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Research Article

SCREENING OF INDICATOR MICROBES, A STRATEGY FOR POLLUTION ASSESSMENT OF KILLIYAR, A RIVULET OF KARAMANA RIVER, THIRUVANANTHAPURAM, KERALA

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ABSTRACT

Pathogenic bacteria are ideal indicator of microbial pollution of surface water because of their quick response to environmental changes. The present study focused on Killiyar to find out the bacteriological characteristics and their monthly variation in the river water. Season wise sampling of water was done at three segments in Premonsoon and monsoon. Analysis of water was done using standard microbiological procedures. The results revealed that the counts of total coliforms and *Escherichia coli* are not within the standard limits so that the downstream segments of river become unsuitable for drinking purposes and outdoor bathing without proper disinfection and treatment of water especially during Premonsoon season. Human induced activities accumulate in the study area and causing disruption in water quality of this precious riverine ecosystem. Immediate safe guard steps are needed for the protection of this riverine ecosystem for present as well as for the future generations.

Keywords: *Escherichia coli*, Indicator bacteria, Killiyar, Total coliforms, Water quality

1. INTRODUCTION

Fresh water resources are reducing day by day at a very faster rate. Pollution in water bodies is a large problem due to rapid urbanization and industrialization. Polluted water is an important vehicle for several pathogens. Microbiological monitoring of river is useful for assessing the river water quality for drinking, irrigation or other purposes [1]. Some microbes can be used as suitable indicators for the assessment of fecal contamination also indicate the presence of other pathogenic organisms like bacteria, virus in water [2, 3]. Coliforms are vaguely represented group of microorganisms has universally used as indicators to assess biological contamination of water. The coliforms are found everywhere in water, soil, vegetation, fish, insects and raw food materials. Non fecal coliform bacteria can grow in water even in presence of low organic content. Coliforms are able to survive for several months in natural tropical water bodies [4]. Coliform count and amount of dissolved oxygen are inversely related. High coliform count found at low dissolved oxygen conditions [5]. The *E.coli* is the predominant bacterial species in human fecal matter and serves as primary indicator of fecal contamination in water. Enumeration of fecal coliform bacteria is important in aquatic microbiology for water quality assessment [6].

Several pathogenic organisms are present in all aquatic ecosystems, but microbiological contamination with faecal bacteria is considered to be a crucial issue in majority of the rivers, therefore the assessment of the presence of pollution indicator bacteria in water helps in human and animal health protection [7; 8 & 9]. Pollution in the Killi River is increasing day by day due to discharge of organic wastes, human excreta, sewage waste, municipal garbage and toxic discharge from factories. River Killi is an important tributary of Karamana River. The present investigation aims to find out the bacteriological characteristics and their monthly variation in the river water.

2. MATERIAL AND METHODS

2.1. Study area

Killiyar a major tributary of Karamana River is located between latitudes 8°40'30"N, 8°27'0"N and longitudes 76°57'E, 77°2'0"E. It originates from Theerthankara near to Panavoor of Nedumangadu Taluk in Thiruvananthapuram District, and flows through Thiruvananthapuram City and merges with Karamana River at Pallathukadavu near Thiruvallam. Killiyar

receives waste water from several factories, hospitals, fish markets domestic city drainage, garbage and agricultural runoff from various cultivations directly without any proper treatment. Three different sites were selected for bacteriological assessment (Table 1).

Table 1: Study sites

Sl.No	Sites	Co-ordinates
1	Vazhayila	(8°32'44.45"N and 76°58'29.90"E).
2	Jagathy	(8°29'33.56"N and 76°57'55.63"E)
3	Pallathukadavu	(8°27'19.55"N and 76°57'31.94"E)

2.2. Microbiological Analysis of water

Season wise sampling of water was done at three segments in Premonsoon and monsoon for microbiological analysis. Six water samples were collected at a time from each segment. The samples were collected in pre sterilized glass containers and transported to the laboratory in ice boxes with in shortest possible time to avoid errors due to multiplication of bacteria.

2.3. Identification and Enumeration of Bacteria

2.3.1. Total heterotrophic bacteria

Enumeration of total heterotrophic bacteria was made by transferring 1ml of water sample at 10^{-1}dilution on a nutrient agar plate by adopting pour plate method and incubated at 37°C for 24 hours. Total heterotrophic bacteria forms white colonies on nutrient agar plates after specific time of incubation.

2.3.2. Total coliform bacteria

Enumeration of total coliform population was made by transferring 1ml of water sample at 10^{-1} dilution on Tergitol -7 agar plates by adopting pour plate method and the plates were incubated at 37°Cfor 24 hours. Lactose fermenters were identified as yellow colonies and non fermenters as blue colonies. TTC is reduced in the bacterial cell to form a red coloured insoluble complex there by producing red colonies [10].

2.3.3. Escherichia coli

Enumeration of *Escherichia coli* was accomplished using pour plate method on MacConkey agar medium. Transferring 1ml of water sample at 10^{-1} dilution on MacConkey agar plates and incubated at 44°C for 24 hours. Pink colonies surrounded by a zone of acid precipitated bile were identified as *E.coli* [11].

All the procedures during the preparations of culture plates were carried out in laminar flow work station to avoid contamination through air.

2.4. Statistical Analysis

Mean and standard deviation were calculated. Two way ANOVA was performed to find out the significant difference in microbial count.

3. RESULTS AND DISCUSSION

Microbiological analysis of vazhayila segment of Killiyar revealed that total heterotrophic plate count of water sample had high density of bacteria during Premonsoon with a mean value of $43.32\pm1.67\times10^{-1}$cfu/ml and a mean value of $25.16\pm1.90\times10^{-1}$cfu/ml during monsoon season. The colony forming units of total coliform bacteria had a high density during Premonsoon season with a mean value of $16.88\pm1.10\times10^{-1}$cfu/ml followed by monsoon season with a value of $11.33\pm1.80\times10^{-1}$cfu/ml. *Escherichia coli* count of vazhayila segment had also moderate number of bacteria during Premonsoon with a mean value of $1.05\pm0.57\times10^{-1}$cfu/ml and a reduction in number during monsoon with a mean value of $0.71\pm0.25\times10^{-1}$cfu/ml (Fig 1-3).

Total heterotrophic bacteria counts of water samples from Jagathy segment was the highest mean value of $54.33\pm2.72\times10^{-1}$cfu/ml in the Premonsoon season. At the same time bacteria showed a reduction in the monsoon season with a mean value of $32.55\pm2.19\times10^{-1}$cfu/ml. Total coliform count was also high during Premonsoon with a mean value of $26.16\pm1.72\times10^{-1}$cfu/ml but monsoon season had lowest number of colonies with a mean value of $16.71\pm1.88\times10^{-1}$cfu/ml. *Escherichia coli* count of Jagathy segment also had the highest number of bacteria during Premonsoon with a mean value of $3.44\pm0.50\times10^{-1}$cfu/ml and lowest in the monsoon as $2.16\pm0.50\times10^{-1}$cfu/ml (Fig 1-3).

Pallathukadavu segment of Killiyar had its highest count of total heterotrophic bacteria during Premonsoon season with a mean value of $61.88\pm1.18\times10^{-1}$cfu/ml and the least count was observed during monsoon with a mean value of $44.77\pm2.07\times10^{-1}$cfu/ml. Total coliform bacteria count was also highest during Premonsoon with a mean value of $34.10\pm1.51\times10^{-1}$cfu/ml and a reduction during monsoon as $23.49\pm1.13\times10^{-1}$cfu/ml. *Escherichia coli* count of Pallathukadavu segment was also higher during Premonsoon with a mean value of

5.10±0.54×10^{-1}cfu/ml and a value of 3.38±0.48×10^{-1}cfu/ml was observed during monsoon season (Fig 1-3).

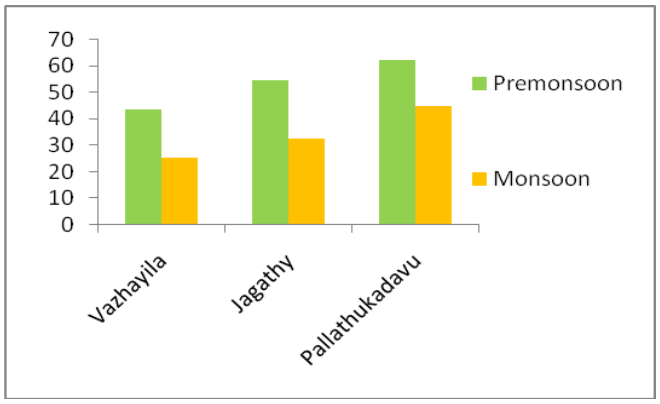

Fig.1: Seasonal variation in the total heterotrophic bacteria count of water samples from three segments of Killiyar at dilution 10 $^{-1}$ cfu/ml

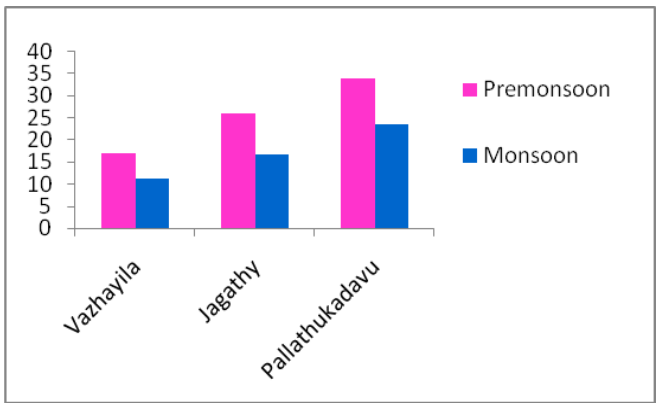

Fig.2: Seasonal variation in the total coliform bacteria count of water samples from three segments of Killiyar at dilution 10 $^{-1}$ cfu/ml

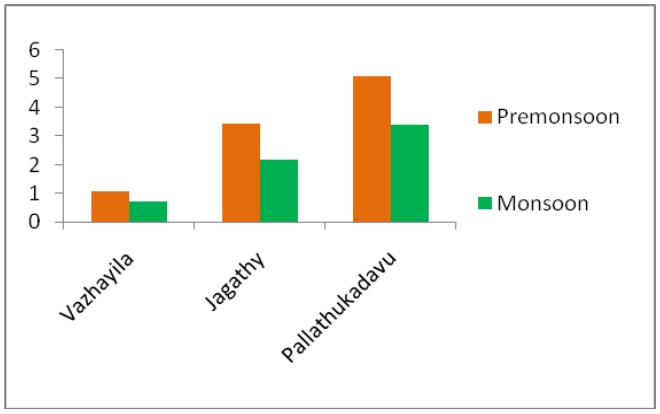

Fig.3: Seasonal variation in the *Escherichia coli* count of water samples from three segments of Killiyar at dilution 10 $^{-1}$ cfu/ml

Table 2: Two way ANOVA results of indicator microbial counts at three segments of Killiyar in Premonsoon and monsoon season

Total heterotrophic bacteria	Between seasons F= 180.737 > Fcrit 18.512; P <0.05 Between sites F= 60.705 > Fcrit 19; P < 0.05
Total Coliform bacteria	Between seasons F = 31.114 > Fcrit 18.512; P <0.05 Between sites F = 30.711 > Fcrit 19; P < 0.05
Escherichia coli	Between seasons F = 7.4829 < Fcrit 18.512; P >0.05 Between sites F = 22.873 > Fcrit 19; P < 0.05

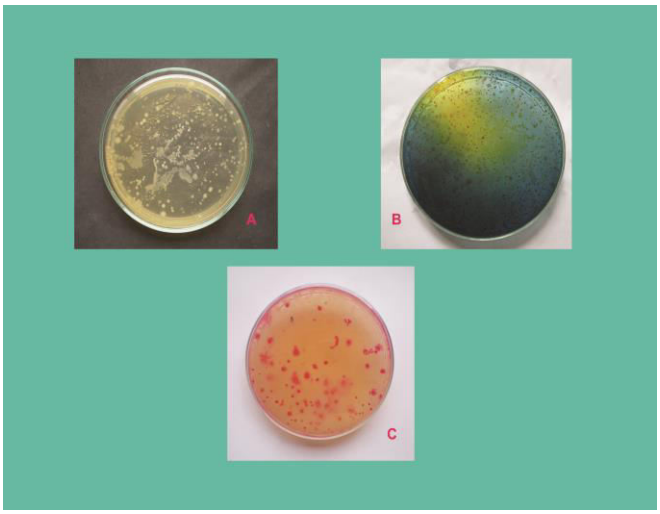

Fig.4: Microbial indicators of Killiyar; A- Total Heterotrophic Bacteria in Nutrient agar; B- Total Coliform in Tergitol- 7 agar; C- *Escherichia coli* in MacConkey agar

During microbial analysis of water from Killiyar lowest density of coliform was reported during monsoon season compared to Premonsoon season from all segment. This might be due to influx of water through the river by heavy rain fall. Stagnant nature of water at Pallathukadavu segment may be a reason for the notable increase in indicator organisms in this site. The city drainage is directly discharged in to the Killiyar just before Jagathy station. Along the banks of the river is dense with human settlement. Sewage and garbage from the houses along the banks of the river also contaminate the river. This might be a reason for the high density of coliforms and other fecal indicators in this segment. At Vazhayila segment of Killiyar laundry activity is very high and other forms of contamination are least

observed in this site. This may be a reason for the lowest indicator bacteria in this site compared to other sites. Total coliforms indicate the degree of pollution [12]. According to rsearchers [13-15] coliforms are very good indicators of fecal contamination. In present study highest count of indicator organisms were noted in the downstream segments of Killiyar. This result showed similarity with the report of scientists [16]. The increased load of pathogens at downstream segment might be due to frequent discharge of city drainage, domestic sewage, agricultural runoff, and wastes from septic tanks. Similar results observed at different sites of River Ganga [17, 18]. According to Ministry of urban development Govt: of India recommended that the desirable limit of fecal coliform at 500 colonies/100 ml and maximum permissible limit at 2500 colonies / 100 ml in major rivers of India. Desirable limit of 1000 colonies of fecal coliforms in 100 ml and maximum limit of 10000 colonies / 100 ml in treated sewage in to water body for reuse [19]. In developing countries approximately 3 millions of children below age of 5years die due to diarrhoeal diseases spread through the ingestion of poor quality contaminated water [9]. Bacteriological analysis of Killiyar during the present study reveals that the downstream segments of the river become unsuitable for drinking purposes and outdoor bathing without proper disinfection and treatment of water especially during Premonsoon seasons.

4. CONCLUSION

The main problems that affect the river water quality is the high degree of pollution, incorporation of high amounts of untreated city sewage is the most responsible factor for the higher population of pathogenic microorganisms. The notable number of indicator organisms at different sampling sites indicated that severe anthropogenic impacts on ecosystem. Knowledge of microbial pollution on aquatic environments is essential for taking decision for the protection of precious river ecosystem. The high values of pollution indicator bacteria detected revealed that the microbiological quality of river water is not acceptable for drinking purpose. For maintaining the health of aquatic ecosystem, it is suggested that in order to prevent the river water from bacterial pollution sewage must be treated before its discharge into the river and also it is necessary to create awareness among people on the problems associated with faecal pollution on water resources.

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