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Biodiversity of cyanobacteria in fresh water ponds of Pudukkottai district, Tamil Nadu, India

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ABSTRACT

Cyanobacteria notably called blue-green algae mostly inhabit moist soils and water. These species constitute a major part of the phytoplanktonic biomass in freshwater ponds. cyanobacteria serve as the significant resource in various applications like medicine, mariculture, feed, fuel, and in combating pollution. Cyanobacterial biodiversity provides various useful insights and is considered an important ecological parameter in freshwater aquaculture. The present research work aims to study the biodiversity of cyanobacteria among 20 different freshwater ponds in Pudukkottai district, Tamil Nadu, India. The samples were collected and pure culture was obtained, followed by maintenance in the BG-11 medium. The species were identified and classified based on the size, shape, and color (morphological features) of the blue-green algae using a trinocular microscope. The physicochemical characteristics such as pH, temperature, biological oxygen demand, chemical oxygen demand, etc., of the freshwater ponds were also studied as they greatly influence the cyanobacterial biodiversity. The abundance of cyanobacteria was seen in a low amount of dissolved oxygen, pH of 8.0 with high oxidizable organic content. About 42 distinct cyanobacterial species were isolated consisting of 25 versatile families of cyanobacteria. Chlorophyceae was found to be predominantly present in the fresh water ecosystem, followed by Cyanophyceae, Bacillariophyceae, Ulvophyceae, and Dinophyceae. The present study revealed the biodiversity of blue-green algae from the fresh water ponds of Pudukkottai District which holds as the baseline data for the more detailed studies in future.

1. INTRODUCTION

The freshwater ecosystem comprises both ponds and lakes in which freshwater ponds constitute a variety of plants, phytoplanktons, aquatic animals, and prokaryotes [1]. The elements in freshwater ponds were mostly dependent on one another for their survival in the environment [2–4]. The blue-green algae (cyanobacteria) were prevalent in freshwater ponds and they maintain the biological balance and water quality. They also possess the capacity to perform carbon assimilation and N_2 fixation and also secrete numerous biologically active substances, ultimately improving the productivity of the environment [5,6]. Particular species of cyanobacteria in the genera *Anabaena*, *Aphanizomenon*, *Microcystis*, and *Oscillatoria* were often formed persistent and extensive blooms in the aquaculture ecosystem [7,8]. Usually, cyanobacteria grow in close association with the number of microorganisms, which include eubacteria, fungi, and protozoans [9,10]. Cyanobacteria play a vital role in converting atmospheric nitrogen into organic forms, such as nitrate or ammonia, and they also perform photosynthesis and ultimately release oxygen as a byproduct which other plants could utilize for growth and survival [11,12] and act as the food source for other organisms like zooplanktons, insects, and snails [13]. Research has shown that only very few species belonging to cyanobacteria were commercially exploited [14,15]. Hence, a detailed research study was necessary on the biodiversity of cyanobacteria to figure out the role of cyanobacteria in the fresh water ponds; also, the diversity profile of cyanobacteria would reveal the extent of pollution and anthropogenic activities in the ecosystem. Various studies on blue-green algae present that the species residing in freshwater ponds include *Microcystis* sp., *Cylindrospermopsis raciborskii*, *Synechococcus* sp., *Planktothrix agardhii*, *Gloeotrichia* sp., *Anabaena* sp., *Lyngbya* sp., *Nostoc* spp., *Oscillatoria* spp., *Schizothrix* sp., and *Synechocystis* sp.

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[19–21]. The physicochemical parameters of the pond water (PW) were also studied as the population diversity hugely depends on the living environment [22]. An in-depth literature survey reveals that no physiological work has been accomplished in the fresh water ecosystem of Pudukkottai district, Tamil Nadu, India. This research work presents the isolation and characterization of different types of cyanobacteria in freshwater ponds of Pudukkottai district, Tamil Nadu, India, and the physical and chemical parameters of the fresh water ponds.

MATERIALS AND METHODOLOGY

2.1. Chemicals and Reagents

BG-11 medium was purchased from Sigma-Aldrich, USA; agar powder, sodium chloride (NaCl), and silver nitrate ($AgNO_3$) were purchased from SRL Company, India, and Fisher Scientific, USA, respectively; sulfuric acid (H_2SO_4) was bought from Merck, USA; methyl red was purchased from SRL Company, India [25].

2.2. Sample Collection and Maintenance

The water samples were collected from fresh water ponds in Pudukkottai district, which is located in Tamil Nadu, India. The concentrated PW samples were collected using plankton net in sterilized bottles of 500 ml capacity at a depth of about 30 cm. After the collection of samples, it was viewed in cavity slide using a trinocular microscope (Labomed Vision 2000 microscope) by wet mount method to identify the different species present in the fresh water ecosystem. This was followed by inoculating the culture in liquid BG-11 medium. Later, the mixed culture was spread in agar plates containing BG-11 medium and the individual colonies were isolated. The mass culture of each colony was carried out by taking a loopful of distinct colonies from agar plates and transferring it to the conical flasks. This process was repeated until axenic cultures were obtained [25].

2.3. Morphological and Species Identification of cyanobacteria

Morphological identification of cyanobacteria was accomplished by spreading an isolated pure culture on glass slides with the help of forceps. The cultures were covered with glass cover slips and their size, shape, color, and other features were observed under low (10×) and high power (100×) objective lens of the trinocular microscope (Labomed Vision 2000 Microscope). Numerous species of cyanobacteria present in fresh water ponds of Pudukkottai district were identified and confirmed using the book written by T.V. Desikachary [16] and also with the help of the "Manual of Freshwater Algae of Tamil Nadu" [17].

2.4. Physical Parameters

All the physical parameters were recorded during the time of collection directly at the site. The pH and temperature were measured using Labline digital pH meter and digital probe thermometer. The color of the sample was determined by a visual comparison of fresh PWs. Total dissolved solids (TDS) and electrical conductivity (EC) values were measured using the RP Scientific TDS and EC meter. Similarly, the turbidity of PW samples was tested using Labtronics Digital Turbidity Meter [18].

2.5. Chemical Parameters

2.5.1. Dissolved oxygen (DO)

The titration method was used to estimate the dissolved oxygen (DO). $MnSO_4$ (1 ml) and alkaline iodide (1 ml) reagent were mixed, which formed a flocculent precipitate. 1.0 ml of conc. H_2SO_4 was added to the precipitate. 50 ml of this solution was transferred to a conical flask and titrated against 0.025 N of $Na_2S_2O_3$ till the color of the solution turned pale yellow. Then, the starch solution (1 ml) was added to give a blue color and the titration was completed by making it into a colorless solution using the following formula:

DO (mg/l) = No. of ml of $Na_2S_2O_3$ solution × 4 [18].

2.5.2. Free carbon dioxide (CO_2)

Exactly, 100 ml water sample was added to the phenolphthalein indicator of two to three drops and then titrated against 0.05 N sodium hydroxide, until a pink color appeared. The free CO_2 was calculated using the following formula:

$$\text{Free } CO_2 \text{ (mg/l)} = \frac{(BR \times n \times 44 \times 1{,}000)}{\text{amount of sample taken (ml)}}$$

where,

B.R. = Burette reading (amount of titrant used);

N = Normality of sodium hydroxide;

44 = Equivalent weight of CO_2 [18].

2.5.3. Biochemical oxygen demand (BOD)

The diluted water sample was prepared by bubbling compressed air in distilled water for about 30 minutes. Ferric chloride solution was added with the diluted water sample and mixed thoroughly with every 1 ml of phosphate buffer, magnesium sulfate, and calcium chloride. Using 1 N NaOH and H_2SO_4, the sample was neutralized to adjust the pH at 7.0. As the DO in the sample was likely to be exhausted, appropriate dilution of the sample was prepared with respect to the expected BOD range. Two sets of dilutions were prepared in a large glass and filled in the BOD bottles in a trough and the contents were mixed thoroughly. One set of the bottle was kept in the BOD incubator at 27°C for 3 days, and the DO content in another set was measured immediately. For blanks, two BOD bottles were filled with the diluted water sample. The DO content was determined immediately in one of the bottles and the other bottle was incubated and the DO content was determined after 3 days using the following formula:

BOD (mg/1) = $(D_0 - D_3)$ * dilution factor

where D_0 = Initial D_0 in the sample and $D_3 = D_0$ after 3 days [19].

2.5.4. Chemical oxygen demand (COD)

Exactly, 20 ml of the sample was taken in a 250–500 ml COD flask. Exactly, 10 ml of copper sulfate solution was added along with a pinch of Ag_2SO_5 and $HgSO_4$, followed by 30 ml H_2SO_4. The contents were then refluxed on a hot plate for at least 2 hours. The flask was removed, cooled, and distilled water was added to make the final volume to about 140 ml. Two to three drops of

ferroin indicator were mixed thoroughly and titrated with 0.01 N (confirm) ferrous ammonium sulfate. At the end point, the blue-green color of contents will be changed to reddish blue. The blank was run simultaneously using distilled water in a similar manner using the following formula:

$$\text{COD (mg/l)} = \frac{(B–S) \times N \times 8 \times 1{,}000)}{\text{sample volume in ml}}$$

where,

B = volume of titrant used in blank;

S = titrant volume of used in sample;

N = strength of the titrant [19].

2.5.5. Estimation of nitrogen, phosphorous, and potassium (NPK)

Estimation of NPK was carried out by the kit procedure (Agrinex Soil Doctor — Rapid N-P-K Testing Kit). Briefly, the water sample was mixed with double distilled water in 1:2 ratio and mixed thoroughly. The sample was kept for 30 minutes for clear water separation. NPK was estimated by taking 5 ml of the PW sample and mixing it with Doctor-N, Doctor-P, and Doctor-K capsule respectively. Only for phosphorus estimation, four drops of trichloroacetic acid (TCA) reagent were added carefully and mixed well. The solution was mixed thoroughly, until the chemical was dissolved. The tube was kept at room temperature for 20 minutes for color development. The color formation was referred with the given reference chart to find out the concentration of the sample [19].

3. RESULTS

3.1. Species Isolation and Identification of cyanobacteria in Fresh Water Ponds

Cyanobacteria population biodiversity in fresh water ponds of Pudukkottai district were identified and characterized. The result concludes that 42 versatile species of 25 different families of cyanobacteria has been distributed in the 20 different fresh water ponds of Pudukkottai district, Tamil Nadu, India. Each and every species has its own significance, varying in size, shape, and color, which distinguishes them to be classified under different families. Among the PWs surveyed, it was seen that PW 5 and PW 6 contain a huge variety of cyanobacterial species belonging to Oscillatoriaceae and Microcystaceae family. Table 1 shows the species isolated from 20 different fresh water ponds. Figure 1 shows the 42 isolated species from the fresh water ecosystem. PW 13 has shown not even a single cyanobacterial population, whereas PWs 2, 4, 7, 8, 10, 11, 12, 14, 19, and 20 were found to contain a single species of cyanobacteria. The other PWs showed fewer cyanobacterial species. The majority of the algal species belonged to the families Oscillatoriaceae, Nostocaceae, Microcystaceae, Scenedesmaceae, and Desmidiaceae. Table 2 shows the family classification of isolated cyanobacterial species in fresh water ponds.

3.2. Physicochemical parameters of fresh water ponds

After the isolation and identification of cyanobacteria, the physicochemical parameters of the ponds were examined as the cyanobacterial biodiversity greatly depends on it. The physical parameter analysis of the fresh water ponds showed that the pH was in the range of 5.3–8.0, temperature varied from 84.5 to 86.6°F, TDS values differed between 87 and 2,655 mg/l, turbidity and EC were in the range of 0.08–0.7 nephelometric turbidity unit and 158–1,433 µmhos/cm. In case of chemical parameters surveyed, DO varied from 2.1 to 10.25 mg/l, free CO_2 ranged from 0 to 9.5 mg/l, the values of NPK lied in the range of 0.2–0.4 mg/l, 0.15–0.5 mg/l, and 0.05–0.2 mg/l, respectively. Also, the BOD and COD values were found to vary from 5.36 to 26.75 mg/l and 12.24 to 48.73 mg/l, respectively. Tables 3 and 4 show the physical and chemical parameters of fresh water ponds, respectively.

4. DISCUSSION

The physical and chemical changes in the environment might influence a particular species and could also induce its growth and abundance in the environment in which they survive [23,24]. The parameters that influence the growth of cyanobacteria in the fresh water ponds include oxidizable matter, nitrogenous content, light intensity, and dissolved oxygen [25–30]. Also, the water level fluctuations might lead to an increase or decrease in the cyanobacterial species diversity. In the present research work, the samples were collected to analyze the biodiversity of cyanobacteria and the dominant species growing in fresh water ponds were noted. Among the 20 different PWs studied, it was seen that PW 5 and PW 6 hold many different species of cyanobacteria and the results may be attributed to the fact that the environment showed high pH values (alkaline = 8.0) and low DO content (2.1 and 2.95 ppm, respectively) compared to all other fresh water ponds having 22.76 and 38.62 mg/l for PW 5 and 21.27 and 32.94 mg/l for PW 6 of BOD and COD values, respectively. The pH value for all the PWs except PW 13 ranged from neutral to slightly alkaline, which lies within the values preferred by the WHO (6.5–8.5) [31]. Neutral to alkaline pH was found to be very suitable for the growth of cyanobacteria [30]. The increased values of BOD, COD, phosphates, and nitrates with low DO and alkaline pH range favored the growth of cyanobacteria compared to any other algae in the ecosystem [32–34]. PW 13 does not contain any cyanobacterial population that could be due to the high acidic nature of PW (pH 5.3), i.e., the reports claim no cyanobacterial population will exist in pH 5–5.5 and the researchers also studied the influence of water quality on cyanobacterial population growth [35,36]. These results conclude that the species growth increases with an increase in pH and BOD, and a decrease in DO values. The temperature was recorded in the range of 84°F–86°F (28°C–30°C) at the time of collection of samples and it was found to be the optimal temperature for the growth of cyanobacterial species [37,38]. The EC is the ability of PW to conduct current which purely depends on the ionic strength of the water ecosystem and it was seen that the values for all the PWs lie within the prescribed limit, i.e., 20–1,500 µmhos/cm [39]. However, the prescribed EC value given by the WHO is 250 µmhos/cm [31]. Total dissolved solid values represent the amount of dissolved substances and their values ≤600 mg/l was regarded as better water quality. Also, the natural waters tend to have values from 30 mg/l to 6,000 mg/l [31]. The presence of adequate amounts of NPK is essential for the cyanobacterial blooms to persist in

Table 1. Cyanobacterial species isolated and classified from 20 different fresh water ponds in Pudukottai district.

S.NO	PW	Name in Pond Place	Longitude and Latitude	Name of algae
1.	PW 1	Thirumalai chamuthiram-Kathirkameshvarar temple.	10˚21′19″ N, 78˚49′42″ E	*Anabaena spbaerica*
				A. arnoldii (aptekarj),
				M. aeruginosa
2.	PW 2	Pudukkottai-Santhanatha swami temple	10.3802˚ N, 78.8135˚ E	*Gloeocapsa nigrescens*
3.	PW 3	Peraiyur-naganathar swami temple.	10.3528˚ N, 78.7564˚ E	*C. turgidus*
				M. robusta
4.	PW 4	Kulathur-Varatharaja Perumal temple.	10.7002892˚ N, 78.5470346˚ E	*A. platensis*
5.	PW 5	Pudukkottai-Sri Brahadhambal temple	10.3915˚ N, 78.8005˚ E	*O. princeps*
				Anabena iyengarii
				N. linckia
				Microsera wollei
				L. aestuarii
				L. majuscula
				O. nigra
				O. curviceps
6.	PW 6	Thiruvarankulam-Arangulanathar temple	10.3561˚ N, 78.8737˚ E	*M. robusta*
				M. aeruginosa
				M. flos-aquae
				M. robusta
				Navicula capitatoradiata
				M. marginata
7.	PW 7	Kumaramalai-Balathandayuthabani	10˚21′53″ N, 78˚43′39″ E	*H. lacustris*
8.	PW 8	Thirumayam-sathyagireeswarar temple	10.2471˚ N, 78.750481˚ E	*S. jurassica*
9.	PW 9	Thirumayam-Kottai sathyamoorthy perumal temple	10.2471˚ N, 78.7508˚ E	*Merismopedia glauca*
				A. pulchra (kutz)
				M. punctata Meyen P. simplex (meyen)Lemm
10.	PW 10	Pudukkotai-Chellayiamman temple.	10˚22′33″ N, 78⁰49′12″ E	*S. subsalsa*
11.	PW 11	Keeranur-Lord Iyyappan temple	10.571564˚ *N*, 78.784416˚ E	*Cylindrospermum stagnale*
12.	PW 12	Alankudi-Chithivinayagar temple	10.37˚ N, 78.89˚ E	*C. lunula (Mull) F. crotomensis (Kittion)*
13.	PW 13	Vadavaalam-Kaliyuga meyya ayyanar temple	10˚25′37″ N, 78˚53′58″ E	Nil
14.	PW 14	Thirumanancheri-Periyanayaki ambika samayathaya suganthi parimaleshwarar temple	11.099957˚ N, 79.556036 E	*C. hirundinella*
15.	PW 15	Malaiyur-Periyanayaki amman temple	10.359119 N, 78.985173 E	*C. humicola*
16.	PW 16	Varappur-Agathishwarar temple	10˚21′2″ N, 78˚28′42″ E	*Ulothrix sp. F. crotomensis (Kitton) Synedra dorsiventralis (ehrenberg) Westella linearis GM smith*
17.	PW 17	Ichadi-Vinayagar temple	10.4254˚ N, 78.8800˚ E	*C. geminella C. pediculus Calothrix sp C. glomerata (L.)*
18.	PW 18	Sempattividuthi-Vinayagar temple	10.04288˚ N, 78.9776˚ E	*S. acuminatus*
				S. denticulatus (lagerheim)
				S. vijugatus
				Cosmorium subprotumidum S. quaquadricauda
19.	PW 19	Ramachandrapuram-Ramar temple	10.244036˚ N, 78.747511˚ E	*N. calcicola*
20.	PW 20	Thuvar-Koothandaramar temple	10˚34′17″ N, 79˚0′57″ E	*S. subsalsa*

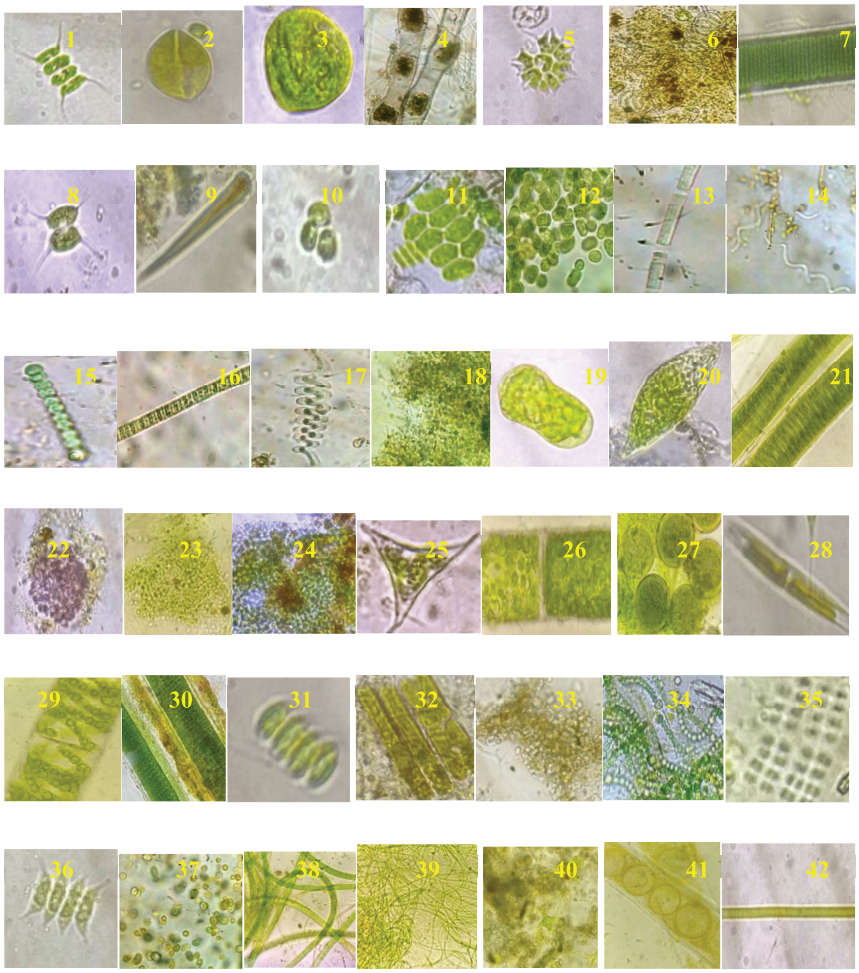

Figure 1. Slide 1. *Scenedesmus quadricauda*; Slide 2. *Cocconeis pediculus*; Slide 3. *Haematococcus lacustris*; Slide 4. *Cylindrocapsa geminella*; Slide 5. *Pediastrum simplex* (Meyen) Lemm; Slide 6. *Arthrospira platensis*; Slide 7. *Oscillatoria curviceps*; Slide 8. *Anabaenopsis arnoldii (*aptekarj); Slide 9. *Calothrix*; Slide 10. *Chroococcus turgidus*; Slide 11. *Scenedesemus arcuatus*; Slide 12. *Scendesmus vijugatus*; Slide 13. *Symploca jurassica*; Slide 14. *Spirulina subsalsa*; Slide 15. *Nostoc calcicola*; Slide 16. Oscillatoria nigra; Slide 17. *A. platensis*; Slide 18. *M. aeruginosa*; Slide 19. *Microcystis marginata*; Slide 20. *M. marginata*; Slide 21. *Lyngbya majuscula*; Slide 22. *M. aeruginosa*; Slide 23. *M. aeruginosa*; Slide 24. *Microcystis flos-aquae*; Slide 25. *Staurastrum pantanale sp. nov*; Slide 26. *Cladophora glomerata* (L.); Slide 27. *Chlorococcum humicola (Naeg ravenhorst)*; Slide 28. *Closterium lunula (Mull)*; Slide 29. *Spirogyra subsalsa (Kuetzing)*; Slide 30. *Lyngbya aestuarii*; Slide 31. *Scendesmus denticulatus (lagerheim)*; Slide 32. *Fragilaria crotomensis (Kitton)*; Slide 33. *Microcystis robusta*; Slide 34. *Nostoc linckia*; Slide 35. *Merismopedia punctata Meyen*; Slide 36. *Scenedesmus acuminatus*; Slide 37. *Aphanocapsa pulchra (kutz)*; Slide 38. *Lyngbya wollei*; Slide 39. *Ulothrix*; Slide 40. *Ceratium hirundinella*; Slide 41. *L. majuscule*; and Slide 42. *Oscillatoria princeps.*

PWs [40]. Depletion of free CO_2 in PWs might trigger harmful cyanobacterial blooms as a result of increased influx of CO_2 due to elevated concentration gradient in the surface water [41]. Hence CO_2 also plays a major role in controlling the growth of cyanobacteria. The free CO_2 values for all the PWs were found to be less than 10 mg/l. Many researchers attempted to study various fresh water ponds in different localities and isolated distinct cyanobacterial species. In the present observation of percentage distribution of cyanobacteria, Chlorophyceae holds the top position having 40%, followed by Cyanophyceae 36%, Bacillariophyceae 16%, Ulvophyceae 4%, and Dinophyceae 4%, i.e., the number of families belonging to the above-said classes were 10, 9, 4, 1, and 1 out of 25 families, respectively. The predominant distribution of Chlorophyceae, followed by Cyanophyceae, was also reported by researchers who studied the phytoplankton diversity in perennial lakes of Coimbatore [29]. Figure 2 shows the percentage composition of cyanobacterial diversity in 20 different fresh water ponds of Pudukkottai district. The algal flora community from temple tanks in Chennai city, Tamil Nadu, India, showed 17 species of algae isolated from the tanks and classified under four different families, including Cyanophyceae, Bacillariophyceae, Chlorophyceae, and Euglenophyceae [42]. The biodiversity and the relationship between the abundance of cyanobacteria concludes that the high concentration of dissolved organic matter occurred as a result of oxygen depletion [43]. The dominant species reported by Okogwu includes *Microcystis aeruginosa*, *Aphanizomenon flos-aquae*, *Oscillatoria limnetica*, and *Anabaena spiroides*, whereas the current study also shows the abundance of *Microcystis* sp. and *Oscillatoria* sp.in the fresh water ecosystem. *Microcystis*,

Table 2. Family classification of identified cyanobacterial species from fresh water ponds.

S. No.	Class	Name of family	Microalgae
1.		*Desmidiaceae*	*Cosmarium quadrum lund*
			C. subprotumidum
			Cosmorium subalatum
			Cosmorium sexnotatum
			S. pantanale sp.nov
2.		*Selenastraceae*	*Kircbneriella obesa*
3.		*Haematococcaceae*	*H. lacustris*
4.		*Closteriaceae*	*C. lunula (Mull)*
5.	*Chlorophyceae*	*Ulotrichaceae*	*Ulothrix sp.*
6.		*Scenedesmaceae*	*Westella linearis GM smith*
			S. acuminatus
			S. denticulatus (lagerheim)
			S. vijugatus
			S. quadricauda
7.		*Zygnemataceae*	*S. subsalsa (Kuetzing)*
8.		*Chlorococcaceae*	*C. humicola*
9.		*Hydrodictyaceae*	*P. simplex (Meyen) Lemm*
10.		*Incertae sedis*	*C. geminella*
11.	*Cyanophyceae*	*Rivulariaceae*	*Calothrix sp.*
12.		*Aphanizomenonaceae*	*A. arnoldii (aptekarj)*
13.		*Chroococcaceae (Nageli)*	*C. turgidus*
			G. nigrescens
14.		*Microcoleaceae*	*A. platensis*
			S. jurassica
15.		*Merismopediaceae*	*M. glauca*
			A. pulchra (kutz)
			M. punctata Meyen
16.		*Spirulinaceae*	*S. subsalsa*
17.		*Microcystaceae*	*M. aeruginosa*
	Cyanophyceae		*M. marginata*
			M. flos-aquae
			M. robusta
18.		*Nostocaceae*	*N. linckia*
			N. calcicola
			C. stagnale
			A. spbaerica
			A. iyengarii
19.		*Oscillatoriaceae*	*O. princeps*
			L. majuscula
			M. wollei
			L. aestuarii
			O. nigra
			O. curviceps
20.		*Bacillariaceae*	*Nitzschia obtusa*
21.		*Cocconeidaceae*	*C. pediculus*
22.	*Bacillariophyceae*	*Fragilariaceae*	*F. crotomensis (Kitton)*
			Synedra dorsiventralis (ehrenberg)
23.		*Naviculaceae*	*N. capitatoradiata*
24.	*Dinophyceae*	*Ceratiaceae*	*C. hirundinella*
25.	*Ulvophyceae*	*Cladophoraceae*	*C. glomerata (L.)*

Table 3. Physical parameters of fresh water ponds.

Sample	pH	Temp (°*F*)	TDS (mg/l)	Turbidity (NTU)	EC (µmhos/cm)	Odor	Color
PW 1	6.7	85.5	87	0.17	158	No	Colorless
PW 2	6.9	86.05	605	0.10	953	No	Colorless
PW 3	6.9	85.25	457	0.09	746	No	Colorless
PW 4	6.7	85.8	339	0.15	553	No	Pale yellow
PW 5	8.0	85.4	323	0.42	284	No	Green
PW 6	8.0	85.4	524	0.11	354	Odor	Pale yellow
PW 7	7.0	84.95	193	0.54	344	Mid odor	Yellow
PW 8	6.6	85.35	585	0.36	927	Odor	Yellow
PW 9	7.4	84.95	1503	0.13	1065	No	Colorless
PW 10	7.5	85.65	1914	0.70	1433	Odor	Yellow
PW 11	6.8	85.45	524	0.05	839	Odor	Yellow
PW 12	6.6	85.65	2655	0.10	1380	Odor	Yellow
PW 13	5.3	85.4	413	0.64	626	No	Green
PW 14	6.8	84.8	411	0.08	716	No	Colorless
PW 15	6.5	86.2	1352	0.26	934	Odor	Green
PW 16	6.9	84.55	1281	0.63	865	Odor	Green
PW 17	6.7	86.2	95	0.28	186	No	Yellow
PW 18	6.9	84.8	742	0.11	1235	Odor	Dark green
PW 19	6.8	86.65	525	0.08	841	Mid odor	Light green
PW 20	6.5	86.05	522	0.55	839	Odor	Pale yellow

Table 4. Chemical parameters of fresh water ponds.

Chemical parameters	DO (mg/l)	FreeCO_2 (mg/l)	N (mg/L)	P(mg/l)	K(mg/l)	BOD (mg/l)	COD (mg/l)
PW 1	9.875	4.5	0.30	0.25	0.150	26.75 ± 3.45	45.00 ± 8.64
PW 2	8.05	2.5	0.40	0.35	0.150	8.93 ± 6.70	12.24 ± 5.23
PW 3	8.65	7.5	0.30	0.45	0.050	23.63 ± 2.65	45.73 ± 1.46
PW 4	4.9	5.5	0.30	0.35	0.150	17.82 ± 2.32	32.74 ± 2.95
PW 5	2.1	4.5	0.30	0.25	0.150	22.76 ± 4.61	38.62 ± 8.37
PW 6	2.95	3.5	0.30	0.35	0.50	21.27 ± 2.37	32.94 ± 1.72
PW 7	9.975	2.5	0.40	0.25	0.10	19.76 ± 1.54	41.34 ± 3.51
PW 8	3.05	0	0.30	0.45	0.150	7.28 ± 2.15	38.86 ± 1.52
PW 9	5.2	0	0.30	0.5	0.10	8.26 ± 7.34	28.46 ± 5.37
PW 10	6.2	9.5	0.30	0.45	0.50	5.36 ± 1.37	18.85 ± 2.01
PW 11	3.2	2.5	0.40	0.35	0.10	15.05 ± 3.85	23.10 ± 1.61
PW 12	8.7	6.5	0.30	0.5	0.20	27.87 ± 7.72	48.73 ± 8.38
PW 13	10.25	2.5	0.40	0.5	0.150	8.20 ± 1.63	17.63 ± 2.95
PW 14	4.9	3	0.40	0.5	0.20	12.85 ± 2.75	28.83 ± 1.95
PW 15	6.75	3.5	0.40	0.5	0.150	10.23 ± 9.54	24.74 ± 6.94
PW 16	8.05	1.5	0.40	0.5	0.100	26.84 ± 2.53	45.85 ± 1.65
PW 17	4.2	2.5	0.30	0.25	0.20	10.63 ± 2.95	22.73 ± 8.93
PW 18	6.9	6.5	0.30	0. 3	0.100	23.97 ± 1.65	47.57 ± 5.97
PW 19	7.5	5.5	0.20	0.15	0.150	12.64 ± 3.56	26.67 ± 1.95
PW 20	8.96	3.5	0.20	0.25	0.10	17.57 ± 1.48	36.94 ± 7.46

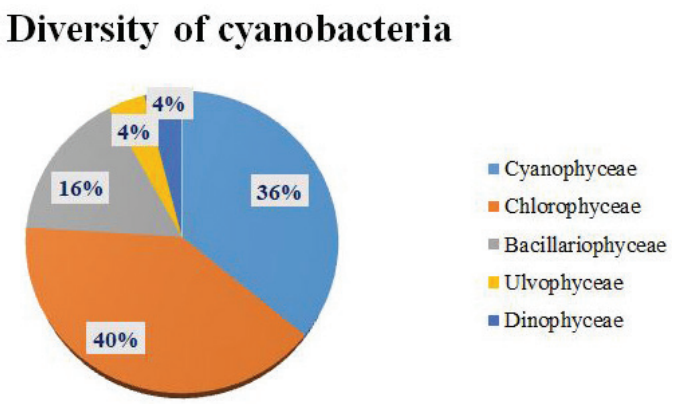

Figure 2. Percentage distribution of cyanobacteria from 20 different fresh water ponds in Pudukkottai district, Tamil Nadu, India.

particularly *M. aeruginosa*, is associated with permanent algal blooms in the fresh water ecosystem and also indicator of organic pollution. The dominance of cyanobacteria was due to their ability to grow in turbid water and also with low light intensity which helps in maintaining the buoyancy [25]. Furthermore, they possessed the capacity to grow exponentially in the wet period where the nitrogenous nutrients were found to be rich. In this present study, families like Naviculaceae, Microcystaceae, Scenedesmaceae, and Fragilariaceae were the representation of eutrophication and anthropogenic activities to a minimal extent. The present research work thoroughly analyzed the biodiversity of cyanobacteria in fresh water ponds of Pudukkottai district; furthermore, physiological, biochemical, and molecular level studies could be beneficial to understand the biodiversity of cyanobacteria in depth and its impact in the fresh water ecosystem.

5. CONCLUSION

Many researchers studied the biodiversity of cyanobacteria in fresh water ponds and no such work has been carried out in the fresh water aquaculture in Pudukkottai district, Tamil Nadu, India. Also, this work highlighted the diversity of cyanobacteria in 20 different PWs by identifying approximately 42 distinct species of cyanobacteria of 25 different families. This paper also showed the physicochemical parameters influence on the species growth in the fresh water ecosystem. Hence, this work could be efficiently used as the basic research study in the analysis of cyanobacterial biodiversity in the fresh water ecosystem.

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7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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There is no funding to report.

9. CONFLICT OF INTEREST

The authors declare no conflicts of interest.

10. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

11. LIST OF ABBREVIATIONS

TDS	Total dissolved solids
EC	Electrical conductivity
PW	Pond water
CO_2	Carbon-di-oxide
DO	Dissolved oxygen
BOD	Biological oxygen demand
COD	Chemical oxygen demand
NPK	Nitrogen, phosphorous and potassium
NTU	Nephelometric turbidity unit

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