Chapter-3

Materials and methods

3.1 Area of study

Kerala, a land of rivers situated in the humid tropics lies between 8º18' and 12º48' North and 74º52' and 77º22' East. Rivers originate from the Western Ghats, out of which 41 flow towards west and the other 3 rivers towards east. Bharathapuzha (Nila River), one of the major rivers of the state flows through Palakkad district. Palakkad, the largest producer of rice in Kerala also known as the granary of Kerala. Palghat gap of 32 to 40 Km in Western Ghats functions as gateway to the northeast monsoon reaches to Kerala. The district has 136257 hectares of hill area including reserve forest Silent Valley. As many as eight rivers flows through these hills especially major rivers like Bharathapuzha and Bhavani. The present investigation for detailed study of algal biodiversity was carried out on Bharathapuzha and Bhavani river in Palakkad district of Kerala.

The Bharathapuzha river is a major west flowing and second longest river in Kerala. It originates from Anamalai hills and lies approximately between 10°26' and 11°13' north latitudes and 75°53' and 77°13' east longitudes (Brijesh 2006). It has got a total area of 6186 Km^2, out of which 4400 Km^2 falls within Kerala and the rest 1786 Km^2 in Tamil Nadu. The river Bharathapuzha is the link of three districts in Kerala namely Palakkad, Malappuram and Thrissur. The Bharathapuzha river basin is well characterized by the major physiographic divisions of Kerala namely the highlands (600-1800 m), the midlands (300-600 m), lowlands (10-20 m) and coastal plains (0-l0 m). It covers the area of an elevation about 1,100 metres above mean sea level, the western Ghats are fed by four major tributaries, the Kalpathypuzha, Gayathripuzha, Thootha and Chitturpuzha, which flow through highly diverse and geomorphologic regions of Kerala. The Kalpathi river basin is the largest sub basin with an area of 1390 m^2, chased by Chittur (1315 m^2). At Parli, Kannadippuzha and Kalpathippuzha

merge and flow westward as Bharathappuzha, eventually emptying into the Arabian Sea at Ponnani. Pallippuram is where the Thootha and Nila rivers meet. The smallest river basin is Thootha river, is rich in water, and after its merger, Nila becomes thicker in flow. Though Bharathappuzha has a large river basin, the water flow is relatively less compared to other long rivers in Kerala because a large portion of the river basin is in the comparatively drier regions of Tamil Nadu and Palghat Gap.

The river is the lifeline water source for a population living in four administrative divisions of Kerala, namely Malappuram, Thrissur and Palakkad districts of Kerala and part of Coimbatore and Thiruppur districts of Tamil Nadu. Rice and coconut are the dominant crops in the coastal regions of the river basin. The river basin experiences a unique climate space from the rest of the state of Kerala may be for its location beginning from the Palakkad plains in the Palghat Gap flanked by mountain ranges of the Western Ghats. Irregularities in the general rainfall and surface temperature of the region have been observed in the last couple of decades. In recent years the river basin is also reported to be facing severe scarcity of water and drought conditions.

Bhavani is an east flowing perennial river flows from the Nilgiri hills of the Western Ghats, through the Silent Valley National Park in Palakkad, and back into Tamil Nadu. The river has 217 Km long and flows through the states of Kerala and Tamil Nadu. It is a major tributary of Cauvery and second longest river in state of Tamil Nadu. It has got an area of 6200S Km^2, out of which 87% in Tamil Nadu, 9% in Kerala and 4% in Karnataka. There are many dams that have been constructed across the river, they are Bhavanisagar dam, Kodiveri dam, Kanjirapuzha dam, Mannarkkad and Siruvani dam. Kanjirapuzha dam is situated in the Palakkad district, irrigates the land of 9173 hectares in Kerala. More than 90% of river water used for

irrigating the agricultural lands. The river Bhavani collects municipal sewage and industrial effluents along its river course and finally joins with the Cauvery River at Erode District. The average annual rainfall of the Bhavani river basin is 811.47 mm. The average discharge of the river at its mouth is 161 m^3/S (Vishnu et al. 2016).

To study the comparative study on the algal biodiversity of Bharathapuzha and Bhavani rivers in Palakkad district, 10 sites were selected for the collection of algae on the different tributaries of river with an approximately 15 to 20 Km distance were selected. The first 8 stations were chosen from the Bharathapuzha river, followed by stations 9 and 10 from the Bhavani river.

1) **Koodallur (Lat N10° 48' 32.4216'' and Long E76° 7' 1.686'')**

   Koodallur situated at Pattambi taluk, bordering Malappuram district. It was very beautiful and calm Kerala village on the banks of Bharathapuzha river. Range of hills and paddy fields make this village as magic place. The place got the name 'Koodal' by the joining of Nila river with Thuthpuzha. The area is extensively exploited for sand mining. Land filling works also undergoing on this area.

2) **Mayannur (Lat N10° 45' 50.328'' and Long E76° 22' 54.912'')**

   Situated in Kondazhi Panchayat in Palakkad district, the station is about 46 m above sea level. From this site onwards Nila river merge with Gayathripuzha and become more wider and thicker. So, this site also called Koottilmukku. Coconut plantations border the river. The river bottom has plenty of colourful rocks. As a result of sand mining there is considerable degradation of the natural habitat. The household wastes are highly discharged to this area.

3) Chittur (Lat N10° 44' 48.9012'' and Long E76° 39' 50.1732'')

The river flows through Thathamangalam and Chittur areas of Palakkad District. It joins the main river Bharathapuzha near Parli. This site also is known as Sokanasini river, the name was given by Thunjathu Ramanujan Ezhuthachan. The average rainfall in the river basin is 1828 mm. This site also faced the dumping of large number of public wastes and the presence of detergents from cattle washing activities.

4) Chulliyar (Lat N10° 33' 46.631'' and Long E76° 49' 16.463'')

Located in Muthalamada Panchayat of Chittur taluk. Chulliyar dam built across Chulliyar river, a tributary of Bharathapuzha. Illegal quarries in this area seriously affecting the nature of water bodies. This river also faced imminent death due to land use pattern. All these activities receding the water level because of decreasing water flow in the river. Surrounding drainage outlets directly open to this area.

5) Kannadi (Lat N10°44' 48.9012'' and Long E76°39' 50.1732'')

Located in Mathur village of Palakkad. The sampling site near to the extensive building construction. This area seriously faced human activities like dumping of household wastes from the neighbouring construction sites. Illegal sand mining is another threat towards this site. This results many pits in the riverbed which lead to the formation of green carpet of Water Hyacinth and other shrubs.

6) Kalpathy (Lat N10°47' 47.2668'' and Long E76°38' 57.6276'')

Along the banks of the river, a small Tamil Brahmin village located. There are many small temples near banks of the river, they are keeping their distinctive culture, temple and mode of worship intact. The river is named after the

Kalpathi Siva temple in Palakkad which is famous for its festival. Many areas of this river exploited to dumping yard of wastes from surroundings. People from the nearby used for washing and bathing. This area was highly affected by flood.

7) **Malampuzha (Lat N10°48' 16.38'' and Long E76°39' 39.5028'')**

Situated at 10 Km away from the Malampuzha dam. Malampuzha dam is built across the river. A printing press is located near the sampling site. The waste from the press is directly discharged into the river. This makes change in colour of water and emanates bad smell too. During summer, continuous sand mining results in extensive growth of shrubs and water hyacinth.

8) **Karimpuzha (Lat N10°55' 17.508'' and Long E76°25' 14.664'')**

Located at Sreekrishnapuram panchayat, Palakkad. The river that gets its waters from Kuntipuzha, Silent valley national park. The area is extensively exploited for washing.

9) **Mukkali (Lat N11°4' 16.2912'' and Long E76°33' 29.092'')**

A small village located in Agali panchayat of Attappadi block. The area is bounded by thick vegetation. Highly undisturbed place due to lack of developmental projects and human activities. But is affected seriously by flood and landslides.

10) **Seenkara (Lat N11°4' 37.092'' and Long E76°34' 11.676'')**

Situated in Attappadi block, bounded by thick vegetation. The bordered areas have banana cultivation. The area is extensively faced flood in previous years.

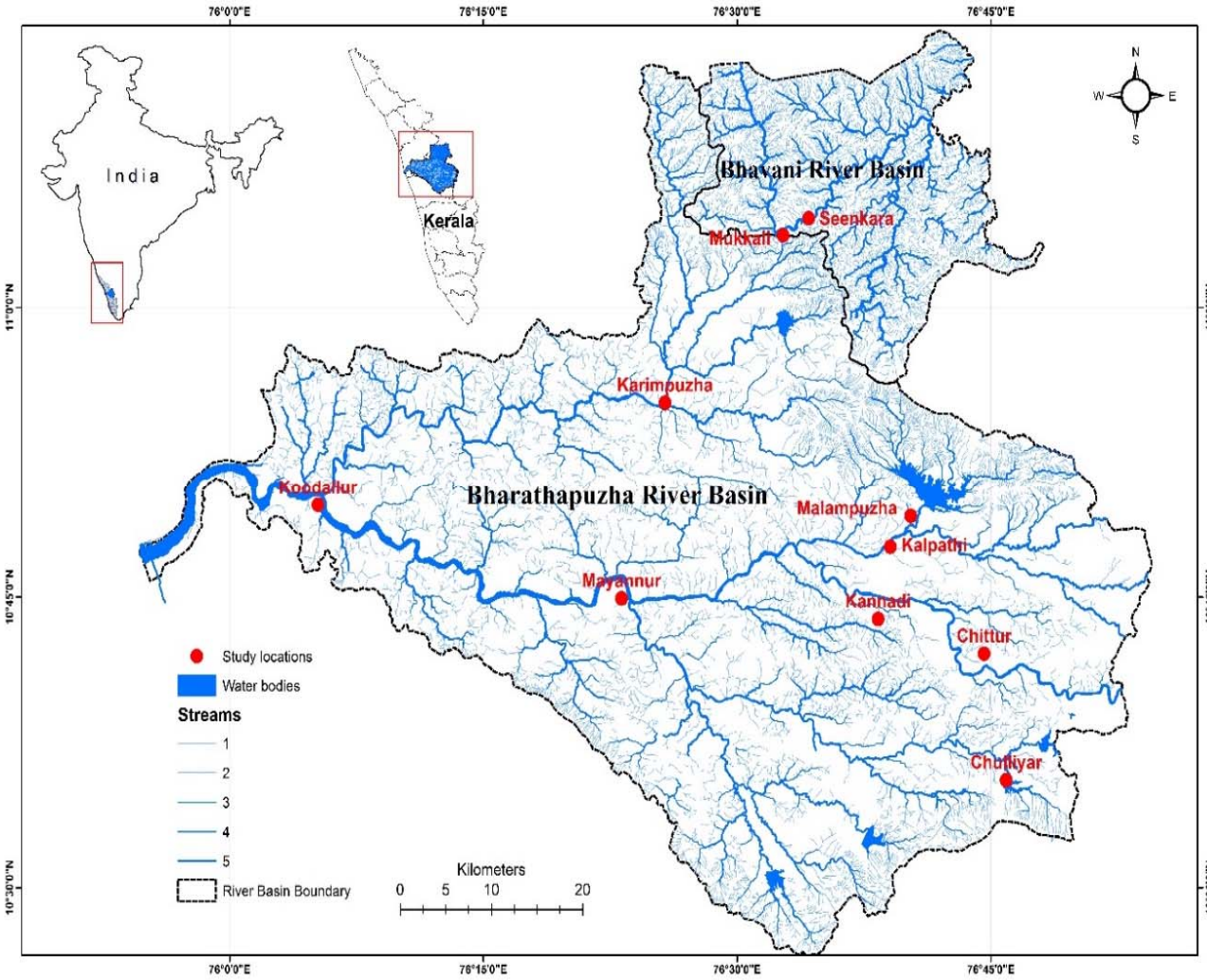

Figure 1. Map of Bharathapuzha and Bhavani river basins in Palakkad district showing sampling sites

1.Koodallur

2. Mayannur

3.Chittur

4. Chulliyar

5. Kannadi

6. Kalpathy

7. Malampuzha

8. Karimpuzha

9. Mukkali

10. Seenkara

Figure 2. Photos of sampling sites from rivers in Palakkad district

3.2 Physico chemical properties of water

Water quality can be defined in terms of physical, chemical and biological characteristics. Natural practices and anthropogenic activities influence the quality of an ecosystem. Systematic and scientific water quality monitoring are required for the analysis of quality of aquatic ecosystems. The occurrence, abundance and structure of the entire biotic community depend on the hydrographic parameters of the riverine ecosystem. Healthy aquatic systems gifted with optimal physico chemical parameters exhibit maximum productivity. A total of 8 different characteristics of water were analyzed in triplicates every month from November 2018 to October 2019. The water samples were collected from 10 study sites between 8.30 and 11.00 a.m. in 2-liter plastic containers and soon carried to the laboratory for analysis without delay. The Physico chemical parameters analyzed were Temperature, pH, Electrical Conductivity (EC), Total Dissolved Solids (TDS) and Dissolved Oxygen (DO) were noted on the spot at the time of collection using standard instruments (digital thermometer, pH pen, EC, and TDS, DO meter). The influence of these parameters together to keeps the aquatic habitat favourable for the existence of life.

3.3 Nutrient Analysis

Study on the distribution and monthly variations of nutrients used as a tool for evaluating the biological status of an ecosystem. The excess nutrient load can be positively correlated with the increased application of fertilizers. For nutrient analysis, water samples collected in polythene bottles are rinsed three times before filling and stored in a refrigerator until nutrient analysis. Samples were analyzed for Nitrate (NO_3), Phosphate (PO_4), and Silicate (SiO_4). Samples were preserved and the methodology for the analysis of the samples as per APHA (1998) and the methodology followed for the study of the models is given in Table 1.

Table 1. Lis of instruments and analytical methods followed for water analysis

Sl. No.	Parameters	Method	Instruments
1	Temperature (^{0}c)	Thermometry	Mercury Thermometer
2	pH	Electrometry	Digital pH meter
3	EC (µmhos/cm)	Electrometry	Digital conductivity meter
4	TDS (µmhos/cm)	Electrometry	Digital TDS meter
5	Nitrate (mg/L)	Colourimetry	UV-visible spectrophotometer
6	Phosphate (mg/L)	Colourimetry	UV-visible spectrophotometer
7	Silicate (mg/L)	Colourimetry	UV-visible spectrophotometer
8	DO (mg/L)	Electrometry	Digital DO meter

The range and the mean along with standard deviation of various physico chemical characteristics of the different stations are studied. It was to test, whether any significant differences in physico chemical factors found between the stations and between months, the data tested with Two-way analysis of variance (ANOVA), using IBM SPSS 21. The values of the various physico chemical parameters were subjected to statistical analysis for the estimation of Pearson correlation coefficient, 'r' using IBM SPSS 21. The significance of correlation coefficient of different parameters was tested at 5% and 1% level.

3.4 Algal taxonomy

Relevant works of literature related to identifying the algal taxa up to the species level were carried out with the help of standard publications, authentic monographs, reference books, articles, and online sources. For the identification of various taxa (Scott and Prescott 1961; Ralfs 1962; Prescott 1982; West and West 1904, 1905, 1907, 1908, 1912; Smith 1920, 1924; Anand 1989, 1998; Prasad and Misra 1992)

were used. Freshwater diatoms of Maharashtra (Sarode and Kamat 1984), Atlas of Diatoms of the Indian Oceans Six Volumes (Desikachary 1959, 1989). Freshwater algal flora of Andaman and Nicobar Islands Vol. I (Prasad and Srivastava 1986). ICAR monograph series on algae, Volvocales (Iyengar and Desikachary 1981), Chlorococcales (Philipose 1967), Cyanophyta (Desikachary 1959), Oedogoniales (Gonzalves 1981; Randhawa 1959; Ushadevi and Panikkar 1994a; Sindhu and Panikkar 1994c, 1995a, 1995b; Sinha and Naik 1997) were used for identification and classification. Taxonomic studies on Euglenophytes (Wolowski 1998; Philipose 1984, 1988) were very helpful in identifying Euglenophytes. The systematic positions of the members of cyanophyceae, chlorococcales, desmids, and bacillariophyceae were arranged according to (Desikachary 1959; Philipose 1967; Scott, and Prescott 1961; Sarode and Kamat 1984) respectively. Electronic reference *viz.* Ph.D. Thesis repositories Science Direct, Shodhganga, and Wiley, were also used. The literature retrieval system Biodiversity heritage library of New York Botanic Garden (https://www.biodiversitylibrary.org) and Digicodes (Digital image collection of desmids).

Algal samples for the systematic analysis were collected from Bharathapuzha and Bhavani river basins in Palakkad district from 10 permanent sampling stations marked in figure 1. Specimens were collected at monthly intervals from these stations of the study area for one year from November 2018 to October 2019 between 8.30 and 11 a.m. Materials are collected in tightly capped plastic bottles and labeled for algal taxonomic studies. One liter water sample from each site was collected and preserved immediately in 4% formalin solution and brought to the laboratory (APHA 1998) at the Department of Botany, St. Thomas' College (Autonomous) Thrissur, Kerala. The temporary slides were prepared from the water samples and observed

under Binocular BIOMED Research Microscope using 4X, 10X, and 100X objectives. In addition, digital photomicrographs were taken for the identification of algal taxa in their original morphology. The taxa were identified with the help of authentic scientific literatures (West and West 1904, 1908; Smith 1920; Venkataraman 1961; Philipose 1967, 1984, 1988; Scott and Prescott 1961; Iyengar and Desikachary 1981; Prescott 1982; Sarode and Kamat 1984; Desikachary 1989; Prasad and Misra 1992; Prasad and Srivastava 1986; Wolowski 1998; Anand 1998; Komarek 2013 and ICAR monograph series on algae (Desikachary 1959). To the identification of the members of Bacillariophyceae, a portion of the concentrated sample was cleaned using concentrated hydrochloric acid, sulphuric acid and potassium permanganate crystals.

3.5 Quantitative analysis of phytoplankton

Phytoplankton were collected from 10 sampling sites were counted under the microscope using the Sedgewick-Rafter counting chamber (Trivedy and Goel 1984). The S-R cell with a rectangular cavity of 1 ml (1 cm^3) is used for the study. The S-R cell was covered with a cover slip without the air bubbles inside. The phytoplankton was counted after the plankton settled down under a compound microscope.

3.6 Community structure

The algal community structure was compared using species richness and evenness, as well as proportional statistics that combined both measures (Shannon-Weiner index). Correspondence analysis is the better algorithm for comparing associations containing counts of taxa across associations.

3.6.1 Diversity Indices

The ecological diversity indices such as species richness (Margalef), Pielou's evenness, Shannon-Weiner index (H) and Simpson's dominance index (1-D) of the

phytoplankton were performed using PAST 3.18 (Paleontological Software Package) in different stations and months with respect to species composition and abundance (Hammer et al. 2001).

3.6.2 Multivariate similarity analysis

Multivariate analysis of ecological data by Canonical Correspondence Analysis (CCA) and Hierarchical Cluster Analysis (HCA) were conducted with the help of multivariate ecological statistical software PAST 3.18 (Hammer et al. 2001) to study on the relationship between physicochemical parameters structure of an algal community. The analysis was carried out to determine the degree of similarity between stations and months in terms of species composition and abundance. The main goal of CCA was to identify the most important physicochemical parameters influencing phytoplankton distribution.

3.7 Phytoplankton as pollution indices

Pollution indices are widely used for the monitoring of qualitative status of water bodies. In the present study, two indices are used to get the biological information based on algae for the assessment water quality-Palmer's pollution index and Boyd's diversity index. Palmer (1969) developed palmer algal genus index to identify and prepare a list of 60 genera and 80 species of algae tolerant to organic pollution. Each genera assigned a particular number based on their tolerance action. He formulates a pollution index scale by adding the number for the assessment of organic pollution. The score value above 20 indicates heavy organic pollution, 15 to 20 represents moderate organic pollution and below 15 means very light pollution.

The diversity index of (Boyd 1981) indicates the order of pollution of water bodies. Number of genera of phytoplankton in the water bodies is the main parameter for calculating the index by the following formula $H = (S–1) / \ln N$.

where H = Boyd's diversity index, S = number of genera of phytoplankton, ln is the natural logarithm and N = total number of phytoplankton. The values > 4 indicates less pollution of water, values 2–3 are characterized as moderate pollution and values < 1 are considered as heavily polluted.

Quality of an aquatic ecosystem is dependent on the physico chemical qualities of water on the biological diversity of the system. The analysis of biological parameters along with chemical factors of water forms a valid method of water quality assessment. Chemical analyses of water provide a good indication of the quality of the aquatic systems.