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Impact of physico-chemical parameters on bacterial population in Mullaiperiyar River water-Theni district, Tamilnadu, India

P. Sivamanikandan[1]* and S. Ahmed John[2]

[1]Research and Development Center, Bharathiar University, Coimbatore 641046, Tamilnadu, India.
[2]Department of Botany, Jamal Mohamed College (Autonomous), Tiruchirappalli-620020 Tamilnadu, India.

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The Mullaiperiyar River is one of the most imperative South Indian rivers. The present study was aimed to investigate the influence of physico-chemical factors in biological changes of Mullaiperiyar River water in Theni district. Water samples were collected from the upstream area (near the Dam - Lower camp, Kullapagoundanpatty, Karunakka muthanPatti), urban stretch area (Surlipatti, Uthamapalayam, Chinnamanur and Veerapandi) and downstream area (Theni-Aranmanaipudur, Vaigai Dam) during the period of December 2013 to March 2014 for the analysis. The samples were analyzed for various physico-chemical parameters like temperature, pH, total dissolved solids (TDS), total hardness (TH), total alkalinity (TA), calcium hardness, magnesium hardness, chloride, nitrate, nitrities, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), iron, and bacteriological analysis was also performed. The Mullaiperiyar River water was natural medium for the growth of bacteria. In this study, bacterial colonies such as *Streptococcus, Escherichia coli, Klebsiella, Proteus, Pseudomonas aeroginosa, Salmonella typhi* and *Shigella dysentery* organisms have been found and reported here. These findings demonstrated the non-portability of mullaiperiyar river water and this scenario alarms the need for appropriate sanitation and drainage systems.

Key words: Mullaiperiyar River, physico-chemical parameters, biochemical oxygen demand (BOD), chemical oxygen demand (COD), *Escherichia coli*, *Salmonella*, non-portable water, sanitation.

INTRODUCTION

Water is essential for all forms of life. The Mullaiperiyar River is one of the longest river of the South India, it originates from the Sundaramalai hills in Western ghats. The people residing in various districts of Tamilnadu and Kerala depend on the Mullaiperiyar River water for domestic and agricultural needs. The objective of the present study was to analyze the physico-chemical and bacterial analysis of Mullaiperiyar River water collected from December 2013 to March 2014. The Mullaiperiyar River is very useful to people of Theni district (Figure 2). According to the 2011 census, population of the Theni district is 1,245,899. Theni town is situated in the south central part of Tamilnadu

*Corresponding author. Email: mphilsiva@yahoo.com. Tel:+91 9626106212.

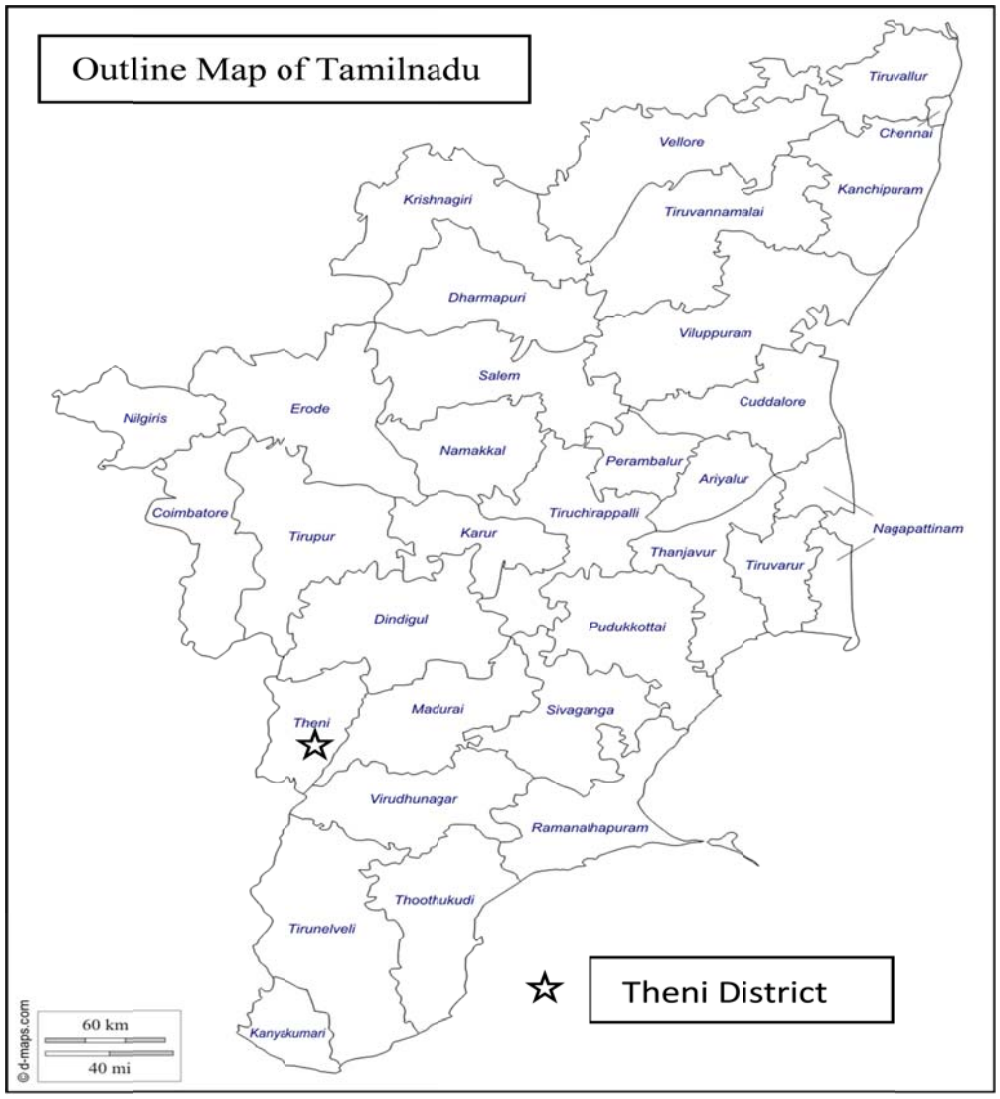

Figure 1. Outline map of Tamilnadu.

(8°.55'N latitude and 70°.07'longitude) (Figure 1). Periyar dam has been constructed at about 100 km upstream from Theni town. The Mullaiperiyar River is directly receiving waste from townships like Gudda lur, Surlipatti, Cumbam, Uthamapalayam, Veerapandi, Theni, Kunnur and Vaigai dam. The present study focuses on the impacts of human activities on the Mullaiperiyar River and an attempt has been made to determine the quality of Mullaiperiyar river water. The Mullaiperiyar river receives large amount of wastes, such as industrial effluents, domestic wastes and polluted by other anthropogenic activities. In India, more than 90% of rural population depends on ground and river waters (Moharir et al., 2002). The pollution in river water leads the fluctuation in physico chemical properties of water. The varying physico-chemical property of the water leads to the accretion of microbial load (Tebutt, 1983). The microbial loads in the water are found to be the source of most of all health problems (Mahbab ul haq, 2002; Subramanian, 2004).The quality of water is directly proportional to human health; hence the water analysis is more important before consumption (Subba Rao and Subba Rao, 1995). The lack of awareness about the water management systems may cause severe effects in availability and quality of river water.

MATERIALS AND METHODS

Area of study

The study area was divided mainly into three areas that included nine stations. Water samples were collected from upstream area of the dam and designated as lower camp (S1), Kullapagoundanpatty (S2), Karunakka muthan Patti (S3); middle of urban stretch area as Surlipatti (S4), Uthamapalayam (S5), Chinnamanur (S6) Veerapandi (S7) and downstream area as Theni Aranmanaipudur (S8) and Vaigai dam (S9) (Figure 2).

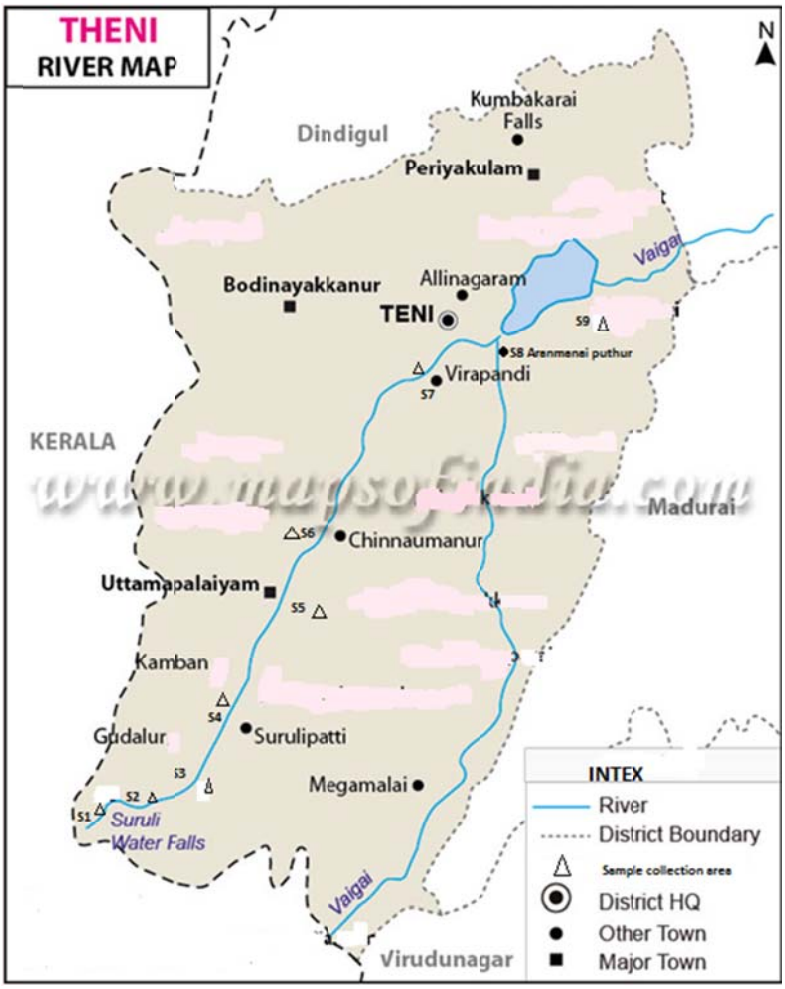

Figure 2. Map of Mullaiperiyar River sampling stations in Theni district. S1: Lower camp; S2: Kullappuram; S3: Karunagamuthan patti; S4: Surlipatti; S5: Uthamapalayam; S6: Chinnamanur; S7: Veerapandi; S8: Aranmanaiputhur; S9: Vaigai dam.

Collection of sample

The Mullaiperiyar River water samples were collected during December 2013 to March 2014 from the lower camp to Vaigai dam for the estimation of physical-chemical parameters and bacterial populations. Samples were collected during the first and third week of every month in sterile one liter polyethene bottles and were analyzed. The bottles were immersed into the water and stoppers were removed underneath. Temperature and pH of the samples were measured at the site using digital pH meter and digital thermometer, respectively. After sample collection, the bottles were kept in ice box and transferred immediately to the laboratory for further analyses.

Analysis of water

The laboratory analysis of Mullaiperiyar samples were done by the standard procedures of the American Public Health Association (APHA, 2005) and World Health Organization (WHO, 2004). Totally, fifteen types of physico-chemical parameters such as temperature, pH, electrical conductivity (EC), total suspended solids, total alkalinity, total hardness, dissolved oxygen, biochemical oxygen demand (BOD), chemical oxygen demand (COD); quantification of calcium, magnesium, nitrate , nitrite, chloride, iron were determined.

Bacteriological methods

Determination of the bacterial population from the Mullaiperiyar River water was analyzed by standard microbiological methods of the World Health Organization (WHO) and Bureau of Indian Standards (BIS, 1998). This bacteriological test was mainly performed to confirm the quality of water. The water samples were serially diluted from 10^{-1} to 10^{-6} dilutions. The enumerations and isolation of total heterotrophic bacterial colonies were done by pour plate method in nutrient agar medium, incubated at 37°C for 24 h. After incubation, the bacterial colonies were recorded by Quebec colony counter, and reported as colony forming units per milliliter (CFU/ml). The enumerations of total coliform were executed by standard plate count method (SPC) and the faecal coliform bacteria were performed by fecal coliform membrane filter (m-FC) agar plate method.

Additionally, the water samples were inoculated in different types

Table 1. Physical and chemical analysis of Mullaiperiyar River water.

Parameters	S1	S2	S3	S4	S5	S6	S7	S8	S9
pH	6.8	6.8	6.9	7.1	7.2	7.3	7.4	7.3	7.6
Temperature	24	24.40	24.45	24.76	25.10	25.32	26.15	26.75	27
Electrical conductivity (µS/cm)	870	895	938	975	990	1110	1156	1395	1725
TDS (mg/L)	1250	1055	975	925	895	95	932	1120	2275
Dissolved Oxygen	8.57	8.15	8.02	7.85	7.13	6.95	6.21	5.95	5.32
Biochemical Oxygen Demand (mg/L)	4.5	5.4	7.0	9.2	13.6	15.5	18.0	35.5	52.6
Chemical Oxygen Demand (mg/L)	7.2	10.8	18.5	25.5	45.2	65.7	85.7	180	240
Total Alkalinity (mg/L)	285	290	290	295	320	355	430	775	870
Total Hardness (mg/L)	242	270	276	310	372	425	610	1120	1750
Calcium Hardness (mg/L)	175	105	85	80	55	47	90	140	210
Magnesium Hardness (mg/L)	130	95	82	75	43	30	75	130	185
Chloride (mg/L)	285	230	195	197	185	160	175	210	355
Nitrate (mg/L)	4.51	4.75	4.92	5.01	5.75	5.95	6.25	6.75	7.15
Nitrite (mg/L)	0.50	0.50	0.52	0.47	0.40	0.40	0.42	0.50	0.52
Iron (mg/L)	6.05	5.45	3.20	2.07	1.05	0.95	0.55	0.42	5.75

Mean values of physical and chemical parameters of Mullaiperiyar River water in Lower camp to Vaigai dam, Theni District (values are in mg/L, except pH, temperature and EC). Here S1- Lower camp, S2- Kullapagoundanpatty, S3- Karunakkamuthan Patti, S4-Surlipatti, S5- Uthamapalayam, S6- Chinnamanur, S7- Veerapandi, S8- Theni Aran manaipudur, S9- Vaigai dam.

of medium like Mac-Conkey agar, Eosin methylene blue (EMB) agar, blood agar, mannital salt agar medium and m-FC agar. All the inoculated plates were incubated at 37°C for 24 h, except for the m-FC agar plates that were incubated at 45°C (APHA). After the incubation, all the plates were observed for size, shape, motility and haemolytic colony (α, β, Y) variations. Presently, the isolated colonies were reassign for standard biochemical tests like Indole, MR-VP, Citrate utilization, triple sugar iron, oxidase, urease, coagulase, catalase, hydrogen sulphide and ONPG (Ortho-nitrophenyl beta –D-galactopyranoside) test methods were used for the identification of the isolated bacterial colonies.

RESULTS AND DISCUSSION

Physico-chemical parameters

The water quality in the Mullaiperiyar River was studied by evaluating the physico-chemical parameters and bacterial population in the nine areas during the period of December 2013 to March 2014. The analyzed physico-chemical parameters showed significant deviation from normal values indicating the presence of constant pollution load in the river. The physico-chemical and microbiological parameters are higher than the acceptable limits of APHA (2005), WHO (2004) and BIS (1998).

The mean value of the physical and chemical parameters of Mullaiperiyar river water was summarized in Table 1. pH and temperature are the most important physical factors that affects the chemical and biological reaction in river water. The pH and temperature value of the water samples measured on the spot using digital pH meter and thermometer were observed with variations of 6.8 to 7.6 and 24 to 27°C in nine stations, respectively. The temperature of water in summer was high due to low water level. The concentration of ionizable component (APHA, 2005) in the water samples was measured by conductivity meter. The electrical conductivity (EC) was recorded with a minimum value of 870 µS/cm at station 1 and maximum of 1725 µS/cm at station 9. Winkler's method measured the dissolved oxygen (DO) in the water sample with high value of 8.57 mg/l at station 8 and a low value of 5.32 mg/l in station 9. The water samples with more dumped sewage waste were observed to have low DO content, which support the growth of pathogenic bacteria and this was due to the presence of high concentration of organic matter and lack of the oxygen in the water (Krishnan et al., 2007).

Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of the water samples were quantified by Winkler and Reflux methods respectively. The BOD and COD of the nine sampling stations were observed between the range of 4.5 to 52.6 and 7.2 to 240 mg/l, correspondingly. The water samples were found with high BOD and COD values due to the increase in organic and inorganic content of water by sewage discharge and public anthropogenic activities (Jeena et al., 2012) content in the water samples was measured by phenol di-sulphonic acid method.

The mean value of nitrate and nitrite in all sample stations were observed at a range of 4.51 to 7.15 and 0.50 to 0.52 mg/l. Synthetic fertilizer waste and human feces and urine can be the major source of water nitrate and nitrite. These increased amounts of nitrate were found to stimulate the aquatic plants and thereby increase the BOD level in the river (Peavey et al., 1985).

Tables 2. Total heterotrophic and total coliform bacterial analysis of Mullaiperiyar River.

Collection area	Total heterotrophic bacteria (total colony count x10^{4}CFU/ml)				Total coliform bacteria (total colony count x10^{4}CFU/ml)			
	December 2013	January 2014	February 2014	March 2014	December 2013	January 2014	February 2014	March 2014
S1	1.6	1.6	1.7	1.6	3.5	3.2	4.0	3.7
S2	1.7	1.8	1.8	1.9	3.8	3.7	4.3	4.5
S3	1.7	1.9	2.0	1.9	4.0	4.5	5.0	4.8
S4	1.9	2.2	2.2	2.2	4.6	4.8	5.8	5.6
S5	2.1	2.2	2.3	2.3	5.5	5.7	6.5	6.8
S6	2.3	2.4	2.4	2.4	6.7	6.5	7.0	7.5
S7	2.5	2.8	2.9	2.8	8.5	7.0	8.3	9.4
S8	3.0	3.1	3.2	3.0	13.6	13.5	12.5	13.8
S9	3.4	3.4	3.2	3.1	14.3	13.8	13.5	14.5

Mean values of total heterotrophic and total coliform bacterial population colony counting in Mullaiperiyar River water from Lower camp to Vaigai dam in Theni district.

Total alkalinity in water samples were measured by acid titration method and the concentration was observed in the range from 285 to 870 mg/l in all stations. The total alkalinity of water samples were reported previously with variation from season to season (Sarma et al., 2002). In this study for the winter season, we found high value of alkalinity presence.

Total hardness of the samples was measured by ethylene diamine tetra acidic acid (EDTA) titration method and was observed in a range from 242 to 1750 mg/l in the nine locations chosen in this study, which was found to contradict the reports of the earlier study in a Mullaiperiyar River (Ramakrishnan et al., 2012). Calcium and magnesium in the samples were measured by EDTA titration method, and the values were observed in the range of 175 to210 and 130 to185 mg/l, respectively.

Chloride was measured by argentometric method and observed within the range of 285 to355 mg/l, which is higher than the reports in an earlier study, which analyzed the presence of chloride in Mullaiperiyar River as 163 to168 mg/l. In the present study, high amount of chloride presence in the river is mainly due to the usage of high potash fertilizer by the farmers, water softener regeneration, presence of discharged rock salt throughout the river flow, animal-human waste discharge and moreover the septic tank discharge. Iron is measured in the range of 6.05 to 5.75 mg/l in all nine stations by phenanthroline spectrophotometric method, and the increasing concentrations of iron found could have direct and/or indirect toxic effects on river biota (Peuranen et al., 1994).

Bacteriological parameters

Bacterial parameters are one of the most important parameters to confirm the river water quality. The bacteriological investigation of Mullaiperiyar River was greatly contaminated with total heterotrophic bacteria, fecal coliform and total coliform bacteria. On the basis of bacterial studies in Mullaiperiyar River water from lower camp to Vaigai dam, the presence of higher concentration of heterotrophic bacterial colonies were found in all nine stations of the river. The mean value of the total heterotrophic and total coliform bacterial colonies of Mullaiperiyar River water is summarized in Table 2. The minimum value of 1.6 x 10^4 CFU/ml and maximum value of 3.1x10^4 CFU/ml were observed at lower camp and Vaigai dam, respectively. In addition, the total coliform bacteria are present minimum in lower camp and maximum at Vaigai dam with the value of 3.5 x 10^4 and 14.5 x 10^4 CFU/ml respectively, during the period of December 2013 to March 2014.

The maximum value of total heterotrophic bacteria and total coliform bacteria, 3.2 x10^4 and 13.6 x10^4 CFU/ml, observed from the station 8 area was due to the agitation of polluted water inflow from another river joined in the middle of Theni-Aranmanai Pudur. The polluted water contains a large amount of organic matter that provides an excellent source of nutrition for the growth and multiplication of microbes as suggested by Thomas (2007).

The total coliform bacteria were identified in MacConkey agar, in which the appearance of small pink colonies (*E.coli*), large pink mucoid colonies (*Klebsiella* pneumoniae) was found. Similarly, in EMB Agar plates, *E. coli* and *Klebsiella* species produced green metallic sheen colonies. *Enterobacter aerogens* produced only pink colour colonies without metallic sheen identified. The faecal coliform bacteria were enumerated by mFC agar plates, with the appearance of colonies in dark blue colour. The high concentrations of *E. coli* forms in the river water was mainly due to municipal sewage disposal and septic tank discharges, which was found to be unhealthy for human consumption (Venkatesharaju et al., 2010). The presences of *Streptococci* were identified in blood agar by the appearance of a small pin point haemolytic colonies. In addition to the identification of the colony and cultural characteristics of the bacterial populations, bio-chemical

Table 3. Characterization of bacterial isolates.

Biochemical test	*Enterobacter aerogens*	*Klebisella pneumoniae*	*Salmonella typhi*	*Shigella dysenteriae*	*Pseudomonas aeruginosa*
Gram stain	-	-	-	-	-
Motility	+	-	+	-	+
Indole	-	-	-	-	-
Methyl red	-	-	+	+	-
Voges-Proskauer	+	+	-	-	-
Citrate utilization	+	+	-	-	+
Urease	-	+	-	-	-
Triple sugar iron	A/A Gas +	A/A Gas +	AL/A Gas -	AL/A Gas +	AL/AL Gas -
Hydrogen sulphide (H_2S)	-	-	+	-	-
Oxidase	-	-	-	-	+
ONPG	+	+	-	-	+
Mannitol	+	+	+	+	-
Catalase	+	+	+	+	+

A = Acid production, AL = alkaline production, + Positive, - Negative. Confirmatory biochemical test result of Gram negative bacteria in Mullaiperiyar River water samples from lower camp to vaigai dam in Theni district.

tests were further used to confirm and identify the bacterial isolates from the water samples of the 9 stations with reference to the Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). The identify-cation of biochemical test is summarized in Table 3. The bacteriological analysis in Mullaiperiyar River water reveals the presence of *E. coli* and other pathogens such as *Klebsiella, Streptococcus, Enterobacter, Pseudomonas aeroginosa, Salmonella* and *Shigella* organisms. Therefore, in this study, we demonstrated the pollution of the Mullaiperiyar River water with domestic sewage, human-animal waste and moreover with septic tank discharge, which could be the probable source of a variety of water-borne diseases such as typhoid, salmonellosis and cholera in accordance with the report of Dhanapaul (2006).

The people from Theni district strictly depend upon the source of water from the Mullaiperiyar river for their agricultural and household activities in their day-today life. Nevertheless, the physic-chemical parameters of the water were found to be below the standards of the WHO. The scenario has been alarmed by the continuous practice of improper sanitation, drainage system and septic tank discharge in the river. In addition, the presence of highly contagious pathogens in the river water warrants the attention of the sanitary department in the district in addition to the public education in practice of hygiene and sanitation.

Conflict of interest

The author(s) have not declared any conflict of interests.

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