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Bacteriological Characteristics of Kodoor River, Kottayam, Kerala, South India – A Seasonal Study

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Abstract: The Kodoor river is one of the major rivers that flows through the Kottayam district of Kerala, South India. In this study the bacteriological characteristics of the Kodoor River were investigated. Five stations of the Kodoor river were chosen for the investigation. In the present work bacteriological parameters were studied in three different seasons viz. monsoon, post-monsoon and pre-monsoon during 2022. An estimation of bacterial production is a crucial step in understanding quantitatively the function and contribution of bacteria in material cycling within the given aquatic habitats. The Total Coliform Count (TCC) and Faecal Coliform Count (FCC) values are high during pre-monsoon period and low during monsoon period. Total Coliform number ranged from 220 MPN/100 ml to 1100 MPN/100 ml during pre-monsoon, 120 MPN /100 ml to 240 MPN /100 ml during monsoon, and 240 MPN/100 ml to 1100 MPN/100 ml during post-monsoon. The coliform bacteria include the genera *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsilla* etc. Highest value of Coliform 1100 MPN/100 ml was observed at station 5 (Kodimatha) during pre-monsoon and the lowest value 120 MPN/100 ml was observed at station 1 (Puthupally) during monsoon period. Faecal contamination is also reported from station 4 (Iriyilakadavu) and station 5 (Kodimatha). The following species of bacteria *E. coli*, *Salmonella sp.*, *Pseudomonas sp.*, *Enterobacter aerogenes*, *Shigelle sp.*, *Vibrio cholare*, *Vibriyo parahaemolyticus*, *Proteus sp.*, *Klebsiella sp.* is isolated from the different stations, mainly from stations 3, 4, and 5. Although the current situation is not grave or alarming, the river water requires intensive monitoring in order to improve its quality for better and more sustainable management.

Keywords: Coliform count, Faecal coliform count, Most probable number

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Introduction

Water is one of the most significant compound on the planet. Water is essential for the survival of all plants and animals. There would be no life on

Earth if there is no water. Water is the only natural material that exists as a solid (ice), liquid, and gas (water vapour). It spans over 70% of the planet

and has a volume of approximately 332.5 million cubic miles. The fact that water makes up between 60 and 95 per cent of all living species speaks volumes about the compound's immense biological relevance. However, because it is salty, 97 per cent of the water on the planet is unfit to drink. Only 3% of the world's water supply is freshwater, with the remaining 77% frozen. Microorganisms that degrade organic matter cannot function without water, disrupting the ecological transmission of matter and energy and shutting down the entire ecosystem.

Except for seawater and brackish water, freshwater refers to any naturally occurring water. A river is a natural water stream that flows towards an ocean, a lake, the sea, or another river. Humans have been enjoying the ecosystem benefits supplied by rivers for generations without knowing how the river ecosystem operates and maintains its vitality (Naiman, 1992). River systems are home to the richest biological variety on Earth, as well as the most intensive human activity. Freshwater variety is under decline as a result of decades of human exploitation of rivers through huge dams, water diversions, and pollution. Freshwater species are significantly more threatened than terrestrial ones. Geometric population growth, combined with fast urbanisation, industrialization, and agricultural expansion, has had a significant influence on the quality and amount of water in India. As a result, the availability and quality of freshwater resources are the most critical of India's numerous environmental issues (CPCB, 2011).

In recent years, there has been a rise in global concern about the quality as well as the quantity of river water. River water quality issues in India have worsened in recent decades, and the situation has now reached crisis proportions. According to river ecology studies, the major Indian rivers are severely polluted, particularly near towns (Srivastava, 1992). Water quality testing is a critical component of environmental monitoring. When water quality is bad, it has an

impact not just on aquatic life but also on the surrounding ecology.

Water quality assessment has emerged as a crucial concern in recent years, particularly as freshwater becomes a scarce resource in the future (Chang *et al.*; 2015). Such river taming and exploitation of riverine resources has frequently resulted in catastrophic deterioration, with serious consequences for human health and the environment (Carpenter *et al.*, 1998). Water quality refers to the relationship between all hydrological features, including physical, chemical, biological, and microbiological indicators, which represent the biotic and abiotic condition of an ecosystem (Tahera Aktar *et al.*, 2016). To assess water pollutants, a variety of scientific procedures and instruments have been created (Dissmayer, 2000). The pH, turbidity, conductivity, total hardness, alkalinity, acidity, dissolved solid, dissolved oxygen, organic and inorganic components, and heavy metals are all tested.

Most aquatic creatures are extremely sensitive to environmental changes, and they respond to pollution in a variety of ways. Death or relocation to another environment are the most dramatic responses (Bassem, 2019). Aquaculture has received a lot of attention in recent decades as a source of food to support the country's rising population. Fish are the most visible component of inland aquatic wildlife and are an excellent source of protein. Sustained exploitation and concurrent protection of fishery resources, as well as fundamental scientific information on biodiversity, are critical (Sone *et al.*, 2000; Shedge, 2008). Fish are an essential indicator of the aquatic ecology and have an exceptional socioeconomic position. Water is critical to the aquatic ecology. Water physicochemical factors are important in fish biology and physiology (Dhawan *et al.*, 2002). Fish are extremely susceptible to changes in water chemistry caused by various human activities in their catchments (Siligato *et al.*, 2002). Many species have become critically endangered, particularly in rivers where freshwater is in great demand. The influence of

anthropogenic activity, habitat degradation, exotic species introduction, water diversions, pollution, and global climate change, on the other hand, is the most significant. Most rivers in the central part of Kerala, such as the Periyar and Muvattupuzha, have poor water quality, but most rivers in the southern portion of Kerala have acceptable water quality. Some rivers in Kerala's northern region, such as the Kadalundi, have high water quality, while others, such as the Chaliyar, have inferior water quality (Jyothi *et al.*, 2021).

Kodoor River is a river between the Kottayam and Alappuzha district of Kerala state, South India. Kodoor River has a long history with the old trading routes between the coastal district Alapuzha and the eastern villages of Kottayam (Kumar, 2006). In the present study bacteriological analysis is done to explore the water quality of selected spots of Kodoor river in order to correlate the effect of pollution on water.

Materials and Methods

Study area:

Kodoor River (Fig. 1) is in between the Kottayam and Alappuzha district of Kerala state, South India. Kodoor River has a long history with the old trading routes between the coastal district Alapuzha and the eastern villages of Kottayam. There was a busy jetty at Puthuppally named Angadi, which means market. The river is originated from the beautiful hills in between Kottayam and Pathanamthitta districts and finally empty into the Meenachil River.

The river is 38 kilometers long and flows through Puthupally, Parekadavu, Kalathikadavu, Iriyilakadavu, Kodimatha, Moolavatom, Nattakom and Pallam. To carry out the present study of water quality of Kodoor river, total 5 sampling stations (Table 1) were chosen within the river basin.

The Puthupally site of Kodoor river is a small town area and Rubber trees are extensively cultivated in vast areas in the river basin. The Parekadavu and Kalathykadavu site of Kodoor

river are small town and many fish markets, slaughter houses and hostels are situated on the bank of the river. A car service station is located near the 4th station, Iriyilakadavu. The fifth station is located near MC road, fish market and boat jetty is also located near to this station.

Sample Collection:

Samples were collected from five stations (Station 1, Station 2, Station 3, Station 4, Station 5) from Kodoor river (Fig. 2) from July, 2020 to August, 2021. The sample bottles were washed with hot water and rinsed several times with distilled water. The water samples were collected from river bank by using sterile 1 L and 200 ml bottles, first the bottles were rinsed with river water then collected the river water. While collecting the water, care was taken to avoid remains in the water.

Bacteriological analysis:

MPN is most commonly applied for quality testing of water to ensure whether the water is safe or not in terms of bacteria present in it. 5 tubes of double strength and 10 tubes of single strength for each water sample was tested. Using a sterile pipette added 10 ml of water to 5 tubes containing 10 ml double strength medium. Similarly, added 1 ml of water to 5 tubes containing 10 ml single strength medium and 0.1 ml water to the remaining 5 tubes containing 10 ml single strength medium. Incubated all the tubes at 37°C for 24 h. If no tubes appear positive re-incubated up to 48 h. Compared the number of tubes giving a positive reaction to a standard chart and recorded the number of bacteria present in it (Ibe *et al.*, 2005).

Isolation of pathogenic bacteria:

1. Inoculated 1 ml of sample into 10 ml sterile brain heart infusion (BHI) broth.
2. Incubated at 37°C for 24 h.
3. Subcultured on to thiosulphate citrate bile sucrose agar (TCBS), xylose lysine deoxycholate (XLD), *Salmonella shigella* (SS)

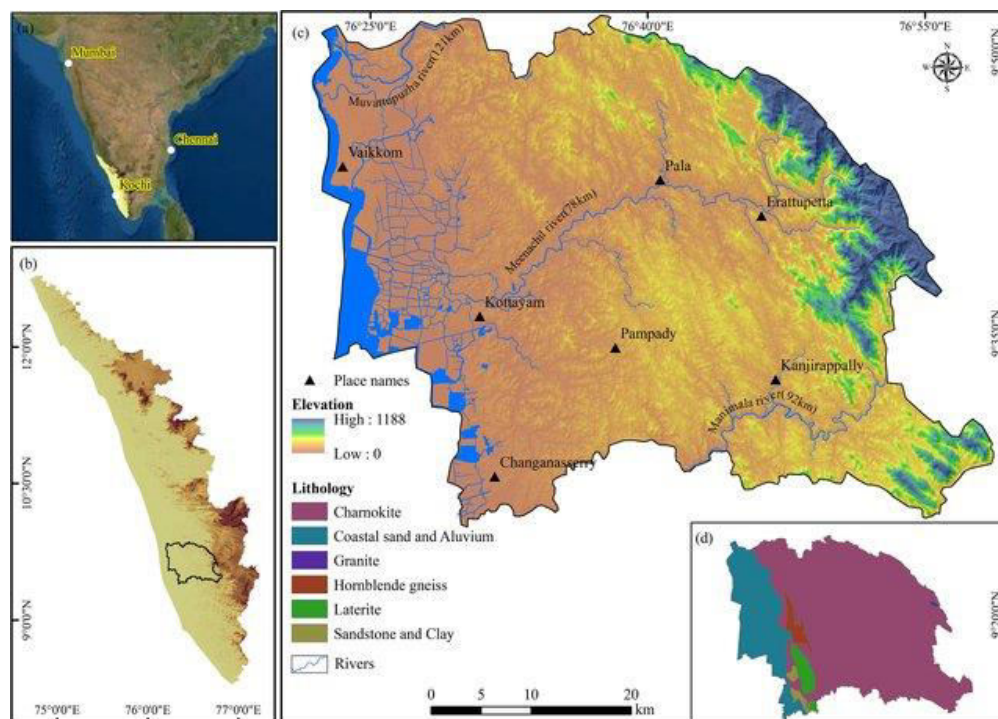


Fig. 1: Location Map of study area (Source: Elevation data of ASTER; Lithology data of Geological Survey of India).

Table 1: Sampling stations

Station No.	Station Name	Location	
		Latitude	Longitude
Station 1	Puthupally	9.55233607 ⁰	76.56619382 ⁰
Station 2	Parekkadavu	9.55171959 ⁰	76.54929419 ⁰
Station 3	Kalithikadavu	9.56128603 ⁰	76.5288436 ⁰
Station 4	Iriyilakadavu	9.58238989 ⁰	76.52937866 ⁰
Station 5	Kodimatha	9.57843345 ⁰	76.51986267 ⁰

agar and eosine methylene blue (EMB) agar plates, respectively.

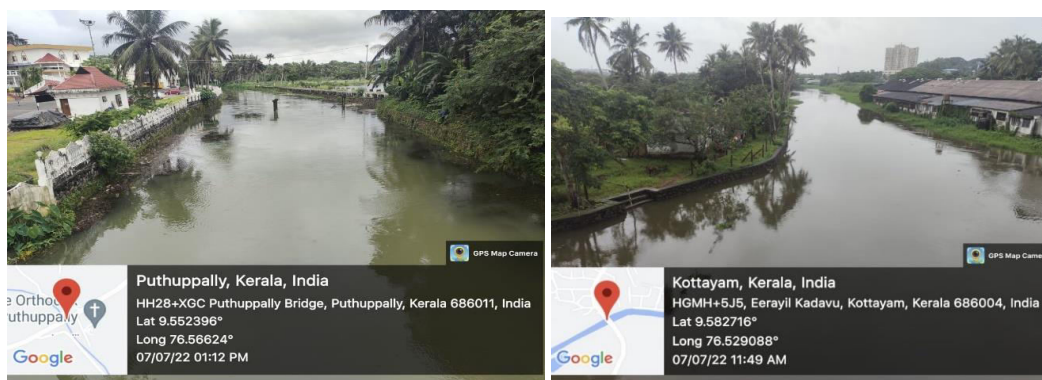
- Incubated the plates at 37°C for 24-28 h.
- Macroscopic and microscopic identification (gram staining)
- Biochemical identification (Indole, Methyl red, Voges-Proskaur, Citrate, Triple sugar iron agar, Motility, sugar fermentation (glucose, sucrose, lactose, mannitol), nitrate, urease,

oxidation fermentation reactions, catalase, oxidase)

On the basis of following cultural, morphological and biochemical characteristics, we have identified the isolated bacterial cultures.

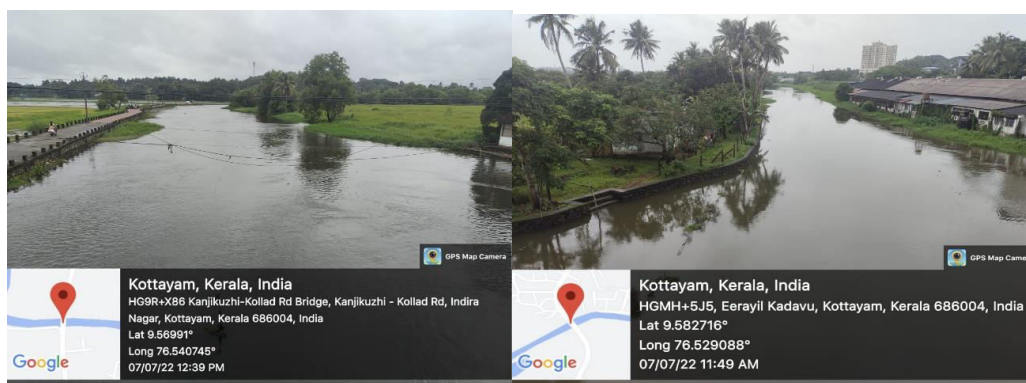
Gram staining:

A thin smear of isolates were prepared and dried. The preparation was flooded with crystal violet (1 min), Gram's iodine (1 min), ethyl alcohol (1 min)



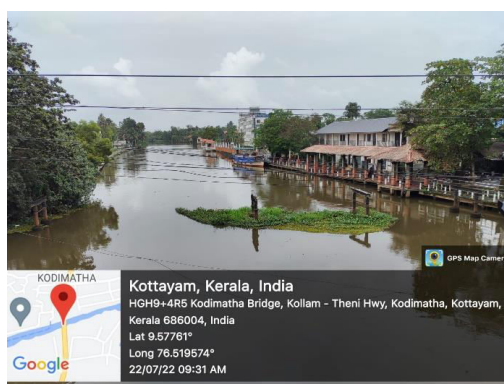
Station - 1 Puthuppally

Station – 2 Parekkadavu



Station- 3 Kalathikadavu

Station – 4 Iriyilakadavu



Satation – 5 Kodimatha

Fig. 2: Location map of sampling stations.

and saffranine (30 sec), respectively with intermittent washing. Then it was air dried and observed under oil immersion. Gram positive bacteria appeared in violet color whereas Gram negative bacteria appeared in pink color.

Sugar fermentation tests:

Sugar fermentation tests were done to detect the

ability of the isolates to ferment sugars – glucose, lactose and sucrose – to produce acid and gas. The acid production was detected by the indicator bromothymol blue that turns yellow and gas production was detected by the presence of bubbles in the Durham's tube. Sugar fermentation media was prepared and Durham's tubes were placed in inverted position. The isolates were

inoculated after sterilizing the media and incubated at 37°C for 24 h.

Indole test:

The indole test demonstrates the production of indole from tryptophan by the enzyme typtophanase. The presence of indole was detected by adding Kovac's reagent, which is composed of para-dimethyl amino benzaldehyde, butanol and Hydrochloric acid. Indole was extracted from the medium into the reagent layer by the acidified butanol component and forms a complex with the p-dimethyl amino benzaldehyde yielding a cherry red color. Inoculated the isolates into peptone broth and incubated at 37 °C for 48 h. Added 0.5 ml of Kovac's reagent without shaking.

Methyl red (MR) test:

The methyl red test was employed to detect the production of sufficient acid during fermentation of glucose. In this test, the pH indicator methyl red detects the presence of large concentration of acidic end products. Methyl red indicator in pH range of 4 will turn red which was indicative of a positive test. With lower H⁺ ion concentrations, the indicator turns yellow and gives a negative test. Prepared glucose phosphate peptone broth and adjusted the pH to 7.6. Sterilized it at 121°C for 15 min. Inoculated the liquid broth with test organism and incubated at 37°C for 48 h. Added 5 drops of methyl red reagent to the inoculated media mixed well and read immediately.

Voges – proskauer (VP) test:

The Voges – Proskauer test determines the capacity of organisms to produce neutral end products such as acetyl methyl carbinol (or acetoin) from the organic acids that results from glucose metabolism. Under alkaline condition and exposure to air, the acetyl methyl carbinol was oxidized to diacetyl which forms a pink colored complex. The test organism was inoculated into 5 ml of sterile glucose phosphate peptone broth. Incubated at 37°C for 48 h. Added 10 drops of VP-1 (Barrits A reagent) and 4 drops of VP-2 (Barrits

B reagent) reagents. Gently shaken the tubes and wait for 15-30 min.

Citrate utilization test:

The test was done to demonstrate the ability of an organism to utilize citrate as a sole source of carbon and energy for growth and ammonium salt as the sole source of nitrogen. This ability depends on the presence of citrate permease enzyme that facilitates the transport of citrate in to the cell. Citric acid is an intermediate metabolic product of Kreb's cycle which oxidizes pyruvate into CO₂. During this reaction, the medium become alkaline. The CO₂ that is generated combines with Na⁺ and H₂O to form Sodium carbonate (Na₂CO₃), an alkaline product. The presence of Na₂CO₃ changes the color of indicator bromothymol blue incorporated into the medium from green to deep Prussian blue. Prepared slants of Simmon's citrate agar medium. Inoculated the slants by streaking on the surface. The tubes were kept at 37°C for 48 h for incubation.

Urease test:

It was the test used to detect the ability of certain bacteria to produce urease enzyme. Urease enzyme breaks down urea into ammonia and CO₂. With the release of ammonia, the medium becomes alkaline as shown by a change in color of the indicator, phenol red to deep pink. Prepared the basal medium without urea. Adjusted the pH to 6.8 – 6.9 and sterilized by autoclaving. Cooled to about 50°C, added urea and allowed to set as a slant. Inoculated it with test organism by streaking over the agar slope and incubated at 37°C for 48 h.

Catalase test:

Most of the aerobes and facultative anaerobes have the characteristic catalase activity. These organisms utilize O₂ to split hydrogen peroxide (H₂O₂) into H₂O and O₂, as H₂O₂ was toxic to their enzyme system. Picked up a colony of bacteria from a plate using a glass rod. Dipped the rod in a test tube containing 3% H₂O₂. A positive test shows effervescence.

Oxidase test:

Aerobic bacteria as well as some facultative anaerobes and microaerophiles exhibit oxidase activity. The ability of bacteria to produce cytochrome oxidase can be determined by the addition of a dye, tetramethyl-p-phenylene diamine dihydrochloride or p-aminodimethyl anilinoxalate. It donates an electron to Cytochrome C, gets oxidized and produces a color. A piece of filter paper was placed in a clean petridish and added a few drops of freshly prepared oxidase reagent. Using a glass rod a colony of the test organism was placed on the filter paper. Looked for the development of the blue-purple color within a second.

Triple sugar iron (TSI) agar test:

TSI slant was prepared and the butt was inoculated by stabbing with the pure culture and then streaked the culture along the slant. The slants were incubated at 37°C for 18-24 h.

Mannitol motility test:

This test is used to identify organisms on the basis of motility and mannitol utilization. Bacterial motility can be observed by the direct examination of the tubes following incubation. If the organism was motile, the bacteria spread out from the line of inoculation and produce growth throughout the tubes. Growth of non-motile organisms was seen only along the stab lines. A mannitol utilizing organism changes the color of the medium from red to yellow. Prepared mannitol agar medium in a test tube. Inoculated the tubes with a pure culture by stabbing the centre of the column of medium to more than half of its depth. Incubated the tubes at 37°C for 24 h.

Spore staining:

Prepared smears of the isolates and heat fixed the smears. Covered the smears with a piece of absorbent paper cut to fit the slide and placed the slide on wire gauze on a ring stand. Saturated the paper with malachite green. Heated the slide until steam can be seen rising from the surface. Removed from the heat and reheated the slide as

needed to keep the slide steaming for about 3 min. As the paper begins to dry added a drop of malachite green to keep it moist. Removed the paper with tweezers and rinsed the slide thoroughly with tap water. Drained the slide and counterstained 45 sec with 0.5% safranin. Washed, blotted and examined under the microscope. The vegetative cells will appear red and the spores will appear green.

OF test:

Inoculated two tubes of OF test medium with the test organisms using a straight wire by stabbing half way to the bottom of the tube. Covered one tube of each pair with 1 cm layer of sterile mineral oil or liquid paraffin (it creates anaerobic condition in the tube by preventing diffusion of oxygen), leaving the other tube open to the air. Incubated both the tubes at 35°C for 48 h. Acid production was detected in the medium by the appearance of a yellow color. In the case of oxidative organisms; color production may be first noted near the surface of the medium.

Results

Bacteriological analysis – pre-monsoon:

Changes in bacteriological parameters in water samples during pre-monsoon are shown in Table 2. An estimation of bacterial production is a crucial step in understanding quantitatively the function and contribution of bacteria in material cycling within the given aquatic habitats. Total Coliform number ranged from 220 MPN/100 ml to 1100 MPN/100 ml. The coliform bacteria include the genera *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsilla* etc. Highest value of Coliform 1100 MPN/100 ml was observed at station 5 and the lowest value 220 MPN/100ml was observed at station 1 (Fig. 3). Faecal Coliform bacteria were recorded only at station 4 and 5 (Table 2, Fig. 3).

Bacteriological analysis –monsoon:

During monsoon total coliform count was minimum at station 1 (120 MPN/ 100 ml) whereas it was maximum at station 5. Faecal Coliform

Table 2: Bacteriological analysis of water samples collected from different sites of Kodoor river – pre-monsoon

Stations	Total Coliform Count TCC (MPN/100 ml)	Faecal Coliform Count FCC (MPN /100 ml)
Station 1	220	0
Station 2	240	0
Station 3	240	0
Station 4	460	120
Station 5	1100	460

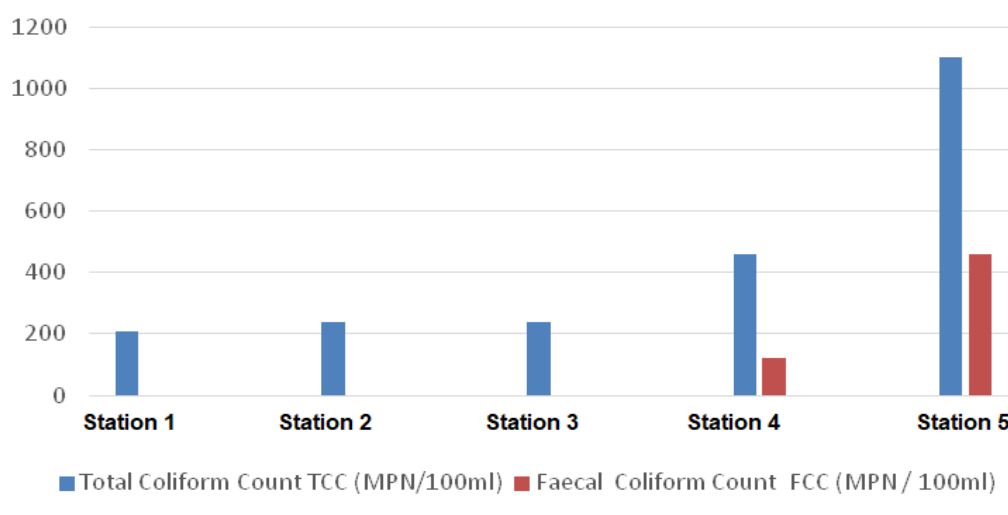


Fig. 3: Total Coliform Count and Faecal Coliform Count of water sample collected from different sites of Kodoor river during pre-monsoon.

Table 3: Bacteriological analysis of water samples collected from different sites of Kodoor river during monsoon

Stations	Total Coliform Count TCC (MPN/100 ml)	Faecal Coliform Count FCC (MPN/100 ml)
Station 1	120	0
Station 2	150	0
Station 3	150	4
Station 4	210	39
Station 5	240	43

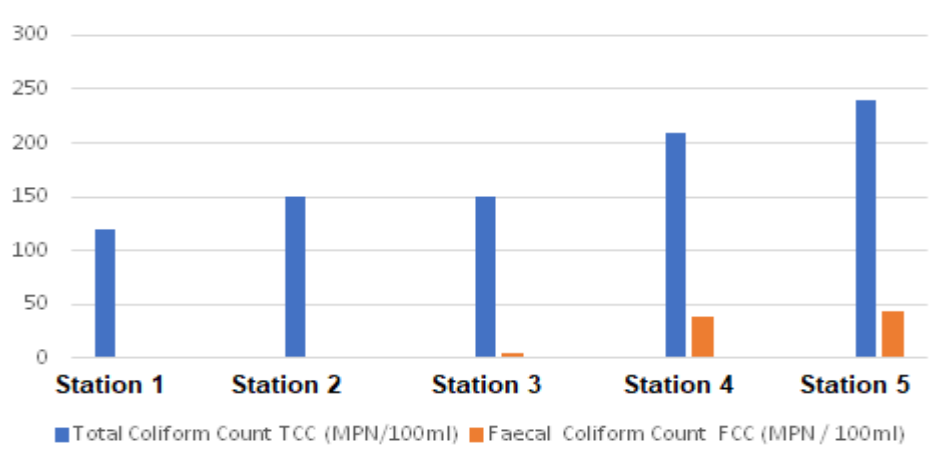


Fig. 4: Total Coliform Count and Faecal Coliform Count of water sample collected from different sites of Kodoor river during monsoon.

Table 4: Bacteriological analysis of water samples collected from different sites of Kodoor river during post-monsoon

Stations	Total Coliform Count TCC (MPN/100 ml)	Faecal Coliform Count FCC (MPN / 100 ml)
Station 1	240	0
Station 2	240	0
Station 3	460	0
Station 4	460	20
Station 5	1100	21

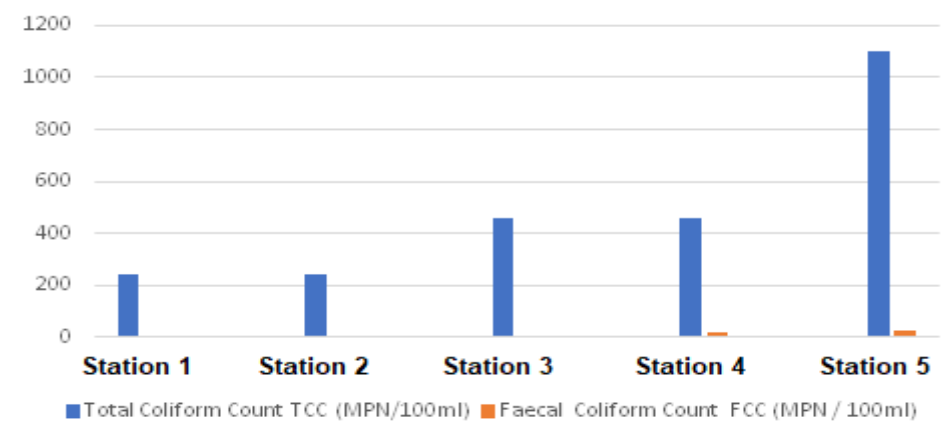


Fig. 5: Total Coliform Count and Faecal Coliform Count of water sample collected from different sites of Kodoor river during post- monsoon.

Table 5: Bacterial isolates from water samples from Kodoor river during pre monsoon

Bacterial isolates	Station 1	Station 2	Station 3	Station 4	Station 5
<i>Pseudomonas sp.</i>	+	+	+	+	+
<i>Escherichia coli</i>	+	+	+	+	+
<i>Enterobacter aerogenes</i>	+	+	+	+	+
<i>Staphylococcus aureus</i>	+	+	+	+	+
<i>Salmonella typhosa</i>	-	-	+	+	+
<i>Shigella sp</i>	-	-	+	+	+
<i>Vibrio cholerae</i>	-	-	+	+	+
<i>Vibrio parahaemolyticus</i>	-	-	+	+	+
<i>Proteus sp.</i>	+	+	+	+	+
<i>Klebsiella sp.</i>	+	+	+	+	+

Table 6: Bacterial isolates from water samples from Kodoor river during monsoon

Bacterial isolates	Station 1	Station 2	Station 3	Station 4	Station 5
<i>Pseudomonas sp.</i>	-	-	-	+	+
<i>Escherichia coli</i>	+	+	+	+	+
<i>Enterobacter aerogenes</i>	-	-	-	+	+
<i>Staphylococcus aureus</i>	+	-	-	-	+
<i>Salmonella typhosa</i>	-	-	-	+	+
<i>Shigella sp</i>	-	-	+	-	-
<i>Vibrio cholerae</i>	-	-	+	+	+
<i>Vibrio parahaemolyticus</i>	-	+	-	+	+
<i>Proteus sp.</i>	-	-	-	-	+
<i>Klebsiella sp.</i>	+	+	+	+	+

Table 7: Bacterial isolates from water samples from Kodoor river during post monsoon

Bacterial isolates	Station 1	Station 2	Station 3	Station 4	Station 5
<i>Pseudomonas sp.</i>	-	+	+	+	+
<i>Escherichia coli</i>	+	+	+	+	+
<i>Enterobacter aerogenes</i>	+	-	-	+	-
<i>Staphylococcus aureus</i>	+	-	-	-	+
<i>Salmonella typhosa</i>	-	-	-	+	+
<i>Shigella sp</i>	-	-	+	-	-
<i>Vibrio cholerae</i>	+	-	+	+	+
<i>Vibrio parahaemolyticus</i>	-	+	-	-	+
<i>Proteus sp.</i>	-	+	-	-	+
<i>Klebsiella sp.</i>	-	+	+	-	-

bacteria were recorded only at station 3, 4 and 5 (Table 3, Fig. 4).

Bacteriological analysis during post-monsoon:

During post-monsoon total coliform count was minimum at station 1 (240 MPN/ 100 ml) whereas it was maximum at station 5 (1100 MPN/ 100 ml). Faecal Coliform bacteria were recorded only at station 4 and 5 (Table 4, Fig. 5).

Bacterial isolates at stations 1-5 during pre-monsoon, monsoon and post-monsoon are shown in Tables 5, 6 and 7, respectively.

Discussion

Nowadays water pollution is considered as one of the most important challenges all over the world. Aquatic pollution cause negative impact for human health and it also affect different organisms. In the present work bacteriological parameters were studied in three different seasons-- monsoon, post-monsoon and pre-monsoon during 2022. An estimation of bacterial production is a crucial step in understanding quantitatively the function and contribution of bacteria in material cycling within the given aquatic habitats. The TCC and FCC values are high during pre-monsoon period and low during monsoon period. Total Coliform number ranged from 220 MPN/100 ml to 1100 MPN/100 ml during pre-monsoon, 120 MPN /100 ml to 240 MPN /100 ml during monsoon, and 240 MPN/100 ml to 1100 MPN/100 ml during post-monsoon. The coliform bacteria include the genera *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsilla* etc. Highest value of Coliform 1100 MPN/100 ml was observed at station 5 (Kodimatha) during pre-monsoon and post monsoon. The lowest value 120 MPN/100 ml was observed at station 1 (Puthupally) during monsoon period. Faecal contamination is also reported from station 4 (Iriyilakadavu) and station 5 (Kodimatha). The species of bacteria *E.coli*, *Salmonella sp.*, *Pseudomonas sp.*, *Enterobacter aerogenes*, *Shigelle sp.*, *Vibrio cholare*, *Vibriyo parahaemolyticus*, *Proteus sp.* and *Klebsiella sp.* were isolated from the

different stations, mainly from stations 3, 4, and 5. The maximum permissible value of total coliforms in drinking water is 1 per 100 ml (ICMR , 1975) and 10 per 100 ml (WHO, 1993). *E. coli* can be used as bio-indicators of aquatic ecosystem dynamics and determination of their occurrence may help to assess the water quality. Presence of coliforms organisms in water is regarded as evidence of faecal contamination as their origin is in the intestinal tract of human and other warm blooded animals. This clearly indicates that the bacterial contamination in the river water is chiefly caused by human excreta and domestic sewage, which is objectionable for drinking purposes (Usharani *et al.*, 2010).

Conclusion

The present study on the water quality assessment of Kodoor River revealed that the bacteriological contamination was significantly high. The study concludes that the Kodoor River is bacteriologically contaminated and is not suitable for bathing and other recreation activities. Also the river water can be used for domestic purposes only after proper disinfection. The presence of total coliforms, faecal coliform, *E. coli*, *Salmonella sp.*, *Shigella sp.* and *Vibrio sp.* have been documented as national primary drinking water regulations (NPDWRs) or primary standards which protect public health by limiting the levels of contaminants in drinking water (EPA, 2002). From the study it is very clear that pre-monsoon and post-monsoon periods, the water quality of Kodoor river is very poor. Bacteriological contamination may cause the spread of various waterborne diseases. So care should taken before using the water for drinking purposes .

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