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Microbial ecology of a tropical freshwater lake

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Abstract

Bacterial groups involved in nutrient cycling in a natural freshwater lake in South India were analysed monthly (1981–1984) with reference to the hydrobiological conditions. The aerobic heterotrophic bacterial counts were low in water (0·08–5·58 × 10^3 ml^{-1}) and high in the sediment (21·00 – 800·00 × 10^3 g^{-1}), commensurate with their organic content. A similar pattern was observed with regard to populations of phospholytic, proteolytic, amylolytic, aerobic and anaerobic cellulolytic, methanogenic and iron bacteria. The bacterial groups in the nitrogen cycle and coliforms were in moderate ranges. The study showed the significance of bacterial activity in aquatic production and decomposition processes. The heterotrophic food chain was shown to play an important role in the trophic dynamics of this 'mesotrophic' lake.

Key words: Indian freshwater lake, bacterial communities, hydrobiological conditions, trophic status.

1. Introduction

Microorganisms, the first link between abiotic factors and the biotic world in aquatic ecosystems, constitute an inherent part of the biocoenoses of water bodies and the character and intensity of bacterial metabolism are the basis of nutrient cycles. Studies on the aquatic bacterial coenoses with reference to hydrobiological conditions have been largely restricted to the temperate zone and Japan in the subtropics[1–4].

While the database on the bacterial populations, associated biochemical activities, natural regulation of microbial communities and environmental conditions and their role in determining the trophic status of waters is extensive in other parts of the world, relevant information from the trophics, particularly on Indian freshwaters, is extremely limited. Most of the Indian aquatic microbial studies pertain to marine environment[5,6] or to fungi from streams and lakes[7,8]. Only recently have some attempts been made at studying the microbiology of natural lakes and fish ponds[9–11], signifying the importance of bacterial communities in trophic interactions. This study examines the dynamics of bacterial populations involved in the nitrogen, phosphorus,

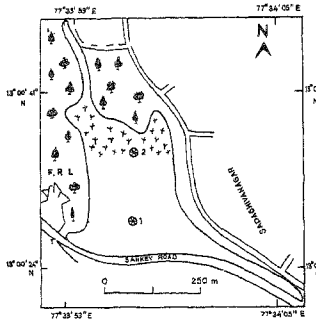

FIG 1 Sketch map of the Sankey lake showing locations of the sampling stations.

carbon and iron cycles in a natural freshwater lake in South India, as part of a comprehensive investigation on its limnology and microbial ecology.

2. Description of the study area

The study was carried out in the spring and rainfed perennial Sankey lake, popularly called 'Sankey tank' in Bangalore (lat. 13° 00′ 24″–13° 00′ 41″ N; long. 77° 33′ 53″–77° 34′ 05″E; altitude 921 masl; Fig. 1). The morphometric features of the lake were: surface area 12·18 ha, maximum length 466 m, maximum width 350 m, maximum depth 6 m, average depth 3·39 m, length of shoreline 1,570 m and shoreline development 1·28. The catchment area on the northern and western shores had abundant terrestrial vegetation, with the northern tip connecting to a seasonal stream during the rainy season. Early 1982 sewage and effluent drains into the lake were closed, and infestations of water hyacinth, *Eichhornia* sp. were cleared. Aquatic macrophytes in the northern zone of the lake comprised *Potamogeton* sp. and *Vallisnaria* sp., along with grassy shrubs in the littoral areas. The water of the lake was being used for boating and garden irrigation, apart from fish culture.

3. Material and methods

Two representative sampling stations were selected (Fig. 1), station 1 being deep (3·5–6·0 m) and station 2 shallower (2·0–4·0 m), with good aquatic vegetation. Hydrobiological studies on water and sediment quality, plankton, bacterioplankton, periphyton, primary production, and organic decomposition were made as per standard methods[12–16]. Monthly analyses of bacterial coenosis were made from January 1982 through June 1984 (aerobic heterotrophic bacteria), February 1982 to January 1983

(total coliforms) and April 1983 to June 1984 for other groups. Water samples (250 ml) were collected from the surface (0.1 m depth) and bottom (1 m above the sediment), in sterile glass bottles (autoclaved at 121°C, 15 psi for 20 min under aseptic conditions). The sediment samples (50 g) were collected using an Ekman grab (15 × 15 cm) and further, a part of the undisturbed surface layers (1 cm) from within the grab was transferred into sterile petri dishes using a spatula under aseptic conditions. They were immediately taken to the laboratory and processed. The surface water samples from the two stations were mixed to serve as one representative sample for surface waters, as also the two bottom waters and two sediment samples.

Standard bacterial enumeration methods of 'dilution plate count technique' with agar media in petri dishes or the 'most probable number method' with liquid media in test tubes were employed (Table I). The dilutions normally used for water samples were 10^0, 10^{-1} and 10^{-2} and for sediments, 10^{-2}, 10^{-3} and 10^{-4}. The plates were incubated at room temperature (25 ± 2°C) for 48 h, the colony-forming units were counted and averages computed. The tubes were normally incubated for 96 h, while those of nitrifiers–I and II were maintained for up to three months. The most probable numbers were read from McCready's table[20] and the bacterial counts expressed as no. ml^{-1} water or no. g^{-1} wet weight of sediment. Sediment samples were dried in a hot air oven at 120°C to constant weight and moisture factors determined for computing bacterial counts on a dry weight basis.

Table I
Methods, media and incubation employed for the enumeration of bacterial populations

Bacterial group	*Method*	*Medium*	*Incubation*
Aerobic heterotrophic bacteria	dpc	Sodium caseinate agar[17,18]	ae
Total coliforms	dpc	Eosine methylene blue agar[18]	ae
Ammonifiers	dpc	Nutrient glucose agar[19]	ae
Ureolytic bacteria	dpc	Agar medium with urea and bromothymol blue as indicator[20]	ae
Aerobic nitrogen fixers	dpc	*Azotobacter* medium with sucrose and Fe–Mo[20]	ae
Anaerobic nitrogen fixers	mpn	Winogradsky medium with glucose and calcium carbonate[20]	an
Nitrifying bacteria – I	mpn	Modified liquid medium with ammonium sulphate[20]	ae
Nitrifying bacteria – II	mpn	Winogradsky medium with sodium nitrite[20]	ae
Inorganic phosphorus-solubilising bacteria	dpc	Agar medium with tricalcium phosphate[20,21]	ae
Proteolytic bacteria	mpn	Nutrient agar with peptone[19]	an
Amylolytic bacteria	dpc	Agar medium with starch and iodine as indicator[20]	ae
Aerobic cellulolytic bacteria	mpn	Hutchinson medium[20]	ae
Anaerobic cellulolytic bacteria	mpn	Imshenetskii medium[20]	an
Methanogenic bacteria	mpn	Barker medium with sodium sulphide[19]	an
Iron bacteria	mpn	Liquid medium with ferrous sulphate[20,22]	ae

dpc – dilution plate count technique, mpn – most probable number method; ae – aerobic, an – anaerobic

Cell morphology and gram behaviour of the heterotrophic bacterial strains isolated during 1982 were studied by differential staining with crystal violet and safranine[18]. The data on bacterial counts were subjected to analysis of variance to assess the significance of variations between months or levels and correlation coefficients were calculated between different parameters to help understand the interdependencies and eco-interactions[23].

Various correlations between the bacterial counts and water and sediment quality parameters were worked out. Accordingly, wherever multiple correlation coefficients were calculated, they have been presented as $R_{1\cdot23}$. Simple correlation coefficients (r_{12}) between one dependent variable (bacterial counts) and the water/sediment quality parameter (independent variable) have been presented at each state as '*r*'. The levels of significance have also been indicated whenever they were found significant, which otherwise are presented to demonstrate the trends of relations between the parameters.

4. Results and discussion

The details of physico-chemical and biological properties of the lake have been described elsewhere[24,25]; however, the ranges of some important parameters are given in Table II to indicate the conditions prevailing in the lake.

Table II
Hydrobiological conditions of the Sankey lake (ranges of parameters)[24,25,32]

Annual rainfall (mm)	770·30–970·00
Atmospheric and water temperature (°C)	20·7–29·2; 20·0–28·5
Water pH and free CO_2 (mg l^{-1})	7·85–9·60, 1·60–20·00
Carbonate and bicarbonate alkalinity (mg $CaCO_3$ l^{-1})	0–64, 68–192
Specific conductivity (μ mho cm^{-1})	112·24–536·80
Nitrate–nitrogen (mg l^{-1})	Traces–0·17
Phosphate–phosphorus (mg l^{-1})	Traces–0·13
Silica (mg l^{-1})	0·01–1·70
Total iron (mg l^{-1})	Traces–0·80
Dissolved organic matter (mg l^{-1})	Traces–13·00
Sediment pH	7·0–8·3
Specific conductivity (μ mho cm^{-1})	134·20–2610·80
Available nitrogen and phosphorus (%)	0·014–0·084; 0·002–0·020
Organic carbon and calcium carbonate (%)	1·86–7·44; 0·67–7·00
Bacterioplankton (no. × 10^6 ml^{-1})	1·26–1·91
Plankton dry weight (g m^{-3})	0·001–1·20
counts (no. × 10^5 m^{-3})	32–12,300
Periphyton dry weight (g m^{-2} d^{-1})	0·001–0·64
counts (no. × 10^4 m^{-2} d^{-1})	23–950
Primary production: Gross, net and respiration (mg C m^{-3} h^{-1})	5·25–561·08, –368·18–318·49; 0·94–769·51
Decomposition rates (% d^{-1})	
Water hyacinth, filter paper and silk	1·1–6·3; 0·1–3·5; 0–1·2

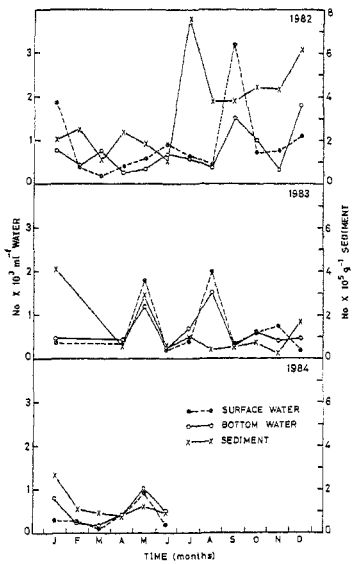

FIG 2. Aerobic heterotrophic bacterial counts in surface and bottom waters and sediment.

4.1. *Aerobic heterotrophic bacteria*

The ranges of heterotrophic bacterial counts in the surface and bottom water and the sediment were 0·08–5·58 × 10^{-3} ml^{-1}, 0·15–3·43 × 10^{3} ml^{-1} and 0·22–8·00 × 10^{5} g^{-1}, respectively (Fig. 2). During the peak monsoon months (July–September), higher counts were recorded in both the water layers. Allochthonous influence on the lake was shown by the positive relation between surface bacterial counts and rainfall (r_{12} = 0·2275). Correlation coefficients between surface bacterial populations and pH, nitrate-nitrogen and dissolved organic matter were 0·0096, 0·4080 and 0·2465, as have also been recorded by other workers[26]. These counts also showed an inverse relation with zooplankton counts (20–2,464 l^{-1}; $r = -0{\cdot}0936$) and a distinct direct relation with phytoplankton (560–11,500 l^{-1}; $r = 4316$; $p<0.05$). The bacterial counts in the sediment showed significant correlation with organic carbon content ($r = 0{\cdot}4970$; $p< 0{\cdot}05$) and specific conductance ($R_{1\,23} = 0.5141$; $p<0.01$), as has been reported[29].

Of the 150 bacterial strains isolated during 1982, 110 were gram-positive bacilli, 17 gram-positive cocci, 11 gram-positive coccobacilli and 12 gram-negative bacilli. A range of 0·5–1·5 million bacteria ml^{-1} water has been proposed for mesotrophic

Table III
Total coliform bacterial counts in surface and bottom waters and the sediment

Date	*Surface water (no ml^{-1})*	*Bottom water (no. ml^{-1})*	*Sediment (no g^{-1})*
1982 Feb	35	15	–
Mar.	20	10	200
Apr	90	40	450
May	20	70	–
June	720	60	300
July	35	15	100
Aug.	100	105	350
Sept	115	125	200
Oct.	15	15	100
Nov	40	30	–
Dec.	165	25	–
1983 Jan.	30	50	200

– Nil.

lakes[28]. Although the heterotrophic bacterial colony-forming units on the agar medium were lesser than this range, it may be mentioned that these numbers represent a fraction of the total bacterial population with a ratio as high as 10,000[29]. Keeping this in view and considering the total bacterioplankton as enumerated by the membrane filter method (1·26–1·91 × 10^6 ml^{-1}; Table II) and the ranges of Central Amazonian lakes[4], the Sankey lake may be categorised as 'mesotrophic'.

4.2. *Total coliform bacteria*

Coliform bacteria have been studied along with heterotrophs in lakes and rivers by several workers, not in the context of productivity, but as a first step in determining the importance of bacteria in aquatic habitats. The surface waters in Sankey lake recorded higher counts (20–165 ml^{-1} with a maximum of 720 ml^{-1} in June 1982) than the bottom (10–125 ml^{-1}) and the sediment counts were in the range 100–450 g^{-1} (Table III). The surface water coliform counts showed a positive relation with rainfall (r = 0·4646; p<0·10), reflecting the allochthonous influence on the lake, but not necessarily that of contamination, as only 1·6 to 8·0% of coliforms were faecal in a similar study published elsewhere[30].

4.3. *Ammonifying and ureolytic bacteria*

The respective ranges of ammonifiers in the surface and bottom waters and sediment were 140–1,320 ml^{-1}, 100–1,400 ml^{-1}, and 12·00–76·00 × 10^3 g^{-1} (Fig. 3a), with significant monthly variations (p<0.01). Correlations were observed between their populations and specific conductance in the surface (r = 0·4095; p<0·10) and bottom waters (r = 0·39645; p <0·10). High counts during rainfall suggested that the rain washings bring in considerable amounts of organic matter, serving as a substrate for ammonification.

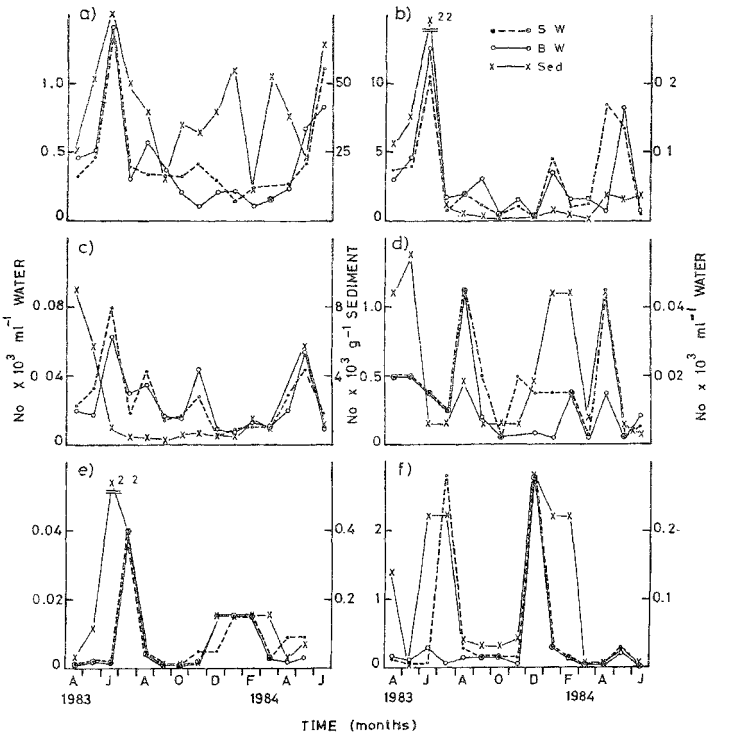

FIG 3 Bacterial populations in the nitrogen cycle: (a) ammonifiers; (b) Urea decomposers; (c) aerobic nitrogen fixers; (d) anaerobic nitrogen fixers; (e) nitrifiers – I; (f) nitrifiers – II in surface bottom waters and sediment.

Wide variations were noticed in the ureolytic bacterial counts of surface and bottom waters and the sediment (5–211 ml^{-1}; 2–250 ml^{-1}; 0·10–22·00 × 10^3 g^{-1}), with significant monthly variations ($p<0·01$). The period April to June showed higher counts during both 1983 and 1984 (Fig. 3b). A positive correlation between the counts and water temperature ($r = 0·4715$; $p<0·05$) was in conformity with earlier observations[31]. The variations in ureolytic bacterial populations were similar to those of ammonifiers with significant correlations among them in bottom water ($r = 0·7840$; $p<0·01$) and

sediment ($r = 0{\cdot}5565$; $p<0{\cdot}05$). Further, the high activity of urea decomposers during April to June coincided with higher decomposition rates of organic substrates observed in the lake[32].

4.4. *Aerobic and anaerobic nitrogen-fixing bacteria*

While the surface and bottom waters showed low populations of aerobic nitrogen fixers (5–80 ml^{-1} and 8–63 ml^{-1}), the sediment counts ($0{\cdot}24$–$9{\cdot}00 \times 10^3$ g^{-1}) were considerable (Fig. 3c). A similar pattern of abundance was observed in the case of anaerobic nitrogen fixers (2–45 ml^{-1} in surface and bottom waters and 75–1,400 g^{-1} in sediment; Fig. 3d). The sediment supported abundant nitrogen fixers and showed maximal numbers during the summer vegetative period, as also recorded by earlier workers[33]. The counts indicate their extent of contribution to the nitrogen budget of the lake through nitrogen fixation, especially in the absence of nitrogen-fixing blue-green algal populations.

4.5. *Nitrifying bacteria–I and II*

The nitrosomonads were low in the water and sediment media (1–40 ml^{-1}; 1–30 ml^{-1}; 8–150 g^{-1} with a high value of 2,200 g^{-1} in June 1983; Fig. 3e). The nitrobacters showed comparatively high counts (1–280 ml^{-1} in surface and bottom waters and 11–2,800 g^{-1} in sediment; Fig. 3f). The latter were higher than nitrosomonads in contrast to earlier reports[34], possibly due to higher level of the substrate (nitrites) made available to nitrobacteria both through ammonia oxidation and denitrification. The nitrifier populations in the surface sediment layers are relatively high for a natural lake[35].

The populations of nitrifier–I and II in surface and bottom waters showed positive correlations with dissolved oxygen concentration ($r = 0.6556$; $p < 0{\cdot}05$) as has been reported earlier[36]. Negative correlations between nitrifier–II counts and conductance in the surface waters ($r = -0{\cdot}4389$; $p < 0{\cdot}10$) and sediment ($r = -0{\cdot}4047$; $p<0{\cdot}10$) observed probably imply that the higher organic content favoured the growth of nitrobacteria in the lake.

4.6. *Inorganic phosphorus-solubilising bacteria*

The phospholytic bacterial counts were in the ranges 5–170 ml^{-1} and $0{\cdot}29$–$14{\cdot}20 \times 10^3$ g^{-1} in surface and bottom waters and sediment, respectively (Fig. 4a), with significant monthly variations ($p<0{\cdot}01$). The counts in surface waters showed positive correlation with the phosphorus content ($r = 0{\cdot}7167$; $p<0{\cdot}01$) and conductance ($R_{1{\cdot}23} = 0{\cdot}7623$; $p<0{\cdot}01$). Thus, the bacterial population seems to be active in solubilising complex inorganic phosphorus compounds, as also evident from high phosphorus levels in the sediment (*see* Table II).

Temperature and nitrogen levels have been found to be influencing the populations of solubilisers[37], which were corroborated in the present study also by the positive correlations between bacterial counts and temperature in the surface and bottom waters ($r = 0{\cdot}4405$ and $0{\cdot}4643$; $p < 0{\cdot}10$) and sediment counts with available nitrogen content ($r = 0{\cdot}6615$; $p < 0{\cdot}01$). A positive correlation between the phosphate

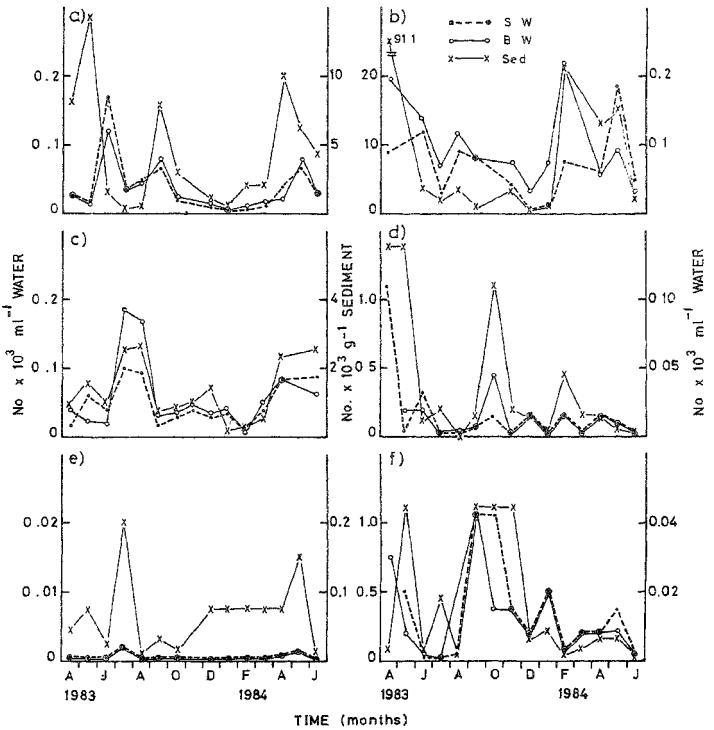

FIG 4. Phospholytic (a), proteolytic (b), amylolytic (c), anaerobic cellulolytic (d), methanogenic (e) and iron bacterial (+) populations in surface and bottom waters and sediment.

solubilisers and heterotrophic bacteria in bottom waters ($r = 0{\cdot}5652$; $p < 0{\cdot}05$) and sediment ($r = 0.4978$; $p < 0.10$) agreed with the earlier observations[38].

4.7. *Proteolytic and amylolytic bacteria*

Proteolytic bacterial populations in the water and sediment media (5–186 ml^{-1}; 32–117 ml^{-1}; 0·48–91·10 × 10^3 g^{-1}; Fig. 4b) showed positive correlations with heterotrophic

bacteria in the water and sediment media ($r = 0{\cdot}5625$, $0{\cdot}0512$ and $0{\cdot}2372$), suggesting that a considerable number of them were proteolytic, as has been reported[39]. Further, significant correlations between their counts and conductance ($r = 0{\cdot}4272$; $p < 0{\cdot}10$ in surface water and $r = 0{\cdot}4280$; $p < 0{\cdot}10$ in sediment) indicate the mineralising action of protein decomposers. The starch decomposers were low in surface and bottom waters (10–102 ml^{-1} and 7–186 ml^{-1}) and considerable in the sediment ($0{\cdot}20$–$3{\cdot}04 \times 10^3$ g^{-1}; Fig. 4c) and exhibited positive correlations with heterotrophic bacterial populations ($r = 0{\cdot}4410$; $p < 0{\cdot}10$ and $0{\cdot}4884$; $p < 0{\cdot}10$ in surface and bottom waters) as has been reported earlier[39].

An alternating pattern of variations between the proteolytic and amylolytic bacteria was apparent (*see* Figs 4b and c). Further, protein decomposers showed a negative relation with sediment organic carbon ($r = -0{\cdot}2171$) while starch decomposers showed a positive relation ($r = 0{\cdot}1583$). It may be deduced that amylolytic bacteria were more active during periods of higher sediment carbon and proteolytic bacteria at other times, thus regulating the carbon–nitrogen ratios of the lake sediment.

4.8. *Aerobic and anaerobic cellulolytic bacteria*

Low populations of aerobic cellulose decomposers were observed in surface and bottom waters (1–15 ml^{-1} in both) and sediment counts varied between 10 and 450 g^{-1}. The anaerobes were higher (1–110 ml^{-1}; 2–45 ml^{-1} 15–1,400 g^{-1}), particularly in the sediment (Fig. 4d). A negative correlation between the aerobic cellulose decomposers in the sediment and conductance was observed ($r = -0{\cdot}4057$; $p < 0{\cdot}10$). Similar negative relations between the anaerobes and conductance were observed in the bottom water ($r = -0{\cdot}4620$; $p < 0{\cdot}10$). These suggest the dependence of their populations on the availability of organic matter and reduction with increased mineral levels, as has also been reported earlier[40].

4.9. *Methanogenic bacteria*

The numbers of methanogens ranged from 1 to 20 ml^{-1} in surface and bottom waters and 10–200 g^{-1} in the sediment (Fig. 4c). The sediment counts were commensurate with the high organic content and lower than those observed in an Indian rural pond[19]. The counts were substantiated by high gas accumulations in the sediment layers, measured by gas displacement method using a funnel, which ranged from 5·62 to 15·13 l m^{-2}. Methanogenesis has been reported to cause 25% of the gas accumulations in the profundal region of an English lake[41]. The results indicate the organic accumulation and intensity of hydrocarbon decomposition in the sediment layers, as also the demand exerted by sediment on the lake's oxygen budget.

4.10. *Iron bacteria*

The bacteria involved in the oxidation of ferrous iron to ferric form were low in surface and bottom waters (1–45 ml^{-1} in both), but considerable in the sediment (20–100 g^{-1}; Fig. 4f). The ferric form in the water medium was influenced by bacterial

activity, as evident from the significant correlation between the two in surface waters ($r = 0{\cdot}4582$; $p < 0{\cdot}05$), as has been reported[42].

The above account details the seasonal and spatial variations in the bacterial populations in the water and sediment media of the Sankey lake. Although the periods of abundance were not the same for all groups, a trend of seasonal variations could be deduced with higher bacterial activity during May–July 1983 and December 1983–February 1984. Various factors influenced their population levels, the important ones being rainfall and water inflow, temperature, dissolved oxygen, nitrogen, phosphorus, specific conductivity and organic carbon content. In turn, the activities of bacterial communities were shown to influence the prevailing chemical conditions of water and sediment media which determine the plankton and production levels.

While only photosynthetic food chain was considered to be important in the earlier studies[43,44], the present investigation revealed the significance of heterotrophic food chain in sustaining the higher trophic levels, by actual quantification of the bacterial populations. This was further evidenced by the high sediment organic matter, zooplankton as also the respiration and decomposition rates (*see* Table II). Trophic classification based on bacterial abundance has been done only in temperate waters[4,30,45]. Considering the levels of bacterial populations and the different aspects already discussed and comparing with the available information on an Indian rural pond[19], the Sankey lake may be classified as 'mesotrophic'.

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