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Microbial Load Induced Degradation of Ithikkara River in Kollam District, Kerala

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Abstract*: The discharge of wastes and chemical compounds into rivers is one of the biggest sources of environmental contamination, due to a lack of domestic and industrial wastewater treatment. Pathogenic microorganisms usually forms a common content of rivers along with sewage that if present in small quantities are not a threat to the health and life of human beings, but in larger quantities can be a pathogenic factor that affects the ecological equilibrium. Ithikkara river is a 56 km long river that originates from the Kulathupuzha in the Western Ghats and flows through the Kollam district, finally emptying into Paravur Lake. Keeping in consideration about the risks of microbial contamination, the physico-chemical and microbiological examination of Ithikkara River was carried out to ascertain their suitability for consumption and presence of microorganisms that could cause water-borne diseases.The objectives of the study was to investigate the seasonal variations of physico-chemical parameters and microbiological analysis such as total microbial count, total *coliform* count and *E.coli* count. For assessment of water quality , the areas such as Alummukku , Anapuzhakkal, Kuzhiyam were selected and investigated .Water samples were collected from the study area and the physico-chemical parameters were analyzed with respect to the seasons following standard methods.The study indicates that there is a pronounced variation of most of the water quality parameters with variations in seasons. Comparison of all stations reveals that high alkaline nature was observed in site 2. This may be due to the discharge of food wastes to the river. The rate of carbon dioxide in all the three stations increases according to seasonal changes from pre monsoon to post monsoon and dissolved oxygen decreases from pre monsoon to post monsoon.The decrease in the DO content could be the result of high microbial load. Microbiological water analysis is mainly based on the concept of fecal indicator bacteria. The study observes that high *E. coli* count were observed in station 3. During the drought period, animals and humans located near the river, depend mostly on rivers for bathing and other purposes. It may be causing faecal contamination of river. The findings from the present study provide a better understanding about the current situation of the river and reminds the necessity for rejuvenating it. The results of this study showed that the bacterial count of all the study sites exceeded than the WHO standard, indicating that the river is becoming increasingly polluted as untreated sewage and agricultural runoff discharges into the water which is not safe for consumption before treatments. Thus, the study suggests that the river water needs urgent measures to control pollution and also to make aware the local people to properly treat the water before using it.

***Keywords*: Total coliforms, Fecal Coliforms, physico-chemical factors, Total Plate Count.**

I. INTRODUCTION

Rivers are the backbone of human civilization that provide freshwater, which forms the basic necessity for human life. Kerala has 44 major rivers, in which 3 flow eastward direction and 41 flow westward direction. All these rivers are now on the verge of destruction due to human activities such as sand mining, dumping of industrial waste and agricultural activities. The thoughtless and unscientific dumping of domestic and industrial wastes, seasonal surface runoff has put strong effect on the river discharge and water quality. The bacteriological quality of drinking water is of paramount importance and monitoring must be given priority (Bharti and Katyal, 2011). Water pollution results in transmission of infectious diseases

such as dysentery, cholera, diarrhea, typhoid, shigellosis, salmonellosis, and varieties of other bacterial as well as fungal, viral, and parasitic infection (Nwachukwu and Ume, 2013). Enteric pathogens are typically responsible for waterborne sickness (Karaboze *et al*., 2003).

In recent decades, the riverine system throughout the world is under severe threat due to human interventions either within the catchment or directly to the river channel (Petts and Calow, 1996). Rivers are highly vulnerable to pollution; therefore, it is important to control water pollution, monitor water quality in river basin (Simeonov *et al*., 2003; Zhou *et al.*, 2006), and interpret the temporal and spatial variations in water quality (Dixon and Chiswell, 1996; Singh *et al*., 2004). Microbiological analysis is a method of analyzing water to estimate the numbers of bacteria present and to allow for the recovery of microorganisms in order to identify them. The number of pathogenic bacteria in river water varies depending on various factors such as the size of the stream, water level, degree of eutrophication of the water course and its tributaries or geographical area through which the river flows and the ability of water course to self-clean. (Lukasz Augustyn *et al.,* 2016). Currently, coliforms and *E. coli* are of great importance among bacterial indicators used in water quality definition and health risk (Giannoulis *et al.,* 2005). The aquatic microbiota is known to be an important factor in the sustainability of natural water ecosystems. However, the microbial community also might include pathogens which result in very serious waterborne diseases in humans and animals (Paruch *et al.,*2019).

Thus, declining river water quality threatens the sustainability of aquatic organisms and its environment and is a major factor contributing to human concerns about the environment. Rapid population growth, land development, urbanization, industrialization, agricultural production, and numerous other socioeconomic activities that occur in and around the river further threatens the sustenance of river ecosystems. Healthy riverine ecosystems are supported by physicochemical properties and its own biodiversity. Thus, the river quality needs regular monitoring and assessment to prevent any disease outbreak and further deterioration. Based on the current situations of Ithikkara river, the present study deals with the assessment of certain water quality parameters from the water samples that were collected from three different sites of Ithikkara river. The present study will provide a baseline water quality scenario of the Ithikkara river.Thoughtless anthropogenic activities in the name of development near the water bodies may worsen the physicochemical and microbiological status of rivers. If these situations continue, microbial contamination may worsen and pose potential health risks in the near term future.

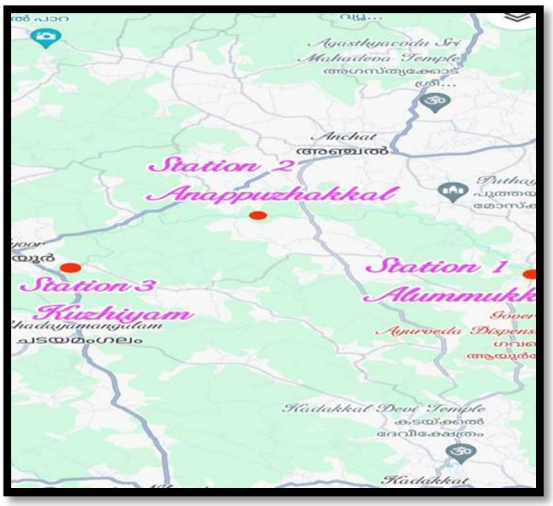
II. MATERIALS AND METHODS

The study area, the Ithikkara river basin, is located mainly in Kollam district, with a small portion extended to the southern neighbour district, Thiruvananthapuram of Kerala State, geographically between 8°47′ – 9°0′ 56″N latitudes and 76°34′27″ – 77°2′25″ E longitudes. The river is bounded by Kallada river basin in the north and Ayirror and Vamanapuram river basin in the south. Water samples were collected during the year, 2023-2024 from the study area and certain physico-chemical parameters such as water temperature,pH,Dissolved Oxygen,Carbondioxide content and primary productivity were analysed following standard methods APHA (2005). The water samples were analyzed as per Indian Standards IS: 1622 - 1981. Water samples were collected using sterile glass bottles and transported in an ice box to the laboratory to be analyzed within 8 hours.The samples were analyzed microbiologically using the following parameters. Total plate count (TPC), Total coliforms (TC), Total number of *E.coli*. TPC technique was done to calculate the number of heterotrophic bacteria within the water sample. This was conducted in two parts by serial dilution of culture sample and agar plating of the sample dilutions to nutrients medium.Using a colony counter the viable cells per ml of original culture is calculated by multiplying the number of colonies counted by the dilution factor. The count is expressed in colony forming units CFU/ml (Colony Forming Units). Bacterial analysis was carried out using Multiple tube fermentation technique. Water samples were collected from three different sites of Ithikkara river for the assessment. These regions are facing a drastic change due to the anthropogenic activities. The region selected for the study are,Site 1.Alummukku ,Site 2.Anapuzhakkal,Site 3.Kuzhiyam.

Multiple Tube Fermentation Technique

It involves inoculating the sample in a suitable liquid medium. After completion of the incubation period, the tubes were examined for gas production by the coliform organisms. The presumptive test was followed by confirmatory test for the positive tubes. Presumptive test: The dilutions to the expected level of bacterial contamination were selected. The tube for each sample as per guide lines for selecting dilutions for estimation of MPN of coliforms were taken. The Durham's vial was put inverted in each test tube and McConkey's broth was put as media. All the samples were shaken vigorously, immediately before removing samples aliquots to inoculate the series of test tubes. Samples were added using sterilized pipettes to the test tubes selected for the test and mixed thoroughly. All the test tubes were placed in an incubator at 35°C - 37°C within 30 minutes. Each tube was examined carefully after 48 hours of incubation. Those having gas in the Durham's vial were recorded as positive. Tubes showing positive test were subjected to a confirmatory test. Confirmatory test: Fermentation tubes were prepared with 10 ml Brilliant Green Lactose Broth (BGLB) medium and Durham's vials. The fermentation tubes with positive results were shaken gently and one loop full of medium was transferred to BGLB medium. The tubes were incubated at 35°C - 37°Cfor 48 ± 2 hours and the tubes with gas formation were recorded as positive. The MPN value for a given samples was obtained by the MPN index table. EC medium was used for the confirmation of faecal coliforms. All presumptive positive test tubes of the coliform test were sub cultured at the end of the 48 hours in to the EC medium and were incubated at 44.5°C for 24 hours in a water bath. Gas formation within 24 hours was considered as positive reaction for faecal coliforms.

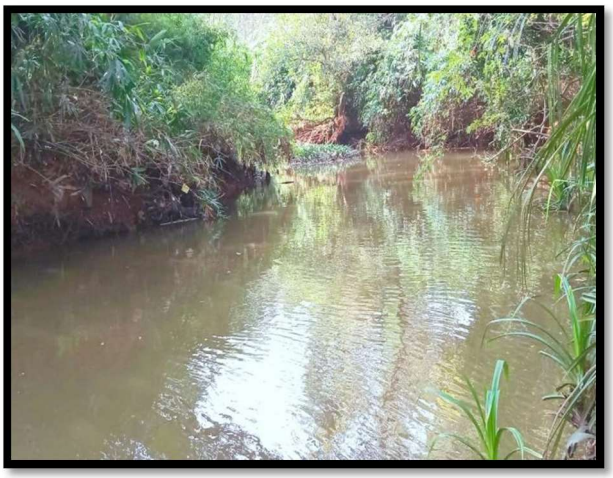
The MPN of faecal coliforms was calculated from the MPN index table.



Map Showing study Area

Photographs showing selected stations of Ithikkara River

Station 1: Alummukku



Station 2: Anapuzhakkal

Station 3: Kuzhiyam

III. Results and Discussions

The study of physico- parameters of selected three stations of Ithikkara river, station 1(Alummukku), station 2(Anapuzhakkal), station 3 (Kuzhiyam) of three seasons were analysed and compared.

Temperature

Based on it, temperature showed a minimum range of 29°C and exhibited maximum range of 33°C. All these stations showed an average temperature range from 29°C - 31°C. A high range of temperature in pre monsoon season was observed in station 2 (32°C) and low range was observed in station 1 (30°C). In monsoon period, the temperature range from 29°C - 30°C. The temperature in the post monsoon period range from 30°C - 33°C. The mean ±standard deviation ranges from 29.66 ± 0.57 to 31 ± 1.73.(Table 1).

pH

From the study, in pre monsoon season the pH ranged from 6.8 to 8. A higher level of pH was observed in station 2 (8) and lower pH showed in station 1 (6.8). In monsoon season lower pH was observed in station 1 (6.9) and higher pH in station 3 (7.5). In post monsoon the temperature ranges from 6.2 to 7.3. High range of pH was shown in the station 2 (7.3) and low in the station 1 (6.2). The average range of pH in all the season ranged from 6.66 to 7.53. In pre monsoon period mean ± standard deviation was about 7.53 ± 0.64. In monsoon period 7.2 ± 0.3 range of mean ± standard deviation observed. In post monsoon 6.66 ± 0.56 range of mean ± standard deviation observed. (Table 1).

Carbondioxide

Carbon dioxide value in pre monsoon season showed the maximum range in station 2 (16.72mg/l) and minimum range was observed station 3 (4.4mg/l). In monsoon season carbon dioxide of water sample was observed maximum in station 1(30.8mg/l) and minimum range was observed in station 3 (16.72mg/l). In post monsoon high range of carbon dioxide was

observed in station 1 (39.6 mg/l) and minimum range was observed in station 2 (29.92mg/l).(Table 1, Fig 1). The maximum range of mean ± standard deviation was found in post monsoon period (34.90±4.84) and minimum range was observed in pre monsoon period (10.85 ± 6.18).

Dissolved Oxygen

Dissolved oxygen of water sample in the pre monsoon season, maximum range was obtained in station 3 (5.12mg/l) and minimum range was obtained in both stations 1 and 2 (4.16mg/l).In monsoon season, high range was obtained in station 2 (4.1mg/l) and low range was obtained in station 3 (2.4 mg/l). In post monsoon maximum dissolved oxygen range was obtained from station 1(4mg/l) and minimum range was obtained from station 3 (1.28mg/l).(Table 1 , Fig 2).The range of mean ± standard deviation was about 2.96 ±1.48 to 4.48 ±0.55. Maximum value was noticed in pre monsoon and minimum obtained in post monsoon season.

Primary Productivity

Gross primary productivity of water sample in pre monsoon ranges from 0.25mgC/m^3/ hr to 0.45mgC/m^3/ hr. Maximum range was obtained from station 2 and lower from station 1.In monsoon season maximum range was obtained from station 1 (0.2mgC/m^3/ hr) and minimum was observed in both stations 2 and 3 (0.05mgC/m^3/ hr).GPP of water sample in post monsoon season was ranges from 0.07mgC/m^3/ hr to 0.8mgC/m^3/ hr . The mean ± standard deviation ranges from mean ± standard deviation ranges from 0.1 ± 0.086 to 0.34 ± 0.04.(Table 1 Fig 3).Net primary productivity of water sample in the pre monsoon season ranges from 0.05mgC/m^3/ hr to 0.12mgC/ m^3 / hr. Maximum range was observed in station 1 (0.12mgC / m^3/ hr) and minimum range was observed in station 2 (0.05mgC/m^3/ hr).NPP of water sample in monsoon season was ranged from 0.3 - 0.36. In post monsoon minimum NPP showed in station 1 (0.38mgC/m^3/ hr) and maximum NPP showed in station 3 (0.52mgC/m^3/ hr).Maximum range of mean ± standard deviation was observed in post monsoon (0.43 ± 0.075) and maximum range was observed in pre monsoon (0.09 ± 0.036).(Table 1 Fig 4).

Microbiological Analysis

Considering the station 1, total plate count or total microbial load was about 1994cfu/ml. Total *coliform* count obtained was 1022cfu/ml and the *E. coli* count obtained was 578cfu/ml. (Table 2.Fig 5).

In station 2 total plate count or total microbial load obtained was 1248cfu/ml. Total *coliform* count obtained was 520cfu/ml and *E. coli* count was about 286cfu/ml. (Table 2.Fig 5).

Considering the station 3,total microbial load obtained was 2688cfu/ml. Total *coliform* range observed was 1278cfu/ml and *E. coli* count obtained was 782cfu/ml(Table 2.Fig 5).

TABLE 1
Seasonal Variations of Physico-chemical Parameters

Parameters	Seasons	Station 1	Station 2	Station 3	Mean±SD
Temperature (°C)	Pre monsoon	30	32	31	31±1
	Monsoon	30	29	30	29.66±0.57
	Post monsoon	30	33	30	31±1.73
pH	Pre monsoon	6.8	8	7.8	7.53±0.64
	Monsoon	6.9	7.2	7.5	7.2±0.3
	Post monsoon	6.2	7.3	6.5	6.66±0.56
Net primary	Pre monsoon	0.12	0.05	0.1	0.09±0.036
Productivity (mgC/m^3/ hr)	Monsoon	0.35	0.36	0.3	0.33±0.032
	Post monsoon	0.38	0.4	0.52	0.43±0.075
Gross primary	Pre monsoon	0.25	0.45	0.3	0.33±0.104
Productivity (mgC/m^3/ hr)	Monsoon	0.2	0.05	0.05	0.1±0.086
	Post monsoon	0.8	0.15	0.07	0.34±0.40
	Pre monsoon	11.44	16.72	4.4	10.85±6.18
CO_2(mg/l)	Monsoon	30.8	29.92	16.72	25.81±7.88
	Post monsoon	39.6	35.2	29.92	34.90±4.84
	Pre monsoon	4.16	4.16	5.12	4.48±0.55
Dissolved oxygen(mg/l)	Monsoon	3.84	4.1	2.4	3.44±0.915
	Post monsoon	4	3.68	1.28	2.96±1.48

TABLE 2
Microbiological Analysis of Selected Stations

Bacteriological parameters of stations	Alumukku (St 1)	Anapuzhakkal(St 2)	Kuzhiyam(St 3)	Method of Analysis	Acceptable Limit As Per IS No:10500:2012
Total Plate Count/ Total Microbial Load	1994 cfu/ml	1248 cfu/ml	2688 cfu/ml	IS 5402:2012	<500 cfu/ml
Total coliforms	1022 cfu/ml	520 cfu/ml	1278 cfu/ml	IS 15185:2016	Absent/ml
Fecal coliforms: E. coli	578 cfu/ml	286 cfu/ml	782 cfu/ml	IS 1622:1981	Absent/ml

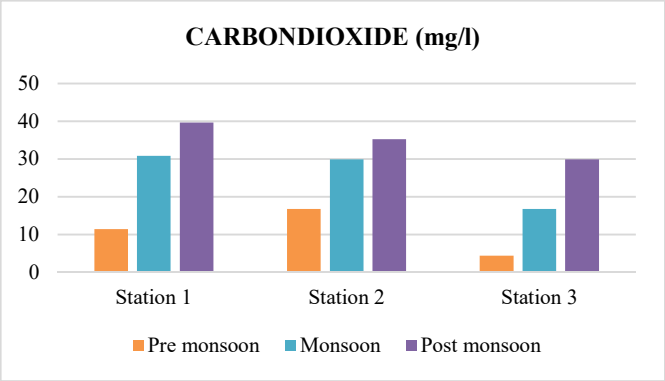


Fig 1. Graph showing seasonal variations of carbon dioxide

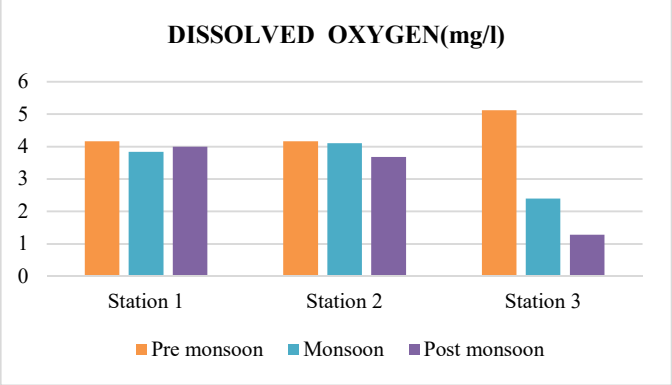


Fig 2. Graph showing seasonal variations of dissolved oxygen

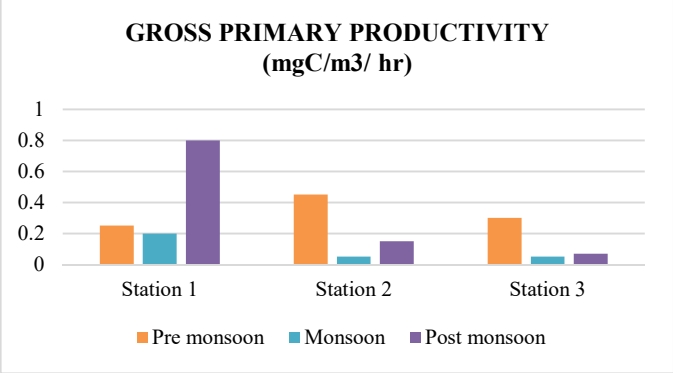


Fig 3. Graph showing seasonal variations of gross primary productivity

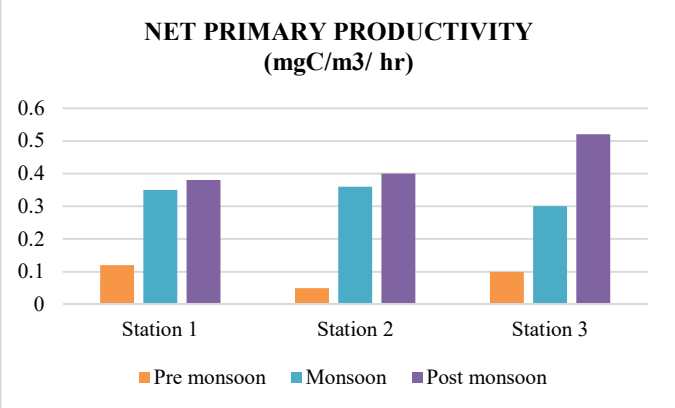


Fig 4. Graph showing seasonal variations of net primary productivity

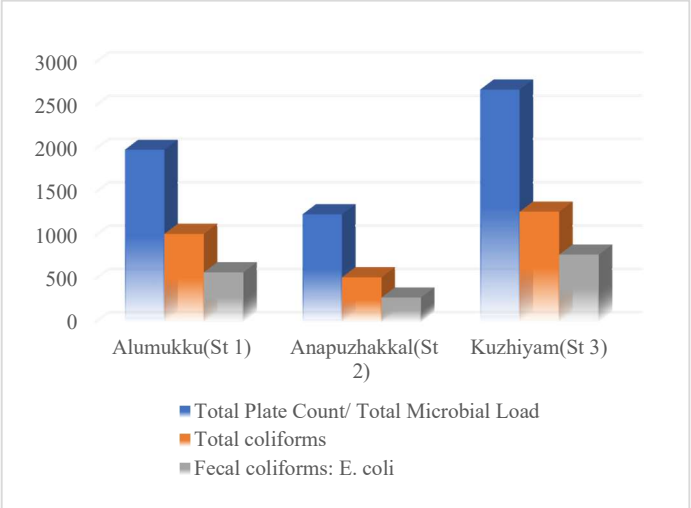


Fig 5. Graph showing microbiological analysis of selected stations

Discussion

Temperature has an important role in maintaining the normal condition of aquatic biota. All organisms have an optimum temperature for their better survival. On the basis of analysis, the temperature ranged between 29°C - 33° C. The maximum temperature was recorded on post monsoon season (33°C) and minimum at monsoon season (29° C).Considering the three stations, high temperature was observed in station 2 (33°C) during post monsoon season and minimum observed in the same station (29°C)during monsoon season. River temperature is a key physical parameter of water quality that exerts an influence on almost every aspect of the ecology of lotic system (Webb and Walsh., 2004).

pH is another important physical property of water. pH is an important indicator of chemical, physical and biological changes in a waterbody and plays a critical role in chemical process in natural waters. A slight variation in the pH can change the normal condition of an aquatic biota. The maximum pH was reported in station 2(8) during pre monsoon season and minimum pH was reported in station 1 (6.2)during post monsoon season. Water with pH ranging from 6 to 9 is usually suitable for growth of organism and results represents that the pH values are within the acceptable limit.(Shrestha .,2006)

Carbon dioxide is another important water quality parameter. Aquatic plants depend on carbon dioxide for life and growth, just as fish depend on oxygen, plant use carbon dioxide during the process of photosynthesis. The maximum carbon dioxide value was recorded in station 1(39.6mg/l) during post monsoon season and minimum carbon dioxide value was recorded in station 3(4.4mg/l)during pre-monsoon season. The increase in carbon dioxide may be due to decay and decomposition of organic matter (Joshi *et al.,*1995).

Dissolved oxygen (DO) is one of the most important indicators water quality. It is essential for the survival of the fish and other aquatic organisms. Oxygen dissolves in surface waste due to the aerating action of winds. Oxygen is also introduced into the water as bi product of aquatic plants

photosynthesis. The maximum range of dissolved oxygen obtained from the station 3(5.12mg/l) during pre-monsoon season and the minimum obtained from the same station 3(1.28mg/l) during post monsoon season. Dissolved oxygen decreased due to increase in temperature and also due to increased microbial activity (Moss .,1972).

Considering the gross primary productivity, maximum range was observed in station 1 (0.8mgC/m^3/ hr) during post monsoon and minimum observed in station 2 and 3(0.05mgC/m3/ hr).To date, many studies have shown that year to year variability of GPP is largely driven by the variations of energy and water related climate variables, including temperature, solar radiation, precipitation as well as CO_2 (Nemani *et al*., 2003). Net primary productivity in the present study ranges from 0.09mgC/m^3/ hr to 0.43mgC/m^3/ hr. Maximum net primary productivity was observed in station 3 (0.52mgC/m^3/ hr) during post monsoon season, and minimum observed in station 2 (0.05mgC/m^3/ hr).

Microbiological analysis is one of the most important analysis to determine the count of microorganisms. Based on the above microbiological analysis of selected three stations ,the maximum range of microbial load was observed in station 3(2688cfu/ml) and minimum range was observed in station 2(1248cfu/ml). Maximum range of total coliforms was observed in station 3(1278cfu/ml) and minimum range was observed in station 2 (520cfu/ml). Maximum range of *E. coli* was observed in station 3(782cfu/ml) and minimum obtained in station 2 (286cfu/ml). All the three bacteriological parameters are maximum in station 3 and minimum in station 2.According to World Health Organization a zero count of *E. coli* per 100ml of water is consider safe for drinking. A count of 1-10 MPN/100ml is regarded as low risk, 11-100 MPN/100 ml is medium risk. Finally an *E. coli* count greater than 100 MPN/100ml is adjudged high risk. (Odonkor and Mahami ,2020). Pathogens are a serious concern for managers of water resources, because excessive amounts of faecal bacteria in sewage and urban run-off have been known to indicate risk of pathogen-induced illnesses in humans (Fleisher *et al.,* 1998). According to the guideline criteria for faecal indicator organisms of (WHO report, 1992) which accept the guide values of the investigated bacteria up to 500/100 ml for total coliforms and 100/100ml for both faecal coliforms and faecal streptococci. E. coli is one of the specific indicators of faecal contamination in tropical and temperate regions. Investigation of the bacterial density of water could provide an approach to assess the reliability of monitoring data (Bayoumi Hamuda & Patko, 2011).The survey of the indicator bacteria along the Ithikkara river revealed that the water is subjected to sewage pollution. Bacteria are ideal markers that denotes the microbial pollution of river waters because of their sudden response to environmental changes. Faecal coliforms are good indicators of faecal pollution and the potential presence of pathogenic agents, that are mainly caused by untreated sewage originating from agricultural land and anthropogenic factors. This study is an attempt to analyze the microbiological contamination and to identify the major role human activities play in altering the water quality of the rivers.

IV. CONCLUSION

The present study indicates the seasonal variation of physico – chemical parameters and microbiological analysis of selected three stations of Ithikkara river, namely station 1(Alummukku), station 2(Anapuzhakkal) and station 3 (Kuzhiyam). The study gives a indication about water quality variations according to seasonal change. The temperature obtained from the three sites ranges in between 29°C to 32° C. Carbon dioxide increases exponentially and dissolved oxygen decreases, it will be negatively affecting the aquatic organisms. Based on the microbiological analysis the total microbial count was very high at each station and other two bacteriological parameters such as total *coliform* count and *E. coli* count are also very high. Therefore, it can be understood that pollution is high in all the three stations. Due to many reasons such as waste disposal, use of river for bathing, silting and other such reasons the natural behaviour of the Ithikkara river was changed. This puts the river and the creatures in it at a great risk. Ithikkara River undergoes environmental deterioration due to the lack of proper scientific management and sustainable practises. The high microbial load in the river renders it to be unfit for human consumption though they can be used for other purposes. The over exploitation of natural resources had left contaminated water, soil, air and exhausted natural resources. Unscientific and unsuitable land use practices have created huge environmental problems in the study area. It is very important to act judiciously to bring it back to its natural state as early as possible. In conclusion, the water of the Ithikkara River is subjected to faecal pollution and monitoring of microbial quality of water is a must to control the spreading of pathogens transmitted by contaminated water. Thus, the Ithikkara river needs immediate measures to control pollution by controlling human activities to prevent sewage from entering the water body.

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