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Faecal indicators and sanitary water quality of shellfish-harvesting environment: influences of seasonal monsoon and river-runoff.

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Incidence of faecal coliforms in shellfish harvesting areas of the southern coastal district of Karnataka was examined for evaluating the sanitary quality in relation to the seasonal monsoon and river-runoff. Faecal coliforms (FC) counts in seawater, mussel tissue and sediments were highest in monsoon. Geometric mean for FC in sea water was below 14 MPN/100 ml, standard set for "approved area" under NSSP classification during fishing season, but the 90th percentile were higher. The tissue *E. coli* levels complied with the EC Directive standard under the "category A", during mussel fishing season commencing in October in the two mussel beds. MPN FC levels were strongly influenced by the seasonal variations in precipitation and river runoff. During the fishing seasons in pre-monsoon and post-monsoon months, the levels were below the limits.

[Keywords: Shellfish sanitation, Perna viridis, rainfall, faecal coliforms.]

Introduction

Among the inhabitants of the coastal ecosystem, the sedentary bivalve fauna are exposed to very high probability of contamination and could act as vectors due to their peculiar feeding habits and bioaccumulation potential. Significance of bivalve shellfish harvesting environment, on the quality of harvest arises from their unique filter feeding behaviour, lack of specificity and selectivity in the filtration process as well as their ability to filter significant quantities of water relative to their size. Shellfish beds are mostly situated in shallow near-shore waters exposing them to contaminants present in land or river run-off or direct sewage discharge, rendering them as potential vectors of water borne pathogens[1]. When exposed to a considerable load of pathogens over certain period, the pathogens are accumulated in their soft tissues[2] at levels hazardous to the consumers. Therefore, the sanitary quality of the coastal waters where bivalve shellfish are harvested is of great public health importance playing a decisive role in shellfish production and marketing[3].

Regulations have been established in many parts of the world which provide a system of classification of shellfish growing/harvesting areas, broadly based on water test results (National Shellfish Sanitation Program, (NSSP)[4] of USA and Canada; Australian Shellfish Quality Assurance Program, ASQAP[5] of Australia) or tissue test results (Council Directive 91/492/EEC[6] of Europe). These classification systems assign the shellfish harvesting areas as approved, restricted and prohibited based on the faecal coliforms and/or *Escherichia coli* levels[1]. Regulatory agencies may close a fishery when contamination is detected.

Recent developments in the sector are indicative of sizeable increase in production of bivalves[7] while the concerns about coastal water pollution from various sources are mounting high. In India, reports on the sanitary quality of the shellfish harvesting waters are very few[8,9,10]. Also little is known about the seasonal trends in the coliform levels of the commercial shellfish harvesting areas located in tropical monsoon regions. Present observation is a preliminary study on the sanitary quality of mussel harvesting areas of the southern coastal district of Karnataka. Attempts had been made to evaluate the impact of seasonal rainfall on the faecal coliform levels in mussels.

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Materials and Methods

Dakshina Kannada (DK) district of Karnataka is naturally endowed with abundant freshwater resources as four rivers traverse the district to join the Arabian Sea (Fig. 1). Nethravati River (annual discharge 12,434 mcm) joins Gurpur River (annual discharge 2,915 mcm) forming the Nethravati-Gurpur estuary in the southern part of the District. Pavanje River (annual discharge 619 mcm) confluence with Sambhavi River (annual discharge 1,253 mcm) forming the Mulki estuary in the northern part of the District. During south west monsoon period June-September) rivers frequently overflow, while during the dry months (December to May) the rivers often experience periods of reduced flow. Two distinct subtidal mussel beds of the District are located, one off Someshwara (12.79ºN; 74.84ºE) at 5.5 km south of Nethravati-Gurpur estuary and the other off Surathkal (13.00ºN; 74.79ºE) at 18.0 km north of Nethravati-Gurpur estuary and 9.0 km south of Mulki estuary[11,12]. Green mussels, seawater and sediment samples were collected on a monthly interval from six to eight sampling points fixed at a depth of 3-4 M. Samples were collected during January-December 2003 from Someshwara and Surathkal mussel beds. A total of 93 seawater samples, 84 sediment samples and 96 mussel samples were collected from Someshwara and 112, 105 and 117 seawater, sediment and mussel samples respectively were collected from Surathkal. Temperature and salinity were recorded using multi-parameter probes (WTW, Germany) and water samples for chlorophyll a (Chl-a) analysis were collected using a reversing bottle operated from a canoe. Chl-a content of the water was measured by spectrophotometry after vacuum filtration and extraction in acetone[13]. Water samples for analysing the Biochemical Oxygen Demand (BOD_5) were collected in BOD bottles, incubated without dilution at 20°C for 5 days and analysed[13].

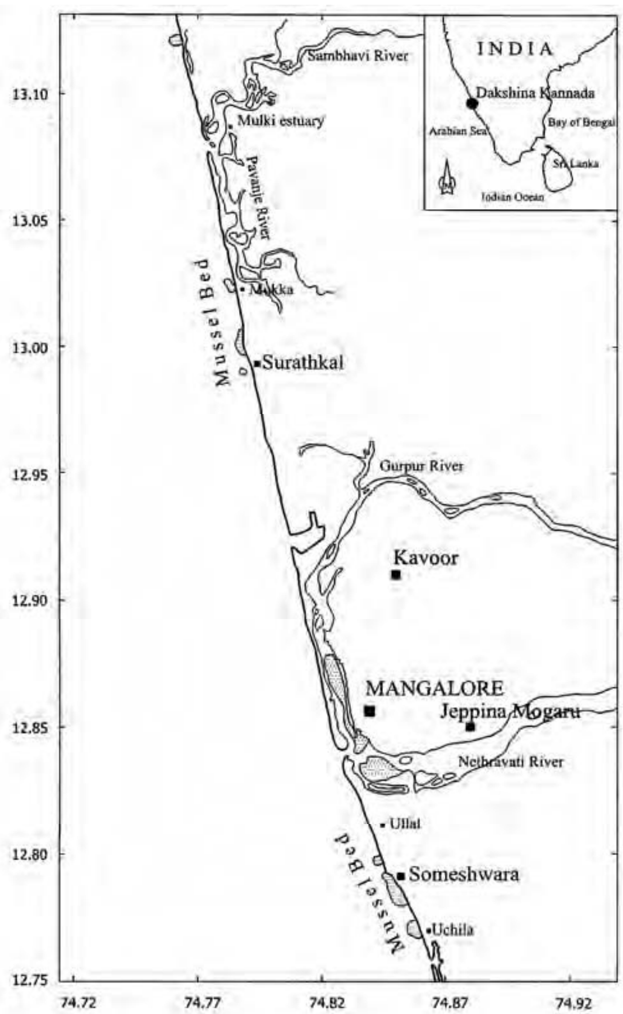

Fig. 1—Map showing the mussel beds off Someshwara and Surathkal along Dakshina Kannada coast of Karnataka.

Seawater samples were analysed for faecal coliforms (FC) and *E. coli* using the most probable number (MPN) technique. This is done in accordance with the statutory method proposed by US FDA NSSP[4]. The geometric mean (GM) and the 90th percentile of the FC levels in shellfish harvesting waters were compared with the NSSP regulations (Table 4). Apart from FC levels of the shellfish waters the coliform levels in the sediment and mussel samples were enumerated. A minimum of 15 mussels were aseptically opened using a flame-sterilized shucking knife and meat and intravalvular fluid removed, homogenized and the diluted homogenates were assayed for coliforms using MPN method for shellfish[14]. The GM of MPN counts of *E. coli* of the mussel samples were compared with the criteria for the shellfish harvesting waters under European Community (EC) Directive 91/492/EEC (Table 5). Bioaccumulation indices were calculated as the ratio of GM of MPN values of *E. coli* in mussel tissue to that of the overlaying seawater[15].

Monthly mean values were obtained for log_{10} transformed MPN counts of FC and *E. coli* from the six-eight sampling points in each mussel beds. For comparing the seasonal variations monthly data were classified into three seasons *viz.*, pre-monsoon (February to May), monsoon (June to September) and post-monsoon (October to January). Seasonal (pre-monsoon, monsoon and post monsoon) variations of coliform counts in each mussel bed was analysed by one-way ANOVA. Site-wise analysis was also carried out within each season using one-way ANOVA. When significant differences between means were detected, post factors were further analysed using a Range test, Student-Newman-Keuls (S-N-K) tests set at 5% significance level to identify homogeneous subsets of means. In each mussel bed the interdependence of the coliform counts of seawater, sediment and mussel tissue were analysed by estimating the Pearson's correlation coefficients. Statistical analysis were carried out using SPSS (13.0) software.

Results

Environmental parameters

Seasonal variations in oceanographic features of the study area are primarily influenced by the prevailing monsoon regime. Southwest monsoon commenced by the last week of May and monthly rainfall ranged from 6 mm in April to 1410 mm (40%) in June (Fig. 2). Southwest monsoon, which accounted for 90% of the total rainfall (3,461 mm), weakened by the end of August. Seasonal variations in surface (S) bottom (B) seawater temperatures in the shellfish harvesting waters are presented in Fig. 3. Mussels were exposed to higher seawater temperatures in April-May and to lower temperatures in August. Seasonal variations in temperature between the seasons were significantly different ($p<0.05$) with highest temperatures in pre-monsoon followed by post-monsoon and monsoon seasons. Analysis of variance also indicated significant site-specific variation between the shellfish waters with respect to water temperature in all seasons. Wide variations in the salinity of shellfish harvesting waters were observed during the period (Fig. 3). Significantly different ($p<0.05$) salinity regime prevailed between the seasons with higher values during pre-monsoon followed by post-monsoon and monsoon. However, between the shellfish waters salinity showed significant variation ($p<0.05$) only during the monsoon season. The Chl a levels peaked in September with increase in primary production in both the sites (Fig. 5).

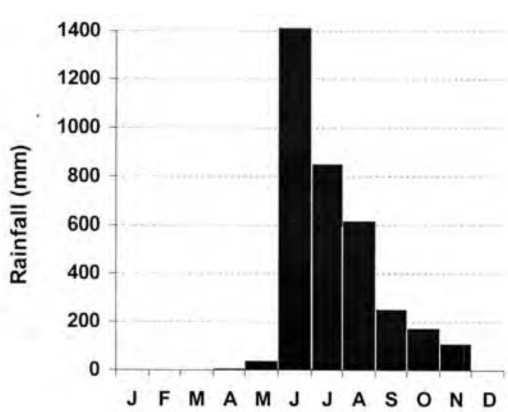

Fig. 2—Variations in rainfall (mm) pattern in the study area.

Coliform levels in seawater

The FC and *E. coli* counts in seawater from the mussel beds are presented in Fig. 4. The mean $\log_{10}$ MPN counts of coliforms in the shellfish waters of Someshwara that encounter more riverine influence was 2.59±1.19 for FC and 2.06±0.88 for *E. coli*/ 100ml. The prevalence of FC (3.20 MPN/100ml) and *E. coli* (2.94 MPN/100ml) were highest in June. In Surathkal shellfish waters, though counts of FC and *E. coli* followed a similar pattern, it presented less variability compared to Someshwara.

Seasonal trends indicated significant differences ($p<0.05$) with higher coliform count during monsoon season compared to pre-monsoon and post-monsoon seasons (Table 1). The mean $\log_{10}$ MPN count of coliforms in seawater between the shellfish waters showed significant differences ($p<0.05$) during monsoon and post-monsoon seasons, while no significant difference was observed in pre-monsoon season.

Coliform levels in sediment

In sediment samples, a reduction in coliform counts were observed following the monsoon at Someshwara and higher values (FC: 2.61±0.52 MPN/100 g and *E. coli*: 2.29±0.46 MPN/100 g) were observed during monsoon. Analysis of variance revealed significant difference ($p<0.05$) in coliforms levels in sediment samples between the seasons. The FC $\log_{10}$ count averaged 2.33±1.13 MPN/100 g and *E. coli* count averaged 2.18±1.08 MPN/100 g in Someshwara. The coliform counts of Surathkal sediments presented less variability with mean values of 2.23±0.96 MPN/100 g for FC and 1.79±0.86 MPN/100 g for *E. coli*. The FC count ranged from lower counts in October and December to 2.73 MPN/100 g in January. *E. coli* counts similarly peaked in January (2.73 MPN/100 g). It was observed that unlike in Someshwara, FC (2.42±1.34 MPN/100 g) and *E. coli* (1.99±1.02 MPN/100 g) counts were higher in post-monsoon season. Between the mussel beds coliform counts showed significant variation ($p<0.05$) during monsoon season whereas, no significant variation was

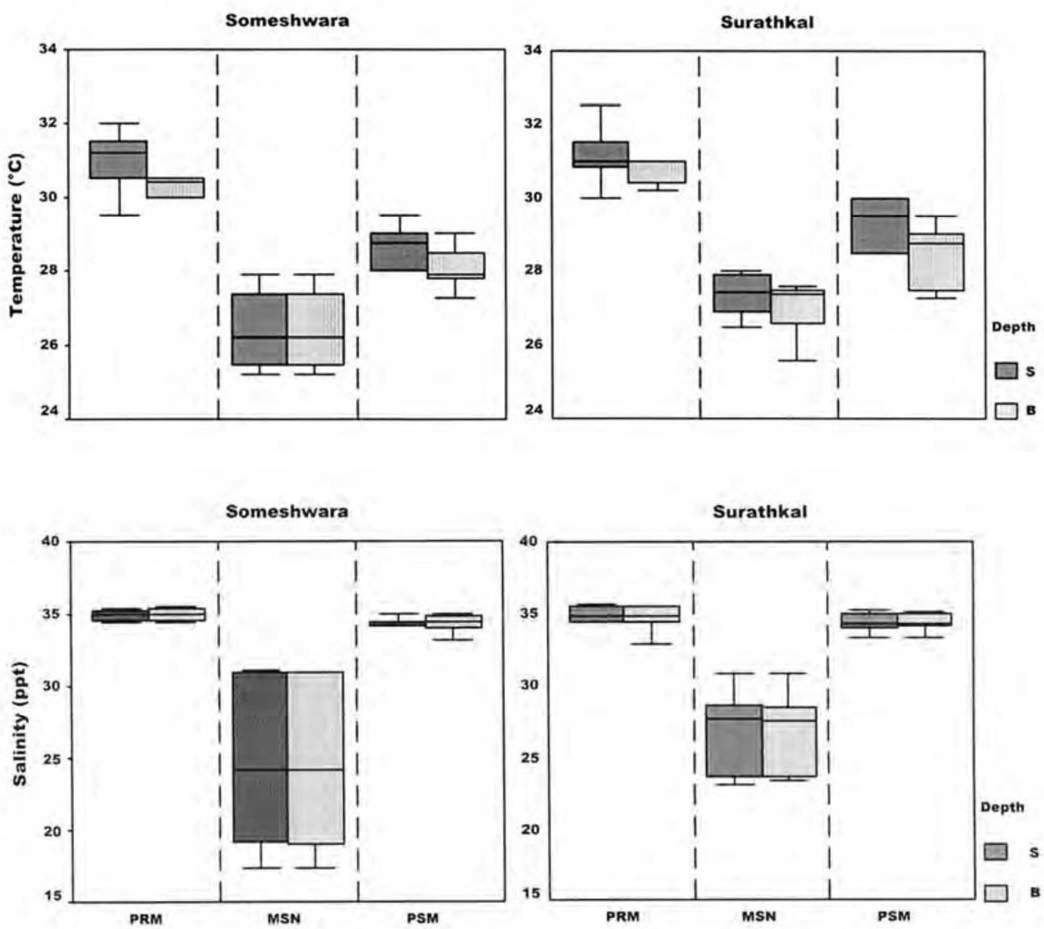

Fig. 3—The box and Whisker summary plot representing (from top to bottom), the maximum, upper-quartile, median, lower-quartile and minimum values of surface (S) and bottom (B) seawater temperature and salinity of the shellfish waters (mean ± SD) during pre-monsoon (PRM), monsoon (MSN) and post-monsoon (PSM) seasons. The box represents the inter-quartile range which contains 50% of values.

observed during the pre-monsoon and post-monsoon seasons.

Coliform levels in mussel tissue

The $\log_{10}$ MPN of FC presented highest monthly variability in Someshwara, registering counts between 1.44 MPN/100 g (February) and 4.04 MPN/100 g (August), averaging to 3.10±1.13 MPN/100 g (Fig. 4). Count of *E. coli* in the tissue samples followed similar pattern ranging between 0.78 MPN/100 g (January) and 2.51 MPN/100 g (August) with a mean of 2.02±0.98 MPN/100 g. Wide seasonal variations were observed in the presence of coliforms in the mussel tissue (Table 1). Variations in coliforms in the mussel tissue were significant ($p<0.05$) between the seasons with monsoon season recording the highest coliform levels of 3.68±0.8 MPN/100 g for FC and 2.39±0.24 MPN/100 g for *E. coli*.

Coliform counts in mussel tissue from shellfish harvesting waters of Surathkal paralleled that of Someshwara except for FC. The mean $\log_{10}$ MPN counts were 2.52±0.90 MPN/100 g for FC and 2.01±0.95 MPN/100 g for *E. coli* in Surathkal. The levels of FC varied from 1.58 MPN/100 g (February) to 3.22 MPN/100 g (August) and *E. coli* from 1.33 MPN/100 g (December) to 2.41 MPN/100g (July). The MPN values of coliforms in tissue samples were significantly higher ($p<0.05$) during the monsoon season, when compared with pre-monsoon and post-monsoon seasons. The mean MPN values during monsoon season were 2.91±0.83 MPN/100 g and 2.29±0.82 MPN/100 g respectively for FC and *E. coli*. Coliform counts between the beds showed significant differences ($p<0.05$) during monsoon and post-monsoon seasons.

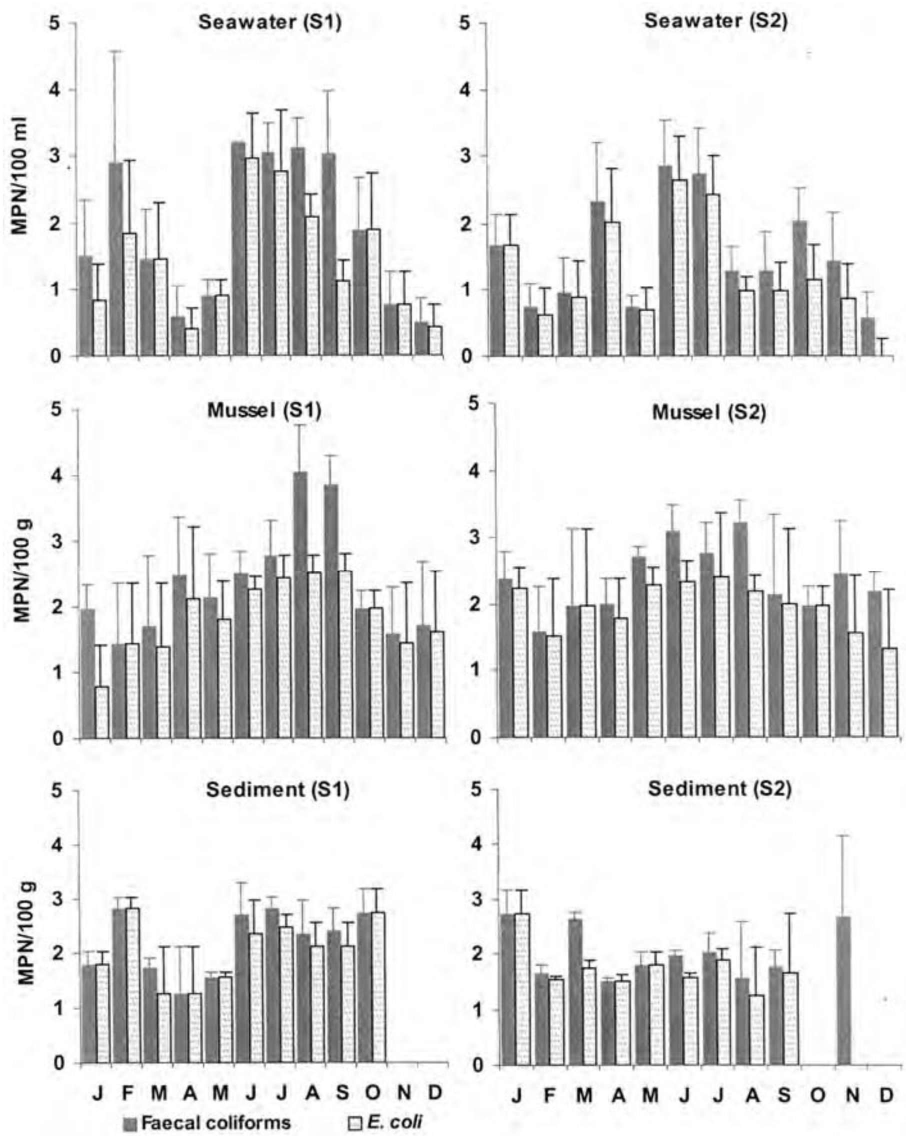

Fig. 4—Variations in faecal coliform and *E. coli* $\log_{10}$ MPN counts (mean ± SD) in shellfish harvesting waters of Someshwara (S1) and Surathkal (S2).

Biochemical Oxygen Demand (BOD)

The mean BOD_5 values of Someshwara and Surathkal mussel beds were found to be below 2 mg/l and it ranged between 0.3 and 4.5 mg/l during the period of study. The spatial and temporal variations in BOD values of the mussel beds are presented in Table 3. Analysis of variance showed significant difference in BOD levels between the seasons ($p<0.05$) in the mussel beds.

Influence of environmental variables on coliform levels

Pearson's correlation analysis revealed that the FC and *E. coli* counts of mussel tissue was directly related ($p<0.05$) to the coliform counts of the shellfish harvesting waters and sediments off Someshwara (Table 2). In the area, significant positive correlation was also noticed between the coliform counts in seawater, sediment and mussel tissue with rainfall, whereas, salinity and temperature had significant negative influence on the coliform counts of seawater, sediment and tissue. In Surathkal, FC and *E. coli* counts in mussel tissue was directly related ($p<0.05$) to the coliform counts in seawater. On the contrary, no significant correlations were observed between the coliform counts in mussel tissue with the levels in the sediment. Rainfall showed strong influence on the coliform counts of seawater and mussel tissue whereas, the coliform counts of the sediment was not

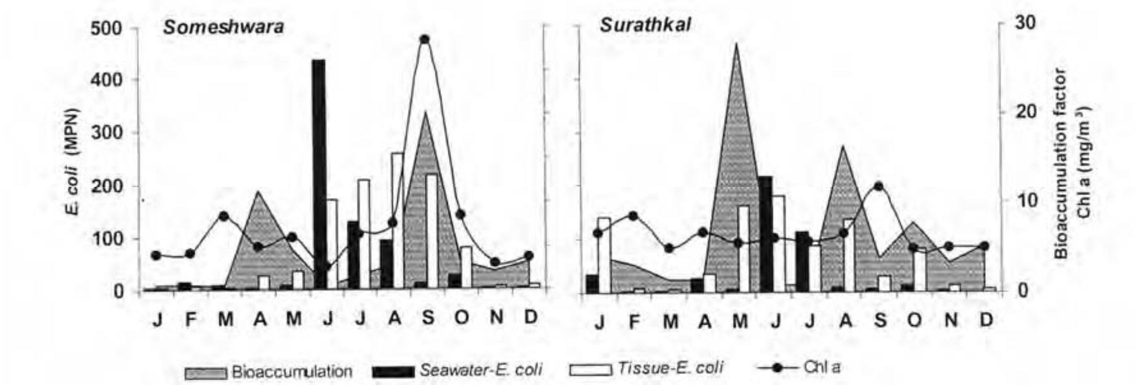

Fig. 5—Bioaccumulation (geometric mean) of *E. coli* by green mussel *Perna viridis* (MPN counts/100 g) in coastal waters (MPN counts/100 ml) with seasonal trends in chlorophyll a (Chl a).

Table 1—Seasonal trends (mean ± SD) in $\log_{10}$ MPN coliform counts of seawater, sediment and mussel samples with results of S-N-K post-hoc tests

Matrix	Season	Someshwara		Suratkal	
		Faecal coliforms	*E. coli*	Faecal coliforms	*E. coli*
Seawater	Pre-monsoon	2.18±0.90^{a}	1.33±0.67^{a}	1.90±0.72^{a}	1.59±0.71^{a}
	Monsoon	*3.11±0.57^{b}	2.59±0.83^{b}	*2.57±0.87^{b}	2.29±0.81^{b}
	Post-monsoon	*1.33±0.66^{a}	1.22±0.61^{a}	*1.62±0.73^{a}	1.18±0.67^{a}
	Annual	**2.59±1.19**	**2.06±0.88**	**2.17±0.86**	**1.87±0.81**
Mussel tissue	Pre-monsoon	2.20±0.96^{a}	1.86±0.94^{a}	2.12±0.95^{a}	1.89±0.96^{a}
	Monsoon	*3.68±0.80^{b}	*2.39±0.24^{b}	*2.91±0.83^{b}	*2.29±0.82^{b}
	Post-monsoon	*1.80±0.84^{a}	1.61±0.93^{a}	*2.30±0.52^{c}	1.80±0.90^{a}
	Annual	**3.10±1.13**	**2.02±0.98**	**2.52±0.90**	**2.01±0.95**
Sediment	Pre-monsoon	2.29±0.85^{a}	2.27±1.02^{a}	2.20±0.50^{a}	1.64±0.16^{a}
	Monsoon	*2.61±0.52^{b}	*2.29±0.46^{b}	*1.91±0.63^{a}	*1.70±0.74^{a}
	Post-monsoon	2.00±1.08^{c}	2.00±1.08^{c}	2.42±1.34^{b}	1.99±1.02^{b}
	Annual	**2.33±1.13**	**2.18±1.08**	**2.23±0.96**	**1.79±0.86**

**Denotes significantly different coliform counts ($p<0.05$) between the two shellfish harvesting environment (column-wise).* [a,b,c]*Seasonal means with the different letters denotes heterogenous seasons (S-N-K post-hoc tests) within the shellfish harvesting environment (row-wise).*

related to the rainfall regime. Of particular importance however was the negative relation of FC and *E. coli* counts of seawater to the salinity and temperature. The coliform counts of mussel tissue were also found to have similar relationship with salinity and temperature except in *E. coli* counts.

Bioaccumulation index

In the study area, mussels accumulated higher loads of coliform bacteria than the levels in the overlaying waters, as indicated by bioaccumulation index above unity in most of the months (Fig. 5). The bioaccumulation index of *E. coli* ranged between 0.4 and 20.2 at Someshwara. The accumulation index was high in April-May and September-October indicating higher load in tissue in comparison with the overlaying seawater. The index in Surathkal indicated wide variations in the range of values compared to that of Someshwara ranging between 0.76 and 27.9 with higher values in May and September.

Comparison of sanitary quality of shellfish waters with standards

NSSP: The GM of FC annual counts observed were 23 MPN/100 ml at Someshwara waters and 19 MPN/100 ml at Surathkal waters (Table 4). These values exceed the FC level of 14 MPN GM/100 ml in seawater approved for unrestricted shellfish harvest or standards set for "approved area"[4].

Table 2—Correlation coefficient (Pearson's) between Faecal Coliforms (FC) and *E. coli* levels in seawater, sediment and mussel tissue

	Water	Sediment	Tissue	Rainfall	Salinity	Temperature
Someshwara (FC)						
Water	1.000	0.611(**)	0.507(**)	0.715(**)	-0.662(**)	-0.558(**)
Sediment	0.611(**)	1.000	0.387(**)	0.475(**)	-0.418(**)	-0.182
Tissue	0.507(**)	0.387(**)	1.000	0.412(**)	-0.328(**)	-0.388(**)
Surathkal (FC)						
Water	1.000	0.118	0.231(*)	0.576(**)	-0.398(**)	-0.303(**)
Sediment	0.118	1.000	-0.163	0.089	-0.010	0.204(*)
Tissue	0.231(*)	-0.163	1.000	0.434(**)	-0.335(**)	-0.400(**)
Someshwara (*E. coli*)						
	Water	Sediment	Tissue	Rainfall	Salinity	Temperature
Water	1.000	0.444(**)	0.435(**)	0.716(**)	-0.649(**)	-0.366(**)
Sediment	0.444(**)	1.000	0.407(**)	0.423(**)	-.356(**)	-0.210
Tissue	0.435(**)	.407(**)	1.000	0.468(**)	-.407(**)	-0.292(**)
Surathkal (*E. coli*)						
Water	1.000	0.380(**)	0.354(**)	.562(**)	-0.355(**)	-0.215(*)
Sediment	0.380(**)	1.000	0.100	0.146	-0.072	0.219(*)
Tissue	0.354(**)	0.100	1.000	0.347(**)	-0.261(**)	-0.160

** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.

Table 3—Monthly variations of BOD (mean± S.D) levels in seawater from the Someshwara and Surathkal mussel beds

Months	Someshwara (mg/l)	Surathkal (mg/l)
Jan	0.8±0.6	1.3±0.3
Feb	0.7±0.7	1.4±0.4
Mar	1.6±1.0	2.0±1.1
Apr	1.8±1.2	0.9±0.5
May	1.3±1.1	1.4±0.7
Jun	0.3±0.2	2.2±0.9
Jul	0.7±0.3	3.4±2.5
Aug	1.8±0.1	1.6±0.4
Sep	4.5±0.2	1.7±0.3
Oct	0.8±0.6	2.5±0.2
Nov	0.7±0.5	1.7±0.6
Dec	0.7±0.7	1.5±0.5
Annual	**1.2±1.1**	**1.8±1.2**

In the subsequent category, requiring depuration or relaying of shellfish prior to sale, the limits of FC are set below the GM of 88/100 ml, with fewer than 10% of samples exceeding 260/100 ml for a five-tube MPN. In Someshwara and Surathkal shellfish waters the GM FC counts were <88/100 ml but in 25% water samples from Someshwara and 12% water samples from Surathkal, the counts exceeded the limit of 260 MPN/100 ml. The samples that exceeded the MPN levels of 260 per/100 ml were drawn mostly during the monsoon months.

Considering the fact that the mussel beds along the coastal waters of DK coast are subjected to bacterial contamination mainly from increased runoff associated with monsoon, the FC in the mussel beds were analysed seasonally and were evaluated for their conformity with the standards.

Seasonal changes in FC counts and its relevance

In Someshwara waters, the GM for FC during pre-monsoon season was 8 MPN/100 ml and during post-monsoon season it was 5 MPN/100 ml (Table 4). These counts were below the 14 MPN/100 ml and in post-monsoon the 90th percentile MPN/100 ml value was lower than 43 MPN, the limit set for "approved area". However, in pre-monsoon the 90th percentile MPN value was higher than 43 MPN. Similarly, the FC levels in seawater from Surathkal were low during pre-monsoon (10 MPN/100 ml) and post-monsoon (13 MPN/100 ml) seasons but 90th percentile MPN value was higher than the limit of 43 MPN (Table 4). When shellfish harvesting waters are exposed to limited degree of pollution the shellfish must be depurated or relayed prior to sale, provided the limits of FC in such conditions do not exceed 88/100 ml and

Table 4—Distribution of faecal coliforms (FC) (MPN/100 ml Geometric mean-GM) in shellfish harvesting waters (Someshwara-S1; Surathkal-S2) based on the limits set by NSSP[4]

Area	Season	FC (GM)	90th Percentile	N	% >260 MPN/100 ml
S1	Pre-monsoon	8	113	33	9
	Monsoon	875	4638	24	83
	Post-monsoon	5	36	36	0
	Annual	23	759	93	25
	Non-monsoon	6	64	69	9
S2	Pre-monsoon	10	80	52	6
	Monsoon	83	1077	30	33
	Post-monsoon	13	112	30	0
	Annual	19	232	112	12
	Non-monsoon	11	90	82	6

US FDA Classification	Geometric mean[4]	90th Percentile	Shellfish treatment required	Criteria
Approved	MPN <14/100 ml	MPN <43/100 ml	None	Acceptable water quality; No significant pollution sources.
Restricted	MPN <88/100 ml	MPN <260/100 ml	Depuration or relaying	Evidence of marginal pollution
Prohibited	No harvest allowed			Evidence of gross pollution

Table 5—Trends in *E. coli* levels Geometric mean (GM) in mussel (MPN/100g tissue) based on the limits set by EC Directive (91/492/EEC)[6]

	Someshwara				Surathkal			
Month	% tissue sample <230	% tissue sample >230	N	*E. coli* (GM)	% tissue sample <230	% tissue sample >230	N	*E. coli* (GM)
Jan	100	-	6	2	100	-	6	142
Feb	100	-	6	7	100	-	11	10
Mar	100	-	6	4	75	25	16	5
Apr	67	33	12	25	100	-	18	35
May	83	17	12	34	83	17	6	160
Jun	33	67	6	169	50	50	6	179
Jul	33	67	6	206	83	17	12	86
Aug	67	33	6	256	67	33	6	136
Sep	50	50	6	217	100	-	6	28
Oct	100	-	6	79	100	-	6	79
Nov	100	-	6	7	100	-	12	13
Dec	100	-	18	8	100	-	12	5
Annual	**80**	**20**	**96**	**25**	**91**	**9**	**117**	**27**
Range				<2-930				<2-930

EC Directive (91/492/EEC)[6]	Geometric mean	Criteria
Category A	<230 *E. coli*/100 g flesh or <300 FC /100 g flesh.	Direct human consumption permitted.
Category B	<4,600 *E. coli*/100 g flesh or <6,000 FC /100 g flesh (in 90% of samples).	Purification in an approved plant for 48 hours prior to sale for human consumption.
Category C	<60,000 FC/100 g flesh.	Relaying for a period of at least two months in clean seawater prior to human consumption.

the estimated 90^{th} percentile do not exceed 260 MPN/100 ml or with fewer than 10% of samples exceeding 260 MPN/100 ml for a five-tube MPN. In Someshwara waters the FC levels in 91% of the pre-monsoon water samples were below 88 MPN/100 ml. Similarly, in Surathkal, the FC levels in 94% of the pre-monsoon water samples and in 100% (all) of the post-monsoon samples were below 88 MPN/100 ml and the estimated 90^{th} percentile was below 260 MPN/100 ml. Therefore, GM and 90^{th} percentile MPN results from Someshwara during pre-monsoon and Surathkal during pre-monsoon and post-monsoon seasons Surathkal were below the limits for “restricted area” classification.

Someshwara (875 MPN/100 ml) and Surathkal (83 MPN/100 ml) waters showed elevated FC MPN values during monsoon season with GM and 90^{th} percentile values well above the limits. It was observed that all the samples from Someshwara and 87% of the samples from Surathkal exceeded the limit during the season. At Surathkal the FC counts were ten fold lower than the GM values of FC in Someshwara during monsoon season.

EC Directive

Comparison of *E. coli* levels in mussel tissue with the European Commission (EC) Directive criteria (91/492/EEC)[6] showed GM levels below 230 MPN/100 g during fishing season, from October through March in Someshwara (Table 5). The *E. coli* GM ranged between 2 and 256 MPN/100 g in Someshwara during diverse seasonal conditions. While the *E. coli* GM in Surathkal was lower, when compared to Someshwara ranging between 5 and 179 MPN/100 g. The *E. coli* levels were below 230 MPN/100 g from September to April in Surathkal, except in 25% of samples in March. Therefore, the majority of the samples collected from the site were consistent with the category A (Table 5) with respect to *E. coli* levels in the tissue. However, during the non-fishing monsoon months (June-September), the *E. coli* levels were above 230 MPN/100 g in a few tissue samples. Alternatively, the *E. coli* levels in mussel tissue in all seasons complied with less than 4300 per 100 g standard for category B.

Discussion

Major rainfall events had significant negative effects on the sanitary quality of the study area demonstrated by higher counts of coliforms in seawater, tissue and sediments during monsoon. Rainfall has been negatively implicated in influencing the coastal bacterial quality by increasing the levels of pathogens through increased surface run-off[9,16-18]. Moreover, in shellfish, rainfall-associated outbreaks of shellfish vectored diseases are frequently described highlighting the importance of rainfall-associated contamination[1]. In the present study, the negative influence of rainfall on the coliform levels in mussel tissue and seawater was probably the result of an increase in non-point sources of sewage brought in by contaminated surface runoff draining into the Nethravati River and eventually reaching the coastal areas. Parallel conclusions were reached in an earlier study[19-22] when higher levels of coliforms were observed in riverine and estuarine waters of Nethravati River and their adjacent coastal areas during monsoon.

Present study indicated an inverse relationship in coliform counts of seawater, mussel tissue and sediments with salinity in Someshwara. However, similar trends were observed only in the coliform counts of seawater and mussel tissue in Surathkal but not in sediments. It should be emphasized that besides increase in the pathogen load brought in, the increase in river run-off temporarily decreases the salinity in river-mouth. Since a positive link between freshwater input and higher FC counts is documented in areas of low salinity/with increase river flow it is likely that freshwater input positively influences the survival rate of FC[9,23].

The negative relationship observed in coliform counts in seawater and mussel tissue with water temperature in the shellfish waters could also be explained with the changes in survival rates of FC with variations in temperature. Sunlight has been indicated as one of the most important inactivating factors on survival of *E. coli* in estuarine waters[24]. Moreover, increase in suspended solids and turbidity in coastal waters can contribute to better survival of FC bacteria by providing an organic substrate as well as protection from light in addition to a mechanism for transport downstream[25]. In coastal waters of Someshwara and Surathkal, a relatively high BOD_5 values were observed during monsoon, which corresponded with the high levels of suspended particulate matter (SPM) and primary production. Some of the earlier studies have also attributed higher BOD values in coastal waters of Dakshina Kannada coast to sewage discharge[12]. The variations in these factors in turn are strongly correlated with rainfall resulting from increased river flow and cooler water temperatures[16].

With the onset of rainfall, significant site-specific differences in the levels of coliforms were observed in sediment samples. Unlike in Someshwara, no substantial increase in the counts in sediment at Surathkal was observed, which may be partly attributed to the release of sediment bound bacteria into the water column in sewage polluted areas due to rough weather conditions in monsoons. In estuarine waters, it is reported that the faecal coliforms that settle down in sediment can be circulated back into the water column in rough weather periods leading to increased coliform levels in water[26]. This cycle may greatly prolong the influence of a contamination event. Other factors contributing to high levels of FC in Someshwara waters are the high degree of urbanization and boating activity contaminating the area with domestic as well as other organic wastes allowing longer bacterial survival. Relatively lower counts of coliforms in sediments at Surathkal suggest reduced sewage pollution with increasing distance from the river mouth due to dispersion, settling and mortality of the bacteria. On the contrary, the presence of FC in the tissue even in the absence of FC in the sediment at Surathkal suggests that, by virtue of their ability to concentrate bacteria, mussels can become actively contaminated from seawater even at considerable distance from the pollution source.

Bioaccumulation was high during warmer periods characterised by the transition from cooler December-January months to warmer summer months and from monsoon to post-monsoon months. However, water temperature alone may not explain accumulation rates since the coliform counts in mussel tissue and seawater was high in low temperature mainly due to monsoon effects. Further, a higher ratio during the warmer months could possibly be attributed to the higher rate of reduction in coliform counts in seawater with rising temperature compared to the rate of reduction in mussel tissue. This is also possible when shellfish accumulates coliforms present in the shellfish waters and then maintain these levels long after the water have been cleared of the microbes. Increase in activity of mussel, such as high filtration rates, enhances the uptake of microbes together with particulate organic matter. Many studies have pointed out the significance of the presence of particles to which microbes can adhere[25,26] thereby greatly enhancing its uptake.

The overall evaluation of faecal contamination of the shellfish environment reveals the occurrence of elevated levels of FC during monsoon months corresponds with the non-fishing periods associated with rough weather conditions prevailing in the region. Though there is a potential health hazard associated with the consumption of mussels without depuration or proper cooking during the season, the probability of such incidence is minimized due to non-availability of mussels to the consumers. Since faecal contamination monitoring needs only to address the harvest seasons of the year, the higher levels in monsoon are of less significance and it was also observed that during the pre-monsoon and post-monsoon seasons the FC counts were below the limits. Therefore, during the fishing seasons in pre-monsoon and post-monsoon the mussel beds off Someshwara and Surathkal can be described as “seasonally classified” with the period when, the classification defined applies. During the peak fishing seasons, in post-monsoon, GM of FC counts from the Someshwara harvesting waters were well below the NSSP limits set for the “Approved” and the tissue levels of *E. coli* were below 230 MPN/100 g in both the mussel beds under “Category A”. This indicates that during the fishing season there is no serious contamination of the mussel beds, except in Surathkal where the accumulation factor in the tissue increased from March. An increase in temperature increases the filtration rate of the bivalves and hence there is a higher probability of bioaccumulation of faecal coliforms from surrounding waters, resulting in higher accumulation in warmer months[15]. Though the study indicates that *E. coli* are efficiently accumulated by the shellfish, it is interesting to note that the levels of *E. coli* even in adverse weather conditions was less than 4600 MPN/100 g suggesting that no specific non-point source of FC is excessively affecting the area. However, such investigations invoke the importance of depuration in probable pollution situation.

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References

1 Lees, D. Viruses and bivalve shellfish, *Int. J. Food Microbiol.*, 59 (2000) 81-116.

2 Burkhardt, W. & Calci, K.R. Selective Accumulation may account for shellfish-associated viral illness. *Appl. Environ. Microbiol*., 66 (4) (2000) 1375-1378.

3 Shumway, S.E., Mussels and public health. In: *The mussel Mytilus: Ecology, Physiology, Genetics and Culture*, edited by Gosling E. (Elsevier Science, Amsterdam) (1992), pp.511-540.

4 NSSP (National Shellfish Sanitation Program), *Guide for the Control of Molluscan Shellfish, Model Ordinance*. Chapter IV. U.S. Department of Health and Human Services, U.S. Food and Drug Administration, Centre for Food Safety and Applied Nutrition, Washington, D.C, USA, (1999) (*available at http://vm.cfsan.fda.gov/~ear/nsspotoc.html).*

5 ASQAP (Australian Shellfish Quality Assurance Program), Operations Manual, (2004) pp.172. (*available at http://www.pir.sa.gov.au/~sasqap/asqap_manualfinal.pdf).*

6 EC (European Commission), Council Directive 91/492/EEC of 15 July 1991 laying down the health conditions for the production and the placing on the market of live bivalve mollusks. Official Journal of the European Communities, No. L268, 24/09/1991, (1991) pp. 1-14.

7 Kripa, V. & Appukuttan, K.K. Marine Bivalves, in: *Status of Exploited Marine Fishery Resources of India*, edited by M.M. Joseph and Jayaprakash, A.A. (Central Marine Fisheries Research Institute, Kochi, India) (2003), pp. 211-220.

8 Pillai, C.T. Microbial flora of mussels in the natural beds and farms. In: Mussel Farming-Progress and Prospects. *Bull. Cent. Mar. Fish. Res. Inst.*, 29 (1980) 41-43.

9 Raveendran, O., Gore, P.S., Iyer, T.S.G., Varma, P.R.G. & Sankaranarayanan, V.N. Occurrence of enteric bacteria in seawater and mussels along the southwest coast of India. *Indian J. Mar. Sci.,* 19 (1990) 282-284.

10 Surendran, P.K., Thampuran, N., Nambiar V.N. & Lalitha, K.V. Microbial ecology of inland water bodies with special reference to fisheries, in: *Riverine and Reservoir Fisheries of India*, edited by Boopendranath, M.R., Meenakumari, B., Joseph, J., Sankar, T.V., Pravin P. and Edwin L. (Society of Fisheries Technologist (India) Cochin), (2002), pp. 6-14.

11 Appukuttan, K.K., Mohamed, K.S., Kripa, V., Ashokan, P.K., Anil, M.K., Sasikumar, G., Velayudhan, T.S., Laxmilatha, P., Koya, K.P.S., Radhakrishnan, P., Joseph, M., Alloycious, P.S., Surendranathan, V.G., Sivadasan, M.P., Nagaraja, D., Sharma J. & Naik, M.S. Survey of green mussel seed resources of Kerala and Karnataka. *Mar. Fish. Infor. Serv., T & E Ser*., 168 (2001)12-19.

12 Gupta, T.R.C. & Bhattacharya, B.K. Effect of sewage on the ecology of Gurpur estuary, Dakshina Kannada, Karnataka, in: *Aquatic Ecosystem* edited by Aravindkumar, ACH Publishing Corporation, New Delhi, (2003), pp. 809-826.

13 Strickland, J.D.H. and Parsons, T.R., A practical handbook of seawater analysis. *Bull. Fish. Res. Board Can.,* 167(1968), pp. 311.

14 APHA, Recommended Procedures for the Examination of Seawater and Shellfish, 4th edition (American Public Health Association, Washington, DC) (1970).

15 Burkhardt, W., Watkins, W.D. & Rippey, S.R. Seasonal effects on accumulation of microbial indicator organisms by *Mercenaria mercenaria*. *Appl. Environ. Microbiol.,* 58 (3) (1992) 826-831.

16 Lipp, E.K., Kurz, R., Vincent, R., Palacios, C.R., Farrah, S.R. & Rose, J.B. The effects of seasonal variability and weather on faecal pollution and enteric pathogens in a subtropical estuary. *Estuaries*, 24 (2) (2001) 266-276.

17 Ackerman, D. & Weisberg, S.B. Relationship between rainfall and beach bacterial concentrations on Santa Monica Bay beaches. *J. Water and Health*, 1 (2) (2003) 85-89.

18 Coulliette, A.D., Money, E.S., Serre, M.L., & Noble, R.T. Space/Time Analysis of Fecal Pollution and Rainfall in an Eastern North Carolina Estuary. Environ. *Sci. Technol.*, 43 (2009) 3728-3735.

19 Sunil, R., Karunasagar, I. & Karunasagar, I. Study of bacteriological quality of water in aquatic habitats of Karnataka Coast. *Conference on Microbiology of the Tropical Seas*. National Institute of Oceanography, Dona Paula, Goa 403 004. India, 13-15 December (2004). Abstract HPM (P)-01.

20 NCDEHNR, Water quality progress in North Carolina 1992-1993, 305(b) North Carolina Department of Environment, Health and Natural Resources, Division of Environmental Management, Water Quality Section, Raleigh, North Carolina. Report, (1994), No. 94-07.

21 Cornax, R., Morinigo, M.A., Romero, P. & Borrego, J. Survival of pathogenic microorganisms in seawater. *Curr. Microbiol.,* 20 (1990) 293-298.

22 Hernroth, B.E., Hansson, A.C.C., Holm, A.S.R., Girones R. & Allard, A.K. Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, *Mytilus edulis*: the First Scandinavian Report. *Appl. Environ. Microbiol.,* 68 (9) (2002) 4523-4533.

23 Anderson, K.L., Whitlock J.E. & Harwood, V.J. Persistence and differential survival of faecal indicator bacteria in subtropical waters and sediments. *Appl. Environ. Microbiol.,* 71 (6) (2005) 3041-3048.

24 Chandran, A. & Hatha, A.A.M. Survival of *Escherichia coli* in a tropical estuary. *S. Pac. J. Nat. Sci*. 21 (2003) 41-46.

25 Pommepuy, M., Guillaud, J.F. Dupray, E., Derrien, A., Le Guyader, F. & Cormier, M. Enteric bacteria survival factors. *Water Sci. Technol*., 12 (1992) 93-103.

26 Davies, C.M., Long, J.A.H., Donald M. & Ashbolt, N.J. Survival of faecal microorganisms in marine and freshwater sediments. *Appl. Environ. Microbiol.,* 61 (5) (1995) 1888-1896.