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ORIGINAL ARTICLE

Prevalence of indicator and pathogenic bacteria in a tropical river of Western Ghats, India

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Abstract The Meenachil, the only river that flows through the heart of the Kottayam district of Kerala state, India was selected for the study. The present study has been carried out with an objective to systematically examine the prevalence of indicator and pathogenic microorganisms and to compare the microbiological quality of the river water during the pre-monsoon and post-monsoon seasons. Water samples from 44 different sites during pre-monsoon and post-monsoon seasons were collected for the analysis. During the pre-monsoon period, the faecal coliform count ranged from 230 to 110,000 MPN/100 ml while there was a variation from 200 to 4600 MPN/100 ml during the post-monsoon period. When the faecal streptococci count was analysed, it ranged from 140 to 110,000 MPN/100 ml during the pre-monsoon and 70 to 4600 MPN/100 ml during the post-monsoon seasons, respectively. All the samples collected were found to have total viable count (TVC) higher than those prescribed by Bureau of Indian Standards (ISI 1991). Total viable counts were found in the range of 1.1 × 102 to 32 × 102 cfu/ml in the pre-monsoon and 1.0 × 102 to 26 × 102 cfu/ml in the post-monsoon. The presence of faecal indicator bacteria, Escherichia coli and potentially pathogenic bacteria, *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Salmonella enterica* in the Meenachil River indicates that the bacteriological quality of the Meenachil River is poor. Moreover, it sheds light to the fact that raw sewage is being dumped into the Meenachil River. Urban runoffs and effluents of rubber factories appear to be the important sources of faecal contamination in the river. From this study, we conclude that these water bodies pose significant public health hazards. Adequate sanitary infrastructure will help in preventing source water contamination. Besides this, public health education aimed at improving personal, household and community hygiene is urgent.

Keywords Pathogenic bacteria · Tropical rivers · Western Ghats · Surface water contamination · Meenachil river

Introduction

Anthropogenic activities result in a significant decrease in surface water quality of aquatic systems in watersheds (Massoud et al. 2006). River inflows contribute many pollutants, thereby tending to induce ecological and hygienic problems (Wang et al. 2007). Escalating water pollution causes not only the deterioration of water quality but it also compromises human wellbeing and the permanence of aquatic ecosystems, economic growth and community affluence (Milovanovic 2007). The surface waters in populous countries have become reservoirs of antimicrobial-resistant pathogenic microbes due to the haphazard use of antimicrobials in human and veterinary medicine and accumulation of faecal contamination through point as well as non-point sources, storm drain infrastructure and malfunctioning

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septic tanks (Ahmed et al. 2005, Economou et al. 2013). The propensity of species dissemination is influenced by a variety of biotic and abiotic factors including geographical area and demography (Randall et al. 2006). Human faecal material is generally considered to be the greater menace to human health as it is more likely to contain human enteric pathogens (Scott et al. 2003). The most important and desired aspect of water quality is its freedom from contamination with faecal matter. Higher the level of indicator bacteria, greater the level of faecal contamination and greater the risk of water-borne diseases (Pipes 1981). A wide range of pathogenic microorganisms can be transmitted to humans via water contaminated with faecal material. These include unicellular parasites (such as the protozoan *Cryptosporidium, Microsporidium, Amoebae*) and enteropathogenic agents such as salmonellas, shigellas, enteroviruses and multicellular parasites as well as opportunistic pathogens like *Pseudomonas aeroginosa*, *Klebsiella*, *Vibrio parahaemolyticus* and *Aeromonas hydrophila* (Karanis et al. 2002; Hodegkiss 1988). It is not feasible to test water for all these organisms. The isolation and identification of many of these organisms are intensely complicated and seldom quantitative (Cairneross et al. 1980; WHO 1983). The most widely used indicators are the coliform bacteria, which may be the total coliform that get narrowed down to the faecal coliforms (FC) and the faecal streptococci (FS) (Kistemann et al. 2002; Pathak and Gopal 2001; Harwood et al. 2001; Vaidya et al. 2001). Concurrently, contamination of water by enteric pathogens has increased globally (Islam et al. 2001; Pathak et al. 1991; Craun 1986). It has been demonstrated that contact with bathing water which has been faecally contaminated enhances the menace of disease (Kay et al. 1994; Fleisher et al. 1993).

The presence of FC as *E. coli* serves as an indicator for the possible presence of other disease-causing pathogens (Rajkumar and Sharma 2013). FC are selected members of the coliform group of bacteria which are able to ferment lactose at 37 °C and are fairly specific for the faeces of warm-blooded animals. The bacteriological parameters of different river systems have been studied by various groups (Badra et al. 2003; Baghel et al. 2005; Arvanitidou et al. 2005; Schets et al. 2008; Jurzik et al. 2010; Chigor et al. 2013).

The bacteriological examination of water has a special significance in pollution studies. It provides a direct measure of the deleterious effect of pollution on human health. On the other hand, over 1.6 million people directly or indirectly depend on water for various purposes such as agriculture, fishing, transportation and recreation. As a result, water-related diseases are very common in the study area, particularly amongst the young children, though no official reports exist on this. The dearth of reports on the bacteriological quality of Meenachil River calls for attention. Therefore, it is important to carry out this study with the primary goal of determining the bacteriological quality of these essential surface waters and to assess the public health risks emanating from the use of the contaminated water. The present study has been carried out with an objective to systematically examine the prevalence of indicator and pathogenic microorganisms and to compare the microbiological quality of the river water during the pre-monsoon and post-monsoon seasons. The river is part of the tourist circuit in Kerala, and has an important role in making the backwater destination of Kumarakom a highly rated one.

Materials and methods

Study area

Meenachil River (length 78 km, area 1272 km^2) originates at an elevation of 1097 m above mean sea level (MSL), in Kerala, in southwestern India. Its watershed extends from 9°25′ to 9°55′N and 76°20′ to 76°55′E (Fig. 1). The general elevation ranges from 77 to 1156 m in the high lands; 8–68 m in the mid lands; and less than 2 m in the low lands. The watershed experiences an average annual rainfall of 3120 mm of which 1646 mm is received after South-West Monsoon (June–September). The river has a total annual yield of 2349 million cubic metres and an annual utilizable yield of 1110 million cubic metres. During monsoon the river can be full and quite often submerge the nearby low lying areas.

Sampling

The sampling sites were selected right from the upstream to downstream of the river. The river originates from Western Ghats grasslands and empties to the largest Ramsar wetland of the state, the Vembanadu Lake. The upstream locations are significant and abound in tourism activities such as contact water sports. In the vicinity of the entire main stream sampling points there are pumping activities for irrigation and drinking purposes. The sampling locations were surrounded by agricultural lands and human settlements. The Meenachil River may be the only river in Kerala, which is characterized by the presence of human settlement right from the source of the river to its cessation at the Vembanad Lake, adversely affecting the river and its basin. The contamination is less till it reaches Erattupetta. Between Erattupetta and Kottayam, the river has to gulp down all the dirt flowing in from the towns like Erattupetta, Bharananaganam, Pala, Kidangoor, Ettumannor and Kottayam. The waste dumping and treatment sites of municipalities are located very close to the main river channel. Majority of the public sewage system opens into the river which in turn results in hepatitis, dysentery, diarrhoea and

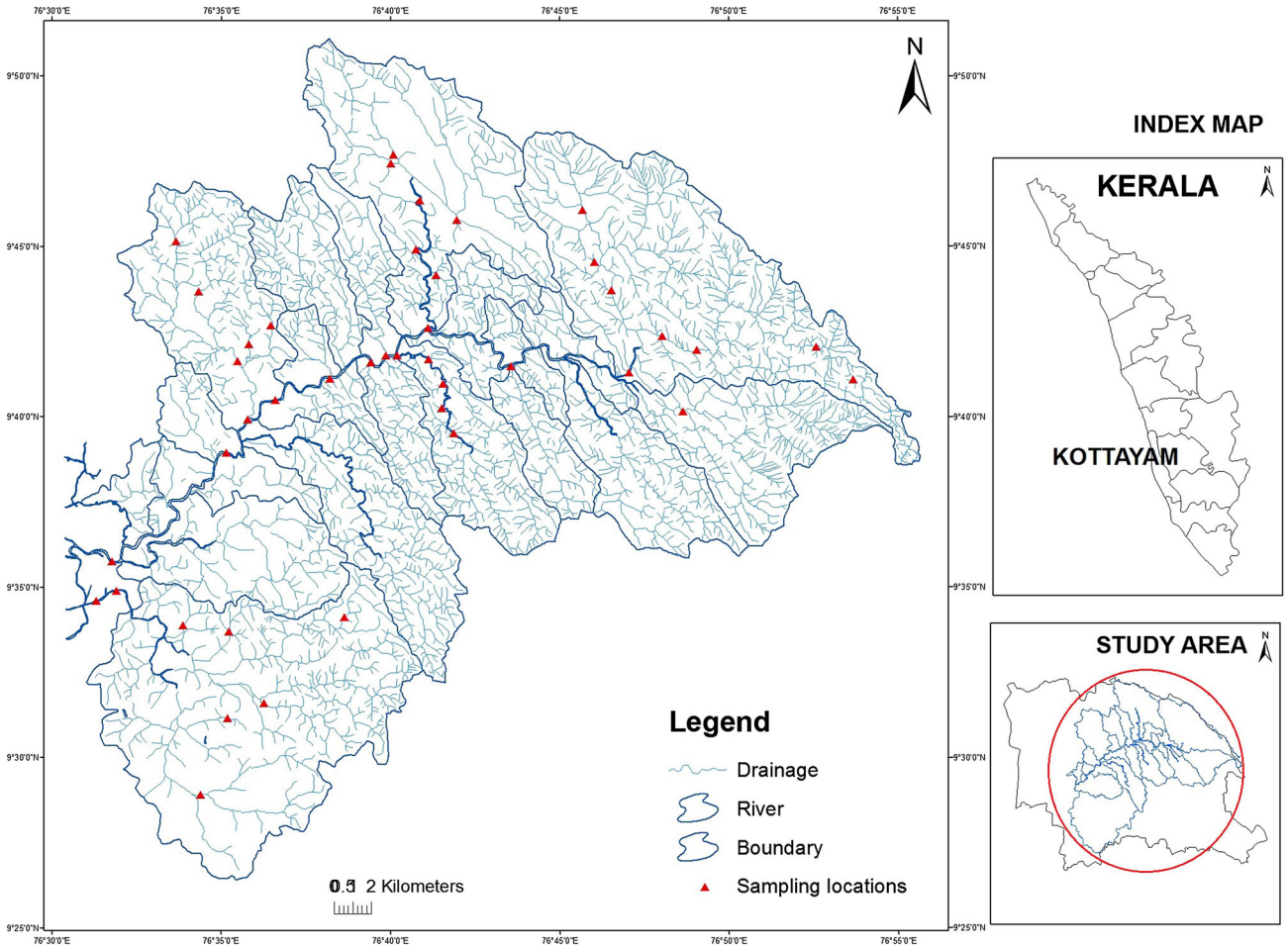

Fig. 1 Location map showing sampling sites

many contagious diseases as per the reports of the public health department.

Water samples were collected from 44 different sampling sites of Meenachil river basin (MRB), in sterile glass bottles, transported on ice to the laboratory and processed within 6–8 h of collection. Samples were collected during the pre-monsoon (February–May) and post-monsoon (October–January) seasons, from the upper (M1–M19), middle (M20–M35) and lower reaches (M36–M44) of the river basin (Fig. 1).

Bacteriological analysis

A three-tube most probable number (MPN) method was used for the isolation of FC and *Escherichia coli* using *Escherichia coli* (EC) broth (Hi-Media Laboratories, India) as medium. 10, 1 and 0.1 ml of appropriately diluted samples were inoculated into respective dilution tubes containing inverted Durham's tubes. Inoculated tubes were incubated at 44.5 °C for 24 h. Loopful of culture from each tube showing growth and gas production were streaked on Eosine Methylene Blue (EMB, Himedia, Bombay, India) agar for the isolation of *E. coli* and incubated at 37 °C for 24 h. Typical *E. coli*-like cultures were isolated, restreaked to ensure purity and corroborated by indole, methyl red, voges proskauer and citrate (IMViC) test. Isolates showing + + − − reaction for IMViC test were confirmed as *E. coli*.

FS were detected by inoculation of water samples into Azide Dextrose broth (ADB) and incubated at 37.5 ± 1 °C for 24–48 h (APHA 1998). Turbidity in ADB was used for the detection of FS after 24–48 h incubation. In order to confirm the presence of *Enterococcus*, positive FS tubes in the presumptive MPN tests were streaked onto Pfizer Selective Enterococcus Agar (PSEA), and incubated at 37 °C for 24 h. After incubation, colonies with black colouration were confirmed as typical *Enterococcus* cells. The source of faecal contamination was identified using FC/FS ratios (US EPA 1978; Geldreich 1974, 1976).

Two methods for isolation of *Vibrio parahaemolyticus* and *V. cholerae* were used. The first was a direct plating procedure, which included inoculating 0.2 ml river water

sample on Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS, Himedia, and Bombay, India) plates, and incubating at 37 °C for 48 h characterization (Chandran et al. 2008). Blue-green colonies were recorded as *V. parahaemolyticus* and yellow colonies were considered as *V. cholerae* and held for further biochemical testing. In the second method, 10 ml of river water samples were inoculated into 40 ml alkaline peptone water for pre-enrichment in a conical flask and incubated at 37 °C for 24 h characterization (Chandran et al. 2008). Flasks showing growth in enrichment broths were streaked onto TCBS agar and incubated at 37 °C for 24–48 h. Typical colonies, whenever present, were isolated, restreaked to ensure purity and maintained on nutrient agar slants for further biochemical.

The cultures were identified according to bacteriological analytical manual (BAM) of United States Food and Drug Administration (USFDA). Cytochrome oxidase (+), Nitrate reduction (+), Voges—Proskauer (−) acid from sucrose (−) and lactose (−), growth in peptone water containing 0 % (2), 3 % (+), 6 % (+) and 8 % (+) NaCl and growth at 43 °C in LIA (+) were considered as *V. parahaemolyticus*. For *V. cholerae*, cytochrome oxidase (+), Nitrate reduction (+) Voges—Proskauer (V) acid from sucrose (+) and lactose (−), growth in peptone water containing 0 % (+), 3 % (+), 6 % (−) and 8 % (−) NaCl and growth at 43 °C in LIA (+) were considered confirmatory.

Pseudomonas species were isolated by adopting spread plate method on pseudomonas agar (HI MEDIA) plate and incubated at 37 °C for 24 h. The inoculant from Brain Heart infusion broth was streaked onto MacConkey agar. *Streptococcus* species were observed by using blood agar swarming test. All the culture media were obtained from Hi-Media Pvt. Ltd., Bombay, India.

Results and discussion

Microbial analyses are presented in Table 1. The bacteriological analysis revealed that all the samples of MRB were contaminated with coliforms, FC and FS. All samples were found to have total viable counts (TVC) higher than those prescribed by Bureau of Indian Standards (ISI 1991). Total viable counts (TVC) were found in the range of 1.1×10^2 to 32×10^2 cfu/ml and 1.0×10^2 to 26×10^2 cfu/ml in the pre-monsoon and post-monsoon, respectively (Table 1). Along the main stream the values of TVC show an escalating trend downstream. Furthermore, the lower reaches show high count of total coliform of 2.5×10^2 to 11.3×10^2 and 1.5×10^2 to 8.5×10^2 in the pre-monsoon and post-monsoon, respectively.

The FC count ranged from 230 to 110,000 MPN/100 ml during the pre-monsoon and 200 to 4600 MPN/100 ml during the post-monsoon period (Table 1). High values of FC were recorded during the pre-monsoon season. The irregular variations in the coliform bacteria due to seasonal changes corroborated the findings of Legendre et al. (1984), Barcina (1986) and Ramanibai (1996). The existence of other members of the FC group (*Klebsiella*, *Enterobacter* and *Citrobacter*) was reported for non-faecal origin (Alonso et al. 1998). The higher FC has indicated the tolerance of high temperature as shown in Table 1. This result coincides with the observation of Ravichandran and Ramanibai (1988).

FS have been proposed as the possible alternative indicator bacteria to *E. coli*. They have greater persistence in water and will not multiply in polluted environments. There is also evidence that these bacteria have a stronger relationship to adverse health outcomes than *E. coli* (Moe et al. 1991). It is clear from our results that the count of FS increases from 140 to 110,000 MPN/100 ml and 70 to 4600 MPN/100 ml during pre-monsoon and post-monsoon, respectively in the MRB (Table 1). The lower reaches evidently displayed high counts of FS.

Population of aquatic microbiota is influenced by many environmental parameters. The increasing presence of pollution indicator bacteria in river water is a frequent hitch in urban and rural areas, often leading to outbreaks of serious water-borne diseases like cholera, dysentery, etc. The bacteriological analysis revealed that the entire sample collected from four different sites of the Meenachil River was contaminated with coliform, faecal coliform (FC) and other pathogenic bacteria. This may be attributed to the large number of pilgrims and tourists who visit the area in summer. Inadequate facilities for sanitation result in pumping untreated sewage into the river. It is clear from the results that the maximum count of FS is observed in the pre-monsoon season. FC and FS ratio is given in the Fig. 2. Probably, the sites with FC/FS ratio above one may have contamination by human faecal matter (Araujo et al. 1989). The Meenachil River may be the only river in Kerala, which is characterized by the presence of human settlement right from the source of the river to its final debouching point at the Vembanad Lake, adversely affecting the river and the river basin. The sewage from hotels and lodges flows into the riverine systems, polluting its freshwater ecosystem. Kistemann et al. (2002) observed that in case of rainfall, the microbial loads of running water may get amplified and reach reservoir bodies swiftly. The above observation indicates that the bacterial contamination increases from the upper reaches to the lower reaches. This may be due to increased anthropogenic activities at different sites along the lower reaches. Rapid development of the townships in the surrounding vicinity of the lower reach may also have added to the increased runoff and to an extent enhancing the degradation of the river water quality. Toilets in the urban agglomerations are located along the river banks and have their outlets into the river systems

Table 1 Seasonal variation of total viable, faecal coliform and faecal streptococci counts

Site no.	FC (MPN/100 ml)		FS (MPN/100 ml)		TVC (cfu/ml)	
	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon
M1	24×10^2	12×10^2	21×10^2	3.9×10^2	11.2×10^2	6.1×10^2
M2	46×10^2	15×10^2	46×10^2	4.3×10^2	13.6×10^2	8.3×10^2
M3	46×10^2	21×10^2	24×10^2	21×10^2	18.4×10^2	9.6×10^2
M4	110×10^2	24×10^2	46×10^2	9.3×10^2	19×10^2	14×10^2
M5	110×10^2	2.3×10^2	46×10^2	2×10^2	1.2×10^2	1.05×10^2
M6	110×10^2	24×10^2	21×10^2	3.9×10^2	1.1×10^2	1×10^2
M7	110×10^2	24×10^2	12×10^2	9.3×10^2	1.15×10^2	1.05×10^2
M8	110×10^2	46×10^2	7.5×10^2	2.1×10^2	1.1×10^2	1.05×10^2
M9	110×10^2	110×10^2	9.3×10^2	4.3×10^2	1.4×10^2	1.1×10^2
M10	46×10^2	24×10^2	7.5×10^2	7.5×10^2	1.2×10^2	1.1×10^2
M11	24×10^2	15×10^2	3.9×10^2	2.8×10^2	1.1×10^2	1×10^2
M12	46×10^2	24×10^2	12×10^2	7.5×10^2	1.6×10^2	1.45×10^2
M13	24×10^2	21×10^2	21×10^2	15×10^2	1.2×10^2	1×10^2
M14	110×10^2	46×10^2	24×10^2	21×10^2	1.8×10^2	1.65×10^2
M15	24×10^2	21×10^2	21×10^2	15×10^2	1.3×10^2	1.15×10^2
M16	21×10^2	9.3×10^2	15×10^2	9.3×10^2	1.1×10^2	1×10^2
M17	9.3×10^2	6.4×10^2	6.4×10^2	4.3×10^2	1.15×10^2	1.05×10^2
M18	110×10^2	15×10^2	46×10^2	7.5×10^2	19.8×10^2	11.1×10^2
M19	24×10^2	21×10^2	21×10^2	15×10^2	21×10^2	13.8×10^2
M20	24×10^2	15×10^2	21×10^2	12×10^2	23×10^2	19×10^2
M21	46×10^2	21×10^2	24×10^2	7.5×10^2	25×10^2	15.4×10^2
M22	46×10^2	24×10^2	24×10^2	15×10^2	27.4×10^2	16×10^2
M23	7.5×10^2	4.3×10^2	7.5×10^2	2×10^2	1.25×10^2	1×10^2
M24	9.3×10^2	4.6×10^2	3.9×10^2	2.8×10^2	1.9×10^2	1.4×10^2
M25	6.4×10^2	3.9×10^2	2.3×10^2	2.1×10^2	1.1×10^2	1×10^2
M26	7.5×10^2	6.4×10^2	4.3×10^2	3.9×10^2	1.8×10^2	1×10^2
M27	3.9×10^2	2.1×10^2	2.3×10^2	2×10^2	1.2×10^2	1×10^2
M28	2.8×10^2	2.3×10^2	1.5×10^2	0.7×10^2	1.2×10^2	1.15×10^2
M29	2.3×10^2	2×10^2	1.4×10^2	1.1×10^2	1.15×10^2	1×10^2
M30	110×10^2	24×10^2	110×10^2	9.3×10^2	1.4×10^2	1.05×10^2
M31	110×10^2	46×10^2	24×10^2	7.5×10^2	1.2×10^2	1.1×10^2
M32	110×10^2	46×10^2	46×10^2	9.3×10^2	1.1×10^2	1×10^2
M33	110×10^2	46×10^2	46×10^2	15×10^2	1.3×10^2	1×10^2
M34	110×10^2	46×10^2	15×10^2	9.3×10^2	1.35×10^2	1.2×10^2
M35	110×10^2	24×10^2	110×10^2	9.3×10^2	1.1×10^2	1×10^2
M36	110×10^2	46×10^2	46×10^2	21×10^2	32×10^2	26×10^2
M37	110×10^2	46×10^2	46×10^2	24×10^2	6.4×10^2	5.2×10^2
M38	46×10^2	21×10^2	24×10^2	4.3×10^2	2.9×10^2	1.7×10^2
M39	110×10^2	21×10^2	21×10^2	6.4×10^2	8.7×10^2	8.4×10^2
M40	24×10^2	24×10^2	15×10^2	7.5×10^2	2.6×10^2	1.5×10^2
M41	21×10^2	9.3×10^2	12×10^2	4.3×10^2	3.4×10^2	1.6×10^2
M42	21×10^2	46×10^2	15×10^2	24×10^2	2.5×10^2	1.8×10^2
M43	24×10^2	46×10^2	21×10^2	24×10^2	3.3×10^2	2.1×10^2
M44	110×10^2	110×10^2	21×10^2	46×10^2	11.3×10^2	8.5×10^2

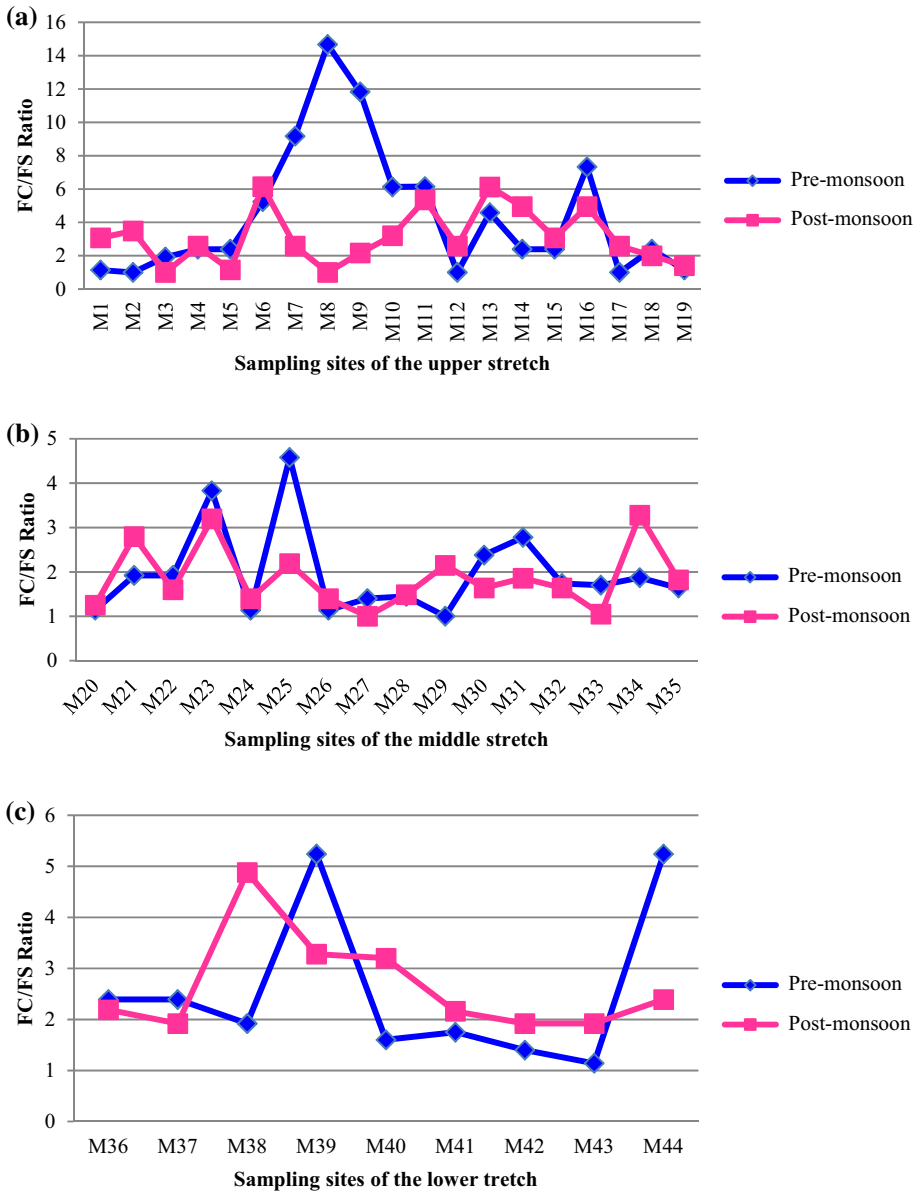

Fig. 2 Faecal colifom (FC)/faecal streptococci (FS) count of the various sites of Meenachil River Basin: **a** upper stretch; **b** middle stretch; **c** lower stretch

(Photo 1). McLellan et al. (2001) stated that faecal pollution indicator organisms can be used to monitor a number of conditions related to the health of aquatic ecosystems and the potential for adverse health impacts among individuals using these aquatic environments. The presence of such indicator organisms may provide indications of waterborne problems and is a direct threat to human and animal health.

The Meenachil river system is the major source of drinking water for the population along the river banks. Drinking water can be contaminated with these pathogenic bacteria, and this is an issue of great concern. However, the presence of pathogenic bacteria in water is sporadic and erratic, levels are low, and the isolation and culture of these bacteria is not straightforward. For these reasons, routine water microbiological analysis does not include the detection of pathogenic bacteria. However, safe water demands that water is free from pathogenic bacteria.

It is universally accepted that higher sewage contamination would lead to increased number of coliform and FC in natural water bodies. Hansen and Bech (1996) clearly suggest that there is a proliferation of allochthonous microflora in the river environment. As inestimable quantities of pathogenic bacteria constitute the microflora of effluents discharged from different anthropogenic activities, quantifying different groups of pathogenic bacteria have to be part of surveys on water quality. For instance, information on occurrence, abundance and distribution of potent human pathogens, *Vibrio cholera* (causing cholera in humans), *Vibrio parahaemolyticus* (gastroenteritis),

Salmonella and *Shigella* sp. (typhoid fever, food poisoning), *Streptococcus* sp. (meningitis and skin infections) and *Pseudomonas aeruginosa* (pulmonary and lung infections) in aquatic environment may prove useful in public health management.

In this context, an attempt was made to identify the bacterial species in the Meenachil river systems (Table 2). The consistently high load of the pollution indicator *E. coli* and its isolation from all the stations indicated that the water body is undergoing severe sewage pollution (Photo 6 and 7). This is due to human interference through settlements along the main reach and mixing of untreated municipality sewage with the river waters. *E. coli* is normally found in human and animal intestines and is the most reliable indicator of faecal contamination in water, which indicates the possible presence of pathogens (Geldreich and Clarke 1966).

All through the study period and at all sites, *E. faecalis* counts were lower than *E. coli* counts. Similar observations were made by Lanusse (1987), Fernandez-Alvarez et al. (1991), Chahlaoui (1996) and Hunter et al. (1999). This is owing to a difference in the rate of decline which is faster for *E. fecalis* (Hunter et al. 1999).

According to WHO (1992) the guideline criteria for faecal indicator bacteria for bathing waters, is: bacteria up to 500/100 ml for total coliforms, and 100/100 ml for both faecal coliforms and *Enterococci*. The survey of the indicator bacteria along the Meenachil river basin revealed that the waters are subjected to sewage pollution and are unfit for bathing. The counts of faecal coliforms (*E. coli*) exceeded 100 per ml all through the sampling stations. This is primarily due to excessive land runoff containing raw sewage and faecal debris, which in turn supports the proliferation of the tested faecal bacteria.

Pathogenic bacteria which may cause serious problem for human health have been studied mostly for their survival in the aquatic ecosystem (Sood et al. 2008; Nagvenkar and Ramaiah 2009; Harakeh et al. 2006; Servais et al. 2007; Sigua et al. 2010). Servaais et al. (2007) studied faecal contamination of the main rivers of the Seine watershed (Seine, Marne, Oise rivers) and found high levels of microbiological pollution when compared to European guidelines for bathing waters. They also found that the discharge of treated urban waste water effluents can significantly degrade the microbiological quality of rivers. The presence of *Pseudomonas* spp. in all the sites in all seasons may be attributed to human activities and sewage discharge to these sites. Pathogenic bacteria such as *S. aureus*, *P. aeruginosa*, and *Salmonella* sp. were isolated and identified. High level of incidence of *S. aureus*, *P. aeruginosa* and *Salmonella* sp. was observed during the post-monsoon season. The relatively high levels of prevalence of pathogenic bacteria during the rainy season suggest high influx of sewage, soil leaching and flooding as well as good survival capabilities of these microorganisms to changing hydrographic parameters (Mohamed et al. 2008). Sigua et al. (2010) too have demonstrated that a positive relationship exists between the variability of faecal contamination levels and agricultural cover. This substantiates the mounting risk of enhanced contamination occurring rapidly during the rainy season as agricultural cover increases.

In Kerala, Hepatitis-A, Typhoid, acute Diarrhoeal Diseases and Cholera are the major water-borne diseases (Shylaja 2009). Leptospirosis, acute dysentery, typhoid fever and acute hepatitis were reported from Kottayam district (John et al. 2004). *Salmonella* is widespread worldwide and is transmitted by ingestion of contaminated food and water. Its presence in river waters makes these waters unfit even for bathing. *P. aeruginosa* is present in the waters of Meenachil river basin but in very minor quantities. The presence of this bacterium poses a risk to swimmers because it is responsible for ear infections. The high incidence of human pathogenic bacteria in the river may indicate their possible presence in fish and other foods derived from this source.

In this study, water collected from majority of the sites were not suitable for domestic uses as it exceeds maximum permissible limits of total coliform and total FC as per the standards of National River Conservation Directorate, India. McLellan et al. (2001) stated that faecal pollution indicator organisms can be used to determine the number of cases related to the impacts on human health as well as health of aquatic ecosystems. The presence of such indicator organisms may provide information regarding waterborne diseases and is a direct threat to human, animal and aquatic organisms. The study clearly exposes the fact that water becomes unhealthy for drinking as well as domestic purposes because of contamination due to industrial and domestic litter. The present study has obviously demonstrated that there is a significant occurrence of bacterial pollution indicators and pathogenic bacterial groups in the Meenachil river. The condition of the river is very alarming.

Conclusion

The detection and isolation of *E. coli* and *V. parahaemolyticus*, *V. cholerae* and *S. enterica* from Meenachil river basin indicates the frequent discharge of sewage containing pathogenic microorganisms into the riverine ecosystem. Moreover, it throws light on the extended survival of these organisms to a detectable level at higher concentrations. The survival and persistence of these bacteria in natural environments is a matter of great concern.

Table 2 Occurrence of bacterial species in various sampling sites

Sampling site no.	*Acinetobacter*	*Aeromonas hydrophila*	*Bacillus subtilis*	*Citrobacter diversus*	*Citrobacter freundii*	*Escherichia coli*	*Enterobacter aerogenes*	*Enterococcus faecalis*	*Klebsiella oxytoca*	*Klebsiella pneumoniae*
M1	+	−	+	−	−	+	+	+	−	−
M2	+	−	+	+	−	+	−	+	−	+
M3	+	−	+	+	−	+	−	+	−	−
M4	−	−	+	+	−	+	+	+	−	+
M5	+	−	+	−	−	+	−	+	−	+
M6	+	−	+	−	+	+	+	+	−	+
M7	−	+	+	−	−	+	+	+	−	+
M8	+	+	+	−	−	+	+	+	−	+
M9	+	+	+	−	−	+	+	+	−	+
M10	−	−	−	−	−	+	−	+	−	−
M11	−	−	−	−	−	−	−	+	−	−
M12	+	−	+	+	−	+	+	+	+	+
M13	+	−	+	−	−	+	+	+	−	+
M14	−	−	+	−	−	+	−	+	−	−
M15	+	−	+	−	−	+	−	+	−	−
M16	−	−	−	−	−	−	−	+	−	−
M17	−	−	+	−	−	+	−	+	−	−
M18	+	−	+	−	−	+	+	+	−	+
M19	+	+	+	−	−	+	−	−	−	−
M20	−	−	+	−	−	+	−	−	−	−
M21	+	+	+	−	−	+	+	+	−	+
M22	−	−	+	−	−	+	+		+	+
M23	+	+	+	+	−	+	+	+	−	+
M24	+	−	+	+	−	+	+	+	−	+
M25	−	−	+	−	−	+	−	+	−	+
M26	+	−	−	−	−	+	+	+	−	+
M27	+	−	−	−	−	+	−	+	−	−
M28	−	−	+	+	−	+	+	+	−	−
M29	−	−	−	−	−	+	−	+	−	−
M30	−	−	+	−	−	+	+	+	−	−
M31	−	−	−	−	−	+	+	+	−	−
M32	+	−	+	−	−	+	+	+	−	−
M33	−	−	−	−	−	+	−	+	−	−
M34	−	−	−	−	−	+	−	+	−	−
M35	−	−	−	−	−	+	−	+	−	−

Table 2 continued

Sampling site no.	*Acinetobacter*	*Aeromonas hydrophila*	*Bacillus subtilis*	*Citrobacter diversus*	*Citrobacter freundii*	*Escherichia coli*	*Enterobacter aerogenes*	*Enterococcus faecalis*	*Klebsiella oxytoca*	*Klebsiella pneumoniae*
M36	+	−	+	+	−	+	−	+	+	+
M37	+	−	+	−	−	+	−	+	−	−
M38	−	−	−	−	−	+	−	−	−	+
M39	−	−	+	−	−	+	−	−	−	+
M40	−	−	+	−	−	+	+	−	−	+
M41	−	−	+	−	−	+	−	+	−	+
M42	−	−	+	−	+	+	+	+	−	+
M43	−	+	+	−	+	+	+	−	−	+
M44	+	−	+	−	+	+	+	−	+	+

Sampling site no.	*Micrococcus* sp.	*Proteus mirabilis*	*Proteus vulgaris*	*Providencia* sp.	*Pseudomonas aeruginosa*	*Salmonella enterica*	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Vibrio cholerae*	*Vibrio parahaemolyticus*
M1	−	+	+	−	+	−	+	−	−	+
M2	−	−	−	−	+	+	+	+	−	+
M3	−	+	−	−	+	−	+	+	+	+
M4	−	−	+	−	−	+	+	+	−	+
M5	−	−	−	−	+	−	+	−	−	+
M6	−	−	+	+	+	+	+	+	+	+
M7	−	−	+	−	+	−	+	−	−	+
M8	−	+	+	−	+	−	+	−	+	+
M9	−	−	+	−	+	+	+	+	+	+
M10	−	−	−	−	−	−	−	−	−	+
M11	−	−	−	−	−	−	−	−	−	+
M12	−	+	−	−	+	+	+	+	+	+
M13	−	+	+	−	+	+	+	+	+	+
M14	−	−	−	−	+	−	+	−	+	+
M15	−	−	−	−	+	−	+	+	−	+
M16	−	−	−	−	−	−	−	−	−	+
M17	−	−	−	−	−	−	−	−	+	+
M18	−	−	+	−	−	−	+	+	−	+
M19	−	−	+	−	+	−	+	+	−	+
M20	−	+	+	+	+	−	+	+	−	+
M21	−	+	+	+	+	+	+	+	+	+
M22	−	−	−	−	+	−	+	+	−	+
M23	−	+	+	−	+	−	+	+	−	+

Table 2 continued

Sampling site no.	*Micrococcus* sp.	*Proteus mirabilis*	*Proteus vulgaris*	*Providencia* sp.	*Pseudomonas aeruginosa*	*Salmonella enterica*	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Vibrio cholerae*	*Vibrio parahaemolyticus*
M24	−	+	−	−	+	−	+	−	+	+
M25	−	+	−	−	+	+	+	+	+	+
M26	−	+	−	−	+	−	−	−	+	+
M27	−	−	−	−	−	−	−	−	−	+
M28	−	−	−	−	−	+	−	+	−	+
M29	−	−	−	−	−	−	−	−	−	+
M30	−	−	−	−	−	+	−	−	+	+
M31	−	−	−	−	−	−	−	−	−	+
M32	−	−	−	−	+	−	−	−	+	+
M33	−	−	+	−	−	−	−	−	+	+
M34	−	−	−	−	−	−	−	−	−	+
M35	−	−	−	−	−	−	−	−	+	+
M36	+	−	−	−	+	+	+	+	−	+
M37	−	−	−	−	−	−	−	+	−	+
M38	−	−	−	−	+	−	+	+	−	+
M39	+	+	+	+	+	+	+	+	+	+
M40	−	+	+	−	+	+	+	+	−	+
M41	−	+	+	−	+	−	−	−	+	+
M42	−	+	+	−	+	+	+	+	+	+
M43	−	+	−	−	−	+	+	+	−	+
M44	+	+	−	−	+	+	+	+	+	+

The public health is at hazard as the population in this region depends on this water body for numerous domestic reasons. Apart from it, this water body sustains major fish and shellfish resources. The people of Kottayam mainly depend on Meenachil river for fish. There is a great chance of food-borne epidemics due to the presence of these pathogenic bacteria in fishes. The observations clearly indicate that all the studied sites of the Meenachil River have been contaminated with water-borne pathogenic bacteria. This may be due to increased anthropogenic and socio-cultural activities at different sites of the Meenachil River. Overall, the bacteriological analysis of the Meenachil River water reveal that the water is polluted by sewage, faecal contaminants and industrial wastes and this water is not appropriate for drinking and recreational purposes. Regular monitoring of microbial contamination in the water of the Meenachil River should be an essential component in future public health protection strategies.

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