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Species Composition and Substrate Specificity of Periphytic Diatoms from Lower and Middle Stretches of the River Periyar in Kerala, India

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Abstract

The present study was conducted to determine the species composition, substrate specificity and station wise distribution of periphytic diatoms from lower and middle stretches of the river Periyar in Kerala, India. Five different substrates, such as log, leaf, root, rock, and wall, were chosen for the study. Algal communities growing on the substrates were sampled for a period of one year. Water quality parameters such as temperature, pH, conductivity, chloride, nitrate, sulfate, phosphate, and dissolved oxygen were also determined. Taxonomic studies revealed the presence of 53 species of Bacillariophyta belonging to 17 families. The results of Principal component analysis suggested that among three seasons, pre-monsoon harboured maximum species and Naviculaceae was the most abundant family. Canonical correspondence analysis illustrated that environmental variables like pH, chloride, and temperature had a significant role in diatoms community composition. Cluster analysis revealed a very high similarity in species composition between log and wall, whereas results of percentage abundance suggested that the diatoms preferred leaf as their suitable substratum. The percentage composition of diatoms was reported maximum at station 4.

Keywords: Bacillariophyta; Periphytic community; Substratum variation; Principal component analysis; Cluster analysis; Naviculaceae

1. Introduction

Periphyton is a micro-ecosystem composed of photoautotrophic algae, chemoautotrophic and heterotrophic bacteria, metazoans, fungi, protozoans, and viruses (Larned, 2010). They are solar-powered biogeochemical reactors commonly found attached to free surfaces of submerged substrata in aquatic ecosystems and play a major role in primary production, nutrient fluxes, and food web interactions (Wu, 2017). Algae usually form the most diverse and abundant component of periphytic biofilm due to its short life cycle, role in primary production, sensitivity to environmental changes and tolerance to adverse conditions (Borduqui and Ferragut, 2012; Dunk *et al*., 2019; Rusanov and Stanislavskaya, 2012). Among periphytic algae, diatoms are considered as the pioneering and efficient colonizers due to its specialized fixation structures and high competitive ability towards the varying environmental conditions (Franca *et al*., 2011). Since the periphyton community composition is greatly affected by the initial colonizing organisms, interspecific and intraspecific competitions among various species as well as by their reproductive strategy; studies specific to periphytic diatoms have become very important in aquatic ecology (Kanavillil and Kurisseryl, 2013). Periphytic assemblages in the lotic ecosystems get much more critical than the planktonic forms due to its stable nature. Periphytic community attached to the submerged substrates act as a micro-ecosystem by forming a biofilm and thus integrate the nutrient and energy fluxes within the periphytic community. But most of the hydrological studies conducted in tropical riverine systems usually concentrate on planktonic forms and the information regarding periphytic assemblages are too limited (Satkauskiene and Glasaite, 2013). The present study aims to understand the species composition and substrate specificity of periphytic diatoms from selected natural substrate along the lower and middle stretches of river Periyar, Kerala.

2. Materials and Methods

Study area

The study was conducted for one year duration (June 2016 – May 2017) from the middle and lower stretches of river Periyar, Kerala, India. River Periyar, one of the longest rivers of Kerala originates from Sivagiri peaks of Western Ghats and has a total length of 244 km. It contributed significantly to the economy of Kerala by providing water for electrical power generation, drinking and agricultural purposes and referred to as the ‘Lifeline of Kerala’. From the middle and lower stretches of river Periyar, five sampling stations were selected. All these stations were localized in Ernakulam district of Kerala, India and are separated by a distance of 10 to 20 Km apart. Station 1 (S1): Pooyamkutty (10° 3’29.59"N, 76° 46’33.88"E); Station 2 (S2): Kuttampuzha (10° 8’37.11"N, 76° 43’16.41"E); Station 3 (S3): Thattekadu (10°082 N, 76°412 E); Station 4 (S4): Aluva (10° 6’56.83"N, 76°21’17.38"E) and Station 5 (S5): Varappuzha (10° 4’10.31"N, 76°16’48.28"E). S1, S2, and S3 were selected from the middle stretches when S4 and S5 were from the lower stretches. The middle stretches are comparatively less polluted when compared to lower stretches and receive mainly domestic, agricultural and laundry wastes. The lower stretches receive a huge amount of industrial effluents, sewage wastes and garbage dumps from the nearby town and industrial areas. Station 5 is also influenced by seawater intrusion during tidal cycles (CPCB, 2000; Joy, 1989).

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Sampling Procedure

From the five selected stations, periphytic algal samples and water samples were collected every month for one year duration (June 2016 – May 2017). From each station, five different natural substrata such as log (fallen limbs and wooden poles of coconut and bamboo trees), leaf, root (water plants such as *Nymphoides indica*, *Persicaria* sp., *Cabomba* sp.), rock, and wall (concrete steps) were chosen from a depth of 2 to 5 cm and a metal frame consisting of 5 cm^2 area (Aloi, 1990) was placed on to the substratum. The periphytic samples were scrapped from the metal frame using brush, blade or scalpel (Biggs and Kilroy, 2000). The scrapped contents were washed into a tray using distilled water and then transferred into a sampling vial. Samples were preserved using 4% formalin and made up to 10 ml using distilled water. 1 ml of this sample was taken and with great care transferred to a Sedgwick rafter counting chamber for enumeration. Sedgwick rafter consists of 1000 cells, each of 1 mm^3 volume and contains a considerable number of algal cells. For convenience, five rows comprised of 250 cells were counted under an inverted microscope (Carl Zeiss Primovert, Germany) equipped with phase contrast and the results were expressed as the number of diatom cells/ cm^2. For identification of the diatom frustules, the preserved samples are homogenized and heated at 100^0C with hydrochloric acid (35%) and hydrogen peroxide (30%) to remove organic matter and then mounted on a microscopic slide using Pleurax, a high refractive index medium (Cruz *et al*., 2006; Duonga *et al*., 2008). Diatom frustules were identified using standard books, literature, and keys (Adhikary and Das, 2012; John and Francis, 2012; Karthick *et al*., 2013). Water quality parameters were also monitored monthly during the study period. Temperature, pH, dissolved oxygen (DO), and conductivity, were measured on-site using a multi-parameter probe (Eutec Cyberscan-650). Remaining phosphate, nitrate, sulfate, and chloride values were monitored in the lab using standard methods (APHA, 2005).

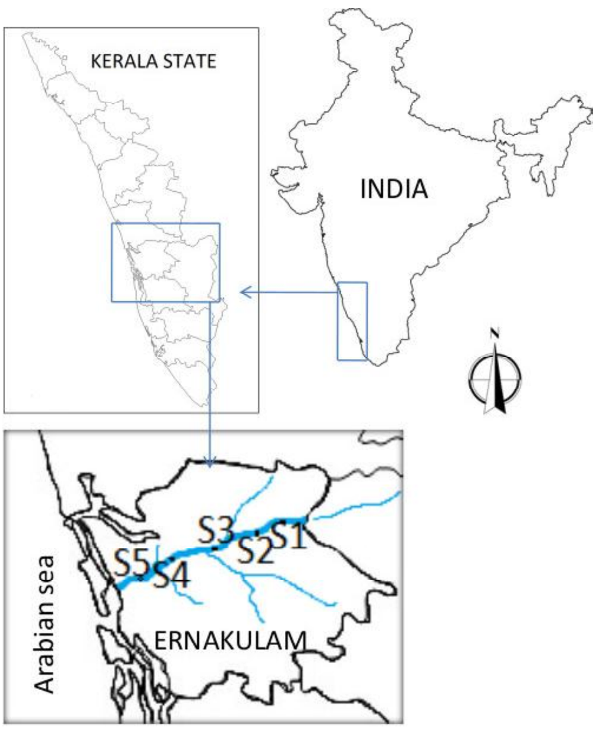

Fig. 1. Map showing the Periyar river, Kerala

Statistical analysis

Statistical data processing and analysis were carried out using the software PAST version 318 (Hammer, 2019). Principal Component Analysis (PCA) was conducted to know the distributional difference of periphytic diatoms among monsoon, pre-monsoon and post-monsoon seasons. Canonical Correspondence Analysis (CCA) was performed to illustrate the relationship between environmental variables and periphytic diatom assemblages. Cluster analysis was performed using the algorithm UPGMA (Bray-Curtis similarity index) to know the percentage of similarity within the substrata and stations regarding periphytic diatom composition.

3. Results

Abiotic factors

The results of the physicochemical parameters monitored have been provided in Table 1. Although station 5 recorded comparatively higher temperatures, the temperature did not show much seasonal variation. pH values showed that station 5 is slightly alkaline compared to other stations. Monsoon period records high DO values, especially in stations 1 and 2. The pre-monsoon period showed a higher concentration of sulfate, nitrate, chloride and conductivity values, especially in lower reaches.

Seasonal distribution of periphytic diatoms

A total of 53 species of diatoms belonging to 17 families were recorded (Table 2). Estimation of percentage abundance of Bacillariophyceae families (Fig. 2) revealed Naviculaceae (26.23%) was the most abundant family, followed by Fragilariaceae (23.57%).

The principal component analysis illustrated that the pre-monsoon period marked higher periphytic diatom composition compared to monsoon and post-monsoon seasons. Periphytic diatom families were represented by vectors radiating from the origin. Vector for Naviculaceae family was diverging more from the origin and showed maximum abundance. Dots represented months on the plot and Convex-hulls were formed for corresponding monsoon, post-monsoon and pre-monsoon periods. The area enclosed by the convex hull shows maximum

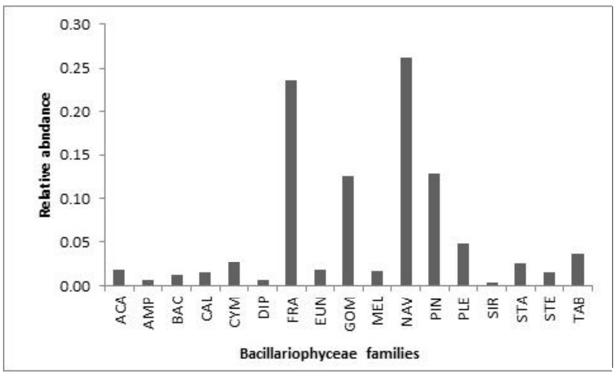

Fig. 2. Percent composition of periphytic Bacillariophyceae families from the study area

Table 1. Physicochemical parameters recorded from different stations of river Periyar (June 2016 – May 2017)

Parameters	Seasons	S1	S2	S3	S4	S5
Temperature (^{0}C)	Monsoon	25.7± 0.31	25.9 ± 0.49	26.4 ± 0.48	27.6 ± 1.0	27.9 ± 1.23
	Post-monsoon	25.0 ± 0.80	26.0 ± 0.77	27.7 ± 1.52	29.5 ± 0.84	30.1 ± 0.62
	Pre-monsoon	26.2 ± 0.61	27.1 ± 1.60	28 ± 1.41	29.7 ± 1.68	30 ± 1.40
pH	Monsoon	6.6 ± 0.22	6.7 ± 0.27	6.9 ± 0.33	7 .0± 0.57	7.4 ± 0.14
	Post-monsoon	6.4 ± 0.36	6.4 ± 0.38	6.6 ± 0.14	6.0 ± 0.18	7.3 ± 0.33
	Pre-monsoon	6.1 ± 0.35	6.0 ± 0.56	6.3 ± 0.57	5.8 ± 0.20	7.5 ± 0.47
DO (mg/l)	Monsoon	8 .0± 0.10	7.8 ± 0.13	7.6 ± 0.17	7.2 ± 0.19	6.8 ± 0.03
	Post-monsoon	8.3 ± 0.61	8.1± 0.41	7.5 ± 0.13	6.8 ± 0.53	6.3 ± 0.40
	Pre-monsoon	7.8 ± 0.42	7.5 ± 0.44	7.1 ± 0.06	6.5 ± 0.29	5.6 ± 0.45
Conductivity	Monsoon	0.01 ± 0.00	0.04 ± 0.05	0.02 ± 0.00	0.02 ± 0.01	24.0 ± 7.52
(mS/cm)	Post-monsoon	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.04 ± 0.01	46.7 ± 6.50
	Pre-monsoon	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.01	49.1 ± 6.22
Phosphate (mg/l)	Monsoon	0.18 ± 0.11	0.16 ± 0.10	0.20 ± 0.16	0.46 ± 0.20	0.66 ± 0.12
	Post-monsoon	0.52 ± 0.19	0.55 ± 0.33	0.69 ± 0.29	1.0 ± 0.25	1.32 ± 0.31
	Pre-monsoon	1.06 ± 0.07	1.07 ± 0.17	1.16 ± 0.14	1.51 ± 0.35	1.6 ± 0.38
Sulphate (mg/l)	Monsoon	0.12 ± 0.01	0.14 ± 0.02	0.20 ± 0.06	0.24 ± 0.15	10.3 ± 4.9
	Post-monsoon	0.25 ± 0.28	0.3 ± 0.36	0.33 ± 0.42	0.46 ± 0.65	40.9 ± 20.01
	Pre-monsoon	0.17 ± 0.10	0.27 ± 0.12	0.20 ± 0.16	0.37 ± 0.17	60.9 ± 37.85
Nitrate (mg/l)	Monsoon	0.28 ± 0.25	0.33 ± 0.31	0.38 ± 0.34	1.23 ± 0.54	0.81 ± 0.58
	Post-monsoon	0.43 ± 0.06	0.45 ± 0.03	0.51 ± 0.08	2.65 ± 0.28	2.96 ± 0.20
	Pre-monsoon	3.60 ± 2.60	5.0 ± 3.75	5.9 ± 3.78	9.81± 2.32	6.95 ± 1.80
Chloride (mg/l)	Monsoon	62.5 ± 25.0	62.5 ± 25.0	75.0 ± 28.9	087.5 ± 47.9	0349.9 ± 107.98
	Post-monsoon	75. 0± 28.9	75.0 ± 28.9	75.0 ± 28.9	100.0 ± 40.81	1487.0 ± 303.7
	Pre-monsoon	99.9 ± 40.8	87.5 ± 47.9	99.9 ± 40.8	112.5 ± 47.86	1928.8 ± 701.8

Table 2. Periphytic diatoms reported from river Periyar

FAMILY	SPECIES	FAMILY	SPECIES
ACHNANTHACEAE (ACA)	*Achnanthes brevipes*	NAVICULACEAE (NAV)	*Navicula protracta*
	A. inflate		*N. microspora*
AMPHIPLEURACEAE (AMP)	*Frustulia frenguelli*		*N. radiosa*
BACILLARIACEAE (BAC)	*Bacillaria paxillifer*		*N. striolata*
	Nitzschia sigmoidea	PINNULARIACEAE (PIN)	*Pinnularia biceps*
	Nitzschia sp.		*P. braunii*
	Tryblionella constricta		*P. divergens*
CATENULACEAE (CAL)	*Amphora ovalis*		*P. gibba*
	Amphora sp.		*P. major*
CYMBELLACEAE (CYM)	*Cymbella affinis*		*P. microstauron*
	C.bengalensis		*P. nodosa*
DIPLONEIDACEAE (DIP)	*Diploneis elliptica*		*P. viridis*
FRAGILARIACEAE (FRA)	*Asterionella* sp.	PLEUROSIGMATACEAE (PLE)	*Gyrosigma acuminatum*
	Fragilaria.capucina		*G. distatum*
	F.virescens		*G. eximum*
	Synedra acus		*G. obtusatum*
	S. ulna		*G. scalproides*
EUNOTIACEAE (EUN)	*Eunotia* sp.		*Pleurosigma lange*
GOMPHONEMATACEAE (GOM)	*Gomphonema angustatum*		*Pleurosigma* sp.
	G. gracile	SURIRELLACEAE (SIR)	*Surirella robusta*
	G.grunowii		*Surirella* sp.
	G. intricatum	STAURONEIDACEAE (STA)	*Stauroneis acuta*
	G. parvulum		*S. anceps*
	G. telegraphicum		*S. phoenicenteron*
MELOSIRACEAE (MEL)	*Melosira granulata*	STEPHANODISCACEAE (STE)	*Cyclotella* sp.
	M. moniliformis	TABELLARIACEAE (TAB)	*Tabellaria flocullosa*
	Melosira sp.		

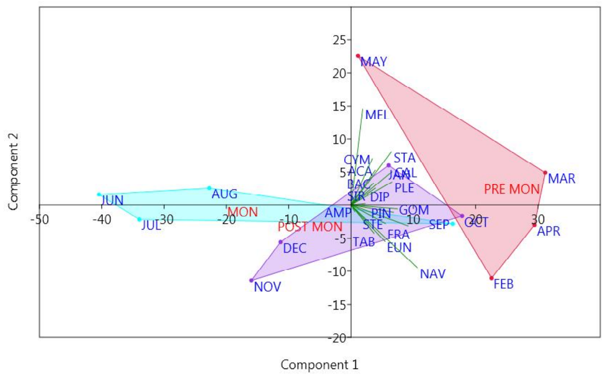

Fig. 3. Principal Component Analysis (PCA) depicting periphytic diatom composition and seasonal abundance. Bacillariophyceae families were represented by the vectors radiating from the origin. Dots on the plot represents months(JUN-June, JUL-July, AUG-August, SEP- September, OCT-October, NOV- November, DEC- December, JAN- January, FEB- February, MAR-March, APR-April) and convex-hull denotes 95% confidence level for corresponding seasons(MON-monsoon, POST MON-post-monsoon, PRE MON- pre-monsoon). Abbreviations for Bacillariophyceae families were provided in table 2.

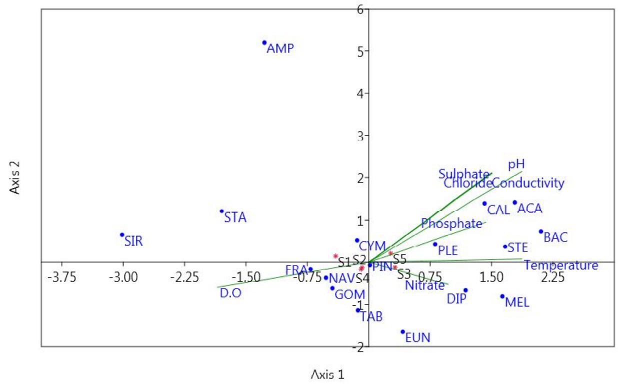

Fig. 4. Canonical correspondence analysis (CCA) ordination plot depicting the relationship between environmental parameters and periphytic diatom assemblages. Environment variables were represented by vectors radiating from the origin. Bacillariophyceae families were represented by dots on the plot (abbreviations given in table2). Stars denote selected stations (S1-station 1, S2-station 2, S3-station 3, S4-station 4, S5- station 5).

variance, and here the pre-monsoon period differs significantly from the rest of the seasons (Fig. 3).

Canonical correspondence analysis (CCA) was conducted to clarify the relationship between environmental parameters and periphytic diatom assemblages. Eight environmental parameters monitored, and 17 families identified were taken into account to perform CCA. Eigenvalues for axis 1 and 2 itself explain 77.65% of the relation between the variables. Vector for dissolved oxygen (DO) is an obtuse angle with other vectors and illustrates the negative relation of DO with all other environmental parameters (Fig. 4). Vectors for conductivity and chloride showed a strong positive correlation; likewise, pH, temperature, phosphate, and nitrate were positively correlated with each other.

Axis 1 has positive loadings for temperature (0.623), pH (0.624), conductivity (0.500), sulfate (0.501), chloride (0.502). Achnanthaceae, Bacillariaceae, Catenulaceae, Diploneidaceae, Melosiraceae and Stephanodiscaceae families also have positive loading with axis1 and clearly defines the role of temperature, pH, chloride, sulfate, and conductivity in the distribution of these families. Axis 1 has negative loadings for DO (-0.616). Families like Amphipleuraceae, Surirellaceae, and Stauroneidaceae also have negative loadings for axis one and clearly define the role of DO in the distribution of these families.

Station wise distribution of periphytic diatoms

Estimation of percentage abundance of periphytic diatoms follows the order station 4 > station 1 > station 5 > station 3 > station 2 (Fig. 5). Maximum numbers of species were reported from station 4 (30.45%), and the minimum was reported from station 2 (11.16%).

Cluster analysis resulted in a dendrogram (Fig. 6) with two groups with a total of 75% similarity. S4 and S1 showed 84% similarity in periphytic diatom composition when S2 and S3 exhibited 83% similarity with each other. S5 forms an outlier from all other stations.

Substrate wise distribution of periphytic diatoms

Estimation of percentage abundance of periphytic diatoms follows the order leaf > root > log > wall > rock (Fig. 7). Periphytic diatoms choose leaf (35.11%) as the most preferred substrate and rock (9.21%) as the least preferred one.

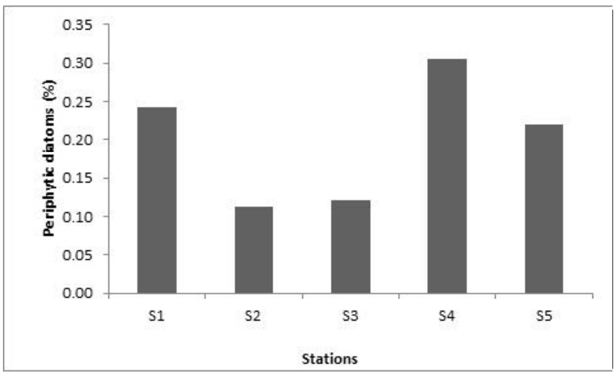

Fig. 5. Percent composition of periphytic Bacillariophyceae from selected stations

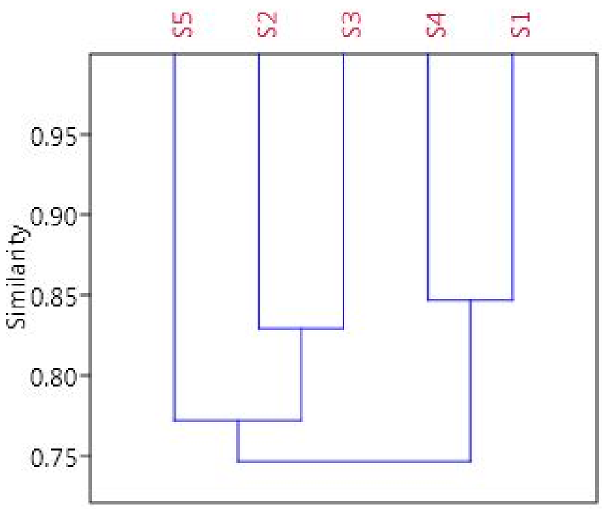

Fig. 6. Dendrogram (UPGMA) based on Bray Curtis similarity index depicting the taxonomic composition of Bacillariophyceae along with different stations of river Periyar

Cluster analysis resulted in a dendrogram with two groups with a total of 73% of similarity. Log and wall showed 93% of similarity in periphytic diatom composition. Rock form an outlier with all other substrata (Fig. 8).

4. Discussion

Periphytic assemblages that grow on the submerged surfaces form a biofilm and are greatly influenced by the physical and chemical nature of the substrates. Physical nature of the submerged surfaces provides a better nutrient-rich microenvironment and thus significantly affects the abundance and composition of the periphytic community (Wu, 2017). Most of the hydrological studies on tropical riverine systems traditionally focussed on the planktonic forms. Any suspended particles in the lotic system ultimately carried out to the sea within few days. Hence the study of attached forms becomes more relevant (Satkauskiene and Glasaite, 2013; Srivastava *et al*., 2019). Habitat wise variation in periphytic microalgal assemblages of Chalakkudy river basin, Kerala was studied by Nasser and Sureshkumar (2014a). Periphytic algae of Pokkali and prawn fields of Vypeen and N. Paravoor, Kerala were studied by Joshi (2010). The present study mainly focussed on the species composition, substrate specificity and seasonal preference of periphytic diatoms of river Periyar.

Taxonomic studies recorded a total of 53 species of periphytic diatoms belonging to 17 different families collected from various substrates. Estimation of percentage abundance in seasonal wise distribution among different families showed the abundance of Naviculaceae (26.23%) family followed by Fragilariaceae (23.57%). Ellaswamy *et al*. (2017) from their studies on tropical aquatic systems has reported the abundance of *Navicula* and *Fragilaria* genera. Species of *Navicula* genera usually seen among pioneering colonizers and are highly correlated to water transparency in the summer season (Franca *et al*., 2011; Felisberto and Rodrigues, 2005; Joshi, 2010).

The principal component analysis illustrates the dominance of periphytic diatoms in the pre-monsoon period. From the loadings of axis one and the area enclosed by the convex hull, the dominance of periphytic diatoms in the pre-monsoon period is clearly defined. The magnitude and orientation of vectors in the ordination space illustrate the dominance of Naviculacae family. Periphytic diatoms are regarded as primary colonizers and are photoautotrophs; they play a major role in primary production at the basal level. Light and temperature contribute significantly to the optimum production and succession of periphytic diatoms, results in their abundance in the pre-monsoon period (Sohani, 2015). The dominance of Bacillariophyceae members in the summer period was reported by Hajong and Ramanujam (2018) from their studies in a tropical lake. In the summer period, increased temperature enhances the rate of decomposition, and the increased nutrient content helps in primary production (Santhanam and Perumal, 2003) results in an abundance of Bacillariophyceae composition. These results reasonably agree with the findings of Ellaswamy *et al*. (2017); Franca *et al*. (2011); Joseph (2017); Kanavillil and Kuriasseryl (2013) and Nasser and Sureshkumar (2014b).

Canonical correspondence analysis extracted those environmental variables that strongly influence the periphytic diatom assemblages. CCA ordination plot clearly defines the role of temperature (0.623), pH (0.624), sulfate (0.501), chloride (0.502) and conductivity (0.500) in the distribution of periphytic diatoms especially at station 5. Dendrogram resulted from cluster analysis showed that station 5 forms an outlier with the rest of the stations. Station 5, Varappuzha is located in the lower reaches of the Periyar river and greatly influenced by sewage wastes, garbage dumps and a wide variety of industrial wastes; this station also receives a considerable amount of seawater in tidal cycles (CPCB, 2000; Joy,1989). All these factors account for increasing the pH, chloride, conductivity and sulfate values at this station. These hydrological conditions at station 5 results in a periphytic diatom composition different from other stations. A positive correlation of periphyton with pH, temperature, chlorides, and nutrients was reported by Oterler (2016). Satkauskiene and Glasaite (2013) from their studies on the Nemunas river reported that Alkaline pH and optimum temperature were the main factors that influenced the abundance of diatoms.

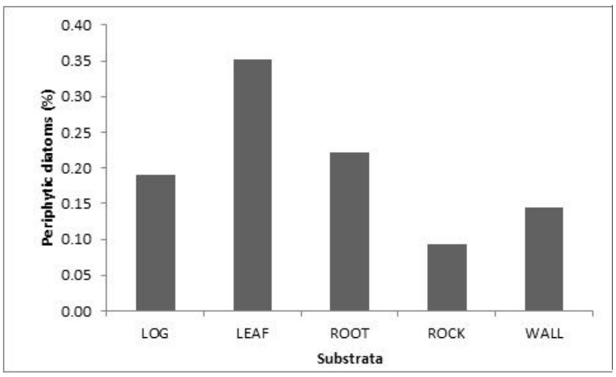

Fig. 7. Percent composition of Bacillariophyceae from different substrata

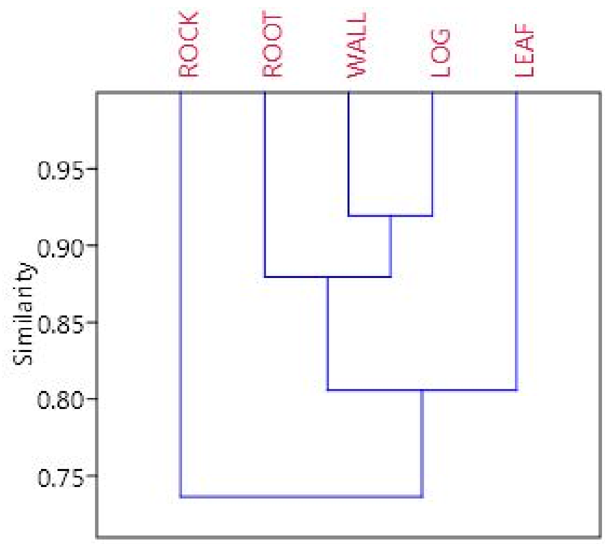

Fig. 8. Dendrogram (UPGMA) based on Bray Curtis similarity index depicting the taxonomic composition of Bacillariophyceae on the varying substrate

The biotic communities in a habitat are mainly influenced by the microclimate and substratum type in that area (Nasser and Sureshkumar, 2014a). Estimation of percentage composition of periphytic diatom along different stations revealed that the maximum number of species were reported from station 4 (30.45%) followed by station 1 (24.20%). Station 4 Aluva, a major commercial town and an important industrial area, is in the lower reaches of river Periyar. The river receives an enormous amount of organic and inorganic pollution load from nearby towns. This resulted in increased nutrient content in the form of nitrate and phosphate in this area. These nutrients help in the rapid multiplication of diatoms (Ansari *et al*., 2015). The higher number of pollution tolerant genera like *Cyclotella*, *Cymbella*, *Lyngbya*, *Melosira*, *Navicula*, *Nitzschia*, *Stauroneis*, and *Synedra* (Palmer, 1969) from lower reaches of river Periyar indicates the eutrophic nature of the river. Sreenisha and Paul (2016) also reported these pollution indicator genera from their studies on Tirur river, Malappuram.

Substrata often play a major role in their colonization and succession of periphytic diatoms. Sessile forms are usually possessed with some specialized features for their attachment with substrata. These attached forms are mostly seen in the productive zones of lotic systems attached to different substrata like a leaf, log, rock, root, and sand. Estimation of percentage abundance on selected substrata of river Periyar revealed that most of the periphytic diatoms choose leaf (35.11%) as their preferred substrate followed by roots (22.27%). This preference is mainly based on the surface area or space for colonization and easy attachment. Many of these periphytic diatoms choose organic substrata than mineral ones. Dendrogram resulted from substrate wise analysis revealed that rock differs greatly in periphytic diatom composition than the rest of the substrate (Fig. 8). Rougher and more stable substrates markedly separated from smoother and less stable substrate in case of diatom abundance (Sharifinia *et al*., 2016).

Diatoms have an important position in periphytic community composition. Periphytic diatoms are efficient colonizers and can withstand adverse conditions. The present study came out with some relevant information regarding community composition, substrate specificity and seasonal preference of periphytic diatoms of river Periyar. Although periphytic diatoms were present in all seasons, they were more abundant in the pre-monsoon period and preferred leaf as their suitable substratum. Multiple interactions of several physicochemical parameters define the periphytic diatom community, and more species were seen in the lower reaches.

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