Seasonal Dynamics of Phytoplankton Productivity and Molecular Diversity in the Kuwano River, Basti, Uttar Pradesh, India

Abstract: ***This research looks at how the seasonal changes influence the phytoplankton population and primary productivity in the Kuwano River, Basti, Uttar Pradesh, on the basis of the changes in the physicochemical characteristics across the various sampling sites (2024-2025). Water samples were taken in five locations in the summer, rainy, winter and spring seasons. (2024-2025). Density of phytoplankton, chlorophyll a and primary productivity were measured together with physicochemical parameters. The statistical tests used were ANOVA, Pearson correlation, the Wilcoxon rank sum test, and CCA. Molecular diversity was evaluated by isolation and amplification of 16S rRNA and 18S rRNA,followed byphylogenetic analysis of these gene sequences. The findings showed that phytoplankton diversity and productivity had significant seasonal changes which were strongly correlated with temperature ($10 - 46$ ℃), nutrient concentrations (PO_4^{3-},NO_3^-,NO_2^-,NH_3), and dissolved oxygen ($2.5 - 10.2\ mg/L$) concentration. Bacillariophyceae (9 genera) and Chlorophyceae (7 genera) turned out to be prevalent groups in nutrient-enriched conditions, whereas hydrological variability was an important contributor to community assemblage organization. Additionally, the phylogeny of phytoplankton species Anabaena cylindrica and Fritschiella tuberosa were determined by MEGA11 software. The results show that nutrient inputs and hydrological activities have a strong impact on the dynamics of phytoplankton and these results present crucial information to the ecological health and management of riverine ecosystems.***

Key Words: Canonical correspondence analysis (CCA),Molecularphylogeny,Kuwano River, Seasonal variation,Chlorophyll-*a*

1. Introduction

Phytoplankton are primary producers and are very important components of aquatic ecosystems. They convert solar radiation into organic biomass through photosynthetic reactions thus supporting biogeochemical cycles and food webs [1], [2]. The seasonal variation is not only in tropical but also in subtropical rivers and wetlands may occurs due to monsoonal cycles and the anthropogenic addition of nutrients and fluctuations in hydrology [3], [4]. Such changes can be linked to environmental changes in terms of temperature, nutrient levels, and dissolved oxygen levels. They, therefore, are major contributors to the dynamics of phytoplankton growth, together with community composition[5], [6]. The increased availability of organic nutrients, especially nitrogen, phosphorus, results in eutrophication thus providing favourable environments to opportunistic species such as the *Oscillatoria* and *Microcystis* which have the potential of imbalancing the ecosystem [7], [8].

Seasonal changes of the structure of phytoplankton communities and biomass abundance are often based on physical and chemical factors. These variations have dry and wet periods that are alternating in tropical and subtropical areas and, hence, affect stability in water flows, as well as nutrient loading[9], [10]. Increased phytoplankton diversity is linked to the influx of nutrients and hydrological mixing during the rainy season and the high phytoplankton biomass is associated with visible water clarity and increased mineral retention during summer periods [11], [12]. Phytoplankton dynamics of estuarine and freshwater systems have been explored at a global scale. As an example, in the Sundarbans (India), seasonal changes in temperature, pH, and nutrient supply produce a substantial effect on phytoplankton productivity, modifying the domination of the seasonal succession of the species of the genus Bacillariophyceae and Cyanophyceae between summer and spring[3].Similar studies in Algeria and Indonesia have emphasized on phytoplankton productivity through nutrient enrichment and thermal regimes thereby supporting algal blooms in favourable conditions [2], [6].

The overall phytoplankton growth is regulated by a complex of biotic and abiotic factors, such as nutrient levels, hydrological situation, and fluctuations of climate [13]. The nutrient enrichment is often the main factor that causes a drastic change in the community structure, leading to the occurrence of algal blooms with cyanobacteria [14].However, hydrological characteristics, including discharge, retention time, and water level changes, also have a strong impact, sometimes more important than those of nutrients [15], [16]. Also, the changes in land use and the seasonal rainfall distribution that alters the amount of pollutants and light can induce the change in the makeup of phytoplankton communities[17], [18].

The phytoplankton biomass and its presence and abundance directly influence the water quality making them ecologically significant. The danger to the abundance of phytoplankton is the increase in temperature, change in the precipitation pattern, and ocean acidification[2]. These threats are worsened by anthropogenic sources of pollution like agricultural runoff and industrial effluents, which eventually result in extreme algal blooms and hypoxia[19], [20]. Such differences have the subsequent impact on phytoplankton growth structure and seasonality and affect the larger aquatic habitat [21].

Sustainability of aquatic biodiversity along with water quality is determined by a thorough understanding of association, that exists between the phytoplankton population and the habitat [5]. In this respect, the studies were carried out on the seasonal alterations in phytoplankton dynamics and productiveness and their relation to the physicochemical parameters during the

different seasons of Kuwano River. The research elucidates on the drivers of the environment underlying in the season and measures the ecological condition of the river. Besides that, the phylogenetic tree that was created using isolated algal strains *Anabaena sp*. and *Fritschiella sp.* gave a molecular validation of the taxonomic classification and evolutionary ties of their groups at the regional blue-green and green algal clades.

2. Materials and methods

2.1 Study Area

The Kuwano river has its origin fromthe Bahraich district;after making a valuable contribution in some districts, it serves as a major water resource in the southern part of the Basti district, Uttar Pradesh, India.It has cultural and religious significance. It provides a habitat for aquatic life and the food chain, contributing to groundwater recharge and supporting irrigation, fishing, aquaculture, and tourism. Presently, the river is facing many challenges, like pollution, untreated waste, water hyacinth (*Eicchornia crassipes*), which causes fatal die-offs in aquatic life, illegal fishing, silting, and loss of flow.

2.2 Sampling Procedure

The study emphasised five different sampling points along the Kuwano River: Shivaghat (S1, 26.931009N, 82.62934E), Atara (S2, 26.878605N, 82.684903E), Amhat (S3, 26.782366N, 82.715375E), Mahson (S4, 26.716965N, 82.774532E), and Lalganj (S5, 26.657102N, 82.822267E), covering a total of 55 km in the Basti District. Each site was around 10 km apart, with subsites located 22 m apart (Fig. 1). The plastic containers, after washing with diluted HCl and deionised water, were used to collect water samples at each location during the summer (May), rainy (October), winter (February), and spring (March) seasons from 2024 to 2025.

Seasonal sampling of phytoplankton was performed from 7:00 to 9:00 AM using a 25 µm mesh phytoplankton net in accordance with established protocols[22],[23].

Phytoplankton primary productivity was assessed using the light and dark water method[22]–[24]. Three hundred millilitre BOD bottles [I (initial), L (light), and D (dark)] were incubated in situ for a duration of 3 to 4 hours. Furthermore, the DO was measured by the Winkler titration method [22].

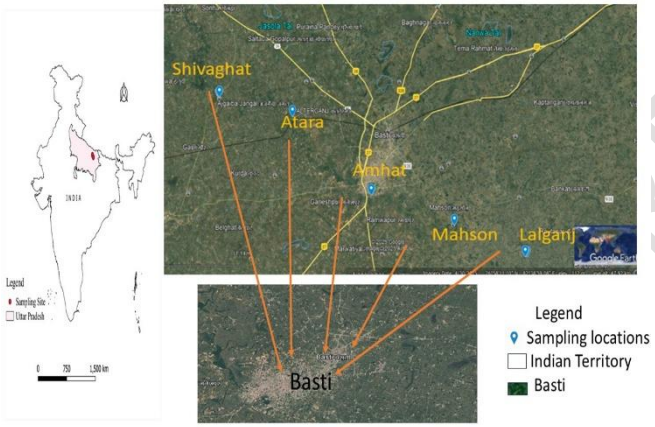

Figure 1: Five sampling sites location map of the Kuwano River, Basti, U.P.

2.3 Physicochemical Parameters

The physicochemical analysis of river water (Table 1) was done through standard analysis [22]. and total nitrogen was estimated by a digestion, distillation, and titration process [25].

2.4 Productivity Measurement

The productivity parameters were computed as

Net primary productivity (NPP) $= \mathrm{DO(L)} - \mathrm{DO(I)}$

Respiration (R) $= \mathrm{DO(I)} - \mathrm{DO(D)}$

Gross primary productivity $\mathrm{GPP} = \mathrm{NPP} + \mathrm{R}$

To estimate the density of phytoplankton, 1 L of water were first taken and preserved. The preservation of the water sample required 2% Lugol's iodine. All samples were allowed to precipitate for a period of 24 to 48 hours and concentrated to a volume of 1 mL. Cell density was measured by counting using a Neubauer chamber hemocytometer under a microscopic magnification of 40x. The cell count in 1 mL was then converted to a count per litre (1 L) using the formula given below. Finally, the entire count of phytoplankton was analysed in 1-liter water sample. [21] – [26].

$$\text{Phytoplankton density (Cells/L)} = \frac{n \times v \times 1000}{V}$$

here,

$n =$ average number of phytoplankton cells of the phytoplankton sample,

$v =$ volume of phytoplankton concentrates (mL),

$V =$ volume of total water filtered (L) [27].

Taxonomic identification was done using standard keys [26]– [28]. To determine the pigment contents (chlorophyll *a*), a vacuum filtration unit using MF-Millipore filter paper (0.22 µm) was used to filter 1 litre sample of water, which was then transferred into a microcentrifuge tube (1.5 ml) having 99.9% methanol sonicated with an ultrasonicator probe (Sonics, Vibra-Cell™) for 10-12 seconds and kept further for 24 hours at 4°C to acclimate. To determine the concentration, the difference in absorbance between 665.2 nm and 652.4 nm

Table 1: Analysis of Physicochemical Parameters for the Kuwano River

S. No	Parameters	Symbol	Analytical Method	Instrument/apparatus
1	Temperature	Temp.	-	Thermometer (HM Digital AP-2)
2	Light Intensity	LI	-	Lux meter (Lumens Gauge tester Lux)
3	Electrical Conductivity	EC	-	EC meter (HM Digital AP-2)
4	Total dissolved solids	TDS	-	TDS meter (HM Digital AP-2)
5	Total suspended solids	TSS	-	Filtration unit
6	pH	pH	-	pH meter (HM Digital AP-2)
7	Ammonia	NH_3	Calorimetric method	Aimil Spectrochem NV
8	Nitrite	NO_2^-	Sulphanilic acid method	Aimil Spectrochem NV
9	Nitrate	NO_3^-	Brucin method	Aimil Spectrochem NV
10	Total Nitrogen	TN	Kjeldahl method	(Gerhardt Analytical System, Germany)
10	Phosphate	PO_4^{3-}	Ascorbic acid method	Aimil Spectrochem NV
11	Calcium	Ca	EDTA Titrimetric method	-
12	Magnesium	Mg	Calculation method	-
13	Iron	Fe	Phenanthroline method	Aimil Spectrochem NV
14	Dissolved Oxygen	DO	Azide modification	-
15	Biological Oxygen Demand	BOD	5-day BOD Test	-
16	Total Hardness	TH	EDTA Titrimetric method	-
17	Phytoplankton Density	PD	Neubauer chamber Haemocytometer	Olympus Microscope
18	Phytoplankton Productivity	PP	Light and dark bottle method	-
19	Chlorophyll a	Chl a	Spectrophotometric method	Remi Spectrophotometer

using the UV-VIS spectrophotometric method was used, formula. [29] was used for further calculation.

$$\text{Chlorophyll}\, a = 16.72A665.2 - 9.16A652.4$$

where$A = \text{Absorbance}$.

The data for temperature (minimum and maximum) along with rainfall for 2024-2025 was gathered from the Indian Meteorological Department (IMD).

2.5 Algal Isolation

In the laboratory, the concentrated sample filtered with a 25 µm plankton net was inoculated onto solidified 0.4% Agar BG-11 medium and incubated under regulatedirradiance (16:8 h light/dark cycle, 4000 lux, and28 ± 2 ℃). After 4-5 days, visibly

distinct algal colonies were picked using sterile inoculation loops and transferred to fresh BG-11 liquid medium for purification through serial dilution and repeated streaking until unialgal cultures were obtained [22].

2.6 Genomic DNA Extraction and PCR amplification

For molecular identification, a standard CTAB method was being utilized to extract total genomic DNA from fresh algal biomass [30]. Further,NanoDrop spectroscopy (Thermo Fisher Scientific) and agarose gel electrophoresis techniques were employed to determinethe DNA concentration and assess its quality[31]. The 16S rRNA and 18S rRNA genes had been amplified using universal (27F/1492R) and microalgal-specific primers.The Polymerase Chain Reaction (PCR) required a thermal cycler (Thermo Fisher Scientific). The preparation of a reaction volume of 50 μL and thesubsequent amplification procedure for PCR were taken into consideration as mentioned [30]−[32]. Agarose gel (1.5%), after staining with ethidium bromide, was used to visualise products of PCR. Positive amplicons were purified for bidirectional sequencing at Biokart lab [30].

2.7 Sequence Analysis

A PCR purification kit (Qiagen, Germany) was used for the separation of PCR positive products following the guidelines of the manufacturer.The bi-directional Sanger sequencing method, using the same primer pair (27F/1492R), had been done for purified amplicons.The sequencing reaction of 10 μL and subsequent amplification were optimized [33]. The ethanol precipitation was used for purification of sequencing reactions and analysed using an ABI 3130 DNA Analyzer (Applied Biosystems, USA). Raw sequences were assembled and edited using MEGA 11 (Molecular Evolutionary Genetics Analysis) software. Algal species identification was carried out by comparing the consensus sequence with reference sequences obtained from the NCBI GenBank database through BLAST. The neighbor-joining method was used to construct the phylogenetic tree to assess the evolutionary relationship between isolated strains [34].

2.8 Statistical Analysis

Each experiment was performed in triplicate, and the obtained data were expressed in mean ± standard deviation. Forrecognising significant differences between biological parameters like phytoplankton productivity, phytoplankton density, chlorophyll *a*, and physicochemical parameters like total nitrogen, phosphorus, pH, water temperature, nitrite, ammonia, nitrate, calcium, magnesium, iron, DO, light intensity, air temperature, and rainfall, single factor analysis of variance was conducted for all data sets (ANOVA).A Wilcoxon rank sum test was conducted to compare the difference in physiochemical characteristics and phytoplankton productivity in the two seasons of sampling. Statistical analyses were performed with the help of Statistical Package (SPSS 24.0, IBM Inc., Chicago, Illinois, USA). The most significant physicochemical variables which influence the phytoplankton diversity were explored using canonical correspondence analysis (CCA) and cluster analysis (PAST 3.06 software). To measure the level of relationships between different parameters, the correlation analysis of Pearson was performed using Excel. The phylogenetic tree was generated with MEGA 11, and the evolutionary relationship was determined.

3. Results and Discussions

3.1 Physicochemical Parameters

The analysis of the physicochemical variables (Table 2) demonstrated that a number of parameters were significantly varying seasonally ($p < 0.02 - 0.05$), as the Wilcoxon test identified (Fig. 2a). The phosphorus level ranged between (4.99 ± 0.91 mg/L) in spring and (1.70 ± 0.11 mg/L) in the rainfall period (Fig. 2b), and there was a significant seasonal difference as it was due to organic and inorganic waste and agricultural run-off. The same experience was gained in the Meenachil River, Kerala[35], as well as in the Ramsar lake in Kerala [36]. The overall levels of nitrogen (TN) were found to be seasonal; the greatest level had been recorded in the spring season where the levels were followed by summer, winter, and rainy seasons (Fig. 2c). This trend is an indication of the combined hydrological and anthropogenic forces acting on the fluctuation of nitrogen. The same tendency was indicated by the Montane River, Western Ghat [37]. The amount of calcium was more during winter due to abiotic precipitation and low temperature, whereas the amount of calcium was low during summer due to high temperatures, which reduced calcium and augmented the biological activity (Fig. 2c).

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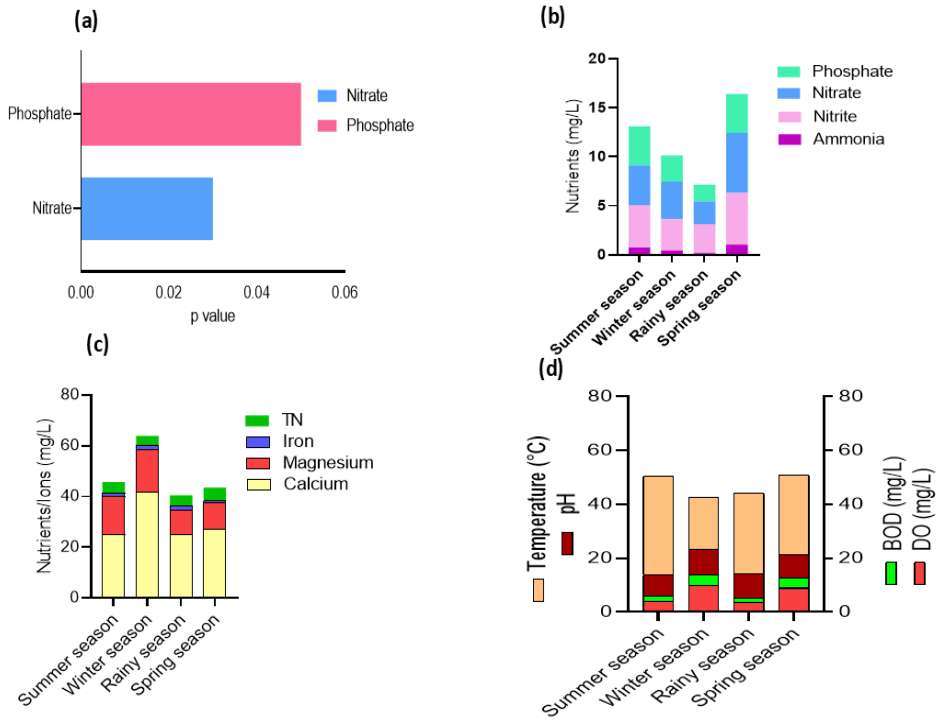

Figure 2:(a) P-value distribution of the phytoplankton productivity (PP), using the nitrate (NO^{3-}) and phosphate (PO_4^{3-}). The differences in all variables were statistically significant ($p < 0.05$) meaning that all of them have a strong influence on the dynamics of phytoplankton. (b) Seasonal variation in nutrients like phosphate, nitrate, nitrite and ammonia. (c) Seasonal variation in total nitrogen (TN), iron, magnesium, and calcium. (d) Seasonal variation in physicochemical parameters like temperature, pH, biological oxygen demand (BOD) and dissolved oxygen (DO)

Table 2: Seasonal change of physicochemical parameters, nutrients, ions, irradiance, phytoplankton productivity (PP), and chlorophyll a of the various sampling sites (S1-S5) of the Kuwano River. Where, [a] ($p < 0.02$) and [b] ($p < 0.05$) are statistically significant, and [c] ($p < 0.07$).

Environmental variables	Seasons	Maximum (Mean)	Minimum (Mean)	Mean ± S. D
pH	Summer	8.6 (S1)	8.0 (S1)	7.97 ±0.36[b]
	Winter	9.7 (S1)	9.0 (S5)	9.65 ±0.52[a]
	Rainy	8.9 (S5)	8.2 (S2)	8.70± 0.45[a]
	Spring	8.3 (S5)	7.5 (S3)	8.55 ± 0.60[b]
Water temperature (°C)	Summer	46 (S5)	36 (S1)	36.57 ± 0.36[b]
	Winter	19 (S1)	10 (S1)	19.31 ± 1.51[b]
	Rainy	31 (S3)	28.1 (S5)	30.16 ± 1.149[b]
	Spring	29.3 (S3)	28.1 (S2)	29.64 ± 6.3[a]
DO (mg/L)	Summer	7.35 (S2)	2.5 (S5)	04.55 ± 1.58[a]
	Winter	10.22 (S2)	8.06 (S3)	09.69 ± 0.822[a]
	Rainy	4.07 (S4)	3.20 (S3)	03.60 ± 0.27[a]
	Spring	10.22 (S1)	7.22 (S4)	08.92 ± 1.06[a]
BOD (mg/L)	Summer	1.9 (S5)	1.2 (S2)	01.71 ± 0.27[a]
	Winter	4.8 (S1)	3.4 (S3)	03.95 ± 0.31[b]
	Rainy	1.8 (S2)	1.3 (S3)	01.64 ± 0.18[b]
	Spring	4.6 (S4)	2.9 (S2)	03.65 ± 0.61[a]
TSS (mg/L)	Summer	1.38 (S1)	0.18 (S3)	00.63 ± 0.55[a]
	Winter	2.14 (S5)	1.22 (S3)	01.61 ± 0.32[a]
	Rainy	0.66 (S1)	0.11 (S4)	00.24 ± 0.21[a]
	Spring	2.32 (S3)	1.43 (S5)	01.91 ± 0.34[a]
NH_3 (mg/L)	Summer	0.9 (S2)	0.6 (S5)	00.74 ± 0.10[a]
	Winter	0.5 (S1)	0.3(S4)	00.44 ± 0.08[a]
	Rainy	0.3 (S3)	0.1 (S2)	00.18 ± 0.07[a]
	Spring	1.3 (S5)	0.8 (S3)	01.04 ± 0.185[a]
NO_2^- (mg/L)	Summer	4.6 (S1)	4.2 (S3)	04.32 ± 0.15[a]
	Winter	3.7 (S5)	3.1 (S4)	03.22 ± 0.22[b]
	Rainy	3.2 (S3)	2.7 (S5)	02.94 ± 0.23[a]

	Spring	5.6 (S4)	5.1 (S3)	05.28 ± 0.17[a]
NO_3^-	Summer	4.6 (S3)	4.0 (S2)	04.06 ± 0.10[a]
(mg/L)	Winter	4.1 (S3)	3.4 (S2)	03.79 ± 0.15[a]
	Rainy	3.3 (S2)	2.1 (S1)	02.32 ± 0.172[a]
	Spring	6.4 (S3)	5.9 (S5)	06.11 ± 0.16[a]
TN	Summer	4.59 (S2)	4.31 (S3)	04.32 ± 0.12[a]
(mg/L)	Winter	3.80 (S1)	2.90 (S5)	03.90 ± 0.19[b]
	Rainy	4.10 (S1)	3.20 (S3)	04.00 ± 0.18[a]
	Spring	5.3 (S5)	4.77 (S4)	04.80 ± 0.22[a]
PO_4^{3-}	Summer	4.44 (S5)	3.2 (S2)	04.94 ± 0.45[a]
(mg/L)	Winter	2.9 (S1)	2.4 (S5)	02.68 ± 0.172[b]
	Rainy	1.9 (S1)	1.6 (S5)	01.70 ± 0.112[a]
	Spring	4.99 (S5)	2.24 S4	04.99 ± 0.91[a]
Fe	Summer	1.26 (S4)	1.23 (S1)	01.23 ± 0.10[a]
(mg/L)	Winter	1.71 (S3)	1.60 (S5)	01.68 ± 0.11[a]
	Rainy	1.68 (S1)	1.51 (S4)	01.68 ± 0.11[b]
	Spring	0.59 (S2)	0.49 (S5)	00.45 ± 0.10[a]
Ca	Summer	27.66 (S1)	22.32 (S3)	25.12 ± 2.25[a]
(mg/L)	Winter	50.91 (S5)	34.66 (S2)	41.76 ± 5.36[a]
	Rainy	25.85(S3)	23.09 (S4)	24.88 ± 0.94[b]
	Spring	34.35 (S3)	19.04 (S4)	41.76 ± 5.36[a]
Mg	Summer	16.78 (S1)	13.16 (S4)	15.02 ± 1.44[a]
(mg/L)	Winter	20.36 (S5)	13.96 (S2)	16.72 ± 2.11[a]
	Rainy	11.32 (S5)	9.20 (S3)	09.87 ± 0.26[b]
	Spring	13.74 (S3)	7.61 (S4)	10.79 ± 2.17[b]
Irradiance	Summer	69000 (S5)	50000 (S1)	61600.0 ± 7088.02[c]
(lux)	Winter	38000 (S4)	25000 (S1)	31000.0 ± 4242.64[c]
	Rainy	42000 (S1)	32000 (S4)	36400.0 ± 3382[c]
	Spring	56000 (S5)	44000 (S2)	52200.0 ±1166.19[c]
EC	Summer	476 (S5)	373 (S3)	417.81 ± 30.51[a]
(µS/cm)	Winter	424 (S3)	309 (S2)	357.98 ± 41.10[a]
	Rainy	447 (S5)	228 (S3)	371.81 ± 77.40[b]
	Spring	597 (S4)	369 (S2)	461.25 ± 75.58[a]
TH	Summer	112.4 (S5)	82.9 (S2)	94.57 ± 10.26[c]
(mg/L)	Winter	72.9 (S3)	61.8 (S1)	66.99 ± 4.28[b]
	Rainy	92.1 (S1)	64.9 (S5)	79.06 ± 9.71[a]
	Spring	85.9 (S4)	54.9 (S2)	67.4 ± 10.13[a]
PP	Summer	5.1 (S2)	4.2 (S4)	4.60 ± 0.36[a]
gc/m^3/Day	Winter	2.1 (S2)	1.2 (S1)	1.76 ± 0.30[a]
	Rainy	2.3 (S3)	1.1 (S5)	1.72 ± 0.40[b]
	Spring	5.9 (S2)	4.7 (S4)	5.06 ± 0.44[b]
Chl a	Summer	0.99 (S2)	0.91 (S1)	0.93 ± 0.03[a]
µg/L	Winter	0.69 (S2)	0.42 (S4)	0.6 ± 0.11[b]
	Rainy	0.42 (S1)	0.33 (S4)	0.38 ± 0.03[a]
	Spring	0.99 (S2)	0.97 (S5)	0.97 ± 0.01[b]

of free CO_2, as well as the respiration of organisms at high temperatures, 7.97 ± 0.36 ℃ and 9.65 ± 0.52 ℃respectively (Fig. 2d). The pH level is also indicated to vary based on the variation in the water level [39]. The higher pH was observed in the hot season in Lake Mboandong, Cameroon. This growth may be due to the fact that there is a lot of microalgal biomass that carries out photosynthesis, that lowers inorganic carbon levels in the water leading to elevated levels of pH by draining alkaline reserves [11]. The winter season showed higher dissolved oxygen as a result of ideal water temperature which enhances oxygen solubility and the rainy season leads to more dilution, which [40]. reported in the Danube River. Electrical conductivity showed significant differences in the river stations that were considered. It was also 461.25 ± 75.58 µS/cmduring spring season and 357.98 ± 41.10 µS/cm during winter season (Fig. 3a). The peak of salinity and an accumulation of salts, organic, and inorganic material in the river in summer represent the reason to consider high conductivity, and the lowering of the value in the river due to the dilution effect of the precipitation [41].

3.2 Phytoplankton productivity and chlorophyll

The Bacillariophyceae, including 9 genera, predominated in phytoplanktonic composition, while Chlorophyceae comprised 7 genera and Cyanophyceae included 6 genera. The mean seasonal abundance of phytoplankton peaked during the spring season at2.91×10^3 cells/L, followed closely by summer at2.86×10^3 cells/L. The rainy season recorded$1.80 \times$

10^3 cells/L, while winter had the lowest at1.40×10^3 cells/L (Fig. 3b). The constant weather of dry season supports an increase of the concentration of plankton, which then increases productivity (Fig. 3b). The drop in the abundance of planktons observed during rainy season can be primarily ascribed to the further dissolution of the important growth nutrients in the area[4],[42]−[44].

A range of studies on the structure of phytoplankton community confirm these findings and shows that there is a strong correlation between important hydrochemical variables. Similar findings were observed by [45].

in the Xuanwu Lake, China. Chlorophyll *a* was recorded to be high in spring season (0.978 ± 0.011 ug/mL), and summer season (0.93 ± 0.03 ug/mL) and lowest in the rainy season (0.3 ± 0.03 ug/mL) and lastly the winter season (0.6 ± 0.1 ug/mL) (Fig. 3d). The described tendency is closely linked to the results of the research of Bohai Sea [46].

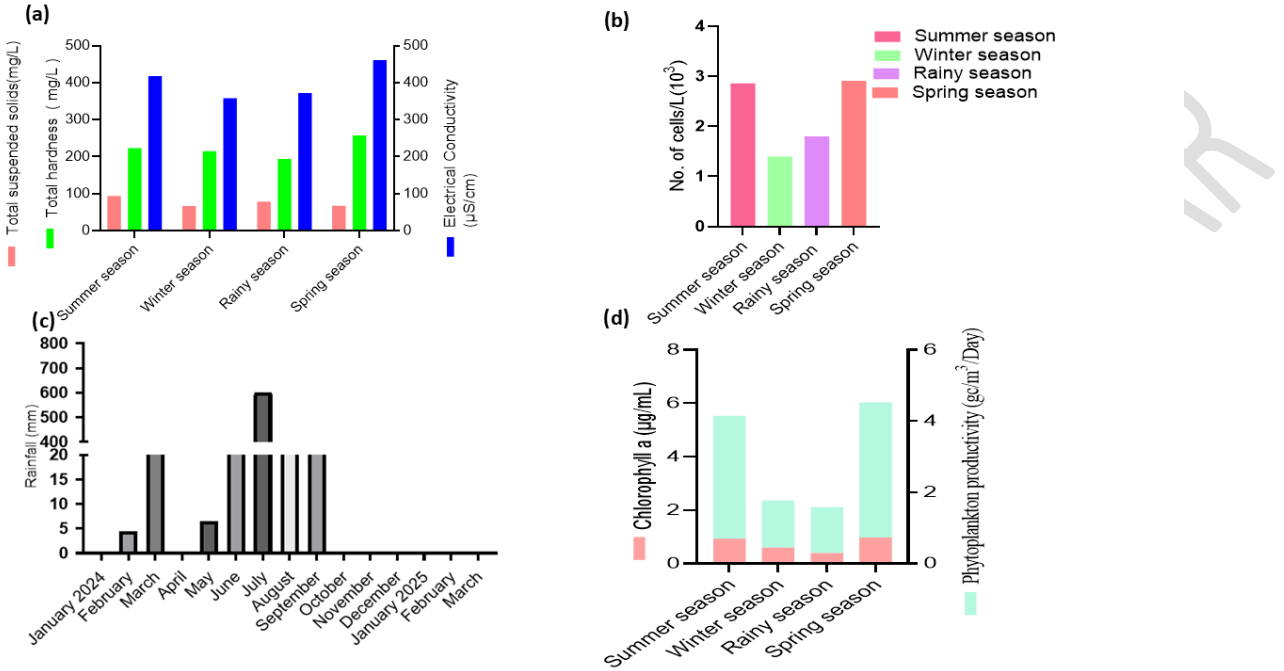

Figure 3: (a) Seasonal variation in physicochemical parameters like total hardness, total dissolved solids, and electrical conductance. (b) Seasonal variation in phytoplankton abundance (cells/L) shows maximum density during spring, followed by summer, rainy, and winter seasons. (c) Seasonal variations in rainfall pattern (January 2024 - March 2025) in Basti district (U. P.). (d) Seasonal variations in chlorophyll a and phytoplankton productivity (PP)

3.3 Phylogenetic Analysis

The phylogenetic relationships of the isolates were inferred based on partial sequence of 16S rRNA gene (Fig. 4a, 4b). The Maximum Likelihood (ML) program was used in order to test the strength of the inferred branching pattern with 1000 bootstrap replicates. The phylogenetic tree (dendrogram) made using 16S rRNA sequences showed that investigated isolates were close relatives to *Stigeoclonium sp.* (HF920645.1, HF920647.1, HF920646.1) and *Caespitella pascheri* (MW678822.1, FN824386.1). The bootstrap values of 54 to 99 ensured the stability of the major clades. A distinct clade containing *Fritschiella tuberosa* (MN428041.1, U83129.2) indicated evolutionary divergence within the Chaetophoraceae lineage. Similarly, the 16S rRNA phylogenetic tree of cyanobacterial isolates (Fig.4b) demonstrated that the strains formed a well-supported monophyletic group with *Anabaena cylindrica* (AP018166.1, CP186034.1) and *Anabaena* sp. PCC 7938 (CP186034.1). High bootstrap values (76 - 100) confirmed the clustering of the studied isolates with reference strains of *Anabaena* and *Hydrocoryne* sp. (KC346266.1, P847267.2). The results are aligned with the earlier studies emphasizing the phylogenetic placement of *Anabaena* sp. within Nostocales based on ribosomal gene sequences [47].

The results also suggested that both green algal isolates (*Fritschiella*sp.) and cyanobacterial strains *(Anabaena cylindrica* complex) retain strong evolutionary linkage with their respective reference taxa, thereby validating the molecular identification. Phylogenetic resolution obtained using rRNA gene markers proved reliable for taxonomic classification at the genus and species levels, in line with recent reports [48].

3.4 Karl Pearson Correlation of physicochemical and biological parameter

Temperature exhibited a pronounced negative correlation with pH ($r = -0.78$) and dissolved oxygen (DO) ($r = -0.71$); same trend was found in the Geum River, Korea [49].

However, it showed a convinced correlation with light intensity ($r = 0.70$) and total hardness ($r = 0.69$). A pronounced correlation has been shown by DO with BOD ($r = 0.91$) and SPM ($r = 0.81$). A significant positive correlation was shown between phytoplankton productivity and light intensity (LI) ($r = 0.84$), ammonia ($r = 0.84$), nitrite ($r = 0.91$), phosphate ($r = 0.80$), and plankton density ($r = 0.94$) (Fig. 5). The significant indicator of algal biomass, Chlorophyll-*a*, exhibited a robust correlation with ammonia ($r = 0.89$), nitrite ($r = 0.89$), nitrate ($r = 0.83$), and phosphate ($r = 0.85$), in agreement with the findings reported in the Geum River research carried out in Korea [49]. In the study phytoplankton density expressed high correlation with light intensity ($r = 0.81$), nitrite ($r = 0.90$), phosphate ($r = 0.81$), and chlorophyll a ($r = 0.94$). Iron showed significantly adverse correlations with biological parameters, including chlorophyll *a* ($r = -0.81$), phytoplankton

density ($r = -0.78$), and phytoplankton productivity ($r = -0.83$). Such trends were also reported from the Tiruchendur coastal in Gulf of Mannar [50].

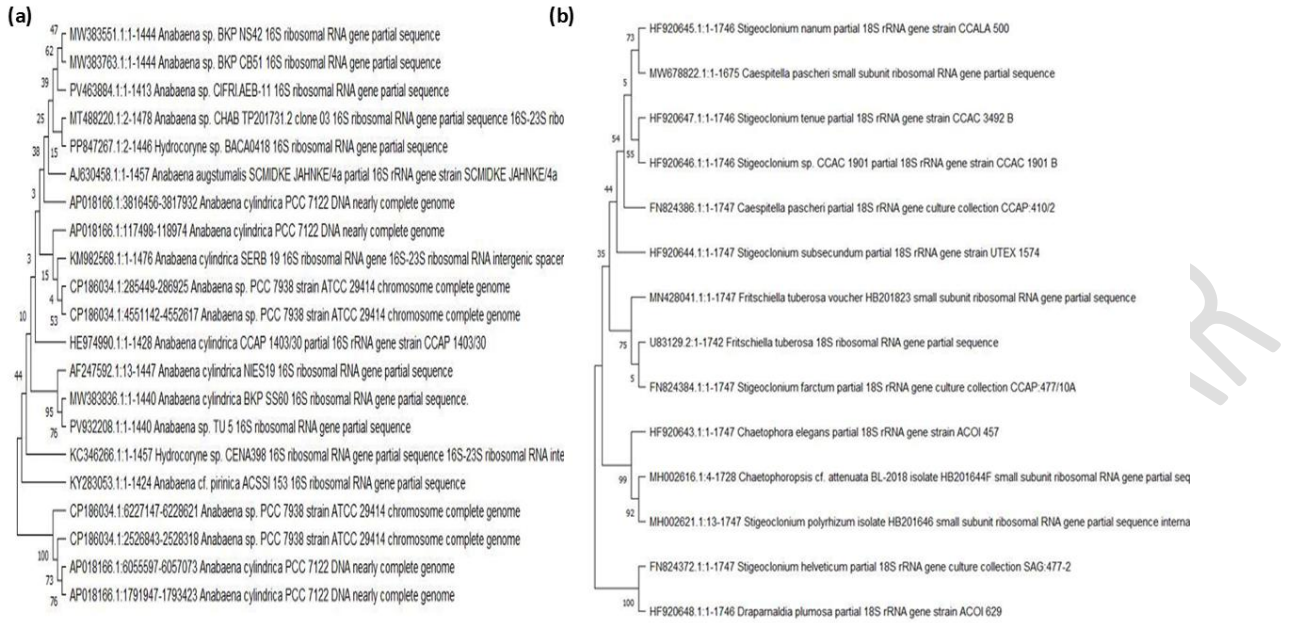

Figure 4: Phylogeny tree of the microalgal isolates (a) *Anabaena cylindrica* and (b) *Fritschiella tuberosa*, created by Maximum Likelihood (bootstrap of 1000) in the MEGA11 software

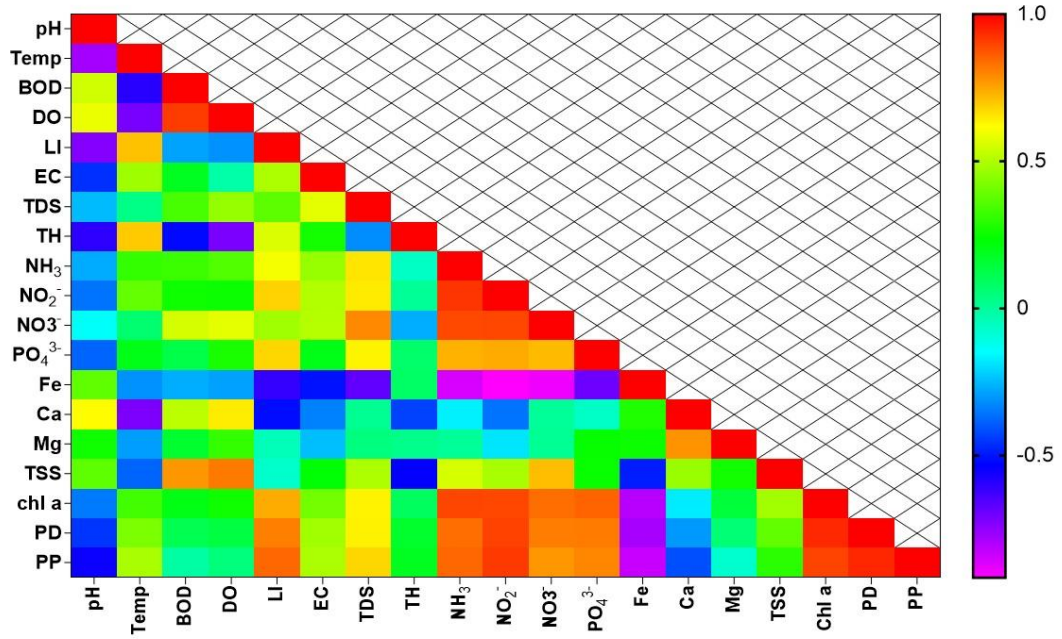

Figure 5: Heatmap showing the correlation matrix among physicochemical parameters and biological parameters of the Kuwano River

3.5 Canonical Correspondence Analysis (CCA)

The clustering of data near the temperature throughout summer indicated that elevated temperatures positively influenced primary productivity and chlorophyll *a*, signifying enhanced phytoplankton productivity. TDS and THwere increased during the rainy season by runoff-driven nutrient and metal input, as indicated by ionic parameters(Fig. 6). Winter had higher alkalinity and mineral content under low-temperature, stable-flow circumstances and was highly correlated with pH, Ca, Mg, and EC.During the spring, nutrient loads such asPO_4^{3-},NH_3,DO,and BOD suggested a larger organic load and resuspension,possibly as a result of increased mixing and biological activity.Findings indicate increased productivity of chlorophyll a and phytoplankton. This dictated the need to consider seasonality as another factor in the aquatic environmental variability and proved that the temporal resolution is essential for water quality monitoring [51]−[53].

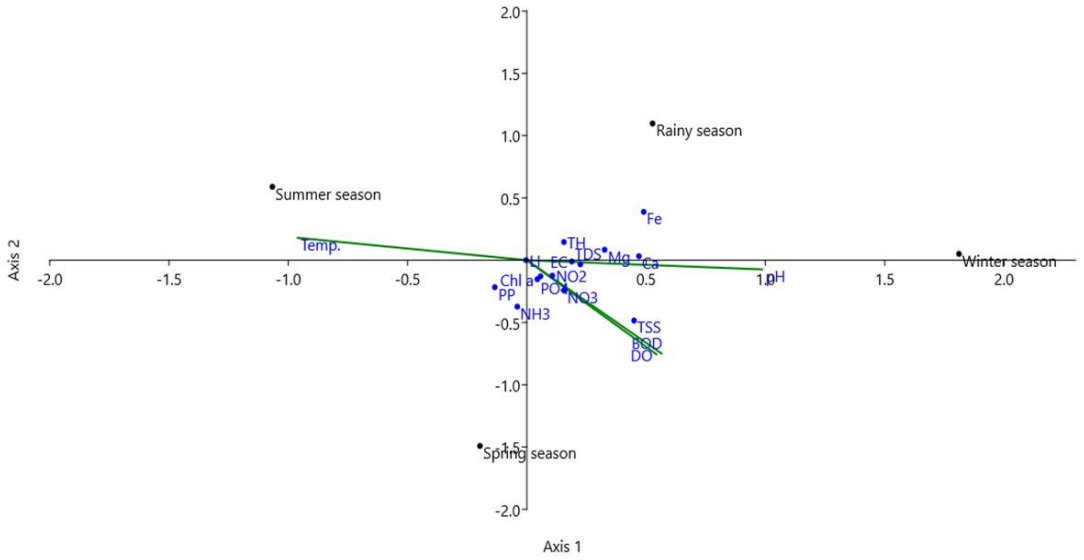

Figure 6: Canonical Correspondence Analysis (CCA) ordination diagram showing the relationship between seasonal variations (summer, rainy, winter, and spring) and physicochemical parameters with phytoplankton productivity. Arrows represent environmental variables (temperature, pH, DO, BOD, TSS, nutrients, and ions), and their direction and length indicate the strength and gradient of influence on seasonal distribution

4. Conclusion

This comprehensive research involved the study of seasonal changes in physicochemical parameters, phytoplankton abundance and productivity as well as algal molecular diversity in the Kuwano River ecosystem. Hydrological changes and anthropogenic effects of agricultural runoff and organic waste input, all demonstrated significant seasonal variation in most of the physicochemical variables ($p = 0.02$). Bacillariophyceae was the most dominant phytoplankton group then Chlorophyceae and Cyanophyceae, and the highest abundance was observed in spring. There was a rise in the chlorophyll a and phytoplankton in the spring and summer due to stable hydrology and nutrient enrichment. The existence of strong positive correlations between the phytoplankton productivity, nutrient contents (ammonia, nitrite, and phosphate) and light intensity was an indication of the crucial role of nutrient-biomass interaction to the ecosystem functioning. The molecular phylogenetic analysis revealed the presence of the algal isolates as *Fritschiella sp*., whereas the isolate of cyanobacteria was grouped with the *Anabaena sp*. Strong bootstrap support values assist the molecular identifications and also show the phylogenetic coherence of the isolates within their species. The Canonical Correspondence Analysis (CCA) showed the effect of seasonal changes on the spatiotemporal distribution of physicochemical and biological variables. This study indicates that there exists a unique relationship between freshwater environmental environment, nutrient cycles and biological productivity.

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Declarations

Authors declare no Conflicts of interest.

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