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Enumeration and Identification of Pathogenic Pollution Indicators in Cauvery River, South India

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Abstract: This study was aimed to estimate current levels of pollution indicator as well as many groups of human pathogenic bacteria and their seasonal variations in different locations of Cauvery river, South India. The samples were collected from 16 different sites along river from Stanley reservoir to estuary regions (Bay of Bengal). The microbiological scrutiny was performed during monsoon (2007), Winter, Summer and Spring (2008) seasons taken for the bacterial analysis of Total Viable Counts (TVC), Total Coliform counts (TC), Total Streptococci counts (TS) and also four different types of pathogenic bacterial load were counts, which are indicator organisms of pollution studies. Total viable counts were found in the range of 6.2-26.0 ($\times10^4$) mL^{-1} in monsoon, 5.2-20.0 ($\times10^4$) mL^{-1} in summer, 4.0-17.9 ($\times10^4$) mL^{-1} in winter and 3.3-15.5 ($\times10^4$) mL^{-1} in spring. The TC was found in the range of 4.1-21.0 ($\times10^3$) mL^{-1}, 3.6-17.0($\times10^3$) mL^{-1}, 2.9-14.1 ($\times10^3$) mL^{-1} and 2.3-12.0 ($\times10^3$) mL^{-1}, for TS, it was 4.3-18.0 ($\times10^2$) mL^{-1}, 3.2-13.0 ($\times10^2$) mL^{-1}, 2.6-11.0 ($\times10^2$) mL^{-1} and 2.0-9.6 ($\times10^2$) mL^{-1} during monsoon, summer, winter and spring, respectively. Counts of EC, SA/SH, SF and PA were in the range of 300-3700 mL^{-1}, 20-280, 20-270 and 40-490 mL^{-1}, respectively. The Cauvery river basin has been facing severe anthropogenic activities, mostly due to religious belief, dense population, municipal sewage and industrial waste confluences etc. A huge bacterial gene pool was obtained after this study which was indicative of immense bacterial diversity in the region.

Key words: Cauvery river, pathogenic bacteria, pollution indicators, coliforms

INTRODUCTION

Rivers are playing major task of an important water resource for our planet. Unfortunately, river is being polluted by indiscriminate disposal of sewerage, industrial waste and plethora of human activities, which affects its physico-chemical characteristics and microbiological quality (Koshy and Nayar, 1999). The lifeline of majority of population in cities, towns and villages are considered sacred. In the recent past, expanding human population, industrialization, intensive agricultural practices and discharges of massive amount of wastewater into the river have resulted in deterioration of water quality. The impact of anthropogenic activities has been so extensive that the water bodies have lost their self-purification capacity to a large extent. Water has played a significant role in the transmission of human disease and indicator microorganisms. Free from contamination with faecal matter is the most important parameter of water quality because human faecal matter

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is generally considered to be a greater risk to human health as it is more likely to contain human enteric pathogens (Scott *et al*., 2003). Coliforms are the major microbial indicator of monitoring water quality. Coliforms bacteria are a natural part of the microbiology of the intestinal tract of warm-blooded mammals including man can be found in their wastes. Total Coliform (TC) and Fecal Coliform (FC) counts are the most widely used bacteriological procedures for assessment of the quality of drinking and surface waters.

Pathogen levels in the water can be estimated by measuring the pathogen indicator organism concentration. Pathogen indicator organisms, often called indicator organisms. (EPA, 2001). Indicator organisms are used as diffuse pollution indicator, as well. Maul and Cooper (2000) was look into to the enterococci and fecal coliform bacteria concentrations to assess the variability of water quality. Aitken (2003) investigated the potential risk of fecal contamination due to diffuse pollution on river catchments and coastal bathing waters using indicator organisms. Indicator organisms are often used as a tool to identify the contaminant sources. The use of bacteria as water quality indicators can be viewed in two ways, first the presence of such bacteria can be taken as an indication of fecal contamination of the water and thus, as a signal to determine why such contamination is present, how serious it is and what steps can be taken to eliminate; second, their presence can be taken as an Indication of the potential danger of health risks that faecal contamination posses (Baghel *et al*., 2005). The higher level of indicator bacteria, elevated level of faecal contamination will be the risks of water borne diseases. The bacteriological parameters of different river systems have been studied by various groups (Badra *et al*., 2003; Arvanitidou *et al*., 2005) and many concerns have been raised recently about the pathogens originating from rainfall runoff (Ferguson *et al*., 2003; Smith and Perdek, 2004). Prevention of river pollution requires effective monitoring of physico-chemical and microbiological parameters (Ramteke *et al*., 1994). Thus, detection and an enumeration of indicator organisms are of primary importance for the monitoring of sanitary and microbiological quality of water (Gunnison, 1999).

Pathogens are disease-causing microorganisms that are a serious threat to public health (Balbus *et al*., 2004). Pathogens considered to be representative of those associated with waterborne disease include enteric viruses derived from human fecal contamination, bacterial pathogens, represented by *Escherichia coli* O157:H7 and the protozoan pathogens *Cryptosporidium* and *Giardia* (Ferguson *et al*., 2003). The bacteriological examination of water has a special significance in pollution studies, as it is a direct measurement of deleterious effect of pollution on human health. The present study focused to envisage and utilize the culturable bacterial diversity of Cauvery river system for its pollution status. Hence, this study was carried out to realize an index of microbial contamination attributes to draw complete overview of all aspects of pollution with in the Cauvery river system of Tamilnadu.

MATERIALS AND METHODS

Study Area

The river Cauvery is among one of the major peninsular rivers of South India. It is located in the Southern part of the Indian sub-continent and its catchment lies between latitude 10°7'N′ -13°28'N′ and longitude 75°28'E - 79°52'E. The river originates from the Brahmagiri range of the Western Ghats and travels 800 km before emptying into the Bay of Bengal. The basin trend NW-SE in direction and extends over an area of about 9×10^4 km^2

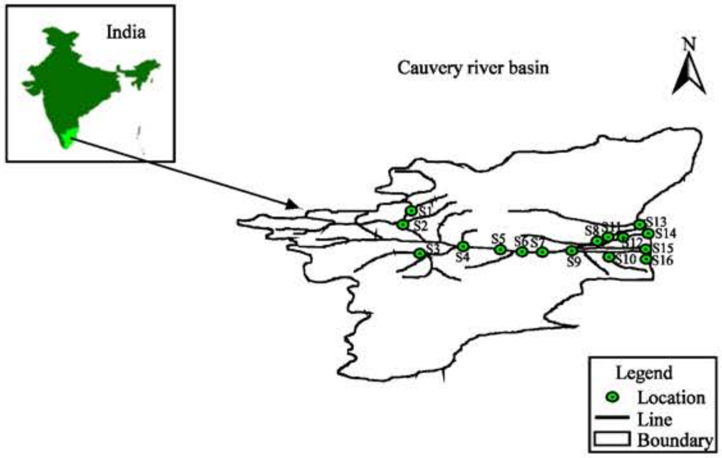

Fig. 1: Map showing the sampling sites (not to scale)

(Fig. 1). The principal tributaries of the river are the Hemavathi, Lakshmanthirta, Kabini, Shimsa, Bhavani and Amaravathi. The basin is characterized by a tropical climate with an average annual rainfall of 1092 mm and an average elevation of about 640 m. Numerous agricultural activities and the large network of transportation in the basin stimulates the growth of nearly 60 urban centers in the basin. It has the oldest diversion dams (locally known as anaicuts) from historical periods as a result of which some of the tributaries disappear into the paddy fields in the deltaic area and only the major tributaries join the Bay of Bengal. Thus, the Cauvery river is very much prone to change by nature and man (Ramanathan *et al*., 1994).

Weather Condition

The South-West monsoon season prevails during June to August while the North-East monsoon season follows from September to December 2008. The post-monsoon season has a brief Winter (January-February) and Summer follows afterwards, characterized by warm humid conditions.

Sampling Sites and Sampling Method

The Cauvery river system of Tamil Nadu was intensively surveyed to select different sites for sample collections. Water samples were collected from 16 different sampling sites from the runoff of Cauvery river between monsoons (rainy) to spring season during 2007- 08. The river drains/received enormous quantities of land materials, urban and rural sewage effluent, industrial and agricultural wastes throughout the year. Different seasonal sampling was carried out during rainy/monsoon 2007 representing (monsoon: September-December), winter 2008 (post monsoon: January-February), Summer 2008 (Summer: March-May) and spring 2008 (pre-monsoon: June-August). Sixteen samples were collected from Stanley reservoir to estuarine area (Bay of Bengal). The sampling locations are Mettur (S1), Bhavani (S2), Karur (S3), Musiri (S4), Upper Anicut (S5), Srirangam (S6), Grand Anicut (S7), Tirumanur (S8), Tiruvaiyaru (S9), Tanjore (S10), Anikkari (S11), Kumbakonam (S12), Pichavaram (S13), Poompuhar (S14), Karaikal (S15), Nagappatinam (S16) shown in Fig. 1. One liter of water sample from each location was collected in to pre-sterilized bottles. All samples were collected with precautions required for microbiological analysis, held on ice in an icebox and transported to the laboratory for microbial analysis (Table 1).

Table 1: Following groups of bacteria were quantified. Specific media (Hi-Media) used for enumerating are listed against them. All the culture media were obtained from Hi-Media Pvt. Ltd., Bombay, India

Specific media	Types of bacteria
Nutrient agar	Total Viable Counts (TVC)
McConkey agar	Total coliforms (TC), *Escherichia coli* (EC)
M Enterococcus agar	Total *Streptococci* (TS), *Streptococcus faecalis* (SF)
Xylose Lactose Deoxycholate (XLD) agar	*Salmonella* sp. (SA), *Shigella* sp. (SH)
Cetrimide agar	*Pseudomonas aeruginosa* (PA)

All the media were prepared with the addition of double distilled water and autoclaved properly. The plates were prepared 5 days prior to sampling. The bacterial population in different samples was estimated by spread plating method on nutrient agar and selective medium plates with 0.2 mL of suitable dilutions. All specific media plates were incubated at 37°C at least for 24 to 48 h and final counts of colonies were noted. All trials were performed in triplicate. When very high counts of any specific group of bacteria were observed in any media at 24 h incubation, water samples held in a refrigerator were diluted 10 or 100 fold and reexamined as above by spread plating method. In this study, to reduce the uncertainties associated with counting the pathogenic bacteria as like organisms for instance *Escherichia coli* like organisms (ECLO), *Streptococcus faecalis* like organisms (SFLO), *Salmonella* like organisms (SALO), *Shigella* like organisms (SHLO) *Pseudomonas aeruginosa* like organisms (PALO) were counted to asses the water quality. Different colonies from the specific media were picked out and characterized by biochemical and morphological tests. We found that approximately 80% of ECLO, SFLO, SALO, SHLO and PALO are EC, SF, SA, SH and PA, respectively. Using this information, nearly true percentage of EC, SF, SA, SH and PA from their LO counts are calculated and presented here.

On the basis of media manufacturer's guide and on the knowledge of innumerable previous analyses, typical colony morphology characteristics of different bacterial groups were recognized and initial enumeration of pollution indicator and pathogenic bacteria was completed. Typical colony characteristics of each group are listed below:

- **McConkey Agar:** All colonies grown on the medium counted as total coliforms. Typical, pink 2-3 mm dia colonies counted as *Escherichia coli* like organisms. All colonies on M Enterococcus agar counted as S*treptococci*. While those typically pink enumerated as *Streptococcus faecalis* like organisms
- **The XLD Agar:** Reddish, small, round, convex colonies with black centers counted as *Salmonella* sp. (SA) and reddish, small colonies without black centers as *Shigella* sp.
- **Cetrimide Agar:** Green colonies on Cetrimide Agar counted as *Pseudomonas aeruginosa*

Similar work was done by Mohandass *et al*. (2000) and Nagvenkar and Ramaiah (2009).

RESULTS

The impact of anthropogenic activities has been so extensive that the water bodies have lost their self-purification capacity to a large extent. The sampling area covered for this study was quite large. Since, we sampled 16 sites in each seasons, the data collection were numerous. Though our sampling locations were chosen to represent pollution gradient in the area, our observations are helpful to understand the seasonal variations of pollution indicators and many human pathogenic bacteria. These observations were used to suggest the non-suitability of Cauvery river for domestic purpose. The Total Viable Counts (TVC) were in order of magnitude of above10^4 mL^{-1} for all sites in all the four seasons, respectively, which was substantially high. All samples were found to have TVC higher than those

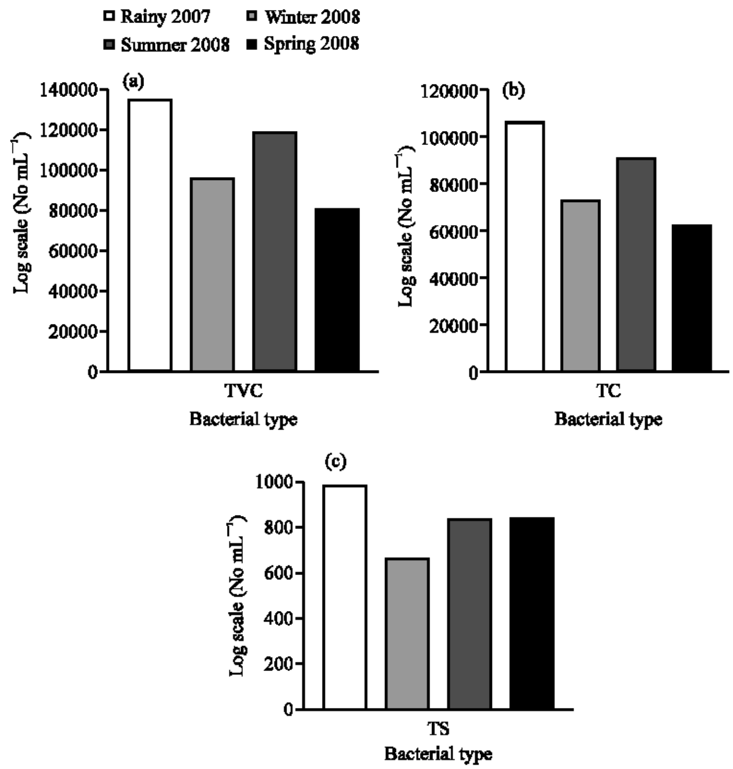

Fig. 2: Pollution Indicator organisms levels in water samples collected from Cauvery river during different seasons. (a) TVC- Total viable count, (b) TC-Total coliforms and (c) TS- Total streptococci

prescribed in Bureau of Indian Standards (ISI, 1991). The TVC was higher in monsoon season as compared to Summer, Winter and Spring in throughout the river. Total Viable Counts (TVC) for water samples were the highest during Monsoon and the least during Spring season (Fig. 2a). In Cauvery river the TVC was in the range of 6.2-26.0 ($\times10^4$) mL^{-1}, 5.2-20.0 ($\times10^4$) mL^{-1}, 4.0-17.9 ($\times10^4$) mL^{-1} and 3.3-15.5 ($\times10^4$) mL^{-1} during monsoon, Summer, Winter and Spring, respectively. The counts increased gradually from upper stretch to lower stretch, i.e., sites of the lower stretch (estuary areas) were found to be more contaminated. Total viable count was high in sampling site Nagappatinam (S16) during all the season i.e., 260000, 200000, 179000 and 155000 mL^{-1} during Monsoon, Summer, Winter and Spring, respectively. The overall TVC was higher during monsoon than other seasons and similar was the case with the TC and TS in Fig. 2b and c.

Commonly, the Total Coliforms (TC) count was also relatively higher in monsoon season than Summer, Winter and Spring season, similarly all the sampling site, outstandingly Upper anicut (S5) and Grand anicut (S7) regions were highest concentration count during Summer season. The TC showed higher in all the seasons i.e., 4.1-21.0 ($\times10^3$) mL^{-1}, 3.6-17.0($\times10^3$) mL^{-1}, 2.9-14.1 ($\times10^3$) mL^{-1} and 2.3-12.0 ($\times10^3$) mL^{-1} in rainy, Summer, Winter and Spring, respectively. Generally, Cauvery river has many holy places (S6, S8, S9 and S12) and dense populated/urban places (S2, S3, S4 and S10) which contributes high level of pollution in all season. But in estuary area high values were found in Karaikal (S15) and Nagappatinam (S16) in the whole year. While, the sampling sites (S15, S16) were prominent

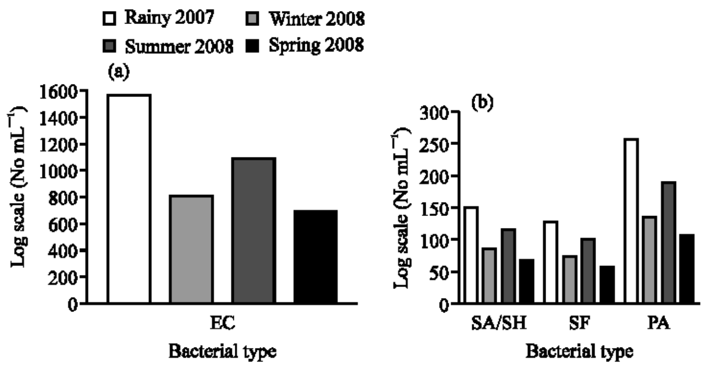

Fig. 3: Pathogenic bacterial levels in water samples collected from Cauvery river during different seasons. (a) EC-*Escherichia coli*, (b) SA/SH-*Salmonella* sp./*Shigella* sp., SF- *Streptococcus fecalis*, PA-*Pseudomonas aeroginosa*

for tourist activity, dense population and used as fishing harbor. Consequently these activities contribute high level of pollution in estuary areas. The TS value was also found higher in the monsoon (rainy) season, which was similar to the findings of TC. The Total Streptococci (TS) counts were higher in sampling site S16 than the other sampling sites in all seasons. In Cauvery river water the TS was in range of 4.3-18.0 ($\times10^2$) mL^{-1}, 3.2-13.0 ($\times10^2$) mL^{-1}, 2.6-11.0 ($\times10^2$) mL^{-1} and 2.0-9.6 ($\times10^2$) mL^{-1} during Monsoon, Summer, Winter and Spring, respectively. Among the sampling station S16 showed higher counts of TC followed by Karur (S3), Karaikal (S15) and other sites.

From the samples four different types of pathogenic bacteria were identified in water during (2007-2008) in different season and were presented. During monsoon (rainy), the counts of EC, SA/SH and SF were generally higher in all sampling site. Similar observations were made by (Nagvenkar and Ramaiah, 2009) in Mandovi and Zuari estuary at Goa, but the counts of SF varied widely between seasons. Similar to most other pathogenic groups, the PA count was obtained in moderate level in most of the sites in all season. Briefly, during Monsoon and Summer, count of human pathogenic *E. coli* was generally high at several locations and was lower during spring season in Fig 3a. It is clear from our result that high count of EC i.e., 5.0-37.0 ($\times10^2$) mL^{-1}, 4.0-24.0 ($\times10^2$) mL^{-1}, 3.0-16.0 ($\times10^2$) mL^{-1} and 3.0-13.0 ($\times10^2$) mL^{-1} in rainy, Summer, Winter and Spring season, respectively. The mean abundance of SA/SH ((*Salmonella* sp.)/(*Shigella* sp.)) were higher during the monsoon followed by Summer, Winter and Spring. The SF (*Streptococcus faecalis)* was also high in wet season in Fig 3b. The range of SF and SA/SH observed in Cauvery river were 5.0-27.0($\times10^1$) mL^{-1}, 3.0-20.0 ($\times10^1$) mL^{-1}, 3.0-12.0 ($\times10^1$) mL^{-1} and 2.0-10.0 ($\times10^1$) mL^{-1}, 6.0-28.0 ($\times10^1$) mL^{-1}, 4.0-22.0 ($\times10^1$) mL^{-1}, 2.0-19.0 ($\times10^1$) mL^{-1} and 2.0-16.0 ($\times10^1$) mL^{-1} in rainy, Summer, Winter and Spring, respectively. Colony forming units of *Pseudomonas aeruginosa* were recorded from all locations in all seasons, but higher in Monsoon and Summer. Interestingly, count of PA was high in sampling site Karur (S3) during all season i.e., 490, 320, 220 and 190 mL^{-1}. Large variations in the abundance of different pathogenic bacterial types were evident in water samples.

DISCUSSION

High levels of pollution indicator bacteria in river water is also a common problem in urban and rural areas that often leads to impairment of beneficial uses. The bacteriological analysis revealed that the entire sample collected from the 16 different sites in Cauvery river water was contaminated with coliforms, total streptococci and some pathogenic bacteria. In the present study, all sites were found to have high TVC. In fact, the water of Cauvery have been used for drinking purposes as part of ritual in most of the region, although the higher TVC values suggest that this practice should be avoided. The TVC value was relatively higher in holy places like Srirangam (S6) and Kumbakonam (S12) which may be attributed to the presence of large population residing at the banks. Like, in Gangetic river sites witness holy dip and mass bathing by a large number of pilgrims as an old age ritual in India, which is a constant source of contamination of water bodies (Semwal and Akolkar, 2006).

The total coliform count was relatively higher in rainy season than Summer and Winter, which suggest the role of precipitation on the sources and extent of microbial pollution. Similar observations were made in Gangetic River by Sood *et al.* (2008). Bhardwaj (2005) observed on TC counts in Cauvery River were in the range of 39-160000 MPN 100 mL^{-1}. Now, the TC counts are higher than the previous cram. As a matter of fact, the banks of Cauvery are more densely populated and face heavy anthropogenic activities as compared to other rivers in South India. The coliform bacterial population was highest in the monsoon season, the pattern which was reported in the earlier studies (Badge and Varma, 1982; Badge and Rangari, 1999). Also, the fact that the number of sub-tributaries falling in Cauvery river which may be responsible for the higher pollution indicators and many pathogenic bacterial count. Sewage contamination of aquatic habitats is detected by enumerating the coliform groups of bacteria (Fujioka, 2002). As is universally accepted, higher sewage contamination would lead to increase numbers of coliforms in natural water bodies. Normally fecal pellets contain several species of bacteria including human pathogens. Hansen and Bech (1996) clearly suggests the proliferation of allochthonous microflora in the river environment. Further, as inestimable pathogenic bacteria will constitute the microflora of effluents discharged from different activities, quantifying different groups of pathogenic bacteria have to be part of such surveys. For instance, information on occurrence, abundance and distribution of potent human pathogens, *Vibrio cholerae* (causing cholera in humans), *Vibrio parahaemolyticus* (gastroenteritis), *Salmonella* and *Shigella* sp. (typhoid fever; food poisoning), *Streptococcus* sp. (meningitis and skin infections), *Pseudomonas aeruginosa* (pulmonary and lungs infections) and *Aeromonads* (septicaemic conditions) in aquatic environment may prove useful in public health management.

In general, the highest abundance of all the examined groups was observed during monsoon. The *E.coli* and coliform were prevalent in river water as well as effluent sample (Ramteke *et al.*, 1994; Ramteke and Tewari, 2002). However, the total coliform counts are higher than those reported from the Mandovi and Zuari river of Goa (Nagvenkar and Ramaiah, 2009) and Gangetic river (Baghel *et al.*, 2005). Apparently, the total coliform counts are lower than those reported from Mumbai waters (Ramaiah *et al.*, 2004) and Geum river of Korea (Kim *et al.*, 2005). Pathogenic bacteria of human health concern have been studied mostly for their survival in the river environment (Baghel *et al.*, 2005; Kim *et al.*, 2005; Nagvenkar and Ramaiah, 2009; Sood *et al.*, 2008). The TS and SF were found maximum in monsoon followed by Summer, Winter and Spring season. Similar finding was observed in river Ganges (Baghel *et al.*, 2005). The EC was also obtained high in rainy season like Mondovi and Zuari river (Nagvenkar and Ramaiah, 2009). Increase the pollution indicators count in Cauvery river during all seasons suggested that the water becomes unfit for drinking as well as domestic purpose because of sewage confluences. The presence of PA in most of the sites of study area during all seasons may be attributed to the fact that the human activities and sewage outfalls to these sites.

Kistemann *et al.* (2002) observed that in the case of rainfall, the microbial loads of running water may suddenly increase and reach reservoir bodies very quickly. The above observation indicates that the bacterial contamination increases from upper stretch to lower stretch. This may be due to increase anthropogenic and socio-cultural activities at different sites of river stretches. Rapid development of town ships and industries in the surrounding vicinity of the river may also have added strains in the runoff and to an extent resulted in the degradation of its water quality. In this study, most of the sites were not suitable for domestic purpose with respect to the maximum permissible limits of TC and TS counts as per the standards laid by National River Conservation Directorate (NRCD), India. McLellan *et al.* (2001) stated that faecal pollution indicator organisms can be used to a number of conditions related to the health of aquatic ecosystems and to the potential for health effects among individuals using aquatic environments. The presence of such indicator organisms may provide indication of water-borne problems and is a direct threat to human and animal health. Present studies on microbial ecology in the runoff of the Cauvery in relation to pollution have clearly revealed that there is significant presence of bacterial pollution indicators and pathogenic bacterial groups; the situation of Cauvery river is not very serious but very alarming.

CONCLUSION

This study aims to understand the concentration dynamics of pathogen indicator organisms in the Cauvery river basin and to understand the impacts of point and non point source of pollution on the water quality of microbial features. Dominance of above pathogenic bacterial genera in Cauvery river basin of Tamilnadu suggests that they may be explored as indicators. Overall bacteriological analysis of Cauvery river basin water revealed that river water was polluted by sewage waste, faecal contaminants and industrial waste that they are not suitable for drinking and other recreational purposes. Therefore, scrutinizing of microbial contamination on periodic basis should be an important component of the protection strategy in this area. Hence, the river needed thorough impoundment. Effective measures could be adopted to prevent spread of diseases through the agency of water and to save the water body from decline.

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