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Potential Exposure Risk Associated with the High Prevalence and Survival of Indicator and Pathogenic Bacteria in the Sediment of Vembanadu Lake, India

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Abstract Prevalence of faecal coliform bacteria and the survival of *Escherichia coli*, *Vibrio parahaemolyticus* and *Salmonella paratyphi* were studied in the water and sediment from Vembanadu Lake in the presence and absence of protozoan predators. The density of faecal coliform bacteria ranged between mean MPN value 5080–9000/100 ml in water and 110,000–988,000/1 g in sediment ($p < 0.01$), which was 110 times greater than in overlying water. The laboratory microcosm studies revealed that *E. coli*, *V. parahaemolyticus* and *S. paratyphi* showed significantly higher survival ($p < 0.05$) potential in sediment than in overlying water both in the presence and absence of protozoan predators. The results indicate that Vembanadu Lake sediment constitutes a reservoir of pathogenic bacteria and exhibits potential health hazard from possible resuspension and subsequent ingestion during recreational activities. Therefore, assessment of bacterial concentration in freshwater lake sediments used for contact and non-contact recreation is of considerable significance for the proper assessment of microbial pollution of the overlying water and the management and protection of related health risk at specific recreational sites. In addition, assessment of the bacterial concentration in sediments can be used as a relatively stable indicator of long-term mean bacterial concentration in the water column above.

Keywords Exposure risk · Indicator bacteria · Pathogen · Survival · Sediment · Vembanadu Lake

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Introduction

Microbial contamination of freshwater resources and associated waterborne infections due to various exposure routes are still a major problem in many parts of the world including India. Untreated or only partly treated wastewaters including industrial, agricultural and domestic wastes released into the rivers and lakes are the major pathway of enteric bacterial pathogens. The long-term survival of these organisms in natural waters constitutes a significant public health concern because of the dangers they pose to humans through consumption and recreation. Faecal pollution originates from a variety of human and non-human sources, but contamination from human faecal material 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). Over 250 million cases of gastroenteritis and respiratory diseases and 5–10 million cases of hepatitis are reported annually from coastal regions all over the world (Clark et al. 2003). Contact with bathing waters with high concentrations of *E. coli* is documented in epidemiological studies as being associated with an increased risk of contracting gastrointestinal disease (Kay et al. 1994; Fleisher et al. 1993). Even though surface water is being getting polluted, all natural systems have got their own natural self-purifying capacities owing to various physico-chemical and biological factors (Abhirosh and Hatha 2005; Abhirosh et al. 2009).

In India, almost three quarters of a billion people live in rural areas without access to safe drinking water and waterborne 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 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 waterborne diseases in the country are unacceptably high www.cseindia.org/programme/health/pdf/conf2006/a1water.pdf. On a global basis, around 2 million deaths per year are attributed to waterborne diseases, and especially to diarrhoea in children (Gordon et al. 2004). Similarly, typhoid fever caused by *S. enterica* serotype typhi and paratyphi is a common infectious disease occurring in all the parts of the world with its highest endemicity in certain parts of Asia, Africa, Latin America and the Indian subcontinent with an estimated incidence of 33 million cases each year (Threlfall 2002).

E. coli is considered a typical faecal indicator bacteria and its presence in natural waters indicates the possible presence of enteric pathogens. *V. parahaemolyticus* is a natural inhabitant of marine and estuarine environments, which has been recognized as an important cause of food-borne illness in Asia and United States and most of the outbreaks are associated with consumption of raw or undercooked shellfish (Daniels et al. 2000). Although international efforts to control diarrhoeal diseases were launched by WHO (Synder and Merson 1982), the Indian subcontinent is still known as the ‘home of cholera’.

The present study has been carried out in Vembanadu Lake that lies 0.6–2.2 m below mean sea level (MSL) along the west coast of India (9°35′N 76°25′E) and has a permanent connection with the Arabian Sea at Barmouth region (Fig. 1). As the north-east monsoon recedes, the area is exposed to tidal incursion of saline water from the Arabian Sea. In order to prevent the saline incursion during certain periods of the year, a salt-water regulator is constructed in the lake. It divides the lake into a freshwater region on the southern part and a saline lagoon on the northern part. 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. On the other hand, over 1.6 million people directly or indirectly depend on it for various purposes such as agriculture,

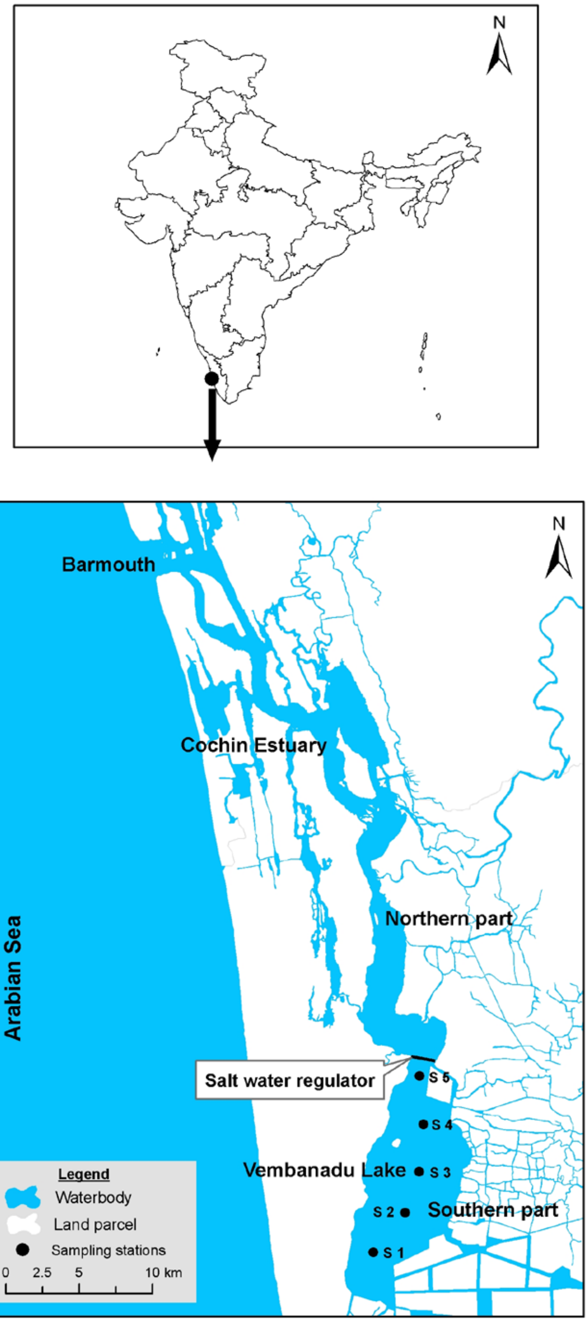

Fig. 1 Map showing Vembanadu Lake and sampling locations

fishing, transportation and recreation. A large population of the area is facing severe scarcity of drinking water because of the saline nature of the groundwater and the lake serves as the only source of freshwater. As a result, water-related diseases are very common in this region, particularly in young children, but none of them were reported officially. The major source of microbial contamination of the lake is from the direct discharge of faecal waste from nearby houses and houseboats, dumping of animal carcasses after slaughtering process and sewage from market place, etc.

The U.S. Environmental Protection Agency, the European Union and Central Pollution Control Board (CPCB) recommend the use of indicator *E. coli* to assess the hygienic safety of recreational waters (USEPA 2000; EU 2006). As hygienic quality of water is of the utmost importance to society, regular water quality monitoring is necessary because prolonged survival and persistence of pathogenic bacteria in natural water resources used as a common source of water by humans would be a public health concern. Usually the hygienic quality of the surface water is assessed only in terms of the density of faecal indicator bacteria (*E. coli*) in overlying water alone and sediment is often overlooked. Thus, the potential of sediment-bound bacteria to pollute the overlying water during recreational activities and the subsequent health risks posed by pathogenic microorganisms are not often considered while assessing the hygienic safety of water.

The southern region of Vembanadu Lake is being increasingly used for contact recreation and other domestic purposes because of the availability of fresh water. However, no regular water quality monitoring programmes have been instituted and the only published work available on the microbial pollution of Vembanadu Lake is by Abhirosh et al. (2008). However, in this study the faecal coliform load in the sediment has not been evaluated. To the best of our knowledge, no published works are available on the health risk associated with the faecal contamination of aquatic sediments in India except the one conducted along the east coast of India by Clark et al. (2003). Nevertheless, they did not evaluate the survival of indicator and pathogens in the sediments. Considering the health risk associated with the increased use of southern region, the present study was undertaken to investigate the prevalence level of faecal coliform bacteria in water and sediment as well as the survival of *E. coli*, *V. parahaemolyticus* and *S. paratyphi* in water and sediment using laboratory microcosm experiments.

Materials and Methods

Collection of Water and Sediment Samples

Monthly water and sediment samples were collected from 5 stations on the southern (enclosed region) part of Vembanadu Lake in sterile plastic bottles. Sediment (subsurface) samples were collected with the aid of a grab sampler and were transported to the laboratory in an icebox and subjected to bacteriological examination within 4 hours of collection. The samples were processed for faecal coliform bacteria.

Bacteriological Analysis

A three-tube fermentation method (MPN) as per USEPA (1978) by using EC broth (casein enzymic hydrolysate—20 g/L, lactose—5 g/L, bile salts mixture—1.5 g/L, potassium phosphate—4 g/L, monopotassium phosphate—1.5 g/L, sodium chloride—5 g/L, final pH 6.9 ± 0.2. Himedia, Mumbai, India) as the medium and incubation at 44.5°C for 24–48 hours, was used to estimate faecal coliform from water and sediment after appropriate dilution of the samples. After incubation, growth and gas production in tubes were recorded and were confirmed as being faecal coliform. The sediment samples were mixed thoroughly, and 10 g (wet weight) were weighed out into 90 ml of sterile water in a conical flask. These were again mixed thoroughly with the help of a sterile magnetic stirrer (KEMI, India) for 30 minutes. Then they were allowed to settle for 10 min prior to withdrawal of the supernatant for the estimation of bacteria. The density of faecal coliform was expressed as most probable number (MPN) per 100 ml of water and per gram of sediment.

Survival Experiments

Test microorganisms Confirmed pure cultures of *E. coli*, *V. parahaemolyticus* and *S. paratyphi* previously isolated from Vembanadu Lake and maintained in the culture collection of the School of Environmental Sciences of Mahatma Gandhi University were used for survival studies. The detailed methodologies for the isolation, characterization and identification of these organisms have been described in our earlier study (Abhirosh et al. 2008). *V. parahaemolyticus* is an indigenous bacterium, which has been selected because of its importance as a leading shellfish-borne pathogen in the developed and developing world.

Preparation of the inocula The inocula were prepared as previously described by Abhirosh and Hatha (2005). Briefly, *E. coli/V. parahaemolyticus* and *S. paratyphi* were grown in tryptone soya broth (TSB) and incubated at 37°C for 24 hours. After incubation, the cells were concentrated by centrifugation at 3000 rpm for 15 minutes and washed twice with sterile isotonic saline. After the final wash, the cells were suspended in the same isotonic saline at a concentration of $10^{8–9}$ colony-forming units per ml.

Microcosm Water microcosms were prepared as previously described by Abhirosh and Hatha (2005). A 100 ml water taken in an Erlenmeyer flask (250 ml capacity) was used as water microcosm. Closed bottle sediment microcosms (Davies et al. 1995) were prepared by taking 100 g sediment (wet weight) into sterile conical flask with 50 ml autoclaved water. Cycloheximide is a eukaryotic inhibitor that was added to individual water and sediment microcosm separately to inhibit protozoan predators at a concentration of 2 g/100 g of sediment and per 100 ml of water (Davies et al. 1995) whenever necessary. Then individual water and sediment microcosms were inoculated separately with *E. coli/V. parahaemolyticus*/*S. paratyphi* (approximately $10^{8–9}$ cfu/ml). After inoculation, the sediment and water microcosms were mixed thoroughly for the uniform distribution of the bacteria in the microcosms. After that all microcosms were incubated (without agitation) at 25 ± 1°C to imitate the temperature of the lake water and sediment. The mean temperature of the study area was around 25°C. The following microcosms were used for the survival study:

– *raw water and sediment supplemented with cycloheximide*: These microcosms were used to evaluate the survival of the test organisms in the absence of protozoan predators;
– *raw water and sediment without cycloheximide*: These microcosms were used to determine the survival of the test organisms in the presence of protozoan predators;
– *autoclaved water and sediment supplemented with cycloheximide*: These microcosms were used to evaluate whether cycloheximide has any negative effect on the test organisms and also to study the survival in the absence of biological factors.

Enumeration techniques The samples from water and sediment microcosms were taken using sterile pipette and sterile spatula and assayed for 27 days using spread plate technique. Prior to sampling the sediment, the overlying water was removed carefully with a sterile syringe without disturbing the surface of the sediment. A total of 10 g sediment was obtained by collecting 1 g each from different portion of the same sediment microcosm. Then this sediment was taken into sterile conical flask containing 90 ml sterile distilled water and mixed thoroughly with the help of a magnetic stirrer for 10 minutes and the supernatant was used. The overlying water was carefully replaced after each sampling. Similarly, after thorough mixing, 1 ml water sample from individual water microcosm were taken into 9 ml sterile distilled water and mixed well with the help of a cyclomixure (REMI, India) for 5 minutes. Enumerations of bacteria from water and sediment were performed after appropriate dilution using spread plate technique on selective media. Eosine methylene blue (EMB) agar, thiosulfate citrate bile salts sucrose (TCBS) agar and xylose lysine deoxycholate (XLD) agar were used respectively for *E. coli, V. parahaemolyticus* and *S. paratyphi* as selective media. All the media were purchased from Hi-Media laboratory, Mumbai, India.

Statistical Analysis

Two-way analysis of variance (ANOVA) was performed to determine the difference in the prevalence of faecal coliform bacteria in water and sediment, as well as the difference in the survival of the test organisms in water and sediment.

Results and Discussion

Prevalence of Faecal Coliform Bacteria

The MPN index of faecal coliform bacteria in the water and sediment during different months is given in Table 1. The abundance of faecal coliform bacteria ranged between mean MPN value 5080–9000/100 ml in water and 110,000–988,000/1 g in sediment. The results also indicated that the prevalence level of faecal coliform bacteria was significantly higher in sediment ($p < 0.01$) than in overlying water. It was substantially high in all the stations (data not shown) both in water and sediment, indicating a high degree of faecal contamination of the water body. In our earlier investigation in the Vembanadu Lake we observed a high abundance of faecal coliform bacteria with mean MPN value ranging from

Table 1 MPN index of faecal coliform bacteria in water and sediment during different months

Months	Water	Sediment
	Mean MPN/100 ml	Mean MPN/1 g
March	6550	807000
April	5620	522000
May	5080	110000
June	6330	782000
July	5900	508000
August	6130	743000
September	9000	988,000
October	5830	768000
November	5880	965000
December	5600	834000
January	5560	927000
February	5280	720000

1718–7706/100 ml in water (Abhirosh et al. 2008). It was also much higher than those recorded in other coastal waters of India such as Cochin region of Vembanadu Lake (Lakshmanperumalsamy et al. 1981; Pradeep and Lakshmanperumalsamy 1986; Hatha et al. 2004), Mondovi and Zuary estuaries of Goa (Row 1981), along the Tamilnadu coast extending between the Pulicat lake and the Cauvery river confluence (Venkateswaran and Natarajan 1987), in the port region of Bhavnagar (Abhay Kumar and Dube 1995) and Visakapattanam (Clark et al. 2003).

In the present study, the density of faecal coliform bacteria in the sediment was 110 times greater than that in the overlying water. However, it was lower compared to the observation made by Stephenson and Rychert (1982) and Ashbolt et al. (1993), who reported even higher concentration of indicator bacteria in sediment than in overlying water in various aquatic sediments, but it was higher than that reported from Indian coastal waters of Visakapatnam (Clark et al. 2003). These results indicated that sediments of different aquatic habitat in various parts of the world are a well-recognized reservoir for enteric microorganisms (Burton et al. 1987; An et al. 2002). Numbers of epidemiological studies have demonstrated that the contact with bathing water subjected to faecal contamination increases the risk of disease (Kay et al. 1994; Fleisher et al. 1993), particularly through the resuspension of sediment-bound bacteria. It also increases the health risk due to the contamination of shellfish bed and subsequent consumption of undercooked seafood due to their ability to concentrate pathogens and toxins during the filter-feeding process (Rippey 1994). The results of the present study is of particular health significance since the present study area presents a major shellfish harvesting water where a potential risk through shellfish consumption could be expected.

The higher load of FC in the lake is possible, as increased commercial and developmental activities in rural areas around the lake have paved the way for a swell in the population, which in turn has increased the volume of wastes. There is no central facility for the effective waste collection and disposal of the waste, and this water body acts as a major sink for all the domestic waste. Besides, most of the houses on the bank of the lake are discharging the faecal waste directly into the lake, as well as many illegal sewers from markets are also entering into the lake. In addition, there is a practice of dumping animal carcasses into the lake at various points. Since the lake is an international tourism destination, the water tourism's contribution to the pollution and contamination of the lake is said to be significant as most of the houseboats operating in the backwaters do not have any sewage treatment facility and the domestic waste including human excreta are dumped directly into the lake. There are not effective measures to control such activities, which could result in gross contamination of the system. A recent article (Srikanth 2009) while addressing the challenges of sustainable water quality management in rural India, reported that 15 major rivers of India, which are the source of water for urban and rural population, are heavily polluted with faecal coliform bacteria. The high microbial contamination of Indian surface water resources would be a public health concern because enteric diseases, skin, ear, and eye infections are frequently reported from exposure to recreational waters contaminated by faecal wastes (Pruss 1998). In addition, according to Planning Commission of India, mortality and morbidity levels due to waterborne diseases are unacceptably high in India where diarrhoea is ranking top in morbidity and 10th in mortality www.cseindia.org/programme/health/pdf/conf2006/a1water.pdf.

Survival of Indicator and Pathogenic Bacteria in Water and Sediment

The survival curves of *E. coli*, *V. parahaemolyticus* and *S. paratyphi* in raw water and sediment with and without the addition of cycloheximide are represented in Figs. 2, 3 and 4 respectively. In raw water and sediment without cycloheximide, they showed relatively high reduction compared to raw water and sediment supplemented with cycloheximide. However, under both experimental conditions they showed significantly high survival in ($p < 0.05$) sediment compared to overlying water. The survival of *E. coli*, *V. parahaemolyticus* and *S. paratyphi* in autoclaved lake water and sediment supplemented with cycloheximide was very high (Table 2). They survived at high density until the end of the experiment and reduced hardly 1 log. The laboratory microcosm study revealed that *E. coli*, *V. parahaemolyticus* and *S. paratyphi* showed higher survival ($p < 0.05$) potential in sediment than in overlying water both in the absence and presence of predators.

This observation is supported by other studies supporting that *E. coli*, *V. parahaemolyticus* and *Salmonella* can survive better in the sediments than in the overlying waters (Burton et al. 1987; Davies et al. 1995). The high survival of these organisms in sediment may be due to the protection offered by the sediment from protozoan predation (Davies and Bavor 2000), availability of nutrients (Craig et al. 2004) and by substratum for adherence (Davies et al. 1995). A greater difference between the densities of bacterial population in the presence of protozoan predators compared to those in their absence was observed for *E. coli, V. parahaemolyticus* and *S. paratyphi* in water and sediment. These results indicated the role of predatory protozoans in the survival of the test organisms. When the survival studies were conducted in autoclaved water and sediment supplemented with cycloheximide (without any biological factors), the death of the test organisms was negligible indicating the specific role of protozoan in the survival of the test organisms and also indicated that cycloheximide had no deleterious effect on the

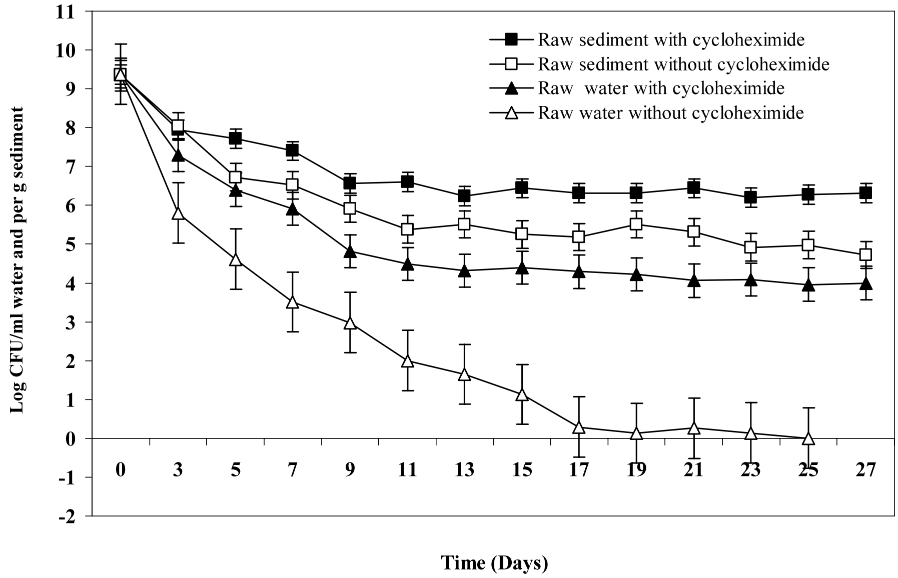

Fig. 2 Survival curves of *E. coli* in raw water and sediment with and without the addition of cycloheximide (mean ± SD, $n = 4$)

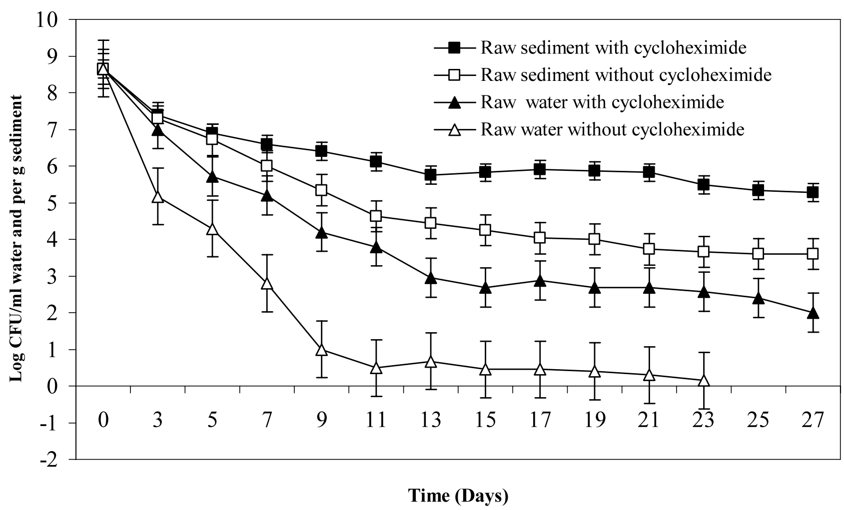

Fig. 3 Survival curves of *V. parahaemolyticus* in raw water and sediment with and without the addition of cycloheximide (Mean ± SD, $n = 4$)

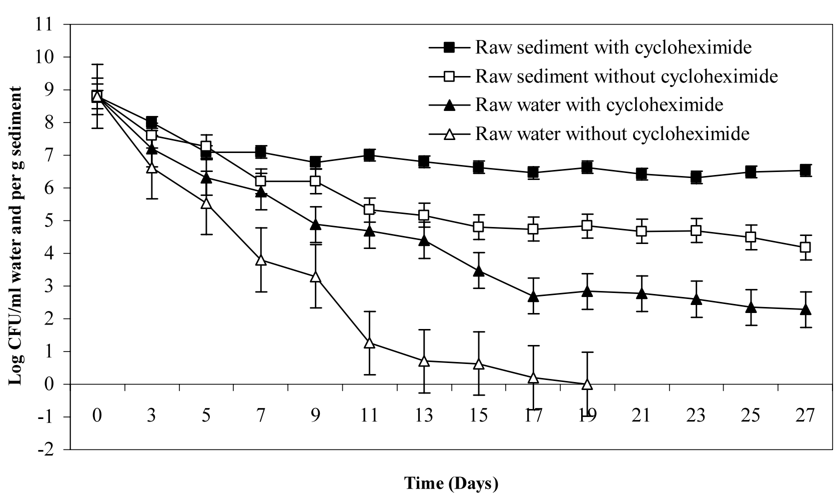

Fig. 4 Survival curves of *S. paratyphi* in raw water and sediment with and without the addition of cycloheximide (mean ± SD, $n = 4$)

Table 2 Variation in the population of test microorganisms in autoclaved lake water and sediment supplemented with cycloheximide

Bacteria	Log population of test microorganisms on		
	0th day	15th day	27th day
*E. coli**	8.823	8.34	8.26
*E. coli***	8.823	7.75	7.25
*S. paratyphi**	8.8	7.94	7.62
*S. paratyphi***	8.8	7.44	6.69
*V. parahaemolyticus**	8.8	8.16	7.34
*V. parahaemolyticus***	8.8	7.44	7.06

*Autoclaved lake sediment

**Autoclaved lake water

test organisms. It also indicates the role of protozoan predation as one of the major natural self-purifying factors in the natural aquatic environments. Once the addition of waste input exceeds the self-purifying capacity of the system, it may cause ecological imbalance and result in gross contamination of the system. The role of protozoan predation on the survival of microorganisms in various aquatic environments has been reported in Hahn and Hofle (2001), Abhirosh and Hatha (2005).

The resuspension of sediment-bound bacteria and subsequent pollution of overlying water exceeding regulatory standards (Obiri-Danso and Jones 1999) through activities such as dredging and boating has been reported in various aquatic environments (Stephenson and Rychert 1982; An et al. 2002). In our earlier investigation, we have isolated different bacterial strains such as enteropathogenic (EPEC) and uropathogenic (UPEC) *E. coli, Salmonella* serotypes such as *S. paratyphi A*, *B*, *C* and *S. Newport, V. cholerae* and *V. parahaemolyticus* (Abhirosh et al. 2008) from this lake water. As we have seen, the faecal coliform density was significantly high in sediments and it is being used as indicator for the presence of pathogens; a large quantum of pathogenic bacteria already detected in the water in our earlier study (Abhirosh et al. 2008) might have settle down in the sediments. The resuspension and subsequent deterioration of water quality by those pathogenic microorganisms are very likely to occur in different parts of Vambanad Lake due to various recreational activities such as dredging, boating and manual collection of shellfish. Mechanical dredging for molluscan shell for cement production is taking place every morning and evening throughout the lake. This is the most risky time for people to come in contact for recreation such as bathing, washing, and any domestic purposes. Well-developed houseboat-based tourism also results in the disturbance of lake's bottom sediment since houseboats are very often propelled with the help of long wooden poles.

The lake also supports rich shellfish resource, which is being harvested on a daily basis and marketed in the nearby areas, where it forms a cheap, popular and protein-rich food item of the local people. The manual collection of shellfish from bottom sediment by the local people may further disturb the sediment layer. Erkenbrecher (1981) suggested that sediments in shellfishing areas could serve as a reservoir for high densities of indicator bacteria and that, potentially, pathogens could pose a health hazard. High concentration and prolonged survival of pathogenic bacteria in sediments from the study area coupled with the filter feeding nature of the shellfishes pose direct or indirect health risk through the consumption of shellfish. Shellfish associated food-borne illness has been reported from India and other Asian countries (Deepanjali et al. 2005). As faecal coliform group is used as an indicator of microbial pollution in various water types, the presence and persistence of this group in bottom sediment must be assessed; otherwise, it would underestimate the actual health risk caused by such pollution.

Conclusions

The results of the present study concluded that the prevalence level of faecal coliform bacteria was significantly higher in sediment compared to overlying water. It was proved experimentally that *E. coli*, *V. parahaemolyticus* and *S. paratyphi* showed better survival potential in sediment than in overlying water both in the absence and presence of protozoan predators. The results indicate that sediments of Vembanadu Lake could act as a reservoir of pathogenic bacteria and exhibit a potential health hazard from possible resuspension and subsequent ingestion during recreational activities. Therefore, assessment of bacterial concentration in freshwater lake sediments used for contact and non-contact recreation is of considerable significance for the proper assessment of microbial pollution of the overlying water and the management and protection of related health risk at specific recreational sites. In addition, assessment of the bacterial concentration in sediments can be used as a relatively stable indicator of long-term mean bacterial concentration in the water column above.

Direct fecal discharge- outlets opens into the lake from toilets

Manual collection of shell fish

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