ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2013; 4(3): 550 - 554

ORIGINAL RESEARCH ARTICLE

Status of Microbiological Population in the Cauvery River Water of Thanjavur District

S. Mathiyazhagan[1], K. Pugazhendy*[2], K. Jayachandran[3], S. Prabakaran[2] and C. Jayanthi[4]

[1]*Bharathiar University, Coimbatore, Tamil Nadu, India*
[2]*Department of Zoology, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India*
[3]*EGS Pillay, PG Research and Department of Biotechnology, Nagapattinam, India*
[4]*Department of Education, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India*

Received 11 Apr 2013; Revised 07 Jun 2013; Accepted 18 Jun 2013

ABSTRACT

Water is one of the abundantly available substances in nature. The surface water is believed to be comparatively clean and free from pollution. Surface water samples from 15 locations have been collected from Kallanai to Thiruvaiyaru in Thanjavur district. The present study deals with the analysis of enumerate the bacterial population in the water samples method and to identify the presence of various genera based on their morphological and biochemical characters. The characters of a colony isolated as size, shape, colour, margin, opacity, elevation, consistency, Gram character and motility. The bacteriological quality of surface water had crossed the upper limits prescribed by WHO. The results found that the water in all these places was found to be unfit for human consumption due to contamination. Pollution control measures and strict enforcement of its laws have been recommended.

Key words: Microbial population, Biochemical study and Bacterial identification.

1. INTRODUCTION

Surface water is generally a good source for drinking purpose because of the purification properties of the soils. Supply of potable water is important to the development of any country [1]. Pollution of natural water may be defined as the addition of certain constituents due to human activities, which would affect its use for domestic, irrigation, industrial and recreational purposes adversely affected to aquatic biota (freshwater). Microorganisms may be found in water; those finding unfavorable conditions grow and multiply to increase their population. The polluted water carries an unpleasant taste or appearance [2].

Microbial population is influenced by anthropogenic activities and industrial activities. The microorganisms are affected by several factors such as sedimentation, ultraviolet rays, temperature, osmotic effects and food supply. Microorganisms have specific gravity slightly more than the distilled water, therefore, they slowly settle down the bottom of water bodies. It has been found that *E. coli* multiply well in autoclaved water at 37°C. Raw water stored at 22°C show increased number of bacteria, but greater number has been recorded in autoclaved waters stored at 22°C as compared to 37°C [3].

The microbes are usually heterotrophic. The digestion of organic matter by these organisms is incomplete, due to which there accumulate acids, bases, alcohols and various gases. Bacterial population is often considered as an important indicator of pollution and eutrophication in aquatic ecosystem. The main bacterial strain in total coliforms and faecal coliforms is *Escherichia coli*, with an average length of 2 - 4 μm and an average diameter of 1 μm [4]. In general, *E. coli* is hydrophilic and its zeta potential, which is a measure of the charge near the surface of the bacterium, varies between 10 and 30 mV [5]. The microorganisms become adapted to heavy metal polluted environments by the acquisition of specific resistance systems [6] and the possibility of using the microorganisms to detoxify such polluted environments are being explored by many researchers [7]. Safe water and sanitation have been promoted as an essential step in reducing water borne diseases [8].

***Corresponding Author:** K. Pugazhendy, **E. mail:** pugalendy@rediffmail.com

2. MATERIALS AND METHODS

Water quality assessment

Frequency of sampling

The present study was carried out for a period of 12 months i.e., from January to December 2011. The 15 samples were collected on monthly basis, throughout the period of study from different localities are presented in (**Fig 1**).

Sampling method and analysis

Water samples were collected as per the guidelines of random sample method. An acid washed one liter polythene bottles were used for water lifting were allowed to run the water for 15 min in order to flush out stationary water. Further, the sample bottles were also flushed with several volumes of water before the samples were collected. As water is dynamic in nature and during sampling, it enters the new environment from its natural environment, its chemical composition may not remain same but may tend to adjust itself according to its new environment and its content alters at very different rates particularly with organic materials. Therefore, the collection of water, pH was measured immediately and for the estimation of dissolved oxygen, samples were fixed with Winkler's reagent. The remaining parameters were analyzed in the laboratory. The samples were analyzed using various analytical methods of APHA, ISI and NEERI. Prior to the analysis of heavy metals, the samples were acidified with HNO_3 so as to prevent adsorption of the metals to the walls of the containers.

Enumeration of bacterial population

Pour plate technique was employed to enumerate the bacterial population in the water samples. One mL of water sample was taken and mixed with 99 mL sterilized distilled water in a 250 mL conical flask and kept in a mechanical shaker (120 rpm) for 15 min. Serial dilutions (10^{-3}, 10^{-4} and 10^{-5}) were prepared. Pour plate technique was followed and nutrient agar medium was used for enumeration of bacteria [9].

Identification of bacteria

The bacterial cultures isolated from nutrient agar plates were grouped to various genera based on their morphological and biochemical characters as given in Bergey's manual of determinative bacteriology [10] after purification by streak plate method. The temperature used for incubation was 37°C for 24 hrs. The characters of a colony isolated as size, shape, colour, margin, opacity, elevation, consistency, Gram character and motility.

Biochemical characteristic study

Bacteria isolated on specific media were subcultured and pure cultures were prepared on nutrient agar slants. These cultures were then studied to identify bacteria at the species level by using their specific biochemical behavior pattern as mentioned in the Bergey's manual of determinative and systematic bacteriology (Volumes I and II) [11].

Various biochemical tests such as IMVIC test (Indole, Methyl red, Voges-Proskauer and Citrate utilization test). Urease test, Gelatin hydrolysis test, Sucrose test, Mannitol test and Lactose test were carried out to confirm the type of bacterial contamination in the samples. Five mL of peptone water was incubated by using nutrient agar slant culture and kept for incubation for 24 hrs at 37°C. After the incubation period, it was used for IMVIC test and triple sugar iron agar test. SIM agar medium was used for indole test. The SIM agar slants were inoculated by streaking the 24 hrs old culture grown in peptone water. The presence of indole was detected by adding Kovac's reagent, which produced a cherry-red coloured layer. MR-VP medium was used for Methyl red test. Red colour showed positive result and yellow colour indicated negative result. Again, the MR-VP medium was used for Voges-Proskauer test. Sterilized Simmon's citrate agar medium was used for citrate utilization test. Simmon's citrate agar slants were prepared and inoculated with 24 hrs old peptone water culture. Growths on the surface of the slant were identified for citrate positive cultures.

For H_2S production SIM agar tubes were used. The SIM agar deep tubes were incubated with peptone water culture and incubated at 37°C for 24 hrs. Formation of black colored precipitate along the line of stab inoculation indicated H_2S production. Nutrient gelatin agar medium was used for gelatin hydrolysis test. The culture was inoculated on nutrient gelatin agar kept at room temperature for 24 hrs. Urea broth was used to determine urease activity. The culture was inoculated on the broth and upon utilization of urea by the bacteria, the red colour of the medium changed to deep pink colour. Sucrose, mannitol, xylulose and lactose tests were used to know the fermentation of sugars by the activity of bacteria. The isolated bacteria were inoculated on peptone water containing sucrose and incubated at 37°C

for 5 days. After the incubation period, two drops of Anderet's indicator were added to the culture. The colour of the medium changed to deep pink indicating positive reaction for sucrose fermentation by the activity of coliform bacteria. The isolates were inoculated on peptone water containing 1% mannitol and incubated at 37°C for 5 days. After the incubation period, two drops of Anderet's indicator were added to the culture. The colour of the medium changed to deep pink, indicating positive reaction for fermentation of mannitol. The isolates were inoculated on mannitol peptone water containing 1% lactose and incubated at 37°C for 5 days for lactose test. After the incubation period, two drops of Anderet's indicator were added to the culture. The colour of the medium changed to deep pink indicating positive reaction for fermentation of lactose. Coliform organisms were identified up to genus level [12]. Pure cultures were characterized on the basis of their morphological, biochemical and physiological characteristics [13].

3. RESULTS AND DISCUSSION

The bacteriological examination of water has a special significance in pollution studies, as it is a direct measurement of deleterious effects of pollution on human health. The great danger to health is the presence of excremental bacteria as contaminated water may convey the causative organisms of diseases [14]. Polluted water contains vast amount of organic matter that serve as excellent nutritional source for the growth and multiplication of microorganisms. Bacteria are the most commonly used microbial tracers because they grow well in aqueous media and are easily detectable [15]. Water bacteriology in distribution systems have received less importance in developing countries, as much time is spent on supplying water in quantity. Microbiological water quality is not given importance in quality assessment as much as physical and chemical parameters.

In the present study, the presence of bacterial isolates in surface water samples in some area indicated undesirable contamination of the samples. The bacteria that were identified from the water samples included *E. coli, Salmonella typhi, Klebsiella pneumoniae, Enterobacter aerogenes, Vibrio cholerae* and *Pseudomonas aeruginosa*. In the areas of Kallanai, Seyyamangalam, Pathirakudi, Agarapettai, Pazhamaneri, Neman, Thirukattupali, Onpathuveli, Kandamangalam, Manathida, Thirupandhuruthi, Thiruvalapohil, adukaveri, Sandrorpalayam and Thiruvaiyaru. *E. coli* was most prevalent and *P. aeruginosa* was least prevalent.

The poor microbiological quality of the drinking water samples recorded in the study has also been observed in other developing countries of Ghana and Nigeria [16]. The occurrence of indicator organisms in the water constitutes a serious threat to the community and they called by the processors and handler in Ghana [17]. In a related study, [18] have reported poor microbiological quality of water in Gaza, which deviated from the recommended limits of the World Health Organization (WHO). The detection of *E. coli, E. aerogenes* and *Klebsiella* sp. implies that the water samples were potentially contaminated from fecal sources and as such were not safe for consumption [19]. [20] have studied the bacteria found in the drinking water samples in Western Nigeria could be due to the sources of water. Detection of coliforms shows the danger of fecal pollution and the consequent hazard of contracting disease through pathogenic organisms.

If an organism is believed to be associated with a disease on disease complex, it is important to identify that organism as precisely as possible using selective media and biochemical characterizations, microorganisms were isolated and characterized [21]. WHO has reported that the occurrence of pathogens or indicator organisms in water sources mainly depends on the intensive physical and chemical characteristics of the catchment area and the magnitude and range of human activities and animal sources that release pathogens to the environment [22]. Disease causing organisms transmitted via drinking water are predominantly of fecal origin, but the coliform count would not constitute much concern without the detection of *E. coli* in the water samples. It has been reported that typical enteropathogenic *E. coli* is a leading cause of infantile diarrhea in developing countries [23]. The risk of water contamination resulting in water borne disease has been noted be high in developing countries, with the reasons being identified as inadequate availability of water; poor quality of water at source; ill-maintained water pipelines and sewer lines; open air defecation; lack of disposal of human; animal and house hold waste; and lack of awareness of good sanitation and personal hygienic practices [24].

Table 1: Total heterotrophic bacterial colony count (×10^4 cfu/mL) in 15 different sampling stations of Kallanai to Thiruvaiyaru in Thanjavur district January 2011 to December 2011

S. No	Sampling stations	Premonsoon			Monsoon			Post monsoon			Summer		
		July	August	September	October	November	December	January	February	March	April	May	June
1	Kallanai	16.5	20.5	17.5	21.5	20.5	20.0	19.0	17.0	14.5	15.0	16.0	15.0
2	Seyyamangalam	18.0	21.0	19.0	23.0	21.0	20.0	21.0	19.0	16.0	17.0	17.5	16.0
3	Pathirakudi	19.0	23.0	19.5	25.0	23.0	24.0	23.0	20.0	16.5	17.5	18.0	17.0
4	Agarapettai	20.0	24.5	20.5	26.0	24.0	23.0	23.5	22.0	17.5	18.5	19.0	18.0
5	Pazhamaneri	25.0	30.0	26.0	31.0	30.0	29.0	30.0	28.0	23.0	24.0	24.5	23.5
6	Neman	26.0	31.0	26.5	31.5	30.5	30.0	30.5	27.0	23.0	24.0	25.0	24.0
7	Thirukattupali	25.5	30.0	26.0	31.0	30.5	27.5	28.5	24.5	22.5	24.5	25.0	23.0
8	Onpathuveli	26.0	31.0	27.0	32.5	31.5	30.0	30.0	27.0	23.0	24.5	25.5	24.0
9	Kandamangalam	27.0	33.0	27.5	34.5	33.5	31.5	32.0	25.0	24.0	26.0	26.5	25.0
10	Manathidal	30.0	34.0	31.0	36.5	34.5	33.5	34.0	29.0	27.5	29.0	29.5	28.0
11	Thirupandhuruthi	32.0	36.0	33.0	37.5	36.5	35.0	36.0	30.0	30.0	30.5	31.0	30.5
12	Thiruvalapozhil	31.0	34.0	32.0	35.5	34.5	33.0	34.0	30.5	27.0	29.5	30.0	28.0
13	Sandrorpalayam	25.5	29.5	26.0	31.0	30.0	28.0	29.0	25.5	22.0	24.5	25.0	23.0
14	Adukaveri	20.0	24.5	21.0	26.5	25.0	23.5	24.0	22.0	18.0	20.0	20.0	19.0
15	Thiruvaiyaru	16.5	20.0	17.0	21.5	20.0	20.0	20.0	15.0	14.0	15.5	16.0	15.0

Table 2: Confirmatory biochemical test results of bacteria in surface water samples from Kallanai to Thiruvaiyaru in Thanjavuir district

S. No	Biochemical tests	*E. coli*	*S. typhi*	*K. pneumoniae*	*E. aerogenes*	*V. cholerae*	*P. aeruginosa*
1	Motility	+	+	−	+	−	+
2	Gram reaction	−	−	−	−	−	−
3	Gas from glucose	+	+	+	+	+	+
4	Acid from lactose	+	−	+	+	−	−
5	Acid from sucrose	+	−	+	+	+	−
6	Indole	+	−	−	−	+	−
7	Methyl red test	+	+	−	−	−	−
8	Voges-Proskauer test	−	−	+	+	−	−
9	Citrate utilization test	−	+	+	+	+	+
10	H_2S	−	+	+	−	−	−
11	Urease	−	−	+	−	−	−
12	Phenylalanine deaminase	−	−	−	−	+	+
13	Arginine dihydrolase	−	+	−	−	−	+
14	Lysine decarboxylase	+	+	+	+	+	−
15	Or n ithine decarboxylase	+	+	−	−	+	−

4. CONCLUSION

On the basis of microbiological studies, it can be concluded that the surface water in Kallanai to Thiruvaiyaru in Thanjavur district and non Industrial area are contaminated due to higher concentration of heterotrophic bacterial colony, which are greater than the WHO permissible limit and cause various health problems like gastroenteritis and urinary tract infections. The results of this study would greatly facilitate the health and sanitary authorities to monitor and control surface water pollution. Public awareness programmed on sanitation, its importance, simple and economical water treatment methods like filtration and boiling would prove beneficial to avoid waterborne diseases in the Thanjavur district.

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