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Increased prevalence of indicator and pathogenic bacteria in Vembanadu Lake: a function of salt water regulator, along south west coast of India

Abhirosh Chandran, A. A. M. Hatha and Sherin Varghese

ABSTRACT

Prevalence of faecal indicator bacteria, *Escherichia coli* and pathogenic bacteria, *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Salmonella* were analysed in Vembanadu lake (9°35′N 76°25′E), along south west coast of India for a period of one year from ten stations on the southern and northern sides of a salt water regulator constructed in Vembanadu Lake in order to prevent incursion of seawater during certain periods of the year. While the northern side of the lake has a connection to the sea, the southern side is enclosed when the salt water regulator is closed. The results revealed the water body is polluted with high faecal coliform bacteria with mean MPN value ranging from 1718-7706/100 ml. *E. coli*, *V. cholerae*, *V. parahaemolyticus* and *Salmonella* serotypes such as *S. paratyphi* A, B, C and *S. newport* were isolated and this is the first report on the isolation of these *Salmonella* serovars from this lake. *E. coli* showed highest percentage of incidence (85.6–86.7%) followed by *Salmonella* (42–57%), *V. choleare* (40–45%) and *V. parahaemolyticus* (31.5–32%). The increased prevalence of indicator and pathogenic bacteria in the enclosed southern part of Vembanadu Lake may be resulting from the altered flow patterns due to the salt water regulator.

Key words | *Escherichia coli*, India, *Salmonella*, Vembanadu Lake, *Vibrio cholerae*, *Vibrio parahemolyticus*

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INTRODUCTION

Environmental pollution, a potential global problem, has rendered waters along the coastline and recreational beaches unsatisfactory for public use. Population explosion and inadequate infrastructure to properly treat and dispose of the sewage, lack of sanitary condition, poverty and over exploitation of natural water has resulted in the discharge of considerable quantities of untreated waste into the natural waters. This organic pollution is especially severe in the coastal waters due to the large density of inhabitants in coastal areas (Scialabba 1998). Direct discharge of domestic waste, leaching from poorly maintained septic tanks, and improper management of farm waste are suspected as the major sources of waterborne disease (Huttly 1990). Sewage effluent contains a wide range of pathogenic microorganisms which may pose a health hazard to human population when they are discharged into the recreational waters (Borrego & Figueras 1997) and the health hazard could be severe in a heavily populated country such as India. In India, almost three-quarters of a billion people live in rural areas without access to safe drinking water and water-borne infections are a major cause of morbidity (Patil *et al.* 2002). Diseases such as enteric fever and diarrhoeal diseases are highly endemic to India and are major public health problems among the children under the age of five years. The Planning Commission in its report 'India Assessment 2002 – Water Supply and Sanitation' acknowledges that mortality and morbidity levels due to water borne diseases in the country are

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unacceptably high (www.cseindia.org/programme/health/pdf/conf2006/a1water.pdf). On a global basis, around 2 million deaths per year are attributed to water-borne diseases, and especially to diarrhoea in children (Gordon *et al.* 2004). Typhoid and paratyphoid fever resulting in an annual incidence of about 17 million cases world wide (Kindhauser 2003) and India has the highest incidence of typhoid, around three million cases each year (Anon, Typhoid, in *The Hindu*, February, 23, 2003).

Faecal coliform, *Escherichia coli* (the predominant member of faecal coliform group) are an operationally defined grouping of enteric bacteria whose presence in natural waters is used as an indicator of recent faecal contamination, and therefore, the possible presence for pathogenic microorganisms (Rhodes & Kator 1998), but their absence does not necessarily guarantee quality of water (Dutka 1973). *E. coli* include several pathogenic serotypes such as enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC) enteroaggregative E. coli (EAggEC) and enteroadhesive *E. coli* (DAEC), which are of public health significance worldwide and are a major cause of acute diarrhea in children in developing countries (Nataro & Kaper 1998; Rodrigues *et al.* 2002).

The presence of human pathogenic bacteria such as *Salmonella*, *V. cholerae*, *V. parahaemolyticus*, and pathogenic serotypes of *E. coli* has been reported from coastal areas (Venkateswaran *et al.* 1989**;** Daniels *et al*.2000; Hatha *et al.* 2004). Among the aquatic microflora *V. cholerae* and *V. parahaemolyticus* are responsible for most infections by *Vibrios* in developing as well as in developed countries (Faruque *et al.* 1998). The occurrence of high concentrations of *V. cholerae*, *V. parahaemolyticus*, *Salmonella* sp and pathogenic *E. coli* in shellfish grown in feacally polluted water and several food borne outbreaks due to the consumption of shellfish from sewage contaminated water has also been reported (Daniels *et al.* 2000). Coastal areas often provide very important recreational and economic resources (Costanza *et al.* 1997), and therefore the trophic status and quality of coastal waters, the safety of shellfish and fish farming waters are a concern for many countries including India.

The region of the Vembanadu Lake where the study has been carried out is known as Kumarakom Lake, which is a lifeline for people around Kumarakom. The population in the study area is scattered in many small islands without any central facility for effective waste collection and disposal, and the Kumarakom Lake acts as major sink for all domestic and industrial waste. Also the number of people using the system for agriculture, fishing, transportation and recreation is much more than the other parts of the Vembanadu Lake. The availability of pure drinking water is very low in this region and this lake water is being used for different domestic purposes. Water related diseases are very common in this region even though most of them are not reported officially. The water quality problem of the lake is further compounded by the construction of a salt water regulator which seriously affects the flow patterns of the lake. Though some published data are available on the water quality of the Cochin region of Vembanadu Lake (Lakshmanaperumalsamy *et al.* 1981; Chandrika 1983; Hatha *et al.* 2004) there are virtually no reports available on the microbial pollution from the present study area, i.e., Kumarakom Lake. Hence the present study has been carried out with an objective to systematically examine the prevalence of indicator and pathogenic microorganisms such as *E. coli, V. cholerae, V. parahaemolyticus* and *Salmonella* at various stations along the southern (enclosed) and northern (open) side of the salt water regulator.

MATERIALS AND METHODS

Description of study area and sampling

Vembanadu Lake, an important Ramsar site, lies 0.6–2.2 m below mean sea level (MSL) along the south west coast of India (9°35′N 76°25′E) and has a permanent connection with the Arabian Sea at barmouth (Figure 1). As the north-east monsoon recedes, the area is exposed to tidal incursion of saline water from the Arabian Sea making the ecosystem predominantly saline. In order to prevent the saline incursion from the Arabian Sea, a salt water regulator was constructed in the lake at the Kumarakom region of the Vembanadu Lake, which divides the estuary into a fresh water lake on the southern side (enclosed when the salt water regulator is closed) and a saline lagoon on the

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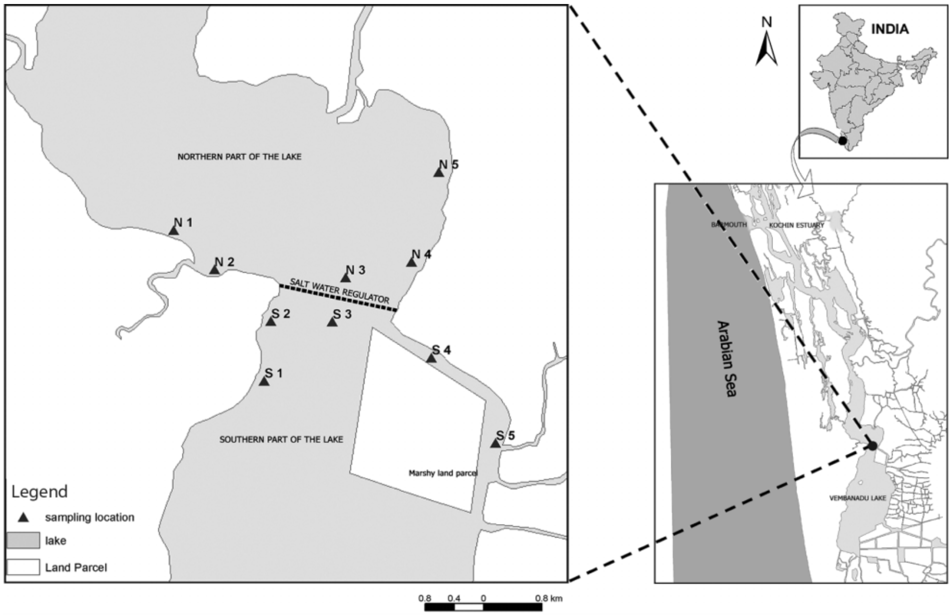

Figure 1 | Map showing Thanneermukkam salt water regulator and sampling locations.

northern side (open to Arabian sea). Every year the shutter of the regulators is closed in December and opens in March and the impact of closure and opening of the regulator has been a topic of endless debate. When the regulator is closed there is virtually no flow of water beyond it making the southern region a static pool. The periodic tidal inflow, which used to flush the water body is completely prevented with the result that the drained water from the surrounding rice fields and human dwellings with heavy load of pollutants remains stagnant in the water body.

Studies were carried out from October 2004 to September 2005 at the Kumarakom region of Vembanadu Lake, where the salt water regulator is constructed and most affected by this structure. Monthly collections of estuarine water samples were made from 5 stations on the southern (enclosed region) and 5 on northern sides (open) of the salt water regulator in sterile plastic bottles. Water samples were transported to the laboratory in an ice box and subjected to bacteriological examination within 2 hours of collection.

Bacteriological analysis

A three tube fermentation method was used to estimate faecal coliforms using EC broth (Hi-media, Bombay, India) as the medium and incubation at 44.5°C for 24–48 hours. Loopful of culture from each tube showing growth and gas production were streaked on Eosine Methylene Blue (EMB, Himedia, Bombay) agar for the isolation of *E. coli* and incubated at 37°C for 24 hours. Typical *E. coli* like cultures were isolated, restreaked to ensure purity and confirmed by indole, methyle red, voges proskauer and citrate (IMViC) test. Isolates showing $++--$ reaction for IMViC test were confirmed as *E. coli*.

Two methods for enumeration of *V. parahaemolyticus* and *V. cholerae* were used. The first was a direct plating procedure, which included inoculating 0.2 ml estuarine water sample on Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS, Himedia, and Bombay) plates, and incubating at 37°C for 48 hrs. 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 estuarine water samples were inoculated into 40 ml alkaline peptone water for pre-enrichment in a conical flask and incubated at 37°C for 24 hours. Flasks showing growth in enrichment broths were streaked onto TCBS agar and incubated at 37°C for 24–48 hours. Typical colonies, whenever present, were isolated, restreaked to ensure purity and maintained on nutrient agar slants for further biochemical characterisation.

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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% (−), 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.

For the detection of *Salmonella*, about 1 or 2 litre volumes of surface waters were filtered through sterile 0.45-μm membrane filters. The filters were cut into pieces and placed into selective enrichment broth such as Tetrathionate broth (TTB, Himedia, Bombay) and Selenite cystine broth (SCB, Himedia, Bombay) following aseptic procedures and incubated at 37°C for 24–48 hrs. After selective enrichment, a loopful of cultures from both SCB and TTB were streaked on to selective media such as xylose lysine deoxycholate (XLD, Himedia, Bombay) agar and Hektoen Enteric agar (HEA, Himedia, Bombay) plates and incubated at 37°C for 24 to 48 hours. Typical *Salmonella* like colonies were picked up restreaked to ensure purity and were maintained on tryptic soy agar (TSA, Himedia, Bombay) slants at room temperature for further biochemical testing.

The stored *Salmonella* cultures were subjected to primary biochemical testing involving reactions in triple sugar iron (TSI, Himedia, Bombay) agar, lysine iron agar (LIA, Himedia, Bombay) slants, indole production in tryptone broth and urease production on Christnsen's urea agar (Himedia, Bombay) were tested. Cultures matching typical reaction of *Salmonella* were subjected to secondary biochemical characterization involving fermentation of carbohydrates such as lactose, sucrose, dulcitol and salicin. Isolates which matched typical biochemical reactions of *Salmonella* were further confirmed by slide agglutination test using *Salmonella* polyvalent 'O'serum (Difco, USA). Serotyping of the strains was carried out at National *Salmonella* and *Escherichia* Centre, Kasauli, Himachal Pradesh, India.

RESULTS AND DISCUSSION

The present study has been taken up considering the modified flow patterns of the Vembanad Lake due to the construction of a salt water regulator and public health importance as a large number of people in this region use this water body for their day to day activities which include fishing for livelihood, transportation and recreation. The MPN index of faecal indicator bacteria such as faecal coliform (FC) at different stations during the period October 2004 to September 2005 is represented in Figure 2. Levels of faecal coliform contamination remained high throughout the study period and ranged from mean MPN value 1718–7706/100 ml and showed spatial variation at different stations along the southern and northern sides of the regulator. The MPN index of FC was higher than those recorded in the Cochin region of Vembanad Lake (Lakshmanaperualsamy *et al.* 1981; Hatha *et al.* 2004) and in Mondovi Zuary estuaries of Goa (Row 1981). The higher load of FC in the study area is possible, as the human population has grown considerably in the last two decades and increased the waste generation. Many illegal sewers, especially from markets and septic tank effluents, directly enter into the estuary. Also, there is a practice of dumping animal carcasses into the lake at various points near the salt water regulator. There are no effective measures to control such activities, which could result in gross contamination of the system, and the self purifying capacity of the system

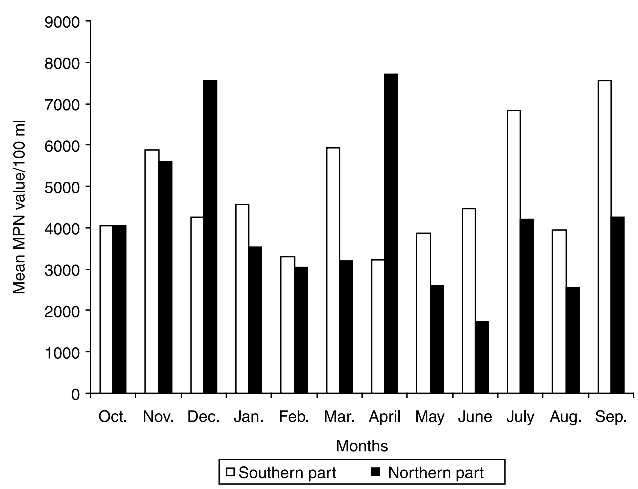

Figure 2 | Prevalence of faecal indicator bacteria in northern and southern part of the salt water regulator in Vembanadu Lake during 2004 to September 2005.

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might cease to function once the waste load into this water body exceeds its carrying capacity.

The monsoon is a very prominent annual climatic feature of Kerala and hence the study period has been divided into three distinct seasons, such as pre-monsoon (February to May), monsoon (June to September) and post monsoon (October to January). Seasonal variation of indicator bacteria at each station during different seasons is represented in Figure 3. The results indicate substantially higher levels of indicator bacteria in the southern part of the lake (enclosed) during the monsoon than the northern region. Higher bacterial population during these months may be due to the increased land run off during the monsoon period resulting in a higher faecal input into the lake from various sources. In our (Abhirosh & Hatha 2005) previous studies on the inactivation kinetics of indicator bacteria in Cochin estuary we could find that sunlight was a major factor affecting the self-purifying capacity of the natural waters. Reduced intensity of sunlight due to overcast conditions during the monsoon as well as increased turbidity from land run off resulting in reduced penetration of sunlight might extend the survival of faecal indicator bacteria. An increase in the faecal coliform level after rainfall was reported previously (Shehane *et al.* 2005). However in the northern region (open) more dynamic environmental conditions prevail (especially salinity) and the monsoon rains may dilute the bacterial load which enters into the lake. During December to March the system is closed and natural

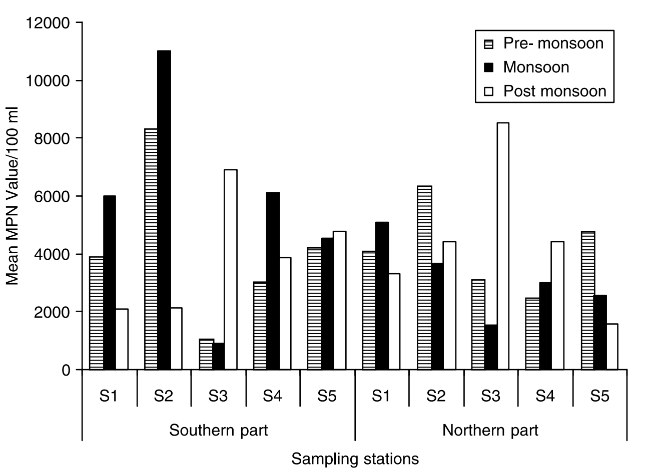

Figure 3 | Seasonal variation of indicator bacteria at different stations in northern and southern parts of salt water regulator in Vembanadu Lake during Oct. 2004– Sept. 2005.

flow is prevented which results in the accumulation of organic load in the southern part of the lake, giving proper environmental conditions for the multiplication of bacteria. The use of this water for harvesting shellfish and recreation during this period may pose serious health risks to the people. After opening the salt water regulator in March, a sudden increase in the MPN index of FC was observed at the stations on the northern part of the salt water regulator. During the summer months of March through May, tidal incursions at the northern part of the regulator, however, resulted in a decrease in FC values possibly due to the high salinity. The seasonal salinity on the northern part of the regulator varied between 0–18 ppt and it has been reported that the increasing salinity causes sublethal stress in *E. coli* and affects the survival of the bacteria (Anderson *et al.* 1979).

High densities of faecal indicator bacteria have been sporadically reported from different coastal regions in India (Pradeep & Lakshmanperumalsamy 1986). Other reports also indicated relatively low levels of total viable counts (TVC) and FC at several locations along the Tamilnadu coast extending between the Pulicat Lake and the Cauvery River confluence (Venkateswaran & Natarajan 1987) in the port region of Bhavanagar (Abhay Kumar & Dube 1995) and Visakapattanam (Clark *et al.* 2003). Faecal pollution of recreational waters may be a health hazard for bathers due to the presence of several microbial pathogens, including bacteria, viruses, fungi and protozoa.

Bacterial pathogens such as *V. cholerae*, *V. parahaemolyticus* and various serotypes of *Salmonella* and indicator bacteria, *E. coli* were isolated and identified from various sampling stations. *Salmonella* serotypes included *Salmonella paratyphi* A, B, C and *S. newport*. The percentage incidences of these specific pathogenic bacteria are represented in Table 1. No significant variation was observed in the prevalence level of specific pathogens at different stations along both sides of the salt water regulator, though there was a slightly higher level of incidence of *Salmonella* at stations in the southern part of the regulator. While *E. coli* were isolated consistently from all stations, the prevalence of pathogenic *Vibrios* varied from 32 to 45%. Prevalence of *Salmonella* was also relatively high (42–57%). The relatively high prevalence of pathogenic bacteria in this important water body suggest high influx of sewage as well as good survival capabilities of

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Table 1 | Incidence of indicator and pathogenic bacteria in the Vembanadu Lake during the study period

Indicator/pathogenic bacteria	Southern part of the salt water regulator Samples tested	Samples positive	% incidence	Northern part of the salt water regulator Samples tested	Samples positive	% incidence
Escherichia coli	212	184	86.7	354	303	85.6
Vibrio cholerae	50	20	40	77	35	45
Vibrio parahaemolyticus	75	24	32	130	41	31.5
Salmonella	75	43	57*	81	34	42**

*Serotypes include S. *paratyphi* A, S. *paratyphi* B, S. *paratyphi* C and S. *newport*
**Serotypes include S. *paratyphi* A, S. *paratyphi* B and S. *paratyphi* C

these microorganisms to changing hydrographic parameters. The analysis of the results also indicated that there is no significant correlation between high levels of FC and incidence of specific pathogens at various stations in the study area.

The association of *V. parahaemolyticus* with freshwater fishes and their ability to survive in association with freshwater plankton under certain environmental conditions is, significant. Recent studies on the Na^+ requirement of *V. parahaemolyticus* and *V. cholerae* have indicated that, in contrast with other marine bacteria, the quantitative requirements for Na^+ for growth vary with the substrate serving as the carbon and energy source in the medium (R. A. MacLeod, personal communication). This would imply that, under certain specific nutrient conditions, the Na^+ requirement of *V. parahaemolyticus* is not mandatory and that the halophile can well survive in conditions where the salt concentration may be equal to or even lower than physiological concentrations. Several workers reported the high incidence of *Vibrio sp*. from the Indian sub continent. For instance, high densities of *Vibrio* sp. were reported from the inshore waters of the east coast (Nair *et al.* 1980) from Cochin back waters (Chandrika 1983) and from the offshore waters of the west coast (Pradeep & Lakshmanperumalsamy 1986; Lokabharathi *et al.* 1987) and from Visakapattanam (Clark *et al.* 2003). However, the prevalence levels were lower when compared to our findings.

In the present study, the prevalence of *Salmonella* was significantly higher than those reported by (Hatha *et al.* 2004) from the Cochin estuary which is the northern part of the Vembanadu Lake system. However, diversity of serovars was limited. While *S. newport* was the predominant serovar, others included *S. paratyphi* A, B and C. Though *Salmonella* cells may enter into viable, but non-culturable state (VBNC) under conditions of stress, their presence would be a health concern especially in shell fish growing waters. The study area is well known for its shellfish resources and many people are engaged in exploiting this important fishery resource for their livelihood. While the flesh is being sold for human consumption in the local markets, the shell is treated to produce powdered calcium carbonate. In many cases the infection due to *Salmonella* is linked to the consumption of seafood (Centers for Disease Control & Prevention 2000), particularly shellfish (Heinitz *et al.* 2000). Though food and water borne outbreaks are common in the study area, no systematic reporting, tracking and monitoring mechanisms are in place in the study area. The *Salmonella* enterica serovars isolated in the present study such as *S. paratyphi* A, B, C and *S. newport* are important human enteric pathogens causing enteric fever by the contamination of water and food. Since humans are the only reservoir of *S. paratyphi* A (Hook 1990), the results are indicative of the contamination of this water body from human excreta. This is the first report of the isolation of these pathogenic strains of *Salmonella* from the Kumarakom region of Vembandu Lake.

The consistently high load of E. *coli* and its isolation from all the stations indicate that the water body is undergoing severe sewage pollution. Although we do not have the information about the serotypes of *E. coli* from the present study, we have encountered more than 40 serotypes of *E. coli* including potential pathogens such as enterohaemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and uropathogenic *E. coli* (UPEC) in our previous studies at the Cochin estuary

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(Hatha *et al.* 2004). We also expect such a wide diversity of *E. coli* serotypes including diarrhegenic strains in the present study area.

CONCLUSION

The detection and isolation of *E. coli* and *V. parahaemolyticus, V. cholerae* and *Salmonella* serovars from Vembanadu Lake indicates the frequent discharge of sewage containing pathogenic microorganisms into the estuary and also the extended survival of these organisms to a detectable level at higher concentrations. The survival and persistence of these bacteria in natural environments is of particular importance to public health as the population in this region is using this water body for several domestic purposes. This water body supports major fish and shellfish resources and the presence of these pathogenic bacteria is still posing a public health concern through related food borne outbreaks. The increased prevalence of indicator bacteria in the southern part of the lake may be due to the altered flow patterns due to the salt water regulator. However, the salt water regulator does not have any significant impact on the prevalence of specific pathogens. The results of the present investigation concluded that the hygienic quality of this water is poor and it may pose severe health risks to the population using the water for different purposes. The hygienic quality of water is of the utmost importance to society, and enforcement of legal measures to control entry of illegal point source effluents into this water body are required for good management of this vital natural resource.

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