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Accumulation of micro-organisms in *Marcia recens* (yellow clam), an edible bivalve of Ashtamudi estuary

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Original Article

Abstract

The Ashtamudi Estuary is one of the unique wetland ecosystems which is composed of fresh and saltwater habitats and has international importance as defined by the Ramsar convention. Though the wetland is noted for its valuable biotic resources, adorable beauty and strong endemicity, the backwater system is under stress due to contamination from waste disposal, industrialization and urbanization. The occurrence of total heterotrophic bacteria and *E. coli* in water, sediment and edible bivalve species, *Marcia recens* (Yellow clam) from three sites *viz*., Neendakara, Dalavapuram and Kureepuzha of the lake is seasonally analysed. Physicochemical parameters such as water temperature, pH, salinity, DO and BOD in surface water samples are measured following standard procedures. TPC and *E. coli* in water, sediment and the clam were analysed using Pour plate and MPN methods, respectively. The results show that counts of TPC and *E. coli* in lake components are comparatively higher in the monsoon season. Even though without spatial variation, analysis indicates slight temporal variation in TPC in water and sediment. Pearson's correlation coefficients revealed a significant positive correlation between TPC and pH. In general MPN *E. coli* values were higher in clams than in sediments and water. There was an increase in accumulation in the clams irrespective of stations during the monsoon season, which exceeded the standards set by European Union for Class A waters. The positive correlation of rainfall with *E.coli* in bivalves shows the negative influence of rainwater on bacterial contamination in the lake. Higher *E. coli* densities in the lake and their bioaccumulation during monsoon season at certain pockets are a matter of concern. To improve consumer health protection, pollution reduction measures and sanitation programmes should be implemented.

Keywords: *Ashtamudi Lake , bioaccumulation, TPC, marcia recens, yellow clam, E. coli*

Introduction

Ashtamudi lake, the second largest of the three major estuarine wetlands of Kerala, is the gateway to backwater transport in the State (RIS, 2002). The water body is palm-shaped with eight arms possessing extraordinary importance for its aesthetic resources, hydrological functions, and diversity of fishes, mangroves, plants and birds. All the arms join into a single channel at Neendakara near Kollam, to enter the Lakshadweep sea. Ashtamudiwetland is the deepest among all the estuaries of the state with a maximum depth of 6.4 m at the confluence zone. The Kallada river originated from the western ghats, passes through the forest land and falls into the Ashtamudi wetland, after covering a distance of about 120 km. The river transfers an average runoff of 76,000 million m^3 of freshwater into the estuary annually (RIS, 2002). The area receives an average annual rainfall of 2400 mm and the humidity during the rainy days is 90% (Saranya and Lancelet, 2020). The lake that acts as a livelihood source for a majority of local people is a prominent tourist place in the state. It is the second biggest fish-landing centre next to the Vembanad estuary and around 30,000 fishermen depend on different facets of fisheries in the wetland for their livelihood (Chackacherry and Jayakumar, 2011). Among the bivalve molluscs of the backwaters, clams form a vital source of meat for human consumption and lime-shell for cement and calcium carbide industries (Appukuttan *et al*., 1988).

Even though the aquatic system has assumed such significance, it is facing serious pollution stress, environmental degradation and shrinkage due to anthropogenic pressures. It receives domestic wastes, septic tank discharges from latrines and untreated sewage from the adjacent Kollam city, faces pollution from Aluminum Industries Ltd., Ceramics and fish and seafood industries, and oil spills from fishing and tourist boats. Also, sewage from nearby tourist resorts is directly disposed of into the lake. Eutrophication of the lake also occurs due to chemical fertilizers and pesticides and the destruction of natural habitat including reclamation of the estuary. All these affect the flora and fauna of the lake.

The possible entry of pathogenic bacteria into the lake through sewage and river runoff poses threat to the fishery resources of the estuary. Such a polluted environment may augment the accumulation of pathogenic bacteria in the food chain via benthic feeders like bivalves. Since many bivalve species can thrive in the most unfavorable conditions, they are capable of reflecting the quality of the environment around them (Baralla *et al.*, 2021). Extensive filter-feeding behaviour, varied geographical distribution and restricted mobility make bivalves useful bio-indicators of the marine environment (Zuykov*et al.*, 2013). The clams, which are collected extensively from the Ashtamudi lake for edible purposes are thus prone to bacterial and viral accumulation. An increase in human population density and the resultant urban developments may affect the estuarine organisms and, consequently the health of humans who consume it (Ashiq *et al.*, 2012). The present study focuses on the microbial contamination of edible bivalve *Marcia recens,* the most predominant species in the Ashtamudi Lake clam fishery zone (Arathi *et al.*, 2018) in terms of total plate count (TPC) and *E. coli*.

Material and methods

Study area

Ashtamudi estuary lies between 8°53′0 19.083′′N to 9°1′11.5′′N latitude and 76°32′6.157′′E to 76°40′15.006′′E longitude. Three sampling stations such as Neendakara (8.9443° N, 76.5402° E) characterized by inorganic pollution, the presence of fishing harbour, large-scale mechanized boats/ trawler traffic and oil spillage, Dalavapuram (8.9487° N, 76.5503° E) characterised by the absence of visible pollution sources and Kureepuzha (8.9182° N, 76.5743° E) characterized by the presence of former waste disposal site of Kollam Municipal Corporation, resorts and sewage dumping were the three sampling stations in the study (Fig. 1).

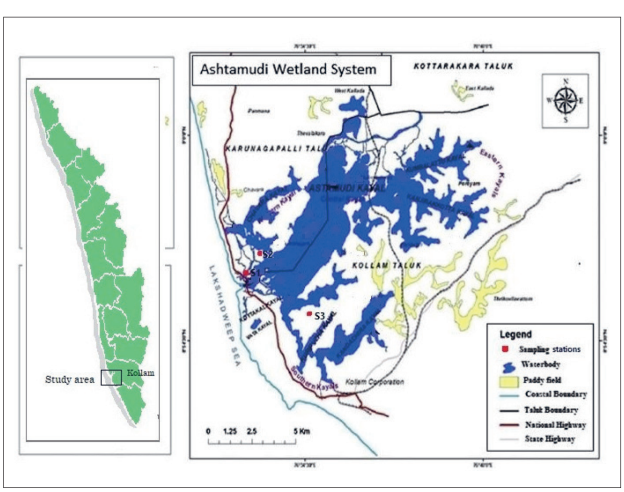

Fig. 1. Location map of the study area

Collection of water, sediment and bivalve samples

The study materials included surface water, sediment and *Marcia recens* (yellow clam).

Seasonal collection of water, sediment and yellow clams (*Marcia recens*) from three sampling stations was performed from April 2019 to February 2020. Sub-surface water samples were collected in 500 ml sterile bottles. Sediment samples were collected with the help of an Eckman's grab and transferred into a clean plastic dish, homogenized with a sterile spoon and transferred to clean polythene bags. Live clams were collected in clean polythene bags with the help of local fishermen. The water, sediment and clam samples were transported to the laboratory in an insulated icebox under aseptic conditions within 4 h and the analysis was initiated on the same day itself.

Measurement of Environmental Factors

Water temperature, pH, salinity, DO and BOD in surface water samples collected in the morning hours was measured in all three seasons following standard procedures (APHA, 2017). Water temperature was measured *in situ* with the help of a thermometer and the samples for DO were fixed immediately after collection with Winkler A and B. pH and salinity were measured immediately after reaching the laboratory. pH was measured using an Alpha 01 Digital pH meter having pH range of 0 to 14 and a resolution 0.01 pH. Salinity was measured with Erma Handheld Digital Refractometer having a measuring range 0-55%. The rainfall data were collected from the report published by the Government of India (CGWB, 2020).

Analysis of water, sediment and bivalve samples for Total Plate Count (TPC)

Analysis of TPC of water, sediment and bivalves was performed using the pour plate method. In the laboratory, the bivalves were cleaned by scrubbing, followed by washing under running water, and shucked with a sterile knife to extract the flesh (Chinnadurai *et al.*, 2016). All the samples were serially diluted with sterile peptone water and 100ml of 10^{-4}, 10^{-6} and 10^{-7} dilutions respectively of water, sediment and tissue samples were plated on petri plates over which sterilized and cooled Plate Count Agar (Hi-Media, Mumbai, India) was poured and mixed uniformly. After solidification, the plates were inverted and incubated at 37^{0}C for 24- 48 hours. After incubation, those plates with 30 to 300 colonies were selected for counting the TPC. The counts are expressed respectively as cfu (colony forming units)/ml for water and cfu/g for sediment and flesh (APHA, 2017).

Analysis of water, sediment and bivalve samples for E. coli

The presence of *E. coli* were determined using 3 tube MPN method (ISO, 2003; APHA, 2017). Decimal dilutions of the samples (10 ml, 1 ml and 0.1 ml) were inoculated into MacConkey Broth (Hi-Media, Mumbai, India) tubes in which Durham's tubes were placed inverted and incubated at 37ºC for 48 h. The number of positive tubes was referred to the MPN table for MPN index/ 100ml of water (APHA, 2017).

Detection of E. coli in the water, sediment and bivalve samples

From the positive tubes, 1ml was transferred to broth and incubated at 45.5ºC for 48 hours. Growth from the positive tubes was streaked on Hi-Crome *E. coli* agar (Hi-Media, Mumbai, India), and incubated at 37 ºC for 24 hours. Typical colonies were further reconfirmed by Indole, Methyl red, Voges Proskauer and Citrate (IMViC) tests and those showing + +–- reactions for IMViC tests were confirmed as *E. coli* (APHA, 2017).

Bioaccumulation Factor (BAF)

Bioaccumulation factors of *E. coli* in the clam were calculated following the method given by Burkhardt *et al.* (1992), where the BAF = Co/Cw. Where, Co = the *E. coli* concentration in the oyster and Cw = the *E. coli* concentration in the water sample. Each bacterial analysis was performed in triplicate.

Statistical analysis

The seasonal data analysis included Pre-monsoon (Feb.-May), Monsoon (Jun.–Sept.), and Post-monsoon (Oct.–Jan.) periods. All mean MPN values for TPC and *E. coli* were converted to log10 values prior to analysis. Statistical analyses of shellfish bio-accumulation were conducted using MS Excel by dividing the bacterial number observed in the clam by that in the water. Analysis of variance (ANOVA) and Pearson's correlation analyses were applied to determine the relationship between microbial bioaccumulation with various seasons and the selected environmental parameters.

Results

Environmental parameters

Selected environmental parameters of Ashtamudi Lake waters are shown in Table 1. The rainfall of the study area during the study period varied between 147.09 mm during pre-monsoon and 1421.3 mm during monsoon (CGWB, 2020). Seasonal mean water temperature fluctuated between 26.7±1.10 °C and 29.9±1.02 °C during monsoon and pre-monsoon seasons with the lowest and highest values at Kureepuzha and Neendakara stations. Seasonal mean pH values ranged from 7.65±0.65 to 8.11±0.30 and salinity from 18.03±0.66 to 19.9±2.32, both during monsoon and pre-monsoon respectively. Station-wise pH values varied between 6.9 and 8.4 and salinity values between 17.30 and 22.01, at both Kureepuzha and Neendakara respectively. Mean DO values varied from 4.38±0.45mg/l during pre-monsoon 5.72±0.55 mg/l during monsoon, and the station-wise values varied from 3.87 mg/l at Kureepuzha to 6.30 mg/l at Dalavapuram. The mean BOD values varied between 1.88± 0.20 mg/l during monsoon and 2.27± 0.15mg/l during pre-monsoon and Dalavapuram recorded the lowest value (1.65 mg/l) while Kureepuzha recorded the highest value (2.30 mg/l).

Total Plate Count in Ashtamudi Lake water

The mean log values of TPC in Ashtamudi Lake in different seasons are shown in Table 2. The TPC in water broadly ranged from 4.717 to 5.533 log10 cfu/ml. The maximum count was observed during the monsoon season (5.553±0.033 log10 cfu/ml) at Kureepuzha and the minimum during the post-monsoon season (4.717±0.033 log10 cfu/ml) at Dalavapuram. Mean TPC in sediment varied between 6.788±0.019 log 10/g at

Table 1. Station-wise seasonal variation of environmental parameters at Ashtamudi lake

	PRM	MON	POM	PRM	MON	POM	PRM	MON	POM	PRM	MON	POM	PRM	MON	POM
	Water temp. (ºC)			pH			Salinity			DO (mg/l)			BOD (mg/l)		
NKA	31.0	28.0	30.0	8.40	8.10	8.30	22.01	18.59	20.0	4.53	5.63	4.80	2.3	2.0	2.2
DPM	29.7	26.2	27.0	8.12	7.95	8.21	20.28	18.20	18.70	4.73	6.30	5.68	2.1	1.65	1.9
KPZ	29.0	26.0	27.1	7.80	6.90	7.10	17.41	17.30	18.10	3.87	5.22	5.17	2.4	2.0	2.15

PRM-Premonsoon, MON-Monsoon, POM-Post monsoon, NKA-Neendakara, DPM-Dalavapuram, KPZ-Kureepuzha

Table 2. Seasonal mean log values of TPC in Ashtamudi Lake water, Kollam, Kerala

Season	NKA	DPM	KPZ	NKA	DPM	KPZ	NKA	DPM	KPZ
	TPC log_{10} water/ml			TPC log_{10} sediment/g			TPC log_{10} *M. recens*/g		
PRM	5.293± 0.001	5.101± 0.006	5.239± 0.006	7.108±0.0001	7.088± 0.00111	7.169±0.0009	8.191± 0.0007	8.130± 0.0086	8.467± 0.0004
MON	5.368±0.001	5.426± 0.003	5.533± 0.033	7.325±0.0001	7.357± 0.0005	7.394±0.031	8.483± 0.0004	8.402± 0.0008	8.569±0.0041
POM	4.833± 0.003	4.717± 0.033	5.122±0.004	6.815±0.0005	6.788± 0.019	7.015±0.0011	8.120± 0.0012	8.070± 0.0041	8.310± 0.0005

PRM-Premonsoon, MON-Monsoon, POM-Post monsoon, NKA-Neendakara, DPM-Dalavapuram, KPZ-Kureepuzha

Table 3. Seasonal Mean S.D MPN of *E. coli* in Ashtamudi Lake water

Season	*E. coli* log_{10} water/ml			*E. coli* log_{10} sediment/g			*E. coli* log_{10} *M. recens*/g		
	NKA	DPM	KPZ	NKA	DPM	KPZ	NKA	DPM	KPZ
PRM	0.80±0.18	1.06± 0.047	1.24± 0.048	0.68± 0.08	0.80± 0.04	0.76± 0.08	0.92± 0.05	0.99± 0.07	0.92± 0.05
MON	2.07±0.057	2.34± 0.026	2.48± 0.02	2.43± 0.03	2.08± 0.03	2.55± 0.03	2.28± 0.04	2.68± 0.03	2.35± 0.03
POM	1.29±0.097	1.51± 0.067	1.44± 0.059	1.07± 0.06	0.89± 0.08	0.89± 0.07	1.09± 0.07	0.92± 0.05	0.97± 0.05

PRM-Premonsoon, MON-Monsoon, POM-Post monsoon, NKA-Neendakara, DPM-Dalavapuram, KPZ-Kureepuzha

Dalavapuram during post-monsoon and 7.394±0.031 log 10/g at Kureepuzha during monsoon. In *Marcia recens,* the TPC values broadly fluctuated from 8.070±0.041 log10 cfu/g at Dalavapuram during post-monsoon to 8.569±0.041 log10 cfu/g at Kureepuzha during monsoon.

ANOVA result showed station-wise variation in TPC values in water and sediment was significant only in *M. recens* ($p < 0.01$). However, highly significant variation ($p < 0.01$) in TPC values was observed in water, sediment and *M. recens* in season-wise analysis (Table 5). Pearson's correlation coefficients revealed a positive correlation of TPC with rainfall ($R^2 = 0.554$) (Table 6).

E. coli in Ashtamudi Lake water

The mean log values of *E. coli* MPN in Ashtamudi Lake in different seasons are shown in Table 3. *E. coli* MPN in water ranged from 0.80 to 2.48 log10 MPN/100 ml. The maximum mean value was observed during monsoon season (2.48±0.02) log10 MPN/100 ml) at Kureepuzha and the minimum during the premonsoon season (0.80±0.18log10 MPN/100 ml) at Neendakara. Mean *E. coli* in sediment varied between 0.68±0.08 log10 MPN/g at Neendakara during premonsoon and 2.55±0.03 log10 MPN/g at Kureepuzha during monsoon. In *M.recens,* the lowest *E. coli* MPN values were 0.92±0.05 log10 MPN/g at Neendakara and Kureepuzha during premonsoon and the same was recorded in Dalavapuram during post-monsoon whereas the highest was 2.68±0.03 log10 MPN/g at Dalavapuram during monsoon.

The bio-accumulation factors (BAF) for *E. coli* ranged between 0.609 to 1.150, with the maximum BAF during the pre-monsoon season and the minimum during post-monsoon season (Table 4). Spatial variation in bioaccumulation factor was visible in the lake and the maximum accumulation was observed at Dalavapuram during pre-monsoon and the minimum at Kureepuzha during monsoon. Station-wise variation of *E. coli* MPN in water, sediment and *M. recens* was not significant ($P > 0.05$) while there was highly significant seasonal variation ($P < 0.01$) in water, sediments and the bivalve (Table 5). Pearson's correlation coefficients revealed a highly significant positive correlation between *E. coli* and rainfall ($R^2 = 0.998$ $p < 0.01$), a highly significant positive correlation was observed for water temperature with pH ($R^2 = 0.997$ $p < 0.01$), salinity ($R^2 = 0.996$ $p < 0.01$), BOD ($R^2 = 0.992$ $p < 0.01$) and highly significant negative correlation with rainfall ($R^2 = -0.965$ $p < 0.01$) and *E. coli* ($R^2 = -0.945$ $p < 0.01$). pH showed highly significant negative correlation with DO ($R^2 = -0.993$ $p < 0.01$), rainfall ($R^2 = -0.983$ $p < 0.01$) and *E. coli* ($R^2 = -0.969$ $p < 0.01$). A highly

Table 4. Seasonal changes in bio-accumulation factor (BAF) of *E. coli* in *M. recens* at Ashtamudi lake

Season	NKA	DPM	KPZ
PRM	1.150	0.934	0.742
MON	1.101	1.145	0.948
POM	0.845	0.609	0.674

PRM-Premonsoon, MON-Monsoon, POM-Post monsoon, NKA-Neendakara, DPM-Dalavapuram, KPZ-Kureepuzha

Table 5. ANOVA comparisons of TPC and *E. coli* of Ashtamudi lake

	F	P value	F	P value
	Stations		Seasons	
TPC in water	1.674	0.209	45.220	7.24E-09
TPC in sediment	0.827	0.449	113.690	5.74E-13
TPC in *M. recens*	6.968	0.004	17.412	2.13E-05
E. coli in water	0.859	0.436	119.574	3.31E-13
E. coli in sediment	0.104	0.902	318.515	5.25E-18
E. coli in *M. recens*	0.067	0.935	410.822	2.73E-19

Table 6. Pearson's Correlation between Environmental parameters and bacterial accumulation in Ashtamudi lake

	WT	pH	Salinity	DO	BOD	Rainfall	TPC	*E. coli*	BAF
WT	1.000								
pH	0.997	1.000							
Salinity	0.996	1.000	1.000						
DO	-0.999	-0.993	-0.992	1.000					
BOD	0.992	0.999	0.999	-0.987	1.000				
Rainfall	-0.965	-0.983	-0.984	0.955	-0.990	1.000			
TPC	-0.316	-0.394	-0.397	0.280	-0.430	0.554	1.000		
E. coli	-0.945	-0.969	-0.969	0.932	-0.978	0.998	0.610	1.000	
BAF	-0.237	-0.316	-0.320	0.200	-0.354	0.483	0.997	0.542	1.000

significant negative correlation of salinity was recorded between rainfall (R^2 = -0.984 $p<0.01$) and *E. coli* (R^2 = -0.969 $p<0.01$). There was a significant correlation between TPC and *E. coli* (R^2 = 0.610 $p<0.05$) and a highly significant correlation with DO and rainfall. *E. coli* in water, sediment and *M. recens* showed a highly significant positive correlation among them (Table 6).

Discussion

Estuarine habitats are generally very productive due to the accrual of nutrients from freshwater runoff. They act as breeding environments for several Prawn and Shrimp species, oysters and fish and offer good inland fishing grounds for the fisherman due to their shallow depth. The large number of fishermen who depend on the inland fishery resources of the Ashtamudi estuary for their livelihood are the immediate stakeholders who suffer from any unwanted change in the quality of the system.

The current study showed that water temperature expresses its peak during the summer months and decreases upon the arrival of southwest monsoon. The seasonal variation in water temperature and pH of the Ashtamudi estuary showing higher values during pre-monsoon is reported as typical of a tropical estuary (Qasim, 2003). In general, pH showed narrow variation between seasons, except a slight decrease during the monsoon period. The extensive buffering capacity of the seawater may be the cause of the change of pH within a very narrow limit in the present study. A higher pH range observed during pre-monsoon period may be due to less mixing with fresh water as reported by CIFRI (2005). Similarly lower salinity was also noted from Kureepuzha station during monsoon season. Babu *et al.* (2010) attributed the freshwater inflow as the reason for the lowering of salinity during monsoon season in the southern region of the lake. Higher values of Dissolved Oxygen and lower values of BOD during monsoon season may be due to the addition of a large quantity of freshwater through the Kallada river. Higher DO values and lower BOD during monsoon season were also reported from the estuary by Babu *et al.* (2010). Sitaram (2014) analysed the water quality parameters for 7 years and found that DO values are less than 4.0 mg/l and the overall water quality in Ashtamudi Lake is deteriorating at a faster rate. Variations in the environmental parameters were generally found influenced by rainfall and tide. The highest temperature in the oyster harvesting water was recorded during pre-monsoon and the lowest was recorded in monsoon. Salinity also increased in pre-monsoon and was the lowest in monsoon due to seasonal rains.

Depending on the level of environmental parameters, benthic organisms accumulate different organic and inorganic pollutants. Recording the extent of microbial bio-accumulation in edible clams is very important in ensuring consumer safety. In the present study, the incidence of TPC was higher in sediments than that in water and higher in shellfish than in both water and sediments irrespective of seasons. This indicates the general trend of microbial accumulation by clams in the lake. Higher TPC in sediments than the water may be because the estuarine sediments play a significant role in the demineralization of organic content which supports the growth of microbes (Swarnakumar *et al.,* 2008) and the lesser dwelling time of microbes in the water column than in sediments. The density of TPC in shellfish samples was higher than that in water and sediments because they accumulate microbes from the surrounding water and maintain their levels for longer periods (Sasikumar and Krishnamoorthy, 2010). High filtration rates in clams may increase the uptake of microbes along with the particulate organic matter from the sediments. This is evident from the higher TPC values during monsoon in all the stations. In general, the occurrence of TPC indicates the presence of heterotrophic bacteria in the lake water due to various human, agricultural and industrial activities. The TPC number recommended for good-quality bivalve molluscs is 500,000/ g (European Communities, 2004) and the counts in bivalves from the current study was found to be below this threshold limit. In the present study, the distribution of TPC in water, sediment and bivalves is found to have slight seasonal variation, but without any considerable variation among stations. *E. coli*, an indicator bacterial species extensively used as a parameter of microbiological quality of water was high in the present study during monsoon season (Odonkor and Joseph, 2013). Their concentrations are the best predictor of swimming-associated gastrointestinal illness (diarrhoea). Some of the harmful types of *E. coli* which are Enterotoxigenic, Enteropathogenic and Enteroinvasive are all generally transmitted through contaminated food and water (Gerba, 2009). In addition to gastrointestinal illness (GI), illnesses such as eye infections, skin irritations, ear, nose, and throat infections, and respiratory illness are also common in people who have come into contact with water contaminated with faeces (Rock and Rivera, 2014). Wcisło and Chrost (2000) observed residual living cells of *E. coli* in freshwater up to about thirty days of the investigation. Better nutritional conditions and the presence of native heterotrophic microflora considerably extend the survival time of *E. coli* in a freshwater environment. It has been reported that substantial populations of faecal coliforms and *E. coli* are harboured in freshwater bottom sediments and bank soils and the resuspension of sediment, rather than runoff from surrounding lands, can create elevated *E. coli* concentrations in water (Pachepsky and Shelton, 2011).

In the present study, the highest MPN $\log_{10}$ value in water and sediments was observed at Kureepuzha and in the shellfish at Dalavapuram. Higher values were recorded during monsoon season irrespective of sampling stations possibly due to the addition of organic pollutants during monsoon and due to comparatively low temperatures as reported by Chinnadurai *et al.* (2016). *E. coli* accumulation by clams varied greatly between seasons and the highest counts were observed during monsoon season. Murali *et al.* (2019) also reported the maximum bacterial load in benthic fauna at Sasthamkotta lake and *E. coli* count was found to increase during monsoon and post-monsoon seasons. In general, higher *E. coli* values in clams than in sediments and water is an indication of bioaccumulation by the clam under study. High levels of *E. coli* in clams were also reported from the adjacent Vembanad Lake by Chandran *et al.* (2008) and Hassan *et al.* (2013). A higher *E. coli* counts at the rate of 1100MPN/100ml was also reported from Vellayani, another freshwater lake in Kerala (Sruthi *et al.*, 2018).

There was an increase in the accumulation of *E. coli* in the clams in stations except the barmouth during the monsoon season which exceeded the standards set by the European Union for Class A (production area) waters (European Communities, 2005; Taylor *et al.*, 2015). The occurrence of high bioaccumulation during monsoon season agreed with the observation made by Chinnadurai *et al.* (2016). The positive correlation of rainfall with *E. coli* counts in bivalves further approves this observation. The negative influence of rainwater has greater implications on the backwater bacterial quality by increasing the number of pathogens. The microbial analysis shows that TPC and *E. coli* were high during the monsoon season in water, sediment and bivalves and the accumulation of *E. coli* in water, sediments and bivalves varied significantly between seasons. Post-monsoon season showed the lowest TPC in water, sediment and bivalves. The predominant counts during the rainy season could be attributed to the release of bacteria from sewages, wastes and domestic contaminants from the incoming monsoon rains that carry a heavy load of waters containing faecal contaminants into the lake ecosystem. Mohamed *et al.* (2013) reported the venerid clams namely *Marcia*, *Mertrix* and *Paphia* as the three important genera in demand in the area and contamination of the clams by pathogenic bacteria need to be taken care of. Thus, this influx could result in the high *E. coli* counts observed during the monsoon period. The bio-accumulation factors (BAF) of *E. coli* varied between 0.609 at Dalavapuram during post-monsoon and 1.150 at Neendakara during premonsoon. Factors statistically associated with *E. coli* accumulation in bivalves were the level of *E. coli* in water, average precipitation and water temperature as reported by Jeamsripong *et al.* (2018) from Phang Nga Estuary Area in Thailand. There was also a highly significant positive correlation among *E. coli* accumulation in water, sediment and bivalves.

Conclusion

The investigation showed that water temperature, pH and salinity attain a peak during the summer season which decreases on the arrival of south-west monsoon. The narrow seasonal variation in pH indicates the extensive buffering capacity of the backwater system. Freshwater addition from the Kallada river causes an increase in Dissolved Oxygen during monsoon season. In general, variations in the environmental parameters were found influenced by rainfall and tide. Depending on the level of environmental parameters, benthic organisms accumulate different organic and inorganic pollutants. The incidence of TPC was higher in shellfish than in both water and sediments irrespective of seasons indicating the general trend of a microbial accumulation from the surroundings water along with the particulate organic matter from the sediments. However, TPC counts in bivalves were found to be below the threshold given by European Union. Higher *E. coli* MPN in water and sediments recorded during monsoon season is possibly due to the addition of organic pollutants during monsoon and due to comparatively low temperatures. Higher *E. coli* values in clams which exceeded the standards set by European Union for Class A water indicate bioaccumulation by the clams. In general, the predominant counts during the rainy season could be attributed to the release of bacteria from sewages, wastes and domestic contaminants from the incoming monsoon rains that carry a heavy load of waters containing faecal contaminants into the lake ecosystem. The study indicates that high levels of *E. coli* in clams do not pose a serious threat to consumers as of now since the clams are thoroughly cooked before local consumption. Their higher densities in the lake during monsoon and their bioaccumulation at certain locations are, however, a matter of concern.

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