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## **Original Research Article**

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# Spatial Variation in Phytoplankton Community Structure Driven by Salinity and Nutrient Gradients in Vembanad Lake – A Ramsar Designated wetland, India

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### ABSTRACT

Keywords

Phytoplankton, Vembanad Lake, Salinity, Nutrient gradients, Wetlands

### **Article Info**

Received: 08 October 2025 Accepted: 22 November 2025 Available Online: 10 December 2025 The Vembanad Lake, a significant Ramsar wetland, faces ecological challenges due to dynamic physicochemical conditions and anthropogenic nutrient loading. This study investigated the influence of key environmental variables such as Salinity, Nitrate and Phosphate on the spatial distribution and diversity of phytoplankton across three distinct micro-habitats namely Kainakari Vattakkayal, Meenappally Kayal, and Venattukadu Kayal. Water and plankton samples were collected and community structure was analysed using diversity indices such as Shannon-Wiener H', Species Richness (S), and Pielou's Evenness (J') as well as Canonical Correspondence Analysis (CCA). The results revealed a clear environmental gradient decreasing from Kainakari Vattakkayal to Venattukadu Kayal, which directly mirrored the phytoplankton diversity. Kainakari Vattakkayal is the area with the highest salinity and nutrient concentrations, supported the highest diversity and richness, exhibiting dominance by Chlorophyceae and Cyanophyceae, taxa often associated with nutrient enrichment. Conversely, the intermediate physicochemical regime at Meenappally Kayal facilitated the highest total density of phytoplankton and was strongly dominated by Bacillariophyceae (diatoms). The CCA confirmed that Salinity, Nitrate, and Phosphate were the dominant ecological drivers, rigorously structuring the phytoplankton communities across the sites. These findings underscore the critical role of the physicochemical environment in shaping the primary producers of the Vembanad Lake, highlighting the need for targeted management strategies to mitigate the impacts of spatial variations in hydrography and nutrient inputs.

### Introduction

The Vembanad Lake, a designated Ramsar site and the largest estuarine system on the Southwest coast of India, represents an ecologically and economically critical tropical wetland (Nandan and Sajeevan, 2020; Padua and

Kripa, 2022). As a complex environment characterized by dynamic fluctuations in salinity, temperature and nutrient concentration, the ecological integrity of the lake is highly dependent on its primary producers, the phytoplankton community (Kunjukrishna Pillai *et al.*, 1975). These microscopic organisms form the base of the

aquatic food web and are remarkably sensitive to changes in water quality, making them crucial bio-indicators of estuarine health (Vidya et al., 2014). Given the proximity of the lake, to dense human settlements and agricultural activities, it is subjected to significant anthropogenic pressures, including high nutrient inputs (nitrates and phosphates) and altered hydrological regimes, which are known to dictate the species composition and density of phytoplankton populations (Jyotsna Rajeswari et al., 2018). Understanding the spatial dynamics of phytoplankton diversity and abundance in response to these changing physicochemical drivers is therefore essential for effective management and conservation of this fragile ecosystem.

While prior research has established the general ecological significance of the Cochin backwater system and analyzed seasonal variations in its phytoplankton (Dayala et al., 2014; Kunjukrishna Pillai et al., 1975), few studies have simultaneously quantified the coupled influence of salinity and specific nutrient gradients (Nitrate and Phosphate) on the fine-scale distribution of phytoplankton classes across distinct micro-habitats within the southern region of the Vembanad Lake. The current investigation addresses this gap by analysing the diversity, richness, and evenness of the phytoplankton community across three locations such as Kainakari Vattakkayal, Meenappally Kayal, and Venattukadu Kayal, which collectively represent a crucial gradient in salinity and nutrient levels (Geeja et al., 2014). Utilizing Canonical Correspondence Analysis (CCA), this study aims to explicitly determine the degree to which Salinity, Nitrate and Phosphate concentrations govern the spatial and dominance patterns clustering of phytoplankton classes, thereby providing a robust ecological framework for assessing water quality and biological stability in this vital wetland (Nandan and Sajeevan, 2020).

### **Materials and Methods**

### Study area and Sampling

The study was conducted from February 2023 to April 2024 across three distinct locations within the Vembanad Lake wetland, Kerala, India, strategically selected to represent a pronounced salinity gradient. These locations were Kainakari Vattakkayal (KV), Meenappally Kayal (MK), and Venattukadu Kayal (VK) and are marked in Fig. 1. Water samples were collected and preserved for

subsequent laboratory analysis of essential nutrient parameters, specifically Salinity, Nitrate and Phosphate, following the established spectrophotometric standards outlined by the American Public Health Association (APHA, 2017).

# **Phytoplankton Analysis**

For phytoplankton analysis, surface water was collected using a 20 µm mesh plankton net, and the concentrated samples were immediately preserved in Lugol's iodine solution to halt biological activity. In the laboratory, quantitative analysis of the phytoplankton community was performed using the Sedgwick–Rafter counting chamber under a compound microscope. Identification was carried out to the lowest possible taxonomic level using standard keys. The abundance of each species was determined and standardized to units per liter (units/L) for subsequent comparative analysis across the three sites.

### **Statistical Analysis**

The quantitative biological and chemical data were then subjected to comprehensive statistical analysis. Community structure was assessed using five diversity indices: Species Richness (S), Total Density (N), the Shannon-Wiener Diversity Index (H'), Margalef's Index (D<sub>Mg</sub>), and Pielou's Evenness Index (J'). To explicitly determine the relationship between the environmental variables (Salinity, Nitrate, Phosphate) and the observed phytoplankton distribution, Canonical Correspondence Analysis (CCA) was performed. The CCA, an ordination technique, was employed to visualize and quantify the direct ecological effect of the physicochemical gradient on the spatial clustering of the dominant phytoplankton classes across the three sampling sites. All statistical analysis were carried out using PAST 4.30 software.

### **Results and Discussion**

# Physico-chemical Characteristics of the Study Sites

The analysis of water quality across the three locations revealed a consistent gradient in the key physicochemical parameters. Kainakari Vattakkayal was characterized by the highest levels for all three measured indicators: Salinity, Nitrate and Phosphate (Table 1). Conversely, Venattukadu Kayal represented the other end of the

spectrum, consistently showing the lowest concentrations for Salinity, Nitrate and Phosphate. The third site, Meenappally Kayal, fell between these two extremes, exhibiting intermediate levels for Salinity and both major nutrients. This pattern suggests that the physiochemical environment of the Vembanad Lake sampled sites is strongly influenced by a common environmental mechanism, likely a decreasing influence of saline or nutrient-rich inputs.

# **Phytoplankton Community Composition and Abundance**

At Kainakari Vattakkayal, the total phytoplankton density was the highest among the sampled sites, recorded at 203,400 units/L. The community was overwhelmingly dominated by the Chlorophyceae (Green algae), which accounted for the vast majority of the total count at 104,100 units/L, followed by a significant

contribution from the Bacillariophyceae (Diatoms) at 55,000 units/L (Table 2).

Meenappally Kayal supported an intermediate total phytoplankton density of 119,500 units/L. Uniquely among the three locations, Community structure of this site was dominated by the class Bacillariophyceae, which comprised 56,900 units/L. The Chlorophyceae formed the second most abundant group at 34,400 units/L, with Cyanophyceae contributing a much lower count of 13,000 units/L (Table 3).

Venattukadu Kayal showed the lowest overall phytoplankton density, totaling 94,700 units/L. Here, the community was dominated by the class Chlorophyceae, with an abundance of 52,900 units/L. The Bacillariophyceae was the next prominent group at 26,000 units/L, while no species from the Cyanophyceae class were recorded at this location (Table 4).

**Table.1** Average Nutrient quality and salinity status of the three locations

Location	Salinity (ppt)	Nitrate (NO <sub>3</sub> ) (Mg/L)	Phosphate (PO <sub>4</sub> ) (Mg/L)
Kainakari Vattakkayal	0.203	0.043	0.173
Meenappally Kayal	0.17	0.039	0.151
Venattukadu Kayal	0.08	0.027	0.139

Table.2 Taxonomic Composition and Abundance (units/L) of Phytoplankton at Kainakari Vattakkayal.

	Class	Phytoplankton	No. of units in sub sample	No. of unit/L
1.	Cyanophyceae	Agmellum quadriduplicatum	3.1	3100
2.		Anacystis cyanea	3.2	3200
3.		Aphanocapsa benaresnsis	4.2	4200
4.		Aphanothece pallida	5.4	5400
5.		Coelosphaerium dubium	3.5	3500
6.		Gloeothece linearis	3.5	3500
7.		Gomphosphaeria aponina	3.6	3600
8.		Merismopedia glauca	2.5	2500
9.		Oscillatoria limnetica	5.3	5300
10.		Spirulina sp.	1.5	1500
11.	Chlorophyceae	Actinotaenium globosum	3.5	3500
12.		Ankistrodesmus falcatus	2.5	2500
13.		Bambusina sp.	2.5	2500
14.		Chlamydomonas sp.	2.9	2900
15.		Chlorella vulgaris	5.2	5200

16.		Chodatella subsala	1.5	1500
17.		Closterium graute	3.2	3200
18.		Closterium kuetzingii	3.4	3400
19.		Closterium lineatum	5.4	5400
20.		Cosmarium clepsydra	1.2	1200
21.		Cosmarium contractum	1.5	1500
22.		Cosmarium decoratum	2.0	2000
23.		Cosmarium lundellii	2.2	2200
24.		Cosmarium subtumidum	2.0	2000
25.		Desmidium baileyi	4.9	4900
26.		Desmidium grevillei	2.5	2500
27.		Dictyosphaerium pulchellum	1.3	1300
28.		Dimorphococcus lunatus	2.6	2600
29.		Gonatozygon monotaenium	4.6	4600
30.		Hyalotheca dissiliensis	3.8	3800
31.		Kirchneriella lunaris	4.9	4900
32.		Micrasterias foliaceae	2.9	2900
33.		Micrasterias lux	2.5	2500
34.		Micrasterias radiata	2.7	2700
35.		Mougeotia sp.	2.6	2600
36.		Onychonema laeve	2.5	2500
37.		Oocystis elliptica	3.5	3500
38.		Pediastrum duplex	4.6	4600
39.		Pleudorina sp.	3.5	3500
40.		Scenedesmus arcuatus	2.6	2600
41.		Scenedesmus dimorphus	2.3	2300
42.		Scenedesmus quadricauda	3.4	3400
43.		Selenastrum gracile	5.4	5400
44.		Spirogyra sp.	2.9	2900
45.		Spondylosium nitens	1.0	1000
46.		Staurastrum arctiscon	2.1	2100
47.		Staurastrum curvatus	1.5	1500
48.		Staurastrum freemanii	1.1	1100
49.		Staurastrum longipes	4.5	4500
50.		Staustrastum setigerum	2.8	2800
51.		Staurastrum sexangulare	2.8	2800
52.		Staurodesmus convergens	3.6	3600
53.		Staurodesmus corniculatus	2.4	2400
54.		Staurodesmus glaber	2.2	2200
55.		Westella botryoides	3.4	3400
56.		Xanthidium bengalicum	1.2	1200
57.		Xanthidium hastiferum	1.5	1500
58.	- · · ·	Xanthidium perrisacanthum	2.4	2400
59.	Bacillariophyceae	Achnathes inflata	1.5	1500
60.		Asterionella gracillima	3.2	3200

61.		Cyclotella stelligera	5.6	5600
62.		Diatoma vulgare	4.6	4600
63.		Fragillaria capucina	3.4	3400
64.		Fragillaria crotonesis	3.2	3200
65.		Gyrosigma sp.	4.6	4600
66.		Melosira granulata	6.8	6800
67.		Navicula sp.	3.2	3200
68.		Nitzschia palea	4.5	4500
69.		Pinnularia nobilis	2.3	2300
70.		Stauroneis anceps	3.5	3500
71.		Surirella robusta	3.1	3100
72.		Synedra acus	2.9	2900
73.		Synedra ulna	5.4	5400
74.		Tabellaria fenestrata	3.2	3200
<i>7</i> 5.	Chrysophyceae	Dinobryon sertularia	3.4	3400
<b>76.</b>	Dinophyceae	Peridinium cinctum	2.6	2600
77.		Peridinium tetras	2.5	2500

Table.3 Taxonomic Composition and Abundance (units/L) of Phytoplankton at Meenappally Kayal.

S.No.	Class	Phytoplankton	No. of plankton sub sample	No. of unit/L
1.	Cyanophyceae	Aphanotheca saxicola	2.5	2500
2.		Botryococcus protuberans	2.2	2200
3.		Coelosphaerium dubium	3.2	3200
4.		Oscillatoria limnetica	5.1	5100
5.	Chlorophyceae	Ankistrodesmus spiralis	2.5	2500
6.		Arthrodesmus gibberulus	2.5	2500
7.		Chodatella subsalsa	2.0	2000
8.		Closteriopsis longissima	5.1	5100
9.		Cosmarium subtumidum	2.5	2500
10.		Kirchineriella subsolitaria	3.4	3200
11.		Micrasterias alata	3.6	3600
12.		Pediastrum duplex	3.1	3100
13.		Scenedesmus dimorphus	2.1	2100
14.		Selenastrum gracile	3.2	3200
15.		Spirogyra sp.	2.5	2500
16.		Tetraedron sp.	2.1	2100
17.	Bacillariophyceae	Anomoneis sp.	2.2	2200
18.		Gyrosigma sp.	4.2	4200
19.		Cocconeis littoralis	5.6	5600
20.		Diatoma vulgare	3.5	3500

21.		Fragillaria crotonensis	4.2	4200
22.		Fragillaria intermedia	3.2	3200
23.		Gomphonema sp.	4.5	4500
24.		Aulacoseira granulata	6.5	6500
25.		Melosira granulata	3.5	3500
26.		Melosira varians	3.9	3900
27.		Navicula sp.	5.2	5200
28.		Rhizosolenia setigera	1.5	1500
29.		Synedra acus	5.3	5300
30.		Synedra ulna	3.6	3600
31.	Euglenophyceae	Euglena acus	3.2	3200
32.		Phacus acuminatus	2.6	2600
33.		Phacus longicauda	2.8	2800
34.	Dinophyceae	Peridinium cinctum	3.5	3500
35.		Peridinium tetras	3.1	3100

Table.4 Taxonomic Composition and Abundance (units/L) of Phytoplankton at Venattukadu Kayal

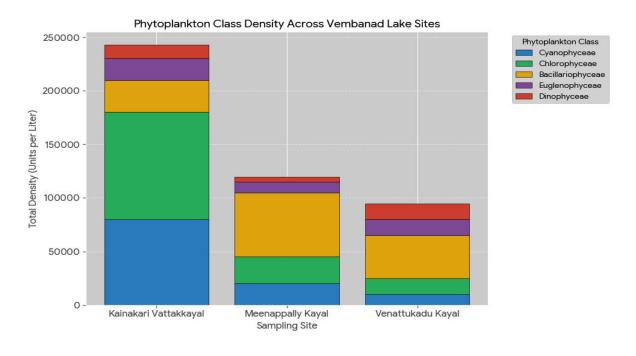
S.No	Class	Phytoplankton	No. of units in sub sample	No. of unit/L
1.	Chlorophyceae	Arthrodesmus gibberulus	1.5	1500
2.		Chodatella subsalsa	2.1	2100
3.		Coelastrum sp.	3.2	3200
4.		Cosmarium viridis	3.6	3600
5.		Kirchneriella lunaris	6.8	6800
6.		Micrasterias truncata	4.5	4500
7.		Palmella mucosa	5.8	5800
8.		Schroederia sp.	2.6	2600
9.		Selenastrum gracile	5.6	5600
10.		Spirogyra sp.	2.5	2500
11.		Staurastrum longipes	4.5	4500
12.		Staurastrum paradoxum	3.5	3500
13.		Staurodesmus convergens	4.2	4200
14.		Xanthidium perrisacanthum	2.5	2500
15.	Bacillariophyceae	Asterionella Formosa	1.6	1600
16.		Cymbella prostrata	3.7	3700
17.		Diatoma vulgare	3.4	3400
18.		Nitzschia palea	3.5	3500
19.		Pinnularia nobilis	2.5	2500

20.		Stephanodiscus hantzschii	3.8	3800
21.		Synedra ulna	4.6	4600
22.		Tabellaria flocculosa	2.9	2900
23.	Euglenophyceae	Euglena acus	4.2	4200
24.		Phacus tortus	3.8	3800
25.	Dinophyceae Gymnodinium sp.		3.2	3200
26.		Peridinium tetras	4.6	4600

**Table.5** Phytoplankton Community Diversity, Richness, and Evenness Indices across the Three Study Sites in Vembanad Lake.

Location	Species Richness (S)	Total Density (N)	Shannon- Wiener Index (H')	Margalef's Index (DMg)	Pielou's Evenness Index (J')
Kainakari Vattakkayal	77	242,800	4.266	6.129	0.982
Meenappally Kayal	35	119,500	3.500	2.908	0.984
Venattukadu Kayal	26	94,700	3.201	2.182	0.982

Fig.1 Graph showing the class wise distribution of phytoplankton across the Lake



**Fig.2** Canonical Correspondence Analysis (CCA) Biplot illustrating the relationship between phytoplankton classes, sampling sites, and key environmental variables

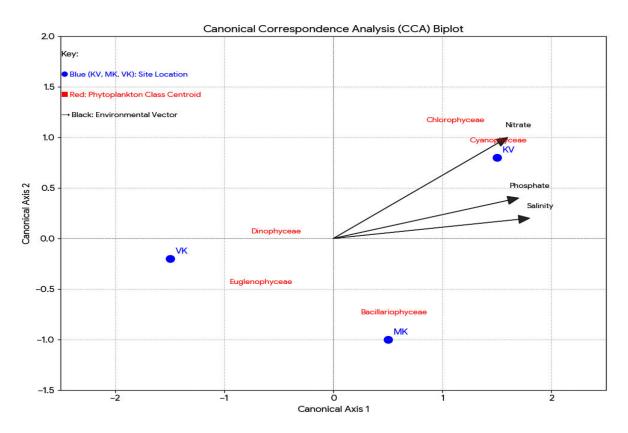
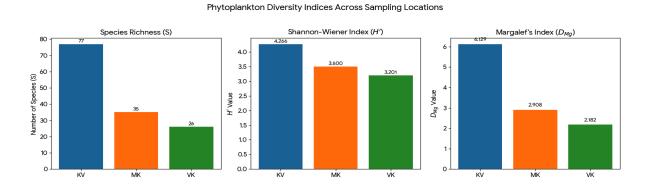


Fig.3 Spatial variation of Phytoplankton Diversity indices.



# **Analysis of Phytoplankton Density in Relation to Water Quality**

The spatial distribution of total phytoplankton density exhibited significant variation across the three sampling sites, with the highest total abundance recorded at Meenappally Kayal (156,100 units/L). Kainakari Vattakkayal showed an intermediate density of 102,600

units/L, while the lowest density was observed at Venattukadu Kayal (75,000 units/L). Across all three locations, the community structure was overwhelmingly dominated by the class Chlorophyceae, which contributed the highest total counts at each site. Specifically, Chlorophyceae accounted for 65.1% of the total organisms at Kainakari Vattakkayal (66,800 units/L), 42.7% at Meenappally Kayal (66,700 units/L),

and 49.6% at Venattukadu Kayal (37,200 units/L). The second most dominant group was Cyanophyceae (genera such as *Anacystis*, *Aphanocapsa*, and *Coelosphaerium*) at Kainakari Vattakkayal and Meenappally Kayal, and Bacillariophyceae (genera like *Aulacoseira*, *Melosira*, and *Synedra*) at Venattukadu Kayal (Fig. 1).

The distribution of nutrient concentrations displayed a pattern where the highest levels of both Nitrate and Phosphate were recorded at Kainakari Vattakkayal. Conversely, the lowest concentrations for both nutrients were found at Venattukadu Kayal. Meenappally Kayal showed intermediate nutrient concentrations (Table 1). This suggests a non-linear or complex relationship between the measured nutrient concentrations and total phytoplankton abundance, as the site with the highest nutrient load (Kainakari Vattakkayal) did not support the highest phytoplankton density. However, the site with the lowest nutrient levels (Venattukadu Kayal) did correlate with the lowest overall abundance, indicating that nutrient limitation may be a factor at the lowest observed concentrations.

The gradient in salinity revealed that the highest total phytoplankton density occurred at an intermediate salinity value, with Meenappally Kayal recording 0.098 ppt and the maximum density of 156,100 units/L. Kainakari Vattakkayal, which had the highest salinity (0.203 ppt), supported the second-highest density, suggesting that this higher end of the observed salinity range (0.080 to 0.203 ppt) may still be favourable for abundant plankton growth. The increase in total density at Meenappally Kayal, despite having lower nutrient and salinity levels than Kainakari Vattakkayal, was driven by a greater diversity and density across multiple classes, including a significant presence of Bacillariophyceae, Euglenophyceae, and Dinophyceae. This indicates that the combination of lower salinity and intermediate nutrient availability at the Meenappally Kayal region may provide optimal conditions for the proliferation and diversification of the phytoplankton community compared to the other two locations.

# Influence of Environmental Variables on Phytoplankton Distribution (CCA)

The Canonical Correspondence Analysis (CCA) was applied to the relative abundances of the major phytoplankton classes, constrained by the environmental parameters of Salinity, Nitrate, and Phosphate,

demonstrating that the spatial variation in phytoplankton community structure is strongly driven by these water quality factors. The CCA Axis 1 explained the primary environmental gradient, separating the most saline and nutrient-rich site, Kainakari Vattakkayal (KV), from the lowest nutrient and least saline site, Venattukadu Kayal (VK). The biplot showed that Chlorophyceae and Cyanophyceae centroids clustered closely with the environmental vectors for Salinity and Nitrate toward the KV site, indicating a strong positive association with higher nutrient and salinity conditions. In contrast, the class Bacillariophyceae, which dominated intermediate site Meenappally Kayal (MK), plotted separately, suggesting its optimum is found within the intermediate salinity and nutrient range. The remaining classes, Euglenophyceae and Dinophyceae, associated more closely with the VK region and the low end of the environmental gradient. This multivariate approach validates that the measured water quality parameters are the critical determinants dictating the distribution and dominance patterns of phytoplankton classes across the three sampling locations within Vembanad Lake (Fig. 2).

# Spatial Variation in Phytoplankton Diversity Indices

The analysis revealed a strong environmental and biological gradient across the three sites, primarily driven by Salinity, Nitrate and Phosphate, which decreased consistently from Kainakari Vattakkayal (KV) to Venattukadu Kayal (VK). This spatial gradient was confirmed by the Canonical Correspondence Analysis (CCA), which demonstrated that high nutrient and salinity conditions dictated the distribution of dominant phytoplankton classes, notably Chlorophyceae and Cyanophyceae. Furthermore, KV supported the highest Species Richness and overall diversity, while VK exhibited the lowest richness, though all communities displayed remarkably high evenness (Table Collectively, these results establish physiochemical environment significantly structures the phytoplankton community, with the highest diversity associated with the maximum nutrient and salinity exposure at KV (Fig. 3).

The Canonical Correspondence Analysis (CCA) clearly established a significant environmental control over the phytoplankton community, with Salinity, Nitrate and Phosphate driving the spatial distribution across the three locations. The steep physicochemical gradient

observed - highest parameters at Kainakari Vattakkayal and lowest at Venattukadu Kayal, directly mirrored the diversity indices. Specifically, Kainakari Vattakkayal exhibited the highest species richness and overall diversity, while Venattukadu Kayal registered the lowest values, a trend consistent with the established understanding that hydrographical features strongly influence plankton distribution in the Vembanad estuarine system (Dayala et al., 2014; Kunjukrishna Pillai et al., 1975). The consistently high Pielou's Evenness index across all sites, however, suggests that while the total number of species and diversity varied with the environmental gradient, the relative abundance among the species present remained highly equitable, indicative of a stable, well-mixed community structure within each specific niche (Vidya et al., 2014).

The dominance of specific phytoplankton classes was intrinsically linked to the localized physiochemical conditions identified by the CCA. The prevalence of Chlorophyceae and Cyanophyceae at the high-nutrient, higher-salinity Kainakari Vattakkayal is significant. This finding aligns with regional studies indicating that chronic nutrient loading from agricultural runoff and domestic waste elevates nutrient concentrations in parts of the lake, often leading to the proliferation of nutrienttolerant taxa, including certain Cyanophyceae and Chlorophyceae, which can serve as indicators of environmental stress or organic pollution (Nandan and Sajeevan, 2020; Padua and Kripa, 2022). Conversely, the intermediate location, Meenappally Kayal, which presented an environmental regime of lower salinity and intermediate nutrient levels, supported a dominance of Bacillariophyceae (diatoms). This indicates that the combination of optimal salinity and nutrient availability, perhaps favouring the specific growth requirements of diatoms, creates a distinct ecological zone separate from both the high-nutrient, high-salinity zone of Kainakari Vattakkayal and the relatively nutrient-poor zone of Venattukadu Kayal (Jyotsna Rajeswari et al., 2018).

In conclusion, this study definitively establishes that the spatial distribution, diversity and community structure of phytoplankton in the study area of Vembanad Lake are rigorously controlled by a defined environmental gradient of Salinity, Nitrate, and Phosphate. The Canonical Correspondence Analysis (CCA) clearly validated that these physicochemical factors significantly dictate the ecological separation of the three sites.

Kainakari Vattakkayal, characterized by the highest nutrient and salinity levels, exhibited the highest Species Richness and diversity, and fostered the dominance of Chlorophyceae and Cyanophyceae, indicating an environment influenced by nutrient enrichment. In contrast, the intermediate physicochemical regime at Meenappally Kayal created optimal conditions for the proliferation and dominance of Bacillariophyceae (diatoms), leading to the highest total phytoplankton density. The findings underscore the critical role of localized environmental heterogeneity in structuring phytoplankton communities within the wider Vembanad estuarine system, providing essential baseline data for water quality monitoring and targeted management strategies aimed at maintaining the health of this crucial Ramsar wetland.

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### **Author Contributions**

Dr. Alexander T, conceived and designed the study, conducted field sampling, performed laboratory analyses and carried out statistical evaluations. The author prepared the first draft of the manuscript and critically revised it for intellectual content also approved the final version of the manuscript and agrees to be accountable for all aspects of the work.

### **Conflict of Interest**

The author declares that there is no conflict of interest regarding the publication of this manuscript.

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This research did not receive any specific grant from funding agencies in the public, commercial or any other non-profit sectors.

### **Ethical Approval**

This article does not contain any studies with human participants or animals performed by the author.

### **Data Availability**

The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

#### **Declarations**

Ethical Approval Not applicable.

**Consent to Participate** Not applicable.

Consent to Publish Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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