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Original Research Article

Isolation and characterisation of Total Streptocci and Faecal Streptococci from Kuppam river basin in South west coast of India

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A B S T R A C T

Rivers are most important natural resource for human development but it is being polluted by indiscriminate disposal of sewage, industrial wastes and plethora of human activities, which affects its physico chemical and microbiological quality. Because of the deterioration of river water quality, it is necessary to monitor the water quality to evaluate the production capacity. Bacteriological analysis of a River in South West coast of India viz. KuppamRiver was undertaken for determining the quality and extent of contamination in the sytem. A total of 16 surface water samples and 33 ground water samples were monitoredfor Total Streptococci (TS) and Faecal Streptococci (FS). The presence of faecal streptococci indicates the presence of faecal pathogens in water. The percentage of samples contaminated with total streptococcal count in surface water samples were 81.25%, 75% and 81.25% in pre-monsoon, monsoon and post-monsoon seasons respectively whereas in groundwater samples it was 78.79%, 78.79% and 75.76% respectively. The pink and red colonies grown on KF- streptococcal agar were analysed and after the complete biochemical analysis, four fecal streptococcal species were identifiedas *E. faecuim*, *E. faecalis*, *E. avium* and *E. durans*. In the case of surface water samples, the percentage of *E. faecuim, E. faecalis* in pre-monsoon, monsoon and post-monsoon seasons were 56.25%, 50%, 50% and 25%, 18.75%, 25% respectively. But in the case of *E. avium* and *E. durans* it was 6.25%, 12.5%, 6.25% and 6.25%, 12.5%, 12.5% in the three seasons respectively. In groundwater samples, the percentage values of *E. faecuim*, *E. faecalis*, *E. avium* and *E. durans* in pre-monsoon were 42.42%, 30.3%, 6.06% and 9.09% respectively whereas it was 45.45%, 33.33%, 9.09% and 3.03% in monsoon season. The value ranged from 3.03% to 48.48% in post-monsoon season.All the fecal streptococcal species isolated from Kuppam River showed varying range of susceptibility to the antibiotics used. Highest susceptibility to different antibiotics used was shown by *E. faecium.* Then the susceptibility was in the range *Ent. faecalis> Ent. durans>Ent. avium.*Hence according to our investigation the surface and ground water of Kuppam River was not of potable quality.

Keywords

Faecal contamination; Total Streptococci; Faecal Streptococci; Potability

Introduction

The lack of basic sanitation and lack of access to safe water supplies constitute the cause of water borne diseases in developing countries (Haruna *etal.*, 2005). The spread of diarrhoeal diseases especially in infants is because of contamination of drinking

water with organisms of faecal origin. Water borne bacterial pathogens most often detected in contaminated drinking water supplies include *Shigella, Salmonella, Campylobacter, Vibrio,* toxigenic *E. coli, Yersinia enterocolitica, Streptococci faecalis* which are also detected in faeces of infected individuals. Faecal streptococci (FS) are consistently present in the faeces of all warm-blooded animals and in the environment associated with animal discharges (Naaz*et al*., 2007). Like faecal coliforms, faecal streptococci are applied as indicators for water pollution.

FS or enterococci are considered as *Streptococcus spp.* that normally occur in faecal matter, which include *E. faecalis, E. faecium, S.bovis, E. avium, S. equines, S. mitis, S. Salivarius* (Leclerc *et al*., 1996). The natural habitat of these organisms is the intestinal tract of man and animals. Enterococci have been studied extensively not only because of their being indicator of faecal contamination and their involvement of food spoilage, but also due to their influence on host physiology and nutrition, and because of their possible role as direct or indirect agent of disease inman (Riaz, 2005). Because of the use of antibiotics in animals can result in the occurrence of antibiotic-resistant bacteria in those animals several attempts have been made to compare the patterns of antibiotic resistance in fecal coliforms with the sources of the isolates.

Kuppam River flows through Kannur district in Kerala in the South West coast of India. The River originates from the Padinalkadu Ghat forests in Karnataka state and it flows almost parallel to the Valapattanam River. Later it joins with the Valapattanam River at Mattool. It has a length of 82 km and it finally empties into the Arabian Sea. The main tributaries of this river are Pakkatupuzha, Alakuttathode, Kuttilolpuzha, Mukkuttathodu and Chiriyatho derivers. The present study attempts to investigate the bacterial species present in the water samples spatially and temporarily.

Materials and Methods

Sample collection

Water samples were collected from Kuppam River basin during the pre-monsoon (Feb- May), monsoon (June-Sept) and post monsoon (Oct-Jan) seasons. The details of sampling stations are indicated in Fig.I. Ground water samples were also collected during this season. A sanitary survey was also conducted to find out the hygienic condition and the pollution sources and the points of contamination of water. Samples were collected in 100 ml sterilized bottles and were immediately transported to the laboratory by keeping in an icebox.

Estimation of total and faecal streptococci

For enumeration of streptococci Most Probable Number (MPN) technique was used (APHA, 2012). Three tubes containing 10ml double strength Azide Dextrose broth was inoculated with 10ml of water samples, while two other sets, each of 3 tubes containing 5ml single strength broth were inoculated with 1ml and 0.1ml water sample, respectively. Well mixed tubes were incubated at 35±0.5°C for24-48hours. Turbidity was noted and interpreted from MPN table (Collins *et al*., 1989). Five ml of single strength Azide dextrose broth was inoculated with 1ml, shaken well and

incubated at 44.5°C for48hours. The tubes that showed growth were considered positive for faecal streptococci. MPN was computed. For further confirmation KF-streptococcal agar was streaked with turbid culture, and incubated at 35±0.5°C for48hours. Pink to dark red colonies were examined, purified and further identified by different biochemical tests.

Biochemical tests for streptococci

For biochemical tests fresh culture was obtained by growing pink or red colonies from the above plates into trypticase soy broth.

For the test using 0.1% methylene blue milk, sterile 0.1% methylene blue milk (5ml), was inoculated with fresh broth FS culture and incubated at 35±0.5°C for 24-72hours. Methylene blue was reduced to leuco methylene blue (colourless) in the case of positive test (Brock and Brock, 1978).

In the case of Arginine dehydrogenase test, four ml of broth (5g peptone, 3g yeast extract, 1g D(+)glucose, 0.016g bromocresol blue and 0.5g arginine per liter) was inoculated with pure fresh FS culture, overlaid with sterile paraffin(viscous) and incubated at 37°C for four days. Tubes with violet colour were considered as positive and with yellow colour as negative. Control tubes containing culture medium base only also gave yellow colour.

While performing Aesculine hydrolysis test, five ml of sterilized Aesculine broth (Peptone 2g, aesculine 0.1g, and ferric citrate 0.05gper 100ml) was inoculated with fresh culture. Olivegreen colour of the medium changed to brown in positive test after 12-hours incubation at 35±0.5°C.

In the case of the test using Acids from carbohydrates, different carbohydrates such as mannitol, sucrose, sorbitol and L-arabinose (1% each) dispensed separately in five ml of autoclaved Phenol red broth (BBL), were inoculated and incubated at35±0.5°C for 24±2hours. Acid production from carbohydrates was indicated by phenol red.

For testing the growth at 10°C and 45°C, five ml of Tryptose soy broth (BBL) was inoculated with fresh pure culture and incubated at10°C in one case and 45°C in other for 24±2 hours.

In the case of analysing the survival at 60°C for 30 minutes, twenty four hours fresh cultures were incubated at 60°C for 30 minutes, and then streaked on KF-streptococcal agar. Dark red colonies after48 hours incubation at 35±0.5°C indicated faecal streptococci.

For testing the growth at pH 9.6,Tryptose soy broth with pH 9.6 was inoculated with fresh pure culture and was incubated at 35±0.5°C for24±2hours. Turbidity in tube was considered as positive test.

In the test for analysing the growth at 6.5% NaCl broth, trypticase soy broth with 6g/L NaCl added was incubated with fresh culture. Turbidity after24±2hours at 35±0.5°C was considered as positive test (Garg and Mital, 1991).

For the antibacterial susceptibility test, Fecal streptococci from KF streptococcal agar plate were streaked on nutrient agar plate to have 24hours fresh culture. A pure culture inoculation was prepared by emulsifying fresh growth in 1 ml sterile 0.1% peptone water. Turbid suspension

was swabbed uniformly on the nutrient agar plate. Four antibiotics such as Penicillin, Methicillin, Tetracycline and Erythromycin were used for the test. The plates were incubated at 37°C for 24 hours. Zone of inhibition were measured and results were interpreted.

Results and Discussion

The MPN Index value ranged between 3 to >2400 MPN/ml. The percentage of total streptococcal count in surface water samples was 81.25%, 75% and 81.25% in pre-monsoon, monsoon and post-monsoon seasons respectively whereas in groundwater samples it was 78.79%, 78.79% and 75.76% respectively. The percentage values of Total streptococcal counts in different seasons are shown in Table 1. The pink and red colonies that grown on KF- streptococcal agar were analysed and four types of pink and red colonies were identified. After the complete biochemical analysis the four species identified were *E. faecuim*, *E. faecalis*, *E. avium* and *E. durans*. In the case of surface water samples, the percentage of *E. Faecuim, E. Faecalis* in pre-monsoon, monsoon and post-monsoon seasons were 56.25%, 50%, 50% and 25%, 18.75%, 25% respectively. But in the case of *E. avium* and *E. durans* it was 6.25%, 12.5%, 6.25% and 6.25%, 12.5%, 12.5% in the three seasons respectively. In groundwater samples the percentage values of *E. faecuim, E. faecalis, E. avium* and *E. durans* in pre-monsoon were 42.42%, 30.3%, 6.06% and 9.09% respectively whereas it was 45.45%, 33.33%, 9.09% and 3.03% in monsoon season. The value ranged from 3.03% to 48.48% in post-monsoon season.

In the case of surface water samples more fecal streptococcal species were found in pre-monsoon season whereas highest bacterial load was seen in post-monsoon season in groundwater samples. In the case of total streptococcal counts, highest percentage values were found in both pre-monsoon and post monsoon seasons in surface water samples. But in the groundwater samples the total streptococcal count was higher in pre-monsoon and monsoon seasons. Also majority of the streptococcal species were found in the downstream region of the river in three seasons. This may be attributed to the fact that these are the mixing regions of the river with the sea and therefore the rate of bacteriological pollution may be high. Also these organisms may be tolerant to the saline conditions prevalent in this area. Microorganisms isolated from surface water and groundwater samples during different seasons were depicted in Tables 2 and 3.Biochemical test results of faecal streptococcal species were depicted in Table 4. Percentage of Fecal Streptococcal species in surface water with respect to different seasons is shown in Fig II and III.

All the fecal streptococcal species isolated from Kuppam River showed varying range of susceptibility to the antibiotics used. Highest susceptibility to different antibiotics used was shown by *E. faecium.* Then the susceptibility was in the range *Ent. faecalis> Ent. durans>Ent. avium*. The lesser the zone of inhibition the lesser will be the susceptibility. Susceptibility of *Ent. faecium, Ent. faecalis, Ent. avium*and *Ent. durans* to various antibiotics was shown in Table 5**.**Zone of inhibition (cm) produced by different Fecal Streptoccal species to Penicillin, Tetracycline, Erythromycin and Methicillin is depicted in Fig IV, V, VI and VII respectively.

Table.1 Percentage values of Total streptococcal counts in different seasons

Sl No.	Nature of Sample	Percentage values of Total streptococcal counts (%)		
		Pre-monsoon	Monsoon	Post monsoon
1	Surface water	81.25	75	81.25
2	Groundwater	78.79	78.79	75.76

Table.2 Microorganisms isolated from Surface water samples during different seasons

Sl No.	Surface water samples	Microorganism isolated		
		Pre-monsoon	Monsoon	Post monsoon
1	KM 1	A, B	A	A, B
2	KM 2	ND	ND	ND
3	KM 3	A	A, C	A, C
4	KM 4	ND	ND	ND
5	KM 5	ND	ND	ND
6	KM 6	A	D	A, D
7	KM 7	ND	ND	ND
8	KM 8	A, B	B	B
9	KM 9	A	A	A, B
10	KM 10	A, B	A	A
11	KM 11	ND	ND	ND
12	KM 12	A, D	A	A, D
13	KM 13	ND	ND	ND
14	KM 14	ND	A	ND
15	KM 15	A, C	A, B, C	A
16	KM 16	A, B	A, B, D	A, B

Note: A- *E. faecuim*, B- *E. faecalis*, C- *E. avium*, D- *E. durans*, ND-Not Detected.

Table.3 Microorganisms isolated from Groundwater samples during different seasons

Sl No.	Groundwater samples	Microorganism isolated		
		Pre-monsoon	Monsoon	Post monsoon
1	KMg 1	ND	ND	ND
2	KMg 2	A	A	A
3	KMg 3	ND	ND	A
4	KMg 4	A, B, C	A, B, D	A, D
5	KMg 5	A, B, D	A, B	A, D
6	KMg 6	A, B	B	A
7	KMg 7	ND	ND	ND
8	KMg 8	ND	ND	ND
9	KMg 9	ND	ND	ND
10	KMg 10	A, B	A, B	A, B, C
11	KMg 11	A, C	A, B, C	A, B
12	KMg 12	ND	ND	ND
13	KMg 13	ND	ND	ND
14	KMg 14	ND	ND	ND
15	KMg 15	A	A, B	A
16	KMg 16	ND	ND	ND
17	KMg 17	A, B	A, B, C	A, B
18	KMg 18	ND	ND	ND
19	KMg 19	ND	ND	ND
20	KMg 20	ND	ND	ND
21	KMg 21	A	A	A
22	KMg 22	A, B	A, B, C	A, B
23	KMg 23	ND	ND	ND
24	KMg 24	A	A	A
25	KMg 25	ND	ND	ND
26	KMg 26	ND	ND	ND
27	KMg 27	A	A	B
28	KMg 28	ND	ND	ND
29	KMg 29	ND	ND	ND
30	KMg 30	A, B	A	A
31	KMg 31	A, B, D	A, B	A, B
32	KMg 32	A, B	A, B	A, B
33	KMg 33	A, B, D	A, B	A, B, D

Note: A- *E. faecuim*, B- *E. faecalis*, C- *E. avium*, D- *E. durans*, ND-Not Detected.

Table.4 Biochemical test results of faecal streptococcal species

Sl no	Strain Code	Acid form				pH 9.6	NaCl 6.50 %	mbm 0.1%	Growth (°C)			Hydrolysis		Strains identified
		so	su	ar	m				10	45	60	Arg	Ae	
1	A	-	+	+	+	+	+	+	+	+	+	+	+	*E. faecuim*
2	B	+	+	+	+	+	+	-	-	+	+	-	+	*E. faecalis*
3	C	+	+	-	-	+	+	+	+	+	+	+	+	*E. avium*
4	D	+	+	-	-	-	+	-	+	+	+	-	-	*E. durans*

Note: so-sorbitol, su-sucrose, ar-arabinose, m-mannitol, mbm-methylene blue milk, Arg-Arginine dehydrogenase, Ae-Aesculine

Table.5 Susceptibility of *Ent. faecium, Ent. faecalis, Ent. Avium* and *Ent. durans* to various antibiotics

Seasons	Zone of Inhibition in cms															
	Penicillin				Tetracycline				Methycillin				Erythromycin			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Pre-monsoon	ND	0.2	0.5	0.3	0.2	ND	ND	0.3	0.1	0.3	1.1	0.7	ND	0.9	1.3	1.6
Monsoon	0.2	0.4	0.9	0.7	0.3	ND	ND	0.2	0.2	0.5	0.9	0.6	0.4	0.9	1.2	1.4
Post-monsoon	0.3	0.8	1.1	1.0	0.2	ND	ND	0.1	ND	0.4	1.3	0.7	ND	1.0	1.4	1.8

Note: A- *E. faecuim*, B- *E. faecalis*, C- *E. avium*, D- *E. Durans*, ND-Not Detected

According to our investigation the surface and groundwater of Kuppam river was not of potable quality. European regulation for drinking water guideline recommends the absence of faecal streptococci in 100ml of drinking water (Collin *et al*., 1988). *Entfaecium* was present in appreciable percentage in this survey as Poucher *et al.* (1991) also indicated that *Ent. Faecium* always represented over 25% of streptococci in different types of faecal samples. *Ent. Faecalis* and *Ent. faecium*are inherently resistant to multiple antibiotics and hence reduce susceptibility to cell wall active agents such as β-lactams. *Ent. Faecalis* and *Ent. faecium*are the predominant enterococcal species associated with clinical infection in human. *Ent. Faecium* strains are less susceptible to β-lactams than *Ent.faecalis*(Herman and Gerding, 1991).

Enterococci are opportunistic pathogen(Geldreich*et al.,* 1990), cause infections of urogenital tract, endocarditis and wound infections in humans. So their presence is a cause of serious concern. As TS and FS were found in surface and groundwater

Fig.I Surface and groundwater sampling stations of Kuppam river basin respectively

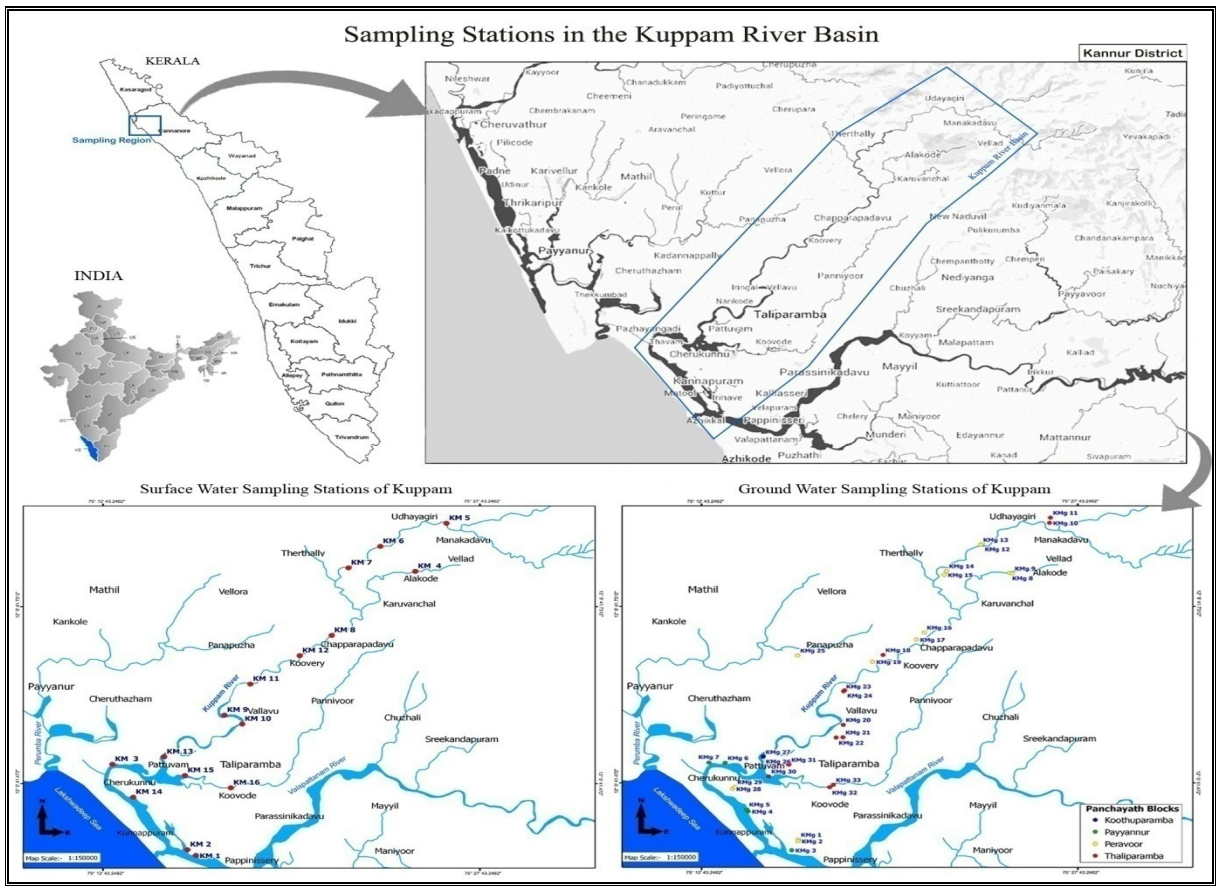


Fig.II Percentage of fecal streptococcal species in surface water during different seasons

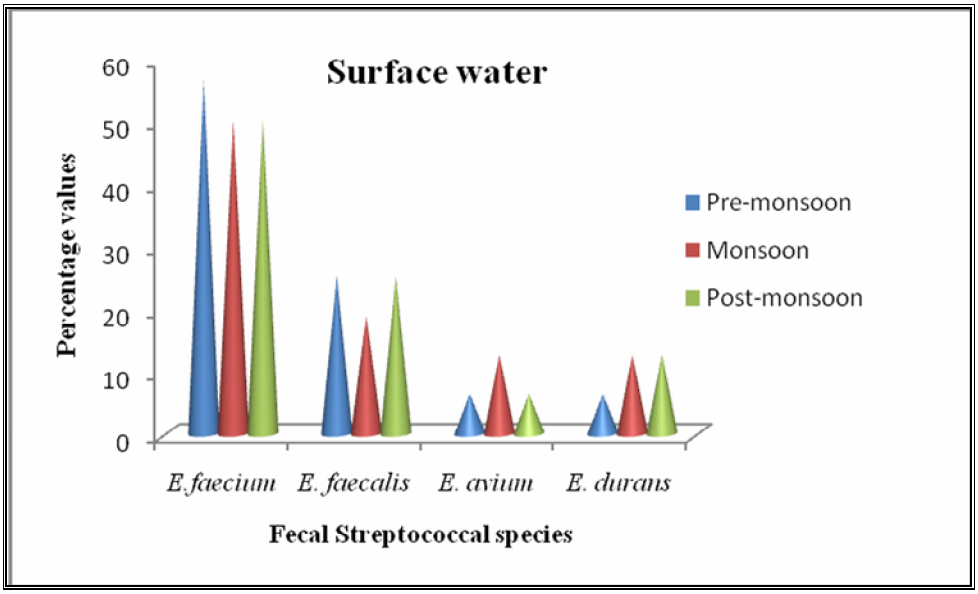


Fig.III Percentage of fecal streptococcal species in groundwater during different seasons

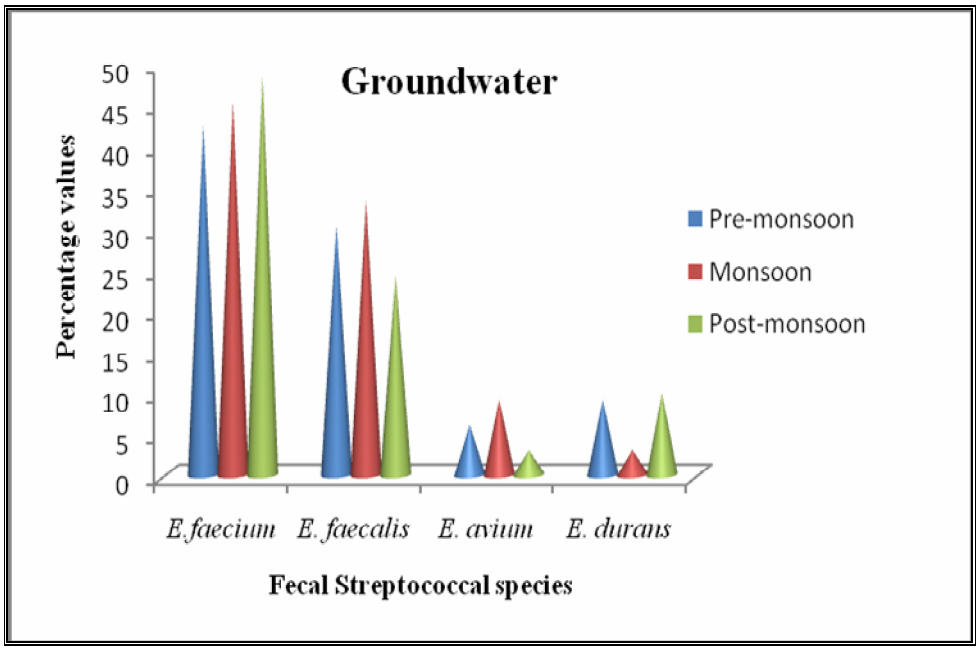


Fig.IV Zone of inhibition (cm) produced by different fecal streptoccal species to Penicillin

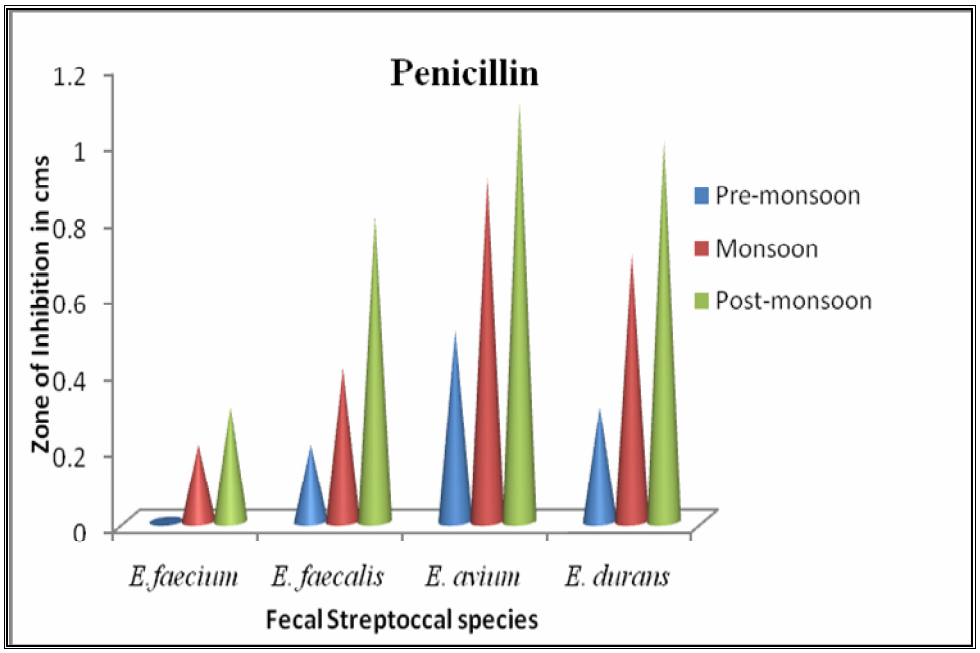


Fig.V Zone of inhibition (cm) produced by different fecal streptoccal species to Tetracycline

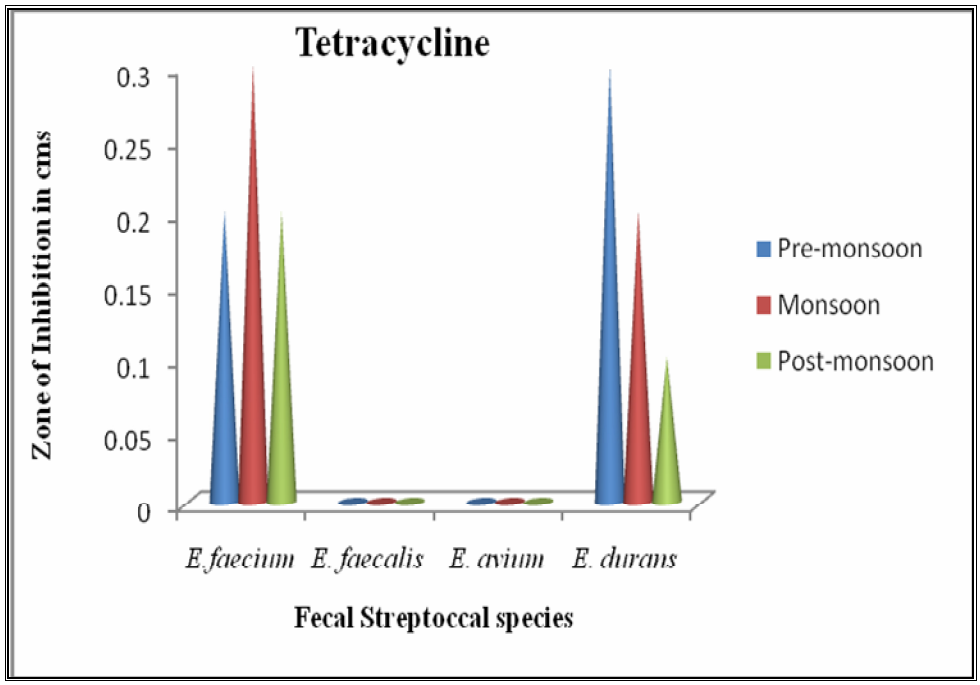


Fig.VI Zone of inhibition (cm) produced by different fecal streptoccal species to Erythromycin

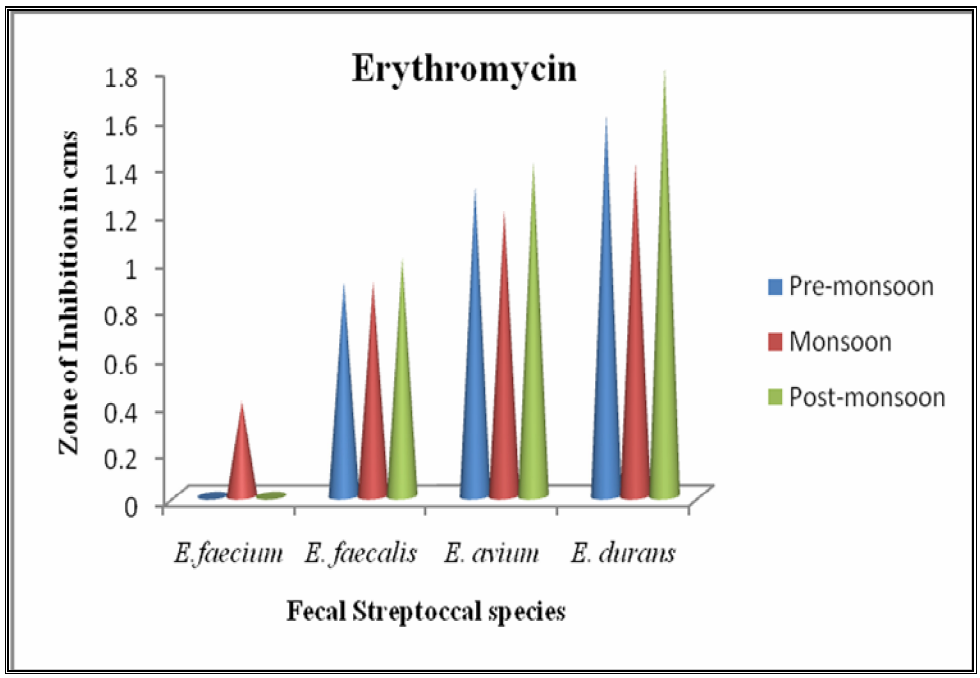
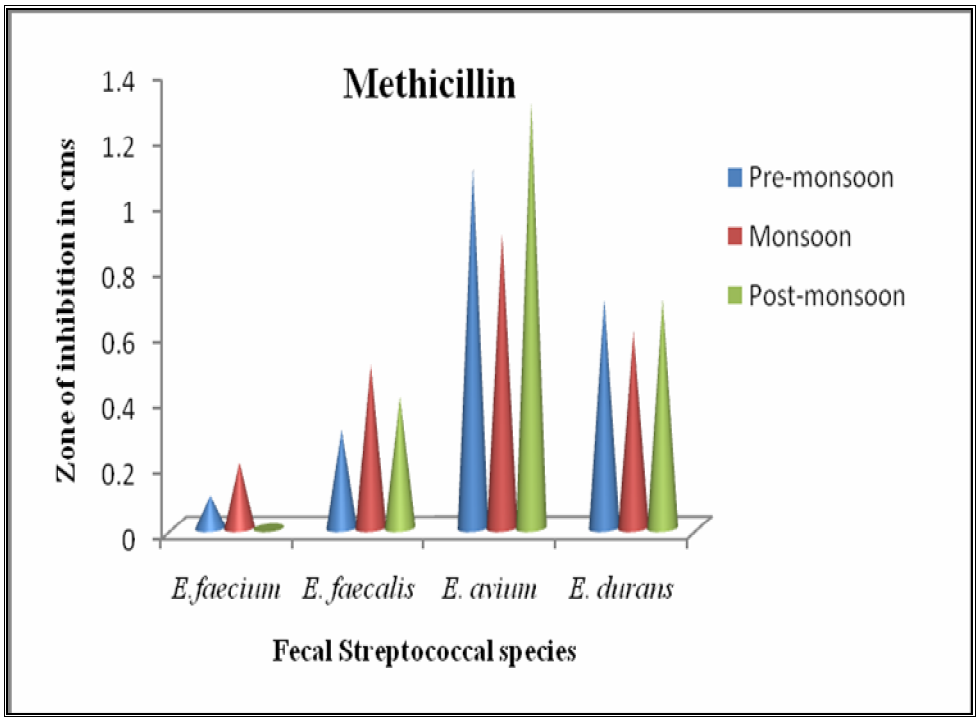


Fig.VII Zone of inhibition (cm) produced by different fecal streptoccal species to Methicillin



samples they may be considered as indicators of pollution and thepresence of other intestinal pathogens may be also present in this river. Enterococci are facultative anaerobe and are able to withstand diverse conditions (Gargand Mital, 1991). Hence their presence in water may cause problems as water is used extensively in dailylife and so proper measures should be taken to regularly monitor water supplies and to provide potable water to general public.

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