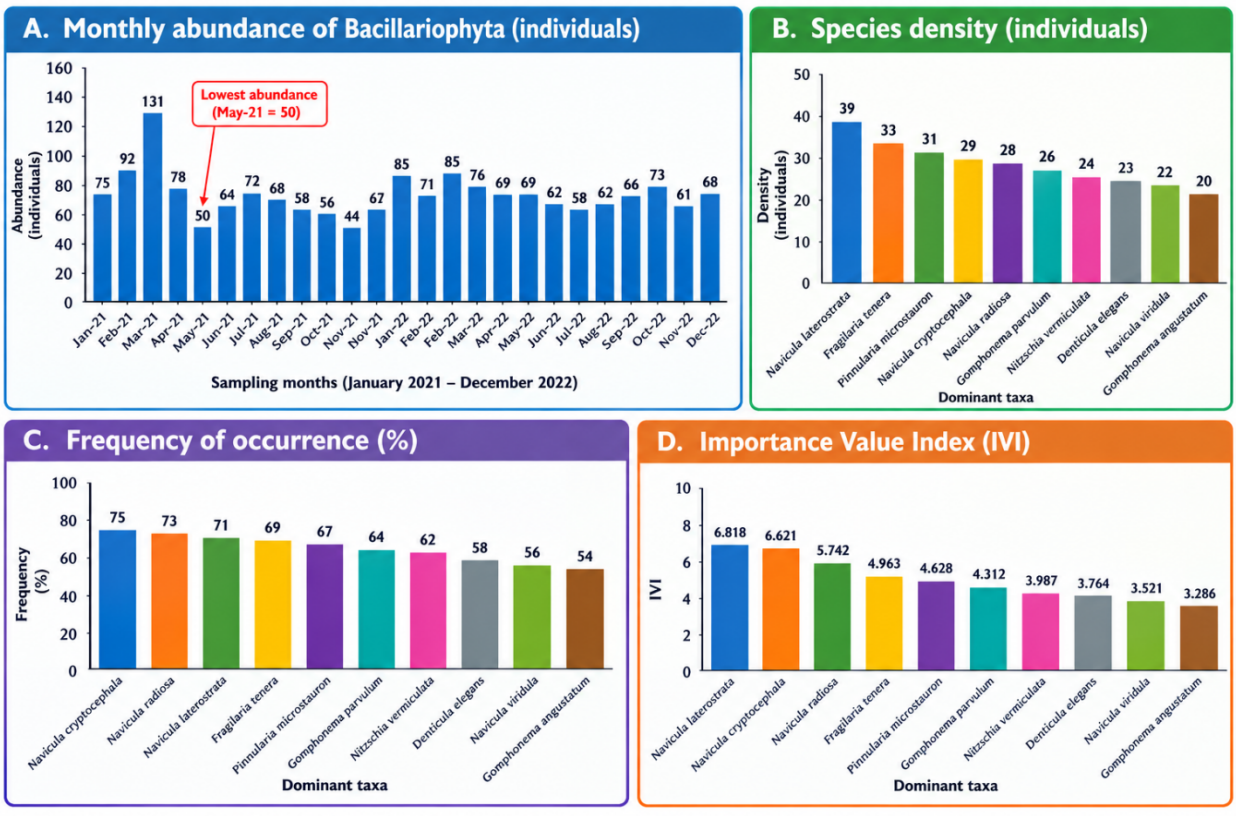


Figure 6. Seasonal and monthly variation in *Bacillariophyta* communities in the Dor River, Abbottabad, Pakistan, during 2021–2022, showing (**A**) monthly cumulative abundance of individual cells/valves, (**B**) dominant species based on abundance, (**C**) frequency of occurrence, and (**D**) Importance Value Index (IVI) of the principal taxa.

The recorded diatom assemblage comprised taxa with different patterns of occurrence and abundance across the sampling records. Some taxa were recorded relatively frequently, whereas others appeared less often, indicating variation in their representation within the overall river assemblage. The occurrence of diatoms across the sampled river sections reflected the heterogeneous nature of the aquatic habitats along the Dor River.

The upper and lower sections of the river differed in their surrounding environments and degree of human influence. These differences provide an environmental context for interpreting the overall distribution of the diatom flora. However, because the results are presented as a general assessment of the river-wide assemblage, the available pooled records do not establish quantitative differences in richness, abundance, or diversity among individual stations. Accordingly, the observed distribution is described as an overall spatial pattern rather than evidence of statistically significant differences or direct causal effects of pollution.

For consistency, all sampling locations are identified using the eight station codes and names shown in Figure 1. Rajoya/Havelian is treated as a single station (S6), and locations not included among the eight mapped stations are omitted from the spatial description. This standardized presentation provides an overall account of *Bacillariophyta* distribution along the Dor River without attributing unverified site-specific differences to particular locations.

3.4. Diversity Indices of Bacillariophyta Communities

The diversity indices of Bacillariophyta communities exhibited pronounced seasonal fluctuations throughout the study period (2021–2022), reflecting temporal changes in community structure and environmental conditions within the Dor River. Monthly analysis indicated considerable variation in species richness, diversity, evenness, and dominance, indicating that the composition of diatom assemblages varied in association with seasonal changes in physicochemical conditions.

Species richness (Taxa_S) varied substantially among sampling months. The highest richness was recorded in December (36 taxa), followed by March (32 taxa) and April (29 taxa), whereas the lowest richness occurred during June (20 taxa). Intermediate richness values were observed in September (28 taxa), November (27 taxa), February (26 taxa), May and July (25 taxa each), January and October (24 taxa each), and August (21 taxa). The greater richness observed during winter and early spring coincided with seasonal physicochemical conditions that may have been suitable for the establishment and coexistence of a diverse diatom flora.

The Shannon–Wiener diversity index (H') demonstrated similar seasonal trends. The highest diversity was recorded in December (H' = 3.355), closely followed by March (H' = 3.329), indicating highly diverse and well-structured Bacillariophyta communities. In contrast, the lowest Shannon diversity was observed during June (H' = 2.725), suggesting reduced species diversity and increased dominance of a limited number of taxa during the summer period.

The Simpson's Diversity Index (1–D) also confirmed these seasonal patterns. Maximum values were recorded in March (0.9604) and December (0.9561), indicating a high probability that two randomly selected individuals belonged to different species. Conversely, lower Simpson values during the summer months reflected reduced community heterogeneity and increased ecological dominance by a few species.

Species evenness remained relatively high throughout most of the study period, with the highest values recorded during March (0.8721) and September (0.8712). High evenness indicates that individuals were relatively evenly distributed among species, suggesting a stable and balanced Algal community. However, evenness declined considerably during June (0.7627), reflecting unequal distribution of individuals and the temporary dominance of a few environmentally tolerant taxa.

The Dominance Index (D) further supported these observations. June exhibited the highest dominance value (0.08105), indicating reduced community complexity and increased ecological dominance by a limited number of species. Elevated dominance during this period coincided with increased water temperature and higher turbidity, together with seasonal hydrological variation.

Species richness estimators, including Margalef's Richness Index, Chao-1, and Fisher's Alpha, demonstrated similar seasonal patterns. Margalef's Index attained its maximum value in December (7.878), reflecting the highest observed species richness. Likewise, the Chao-1 estimator (44.27) suggested the presence of additional rare taxa within the community, indicating that the actual species richness may exceed the observed diversity. Fisher's Alpha also reached its highest value during December (23.57), followed by May (19.90), September (19.31), and August (19.29), confirming December as the period of maximum ecological diversity.

Overall, the diversity indices consistently demonstrated that Bacillariophyta communities attained their greatest diversity, richness, and ecological stability during late winter and early spring. In contrast, reduced diversity during the summer months coincided with elevated temperatures, increased turbidity, and seasonal hydrological disturbances, together with greater dominance of a smaller number of taxa. These findings indicate an association between seasonal environmental variability and changes in freshwater diatom community structure.

3.5. Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was performed to investigate the relationships between Bacillariophyta diversity and environmental variables and o explore the major environmental gradients associated with seasonal variation within the Dor River ecosystem. The multivariate analysis effectively reduced the complexity of the dataset and revealed the major environmental factors associated with variation in species distribution and community composition shown in Figure 7. The first principal component (PC1) explained 27.88% of the total variance, whereas the second component (PC2) accounted for an additional 14.65%, resulting in a cumulative variance of 42.53%. Incorporation of the third principal component (PC3) increased the cumulative explained variance to 54.83%, indicating that the first three components successfully captured more than half of the total ecological variability observed during the study period as shown in Figure 7.

Subsequent principal components contributed progressively smaller proportions of variation. The first six principal components collectively explained 81.58% of the total variance, while the first eight components accounted for more than 90% of the overall variability. The complete dataset was represented by twelve principal components, with the cumulative explained variance reaching 100%, demonstrating that the PCA effectively summarized the multidimensional environmental dataset.

The ordination plot revealed distinct seasonal clustering of sampling months, indicating strong temporal variation in physicochemical characteristics and Bacillariophyta community composition. Winter and early spring samples were associated with greater species richness, higher diversity indices, and particular combinations of the measured physicochemical variables, whereas summer and monsoon samples were positioned separately along environmental gradients associated with elevated water temperature, increased turbidity, altered nutrient concentrations, and seasonal hydrological variation.

The distribution of species within the PCA ordination demonstrated that dominant taxa, particularly species belonging to the genera *Navicula*, *Fragilaria*, *Pinnularia*, and *Gomphonema*, were closely associated with favorable physicochemical conditions characterized by moderate temperature, stable pH, lower turbidity, and balanced ionic composition. Conversely, several environmentally tolerant taxa were associated with periods of

increased conductivity, suspended solids, and seasonal runoff, indicating their occurrence under environmental conditions characterized by increased conductivity, suspended solids, and seasonal runoff.

Overall, the PCA indicated that seasonal fluctuations in physicochemical parameters were associated with variation in Bacillariophyta community dynamics in the Dor River. Environmental variables including temperature, electrical conductivity, alkalinity, turbidity, nutrient availability, and precipitation contributed to the environmental gradients observed in the PCA and were associated with variation in species composition and temporal patterns. Similar multivariate relationships have been reported by recent ecological studies, where PCA has been used in previous ecological studies to identify temperature, nutrient availability, electrical conductivity, and hydrological variability as important environmental variables associated with variation in freshwater Algal communities. The present findings highlight potential relationships between physicochemical variables and *Bacillariophyta* diversity. As shown in Figure 7, PCA provides an exploratory representation of patterns in the relationship between environmental variables and diatom community composition, supporting its use in freshwater ecosystem assessment and environmental monitoring. However, these patterns should be interpreted as associations rather than evidence of direct causal effects or statistical significance.

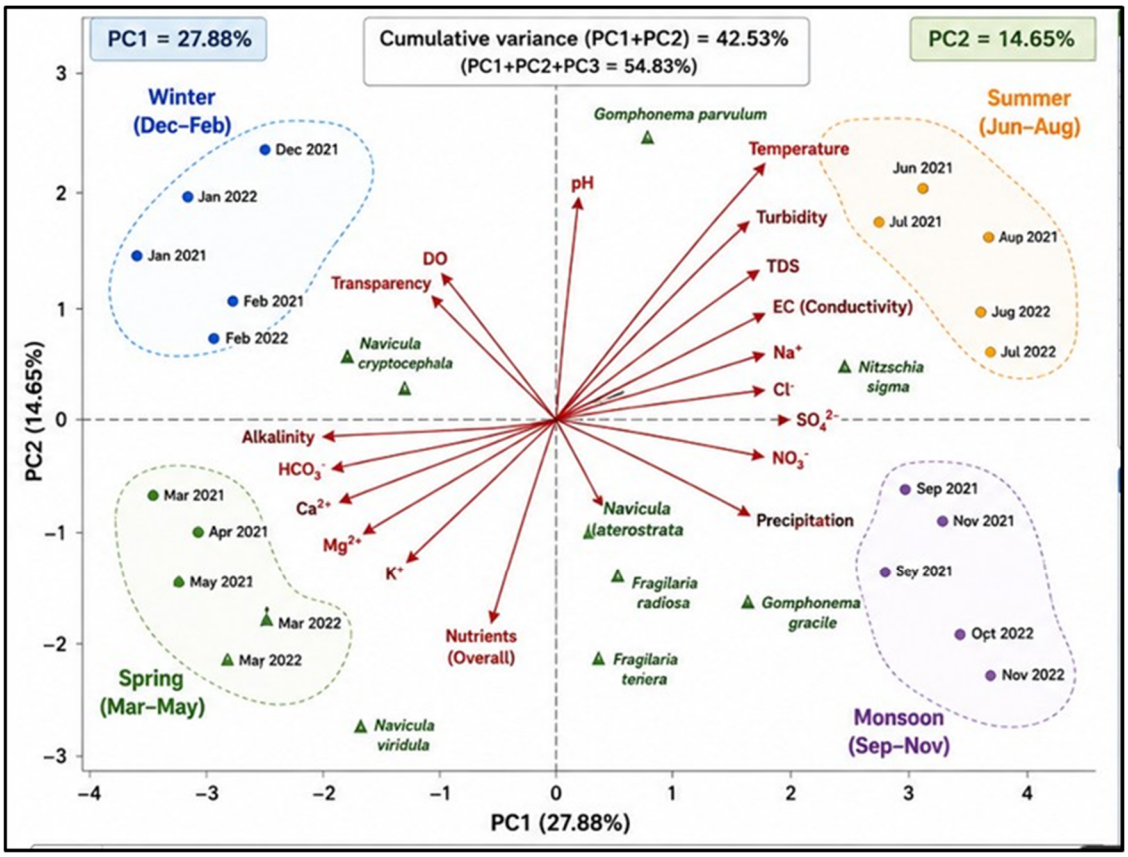


Figure 7. Graphical representation of the Principal Component Analysis (PCA) illustrating the relationships between Bacillariophyta communities and physicochemical water quality parameters in the Dor River, Abbottabad, Pakistan, during 2021–2022.

3.6. Seasonal Variation in Physicochemical Characteristics of the Dor River

The physicochemical characteristics of the Dor River exhibited pronounced seasonal variations throughout the study period (2021–2022), reflecting the combined influence of natural hydrological processes and anthropogenic activities. Monthly monitoring revealed considerable fluctuations in major water quality parameters, including pH, electrical conductivity (EC), total dissolved solids (TDS), alkalinity, turbidity, temperature, major ions, and precipitation. These seasonal changes coincided with temporal variation in the structure and distribution of Bacillariophyta communities.

The pH of the river remained relatively stable throughout the study period, ranging from 7.1 in June to 7.9 in March, indicating slightly alkaline conditions that are generally favorable for freshwater diatom growth. The consistently alkaline nature of the river suggests good buffering capacity and suitable environmental conditions for the development of diverse Bacillariophyta assemblages shown in Figure 8.

Marked seasonal variation was observed in the concentrations of major cations. Calcium (Ca) concentrations ranged from 44.6 ppm in September to 105.5 ppm in April, whereas magnesium (Mg) varied between 11.3 ppm in July and 26.4 ppm in March. Elevated concentrations of calcium and magnesium during late winter and early spring likely reflect increased mineral weathering and groundwater contributions following seasonal precipitation. These minerals contribute to water hardness and may be ecologically relevant to freshwater community structure.

Potassium (K) and chloride (Cl) also exhibited seasonal fluctuations, with the highest concentrations recorded during July (23.3 ppm and 4.1 ppm, respectively) shown in Figure 8. he increased concentrations during the monsoon period may be associated with surface runoff, agricultural drainage, and localized anthropogenic inputs, which transport dissolved ions into the river system. Similar seasonal enrichment of dissolved ions has been reported in rivers influenced by agricultural and urban catchments.

Bicarbonate (HCO_3^-) concentrations varied considerably throughout the year, reaching a maximum value of 230.1 ppm in March before declining sharply to 33.2 ppm in July. Likewise, sodium (Na) concentrations increased during the monsoon and winter months, with the highest values (11.9 ppm) recorded in July and January. These fluctuations reflect seasonal changes in river discharge, weathering intensity, and watershed runoff.

Nutrient concentrations remained within acceptable ecological limits during most of the study period. Nitrate (NO_3^-) reached its highest concentration (6.6 ppm) in December and decreased to 0.14 ppm during July, whereas sulfate (SO_4^{2-}) exhibited comparatively minor seasonal variation. The relatively low nutrient concentrations indicate that the river remains moderately productive despite increasing anthropogenic influences in downstream reaches shown in Figure 8.

Electrical conductivity (EC), an important indicator of dissolved ionic content, ranged from 331.1 μS cm^{-1} in December to 444.1 μS cm^{-1} in October. The elevated EC values observed during the post-monsoon season suggest increased concentrations of dissolved minerals resulting from runoff, evaporation, and anthropogenic inputs. Seasonal variation in EC corresponded closely with fluctuations in major dissolved ions, confirming changes in the ionic composition of the river water.

Alkalinity exhibited marked seasonal variation, with the highest values recorded during March (161.1 ppm) and April (155.2 ppm), whereas the lowest concentration (33.9 ppm) occurred in July during the peak monsoon season. The decline in alkalinity during periods of high river discharge is likely associated with dilution effects caused by rainfall and snowmelt.

Turbidity showed a clear seasonal pattern, increasing substantially during the monsoon months. The maximum turbidity (48.6 NTU) was recorded in July, indicating enhanced sediment transport and suspended particulate matter resulting from heavy rainfall and catchment erosion. Elevated turbidity during the rainy season reduces light penetration into the water column and may adversely affect photosynthetic activity and diatom productivity.

Total Dissolved Solids (TDS) also exhibited seasonal fluctuations, with maximum values recorded during June (211.1 ppm) and December (198.7 ppm). The observed variation may reflect seasonal differences in dissolved mineral content, runoff, and evaporation. Water temperature followed the expected annual climatic cycle, ranging from 11.86 °C in March to 34.02 °C in August. These temperature fluctuations coincided with seasonal changes in Bacillariophyta abundance and community structure shown in Figure 8.

Monthly precipitation data demonstrated pronounced seasonal variability, with the highest rainfall recorded during October (271.52 mm) and September (266.04 mm), whereas February (30.17 mm) and December (48.59 mm) received the lowest precipitation. Increased rainfall during the monsoon and post-monsoon periods substantially altered river discharge, nutrient transport, sediment loading, and water chemistry, thereby influencing the temporal dynamics of diatom communities.

Overall, the observed seasonal variation in physicochemical parameters indicates that the ecological condition of the Dor River reflects the interaction of climatic variability, watershed hydrology, and anthropogenic activities. Seasonal changes in water chemistry were closely associated with fluctuations in Bacillariophyta diversity and abundance, emphasizing the importance of integrated physicochemical and biological monitoring for evaluating freshwater ecosystem health. The observed variations in Bacillariophyta diversity and community composition were associated with the measured physicochemical characteristics of the Dor River. However, these patterns should be interpreted cautiously because established diatom-based water-quality indices and several direct pollution indicators were not included in the present study. Therefore, the findings provide an ecological assessment based on the measured environmental variables rather than a definitive quantitative classification of pollution or overall ecological status.

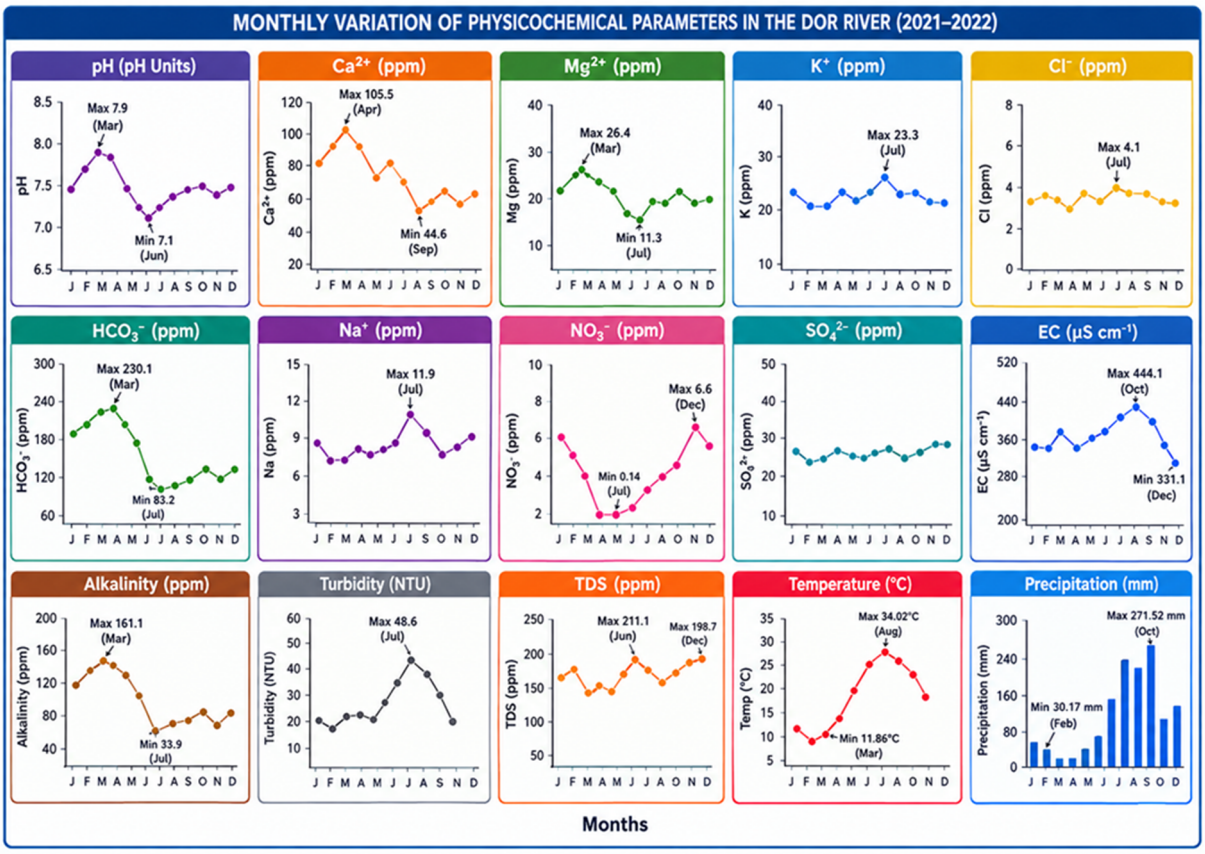


Figure 8. Seasonal variation in the physicochemical characteristics of the Dor River, Abbottabad, Pakistan, during 2021–2022, illustrating monthly fluctuations in water temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), turbidity, alkalinity, major ions, nutrients, and precipitation.

4. Discussion

Comparable patterns of freshwater Algal diversity have been reported from other tropical and subtropical freshwater ecosystems. Ref. [16] documented 28 freshwater micro Algal species representing eight genera in the Kothandaramar Temple Tank, India, and reported seasonal fluctuations in community composition. Similarly, [17] identified 72 Algal species belonging to four major classes from the Kadambrayar River and reported reduced ecological quality in downstream sections associated with eutrophication and anthropogenic pollution. Although the species richness recorded in the present investigation was comparatively lower, the documentation of 46 *Bacillariophyta* taxa provides important baseline information on freshwater diatom diversity in the Dor River and contributes to the limited knowledge of freshwater diatom communities in northern Pakistan. The present findings therefore provide a useful reference for future biodiversity assessments and ecological monitoring of the river.

The seasonal patterns observed in the Dor River were also comparable with those reported from other freshwater ecosystems. Ref. [18] reported marked seasonal fluctuations in *Cyclotella meneghiniana*, *Gomphonema gracile*, and *Nitzschia palea* in association with changes in nutrient availability and water-quality conditions. In the present study, Bacillariophyta abundance varied considerably among months, with the highest cumulative number of individual cells/valves recorded in March, followed by February and December, whereas comparatively lower values were observed in May, October, and June. Species richness and diversity indices also varied seasonally, with higher Taxa_S and Shannon diversity during December and March and comparatively lower diversity during June. These temporal patterns corresponded with the seasonal changes observed in the physicochemical characteristics of the Dor River. The occurrence and relative abundance of different taxa across months therefore indicate temporal variation in the Bacillariophyta assemblage in relation to changing environmental conditions.

Seasonal variation in community diversity was further reflected by the ecological indices. Ref. [19] observed pronounced seasonal fluctuations in micro Algal diversity, with higher Shannon diversity and lower dominance during environmentally stable periods. Similarly, the present study recorded comparatively high Shannon and Simpson diversity during March and December, whereas dominance was highest during June. Evenness was also relatively high during March and September, indicating a comparatively balanced distribution of taxa during these

periods. The lower diversity and comparatively higher dominance observed during some months may reflect changes in the relative contribution of individual taxa to the community. These observations suggest that seasonal changes in the river environment were associated with temporal shifts in Bacillariophyta diversity and community structure. However, because no inferential statistical test was applied, these patterns are interpreted as ecological associations rather than statistically significant differences.

The relationship between diatom communities and environmental conditions was further examined through the measured physicochemical variables and PCA. Similar findings have been reported by [20], who demonstrated that variations in water chemistry were associated with changes in freshwater micro Algal communities, particularly in environmentally stressed ecosystems. Likewise, [21] reported that temperature, electrical conductivity, pH, nutrient availability, and hydrological conditions were important environmental variables associated with Algal diversity and distribution in tropical freshwater ecosystems. In the present investigation, seasonal variation in temperature, pH, electrical conductivity, turbidity, TDS, alkalinity, nutrients, and other measured water-quality parameters corresponded with changes in Bacillariophyta composition and diversity. The PCA further indicated multivariate associations between the diatom assemblages and physicochemical variables, with the first two principal components accounting for 42.53% of the total variation. Overall, the findings indicate that seasonal hydrological processes and water-quality characteristics were associated with variation in the composition and ecological dynamics of Bacillariophyta communities in the Dor River. These results highlight the value of continued diatom and water-quality monitoring for understanding temporal and spatial changes in freshwater ecosystems.

5.1. Conclusions and Recommendations

This study documented the diversity and seasonal distribution of Bacillariophyta in the Dor River from 2021 to 2022, with 46 taxa recorded during the study period. The families Bacillariaceae, Gomphonemataceae, and Naviculaceae showed comparatively high species richness, while Naviculales represented the most species-rich order. Seasonal variation in Algal abundance, frequency, and community composition was evident, with relatively high values observed during winter and pre-monsoon months, particularly December, February, and March, whereas lower diversity was generally observed during the mid-summer period. These temporal patterns indicate that seasonal changes in physicochemical conditions and hydrological characteristics were associated with changes in the structure of the Bacillariophyta community.

Based on density, frequency, and Importance Value Index (IVI), *Navicula laterostrata* and *Fragilaria tenera* were among the prominent taxa recorded during the study period. Their occurrence across multiple sampling periods suggests that these taxa may have ecological significance within the studied river system. However, their presence should not be interpreted as definitive evidence of specific pollution levels or water-quality status without the application of established diatom-based water-quality indices and additional pollution-related measurements.

The diversity indices demonstrated clear temporal changes in community structure. December recorded the highest observed richness, with 36 taxa, together with comparatively high values of Shannon's diversity, Margalef richness, Fisher's alpha, and Chao-1 estimates. High diversity and evenness during March and April suggested a relatively balanced community during these periods. In contrast, June showed comparatively lower diversity, lower evenness, and higher dominance, indicating a less evenly distributed community that may be associated with seasonal environmental variation and the increased dominance of a limited number of taxa. These patterns demonstrate the dynamic nature of the Algal community in response to seasonal changes and associated environmental conditions.

Principal component analysis further demonstrated temporal variation in the physicochemical characteristics of the river and their association with changes in Algal community structure. The observed patterns may reflect the combined influence of natural seasonal processes, hydrological variability, resource availability, and possible anthropogenic influence. However, because established diatom-based water-quality indices and several direct pollution indicators were not included in the present study, the observed Algal patterns cannot be used alone to confirm pollution status, quantify pollution levels, or establish direct causal relationships between human activities and changes in water quality.

Overall, the study provides baseline information on the diversity, seasonal distribution, and community structure of Bacillariophyta in the Dor River. The findings emphasize the ecological value of continued monitoring of Algal communities and physicochemical conditions as part of freshwater ecosystem assessment. Future investigations should incorporate validated diatom-based water-quality indices, additional chemical and microbiological pollution indicators, and more detailed spatial and statistical analyses to strengthen assessment of water-quality conditions and potential anthropogenic influences in the river.

5.2. Recommendations

1. **Regular monitoring:** Seasonal and long-term monitoring of Bacillariophyta and physicochemical parameters should be continued to identify changes in Algal community structure and environmental conditions.
2. **Expanded water-quality assessment:** Future studies should include established diatom-based water-quality indices together with additional parameters such as nutrients, organic pollutants, heavy metals, microbial indicators, and other relevant pollution-related variables where appropriate.
3. **Improved spatial assessment:** Sampling should be expanded to include additional upstream, midstream, and downstream locations to better characterize spatial variation in Algal communities and environmental conditions.
4. **Integrated statistical analysis:** Future research should combine diversity indices, multivariate statistical approaches, and spatial analyses to better evaluate relationships between Algal assemblages and environmental variables.
5. **Anthropogenic influence:** Areas affected by domestic, agricultural, and other human activities should be monitored carefully; however, their effects on Algal communities should be evaluated using appropriate environmental measurements rather than inferred solely from Algal composition.
6. **Future indicator studies:** The recurrent occurrence and abundance of taxa such as *Navicula laterostrata* and *Fragilaria tenera* warrant further investigation to determine their potential usefulness as ecological indicators under locally validated environmental conditions.
7. **Conservation and management:** The baseline information generated by this study may support future freshwater monitoring and management programs aimed at maintaining ecological conditions and protecting the biodiversity of the Dor River.

Author Contributions

Z.A.: Original draft writing, formal analysis, methodology, and conceptualization; G.M.S.: Writing, reviewing, editing, and supervision. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

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Informed Consent Statement

Not applicable. This study did not involve human participants.

Data Availability Statement

All data generated or analyzed during this study are included in this published manuscript and its supplementary information files.

Conflicts of Interest

The authors declare no competing interests.

Use of AI and AI-Assisted Technologies

During the preparation of this work, the authors used ChatGPT (OpenAI) to assist with language editing, improving manuscript clarity, and refining responses to reviewers. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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