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Assessment of Bacterial Load in the Fresh Water Lake System of Tamil Nadu

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A B S T R A C T

Microbial study was done on two freshwater systems located in Viluppuram District of Tamil Nadu. Total bacterial density ranged from 6.2 - 14.4×10^7 cfu/lit in Mukaiyur lake and from 5.4 - 8.6×10^6 cfu/lit in Tirukoilur lake. Maximum Total Bacterial Density was noticed in November in both systems. Total coliform ranged from 165 - 1635/100 ml in Mugaiyur lake and from 315 - 521/100 ml in Thirukoilur lake. Mukaiyur lake recorded 22 bacterial species belonging to 16 genera, while Thirukoilur lake recorded 28 species belonging to 18 genera. In both the systems there were 11 bacterial perennial species occurred during study period.

Introduction

Microorganisms are characterized by tremendous metabolic versatility. Many important ecosystem processes rely entirely on microbial activities (e.g. nitrogen fixation). Moreover, viruses can cause bacterial diversification through gene transfers 'Weinbauer and Rassoulzadegan - 2004' which in turn, has the potential to influence ecosystem functioning and the services mediated by microorganisms.

Eventhough great strides have been made in describing microbial diversity by means of modern analytical methods and a range of essential microbial-mediated processes has also been measured. Most assessments of microbial community structure and the activities that impact ecosystem functioning have been examined outside the conceptual framework of ecology and, with few exceptions, relationships between microbial biodiversity and ecosystem functioning have not been explicitly addressed.

Given the many competitive and facilitative interactions known among bacteria, changes in their biodiversity potentially have enormous scope to affect ecosystem processes (Heard and Richardson, 1995). Hence the present study was attempted to analyse the bacterial diversity in a freshwater system located in Villuppuram District, Tamil Nadu.

Materials and Methods

The freshwater systems chosen for the present study are Mugaiyur lake and Tirukoilur lake located in Villupuram District of Tamil Nadu (Plate 1). Of these one is located in Mugaiyur village and referred to as Mugaiyur lake (Plate 2). The second lake is located in Tirukoilur town and referred to as Tirukoilur lake (Plate 3). Both the lakes are located at an elevation of about 85' MSL. The distance between two lakes is about 16 km and hence the general wealth conditions are almost similar to both lakes. However, both the lakes depend on varied water sources even though both are perennial. While Mugaiyur lake is rainfed, the Tirukoilur lake is purely river fed (Penniyar river of Villupuram District).

The water samples were collected by using aseptically broad mouthed sterilized glass containers, transferred to ice boxes and immediately brought to the laboratory for culturing within 6 hours. In addition, water samples were also collected in sterilized bottles for bacterial studies and transferred immediately to the laboratory in compact thermo- cold ice boxes with minimum exposure to light, temperature and undue shaking for further analyses. Water samples for both the systems were collected between 7:30 and 8:30 am.

WHO (1985) guidelines were followed for the frequency of sampling. Total bacterial count (TBC) was estimated by standard plate count using nutrient agar, while Most Probable Number (MPN), Total Coliform (TC) and faecal *Streptococci* (FS) were carried out by multiple tube method using Mc Conkey's and sodium azide broth respectively as recommended by ISI (1982), WHO (1985) and APHA (2000). Other biochemical tests were also done for isolation and identification of different bacterial species by using selected media and tests like IMVIC, Gram stain, fermentation, nitrate reductase, oxidase, citrate, catalase and H_2S, as recommended by Jacobs and Gerstein (1960), Sirockin and Cullimore (1969), Crueckshank *et al*. (1975) and APHA (1985).

Fluctuations of Microbiological Variables

Bacterial Abundance and Diversity (Tables 1-4)

Total Bacterial Density (TBD)

The Total Bacterial Density in the surface water of Mugaiyur lake ranged from 6.2 to 14.4×10^7 cfu/l and for the Tirukoilur lake from 5.4 to 8.6×10^6 cfu/l. For the bottom water, Mugaiyur lake recorded levels ranging from 6.4 to 14.8 × 107 cfu/l and for the Tirukoilur lake from 6.0 to 8.8×10^7 cfu/l. The bottom water TBD of both systems followed the same pattern as that of surface water recording minimum levels in January and maximum in November. However a comparison of TBD levels reveals that bottom water recorded higher levels than surface water in both the systems (Tables 1 and 2). Similar observations were also noticed by Shimna (2012), Sankar Rao (2013) and Mugilan (2014). The maximum TBD noticed during November in present study is due to the rains resulting in the mixing of waters along with run-off water entering the systems bringing nutrients for enabling the growth of the organisms while the minimal values noticed in January may be due to the lower water temperature which is unsuitable for the growth of bacteria. Similar observations were also recorded by Shimna (2012), Sankarrao (2013) and Mugilan (2014). A perusal of the concentrations of Oxidable Organic Mater, Ammonia nitrogen, Nitrate-nitrogen and Phosphate (OOM, NH_3-N, NO_3-N and PO_4)

levels in the waters of both the systems reveals a higher concentration during the rainy season which could also be a reason for the highest TBC shown during this season. In the present study, correlation between TBC and oxidizable organic matter, suspended solids and ammonia showed a positive relationship indicating their effect on bacteria. The differences in bacterial count noticed during the different seasons of the year can also be attributed to the differences in temperature and suspended solid levels as suggested by many workers (Seki, 1972; Fukami *et al*., 1983; Sivakami *et al*., 2011, 2012) in addition to oxidizable organic matter and nutrients (Sivakami *et al*., 2012; Sankarrao, 2013).

A comparison of the nutrient levels in different seasons also reveal that the TBC was high when the nutrient levels were also high. Correlation between TBC and nutrients like phosphates, nitrates and sulphates also a revealed positive correlation in both the systems. However, Jones (1971), suggested that temperature, pH and oxygen are the main limiting factors controlling the vertical bacterial population. A comparison of TBC among the two systems reveals that Mugaiyur lake recorded higher levels than Tirukoilur lake. This is probably due to higher amount of suspended solids, oxidizable organic matter as well as nutrients in this system.

Total Coliform Count

Total Coliform Count (TCC) in the surface water of Mugaiyur lake was found to range from 165 to 1635 per 100 ml and from 315 to 521 per 100 ml in the Tirukoilur lake. The bottom water TCC levels in Mugaiyur lake ranged from 205 to 1685 per 100 ml and from 330 to 585 per 100 ml in Tirukoilur lake. Both the surface and bottom waters in both the systems followed the same pattern by recording minimal levels in January and the maximum in November (Tables 1 & 2).

In the present study, the maximum TCC that was recorded in November in both systems can again be attributed to entry of water into the system due to rains resulting in surface run-off entering the systems resulting in suspended solids, OOM and nutrients. Correlating TCC with suspended solids, OOM and nutrients (NO_3, NH_3, SO_4, PO_4) reveals a positive correlation in both the systems. The higher TCC levels noticed in the bottom waters when compared to surface waters can again be attributed to increased amount of Suspended Solids and Oxidable Organic Matter (SS and OOM) and nutrients in the bottom water when compared to the surface water.

Among the two systems, Mugaiyur lake recorded a higher level when compared to that of the Tirukoilur lake which may be due to higher amount of suspended solids, temperature, OOM and nutrients like PO_4, NO_3 and NH_3. Literature reveals that similar findings were also suggested by Kumar and Shaka (2009), Petter *et al.* (2012), Shimna (2012), Sankar Rao (2013) and Mugilan (2014).

Faecal *Streptococci* (FS)

Faecal *Streptococci* in the surface water of Mugaiyur lake was found to range from 82 to 482 per 100 ml and from 102 to 252 per 100 ml in the Tirukoilur lake. The bottom water Faecal *Streptococci* count in Mugaiyur lake was found to range from 92 to 562 per 100 ml and from 132 to 257 per 100 ml in Tirukoilur lake. The bottom water Faecal *Streptococci* count followed the same pattern of surface waters in both the systems recording minimum level in January and maximum in November (Tables 1 and 2).

Table.1 Bacteriological Studies in Mugaiyur Lake Water

Month	Total Bacterial Density (TBD) (per 100 ml)				Total Coliforms Count (TCC) (per 100 ml)				Faecal *Streptococci* Count (FSC) (per 100 ml)			
	Surface water		Bottom water		Surface water		Bottom water		Surface water		Bottom water	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	6.2	6.7	6.4	6.9	165	225	205	295	112	82	142	92
February	6.7	6.9	6.7	7.0	205	235	335	315	142	102	152	122
March	7.6	7.6	7.8	7.8	305	345	385	375	162	142	172	161
April	7.8	7.8	7.9	8.0	345	375	410	405	170	151	182	182
May	8.1	8.2	8.0	8.4	375	435	485	445	172	162	192	192
June	8.4	8.7	8.2	8.9	390	485	505	515	177	172	205	192
July	9.1	8.8	8.4	9.0	410	510	555	585	181	182	220	212
August	10.6	9.0	10.8	9.1	465	535	605	665	184	197	246	231
September	10.8	9.2	11.0	9.8	605	585	635	735	242	242	262	252
October	13.2	12.9	13.4	13.2	805	735	825	985	312	302	352	332
November	14.4	13.6	14.8	13.9	1445	1635	1805	1685	482	442	562	562
December	11.4	11.2	11.9	11.8	865	945	925	1035	362	352	402	392

Table.2 Bacteriological Studies in Tirukoilur Lake

Month	Total Bacterial Density (TBD) (per 100 ml)				Total Coliforms Count (TCC) (per 100 ml)				Faecal *Streptococci* Count (FSC) (per 100 ml)			
	Surface water		Bottom water		Surface water		Bottom water		Surface water		Bottom water	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
January	5.4	6.0	6.0	6.6	315	325	330	336	102	109	122	141
February	5.8	6.2	6.2	6.8	345	341	350	371	107	117	127	132
March	5.9	6.8	6.4	7.0	360	365	366	375	109	119	132	137
April	6.1	6.9	6.5	7.2	385	381	390	396	112	123	134	147
May	6.4	7.1	6.7	7.4	390	396	401	405	123	128	139	152
June	6.7	7.3	7.1	7.5	410	401	422	411	142	132	147	157
July	6.8	7.5	7.4	7.7	415	422	430	455	172	155	177	187
August	7.0	7.6	8.0	7.9	445	449	460	471	192	162	197	192
September	7.1	7.8	8.1	8.2	465	482	490	495	202	192	217	212
October	7.4	8.1	8.3	8.4	475	498	516	524	212	222	227	237
November	8.6	8.2	8.8	8.5	490	521	584	585	232	252	247	257
December	7.2	7.8	7.4	7.5	415	435	425	465	176	182	217	207

Table.3 Seasonal Changes in Bacterial Composition of Mugaiyur Lake

No.	Species	Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1.	*Aerobacter aerogenes*	2013	+	+	+	+	+	+	+	+	+	++	+++	+++
		2014	+	+	+	+	+	+	+	+	+	++	+++	+++
2.	*Aeromonas hydrophila*	2013	+	+	+	+	+	+	+	+	+	++	++	++
		2014	+	+	+	+	+	+	+	+	+	++	++	++
3.	*Bacillus cereus*	2013	+	+	+	–	–	–	+	+	+	++	++	++
		2014	+	+	+	–	–	–	+	+	+	++	++	++
4.	*Bacillus firmus*	2013	++	++	++	++	++	++	++	++	+++	+++	+++	+++
		2014	++	+	++	++	+++	++	++	++	++	+++	+++	++++
5.	*Bacillus subtilis*	2013	+++	+++	+++	+++	++	++	++	++	+++	++++	++++	++++
		2014	+++	+++	++	+++	+++	++	+	++	++	++++	++++	++++
6.	*Clostridium perfringens*	2013	+	–	–	–	–	+	+	+	+	+	++	++
		2014	+	–	–	–	–	–	+	+	+	+	++	++
7.	*Enterobacter aerogenes*	2013	+	+	+	+	+	+	+	+	++	++	++	+
		2014	+	+	+	+	+	+	+	+	++	++	++	+
8.	*Enterobacter cloacae*	2013	+	+	+	–	–	–	–	+	+	+	+	+
		2014	+	+	+	–	–	–	–	–	+	+	+	+
9.	*Erwinia rhapontici*	2013	+	–	–	–	–	–	–	–	–	+	+	+
		2014	–	–	–	–	–	–	–	–	–	–	–	–
10.	*Escherichia coli*	2013	++++	++++	+++	+++	++++	+++	+++	+++	++++	++++	+++++	+++++
		2014	++++	++++	+++	+++	++++	+++	+++	+++	++++	++++	++++	++++
11.	*Flavobacterium johnsoniae*	2013	+++	+++	+++	+++	+++	++	++	++	+++	+++	+++	+++
		2014	+++	++	++	++	++	++	++	+++	++	+++	+++	+++
12.	*Klebsiella pneumoniae*	2013	+	+	+	–	–	+	+	+	++	++	++	+
		2014	+	+	+	–	–	+	+	+	++	++	++	++
13.	*Proteus mirabilis*	2013	+++	+++	+	++	++	++	++	++	++	++	+++	+++
		2014	++	++	+	+	+	+	+	+	+	+	+++	+++
14.	*Proteus vulgaris*	2013	+	+	+	+	+	–	–	+	+	+	+++	+++
		2014	+	+	+	+	+	–	–	–	–	+	+++	+++

Table-3 continued….

No.	Species	Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
15.	*Pseudomonas aeruginosa*	2013	+++	+++	++	++	++	++	++	++	+++	++++	++++	++++
		2014	+++	+++	++	++	++	++	++	++	+++	++++	++++	++++
16.	*Salmonella typhi*	2013	+	–	–	–	–	–	+	+	+	+	+	+
		2014	+	–	–	–	–	–	–	+	+	+	+	+
17.	*Serratia marcescens*	2013	+	+	+	+	–	–	–	–	–	+	+	+
		2014	+	+	+	+	–	–	–	–	–	+	+	+
18.	*Shigella sonnei*	2013	–	–	–	–	–	–	–	–	–	–	–	–
		2014	–	–	–	–	–	–	–	–	+	+	+	+
19.	*Staphylococcus aureus*	2013	++	++	++	++	+	++	++	+++	+++	+++	++++	++++
		2014	++	++	++	++	+	++	++	+++	+++	+++	+++	++++
20.	*Streptococcus faecalis*	2013	+	+	+	+	+	+	+	++	++	++	++	++
		2014	+	+	+	+	+	+	+	++	++	++	++	++
21.	*Vibrio alginolyticus*	2013	–	–	–	–	–	–	–	–	–	–	–	–
		2014	+	+	–	–	–	–	–	–	–	+	+	+
22.	*Vibrio cholerae*	2013	+	+	+	–	–	–	+	+	+	+	+	+
		2014	+	+	–	–	–	–	+	+	+	+	+	+

'+' represents Presence
'–' represents Absence

Table.4 Seasonal Changes in Bacterial Composition of Tirukoilur Lake

No.	Species	Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1.	*Aerobacter aerogenes*	2013	++	++	+	+	+	+	+	+	+	+	+	+
		2014	++	++	+	+	+	+	+	+	+	+	+	+
2.	*Aeromonas hydrophila*	2013	+++	+++	+++	++	++	++	++	+++	+++	+++	++++	++++
		2014	+++	+++	+++	++	++	++	++	++	+++	+++	+++	++++
3.	*Alcaligenes denitrificans*	2013	+	+	–	–	–	–	–	–	++	++	++	++
		2014	–	–	–	–	–	–	–	–	–	+	+	+
4.	*Bacillus cereus*	2013	+	+	+	+	+	+	+	+	+	+	+	+
		2014	+	+	+	+	+	+	+	+	+	+	+	+
5.	*Bacillus megaterium*	2013	+	++	++	++	+	++	+	++	++	++	++	++
		2014	+	++	++	+	+	++	+	++	++	++	++	++
6.	*Bacillus subtilis*	2013	++	++	++	++	++	++	++	+++	+++	+++	+++	+++
		2014	++	++	++	++	++	++	++	++	+++	+++	+++	+++
7.	*Chromobacterium* sp.	2013	+	+	–	–	–	–	–	–	–	+	+	+
		2014	+	+	–	–	–	–	–	–	–	+	+	+
8.	*Clostridium perfringens*	2013	+	+	–	–	–	–	–	+	+	+	+	+
		2014	+	+	–	–	–	–	–	+	+	+	+	+
9.	*Enterobacter aerogenes*	2013	++	++	++	–	–	–	–	++	++	++	++	++
		2014	++	++	++	–	–	–	–	++	++	++	++	++
10.	*Erwinia rhapontici*	2013	–	–	–	–	–	–	–	–	+	+	+	+
		2014	–	–	–	–	–	–	–	–	+	+	+	+
11.	*Escherichia coli*	2013	+++	+++	+++	+++	+++	+++	+++	+++	++++	++++	+++	+++
		2014	+++	+++	+++	+++	++	++	++	+++	++++	++++	+++	+++
12.	*Flavobacterium johnsoniae*	2013	+	+	+	+	+	+	+	+	+	+	+	+
		2014	+	+	+	+	+	+	+	+	+	+	+	+
13.	*Klebsiella pneumoniae*	2013	+	+	+	+	+	+	+	+	+	+	+	+
		2014	+	+	+	+	+	+	+	+	+	+	+	+
14.	*Proteus mirabilis*	2013	+	+	+	–	–	–	–	–	–	+	+	+
		2014	+	+	–	–	–	–	–	–	–	+	+	+

Table-4 continued….

No.	Species	Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
15.	*Proteus vulgaris*	2013	+	++	++	++	+	++	++	+++	+++	+++	+++	+++
		2014	+	++	++	++	+	++	++	+++	+++	+++	+++	+++
16.	*Pseudomonas aeruginosa*	2013	++++	++++	++++	+++	++	++	+++	+++	++++	++++	++++	++++
		2014	++++	+++	+++	+++	++	++	+++	+++	++++	++++	++++	++++
17.	*Pseudomonas agarolyticus*	2013	+	+	+	+	–	+	+	+	+	+	+	+
		2014	–	+	+	+	–	+	+	–	+	+	+	+
18.	*Salmonella typhi*	2013	+	–	+	+	–	–	–	+	+	+	+	+
		2014	+	+	+	+	–	–	–	–	+	+	+	+
19.	*Serratia marcescens*	2013	–	–	–	+	+	+	–	+	+	+	–	–
		2014	+	+	+	–	–	–	–	+	+	+	+	+
20.	*Shigella flexneri*	2013	–	–	–	–	–	–	–	–	–	–	–	–
		2014	+	+	+	+	+	–	–	–	++	++	++	++
21.	*Shigella sonnei*	2013	+	+	+	+	+	–	–	–	++	+++	+++	++
		2014	+	+	+	+	+	–	–	–	++	+++	+++	++
22.	*Staphylococcus aureus*	2013	+	+	+	+	+	+	+	+	++	++	++	++
		2014	+	+	+	+	+	+	+	+	++	++	++	++
23.	*Streptococcus bovis*	2013	++	++	++	+	+	+	+	+	++	+++	+++	+++
		2014	++	++	++	+	+	+	+	+	++	+++	+++	+++
24.	*Streptococcus equinus*	2013	+	+	+	–	–	–	–	+	+	+	++	++
		2014	+	+	+	–	–	–	–	+	+	+	++	++
25.	*Streptococcus faecalis*	2013	++	++	++	+	–	–	+	++	++	++	++	++
		2014	++	+	++	+	–	–	+	++	++	++	++	++
26.	*Vibrio alginolyticus*	2013	+	+	+	–	–	–	–	–	–	++	++	++
		2014	+	+	–	–	–	–	–	–	–	++	++	++
27.	*Vibrio cholerae*	2013	–	–	+	–	+	–	–	–	–	++	++	++
		2014	–	–	+	–	+	–	–	–	–	++	++	++
28.	*Vibrio parahaemolyticus*	2013	+	–	–	–	–	–	+	+	+	+	+	+
		2014	+	–	–	–	–	–	+	+	+	+	+	+

'+' represents Presence; '–' represents Nil

Vasconcelos and Swartz (1976) reported that viability of faecal and coliform bacteria is inversely proportional to temperature, since high temperature enhances the growth of such bacteria in water. In the present study also both the systems recorded the maximum counts during rainy season (November) when temperature was low. Statistical correlation of Faecal *Streptococci*, total coliform count and total bacterial count with temperature showed a negative correlation (Annexures 1 to 10) thus showing their interrelationship.

Bacterial Species Diversity

Tables 3 and 4 reveal in Mugaiyur lake a total of 22 species were noticed which belonged to 16 genera while in the Tirukoilur lake, a total of 28 species belonging to 18 genera were recorded with both the systems recording 11 perennial species each. Among the perennial species, seven species (*E. coli*, *S. aureus*, *B. subtilis, A. aerogenes*, *F. johnsomae, P. aeruginosa* and *A. hydrophila*) were common to both the systems while one species was unique (*Enterobacter cloacae*) to Mugaiyur lake while for Tirukoilur lake five were unique (*Alcaligenes denifrificans, Bacillus megaterium, Shigella flexneri, Streptococcus bovis* and *S. equines*). Further, in both the systems *E. coli* and *P. aeruginosa* were the dominant species in terms of count even though in Mugaiyur lake *F. johnsomae* also dominated while in Tirukoilur lake, *A. hydrophila* also dominated. With regard to the least dominant species. Mugaiyur lake recorded *S. sonneri* and *V. alginolyticus* while in Tirukoilur lake it was *E. rhapontici* and *E. nurabilis* (Tables 3 and 4).

With regard to their most favourable period of occurrence in maximal numbers, total bacterial count as a whole in both the systems recorded higher levels during the period between October and December even though each species appeared to occur in highest counts in different months of the year. Thus both the systems showed both uniqueness and similarity between them. Between the two systems, Tirukoilur lake recorded higher diversity in terms of both species as well as genera. This variation may be due to the differences in nutrient levels as reported by other workers (Lim and Flint, 1989; Bogosian *et al.*, 1996; Shimna, 2012; Sankar Rao, 2013; Mugilan, 2014).

In the present study, the presence of *Vibrio, Aeromonas, E. coli, Enterobacter, Staphylococcus* species *etc.* indicates the presence of significant levels of microbial pollution. The presence of *E. coli* in both the systems possibly indicates faecal contamination. According to Kataria and Ambhore (2012) the presence of *E. coli* indicates potentially dangerous contamination requiring immediate attention. Hence this requires immediate attention if such systems are used for the betterment of man.

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