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EVALUATION OF MICROBIAL CONTAMINATION FROM SAMATHIRAM LAKE, MARIYAMMAN KOVIL, THANJAVUR, TAMIL NADU

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Abstract — *Lake water is generally hydrostatic water and more prone to eutrophication, which is further exacerbated by the plant nutrients inputs from industrial wastewater, domestic sewage and agricultural runoff. It is hard to solve the eutrophication of lake solely relying on the self-purification of lake water, and Lake Eutrophication is urgent to be solved. The world we live in is one full of microbes. Microbes, whether they are good, bad, or benign, are certainly everywhere. This includes on our body, in our homes, far below the earth's surface and up to the atmosphere, in cold, cool, warm and hot and very hot places, and even in places without oxygen. Our body temperature and wealth of nutrients provide an ideal home for these micro-organisms to thrive. Microorganisms always live in water. Lake water quality is as important as the quantity. Its poor quality harmfully affects the plant growth and human health. So monitoring the water quality is essential for survival of organism. Keeping this in view, in the present study was to analyze the lake water microbial contamination from Samathiram Lake, Mariyamman Kovil, Thanjavur, Tamil Nadu.*

Keywords- *Eutrophication, microbes,Microorganisms, Samathiram Lake, Microbial contamination,*

I. INTRODUCTION

Aquatic ecosystem contains characteristic communities of microorganisms and non indigenous species that are introduced into such environments, which commonly decline in abundance and ultimately disappear. The factors regulating the composition of aquatic microbial community would be useful in predicting the persistence and behaviour of human, animal and plant pathogens in natural water (Yigal Henis et al., 1989). Water contaminated with fecal matter have the capability to pose serious health risks for shell fish consumers and swimmers and major economics losses for shell fish harvesting and business (Trevette et al., 2005). Bacterial, viral and protozoan pathogens can be introduced into waters in various ways, including leaking septic tanks, sewer malfunction contaminated storm drains, runoff from animal feedlots, human fecal discharge and other sources (Aslan- Yilmaz et al., 2004).

Due to the impacts of human life and pollution, nitrogen, large amounts of phosphorus and other nutrients entered into the lake (Liu et al., 2008). This causes rapid growth of algae and other plankton, decreased dissolved oxygen in water bodies, deteriorated water quality, and massive death of fish and other organisms; this phenomenon is known as water bloom of lake water. The major components that cause lake water bloom were phosphorus, nitrogen and organic carbon (Dittman *et al.*, 2007). The microbial water purification and restoration rely mainly on microbial metabolism for the purpose of deaminase, dephosphorization and carbon transfer to purify and restore water bodies (Pecharaply et al., 2007; Lee, 1994).

Lake water is generally hydrostatic water and more prone to eutrophication, which is further exacerbated by the plant nutrients imputs from industrial wastewater, domestic sewage and agricultural runoff. It is hard to solve the eutrophication of lake solely relying on the selfpurification of lake water, and Lake Eutrophication is urgent to be solved (Frijters *et al*., 2007).

Water is a unique natural resource among all sources available on earth. It plays an important role in economic development and the general wellbeing of the country. Ground water is one of the earth‟s widely distributed, renewable and most important resources which exist in the zone of saturation, so it may be fresh or saline. Ground water quality is as important as the quantity. Its poor quality harmfully affects the plant growth and human health. So monitoring the water quality is essential for survival of organism. Keeping this in view, in the present study was to analyze the lake water microbial contamination from Samathiram Lake, Mariyamman Kovil, Thanjavur, Tamil Nadu.

II. MATERIALS AND METHODS

Collection of pond water sample

The lake water collected from Samathiram Lake, Mariyamman Kovil, Thanjavur, Tamil Nadu in the month of January 2017.

Identification of bacterial strains

The identification of water bacterial strains was carried out based on morphological characteristics and biochemical tests.

Isolation of bacteria

Isolation of water bacteria was performed by serial dilution and pour plate. 1 ml of water sample was serially diluted in sterilized distilled water to get a concentration 10^{-3}.

Cultivation of Bacteria

Preparation of bacterial broth culture:

Suspend 5.00 g of Animal's tissue, 5.00g of Sodium chloride, 1.50g of Beef extract, and 1.50g of Yeast extract in 1000 ml distilled water. Heat to boiling and dissolve the broth completely. Sterilize by autoclaving at 15 Ibs pressure (121˚c) for 15 minutes. Mix well and pour into sterile test tube and inoculate the **soil** sample. The inoculated tube was incubated at 37ºC for 24 hours. After completion of incubation period, when growth was observed the tubes were kept into 2-8˚C until use.

Media preparation for isolation of bacteria

Suspend 5.00 g of Animal's tissue, 5.00g of Sodium chloride, 1.50g of Beef extract, and 1.50g of Yeast extract and 15g of Agar in 1000 ml distilled water. Heat to boiling and dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure (121˚c) for 15 minutes. Petri plates were prepared by pouring 30 ml of Nutrient medium for bacteria. The 25µl of soil sample was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The inoculated plate was incubated at 37ºC for 48 hours. After completion of incubation period, when growth was observed the tubes were kept into 2-8˚C until use.

Bacterial colony counting

Plate was carefully observed under the digital colony counter to detect and calculate the number of colonies formed. The number of colonies was calculated according to dilution ratio and defined as the number of colony forming units (CFU) per milliliter.

$$\text{CFU/ml (cfu/ml)} = \frac{\text{Number of colonies x dilution factor } (10^3 = 1000)}{\text{Volume of culture plate (25µl convert into ml = 0.025ml (25/1000))}}$$

Microscopical examination

The morphological analysis of Microorganism was examined by using a sterile loop to pick culture from the culture plate were placed on a microscope slide, covered with a cover slip and observed under the microscope for structure.

Gram Staining

The Gram stain is a differential stain that allows classifying bacteria. Gram +ve as well as Gram –ve cells take up the deep violet color of the primary stain crystal violet. When treated with a mordant (Iodine) a crystal violet-Iodine (CVI) complex is formed resulting deep purple color in all cells. When alcohol is applied, it quickly removes the crystal violet-Iodine complex of Gram –ve cells with ease. This is attributed to the dissolving of excess lipids present in the outer membrane of Gram –ve bacteria. The complex takes a longer time to be removed in Gram +ve cells due to thick and impermeable peptidoglycan layer in their walls. Thus controlled treatment of alcohol only decolorizes the entire Gram –ve cells while the Gram positive retains purple color. A counter stain such as safranine is then used to stain the colorless Gram negative cells.

Motility test using hanging drop slide

The motility test was performed to differentiate motile bacteria from non-motile oneOne drop of cultured broth was placed on the clean cover-slip and was placed inversely over the concave depression of the hanging drop slide to make hanging drop preparation. Vaseline was used around the concave depression of the hanging drop slide for better attachment of the cover-slip and to prevent evaporation of the fluid by air current. The hanging drop slide was then examined carefully under high power objective (100X) of a compound light microscope. The motile and non-motile organisms were identified by observing motility in contrasting with swinging movement of bacteria (Merchant and Packer, 1967).

Biochemical characterization

All required media for biochemical tests were prepared in respective test tubes, flasks and petri dishes. All biochemical tests were performed with specific requirements for each test as outlined in Bergey's Manual of Determinative Bacteriology (1989).

III. RESULTS AND DISCUSSION

The water contamination, the outcome of rapid development of industry, has resulted in contamination of all kinds of natural water system. This contamination is not only confined to highly industrialized countries, where the population

explosion has given rise to complications such as increasing amounts of waste, waste water and other types of contaminants which have endangered living organisms dependent on this vital resource. Water acquires bacteria from air, soil, sewage, organic wastes, dead plants and animals. Almost any organism may thus be found in water at any time. However, most of the bacteria find condition unfavourable and soon die and survivors contribute natural flora of that water body. Coliform bacteria also consists of an artificial grouping of organisms believed to be associated with fecal pollution but in reality may also include environmental organisms from soil, vegetation and decaying organic matter (Geldreich and Bordnee, 1962).

Enumeration of fecal coliforms, *E coli* and /or *Enterococcus* Sp. has generally been used to assess microbial water quality. These micro organisms share a common feature, they all can inhabit the intestines of warm-blooded animals, including wildlife, livestock and humans and therefore can be excreted in the feces of these animals. Although these have been some association between high levels of indicator bacteria and disease outbreak (Chou et al., 2004), there is little or no prediction of species sources of contamination or correlation with human pathogens when using these indicators (Gonzalves and Joshi, 1964).

The most basic technique used for classifying bacteria is based on the bacterium's shape and cell arrangement. The most ordinary shapes of bacteria include rod, cocci (round), and spiral forms. Cellular arrangements occur singularly, in series, and in groups. Some species have one to numerous projections called flagella which enable the bacteria to swim and move. Cocci or coccus for a single cell are round cells, occasionally flattened when being adjacent to each other. Cocci bacteria can exist individually, in pairs, in groups of four, in chains, in clusters or in cubes consisting of eight cells. Bacilli are rod-shaped bacteria which also can occur individually, in pairs, or in chains (Prabakar et al., 2010).

Bacteria classification plays important role in yielding information for disease control. Bacterial species are usually sub-grouped to different types and is used for many crucial pathogenic bacteria such as *Salmonellae*, *E Coli*, and *Vibriones* (Frank, 2009). H.C. Gram in 1884 discovered the Gram stain classification remains an important and useful technique until today. This technique classifies bacteria as either Gram positive or negative based on their morphology and differential staining properties (Prabakar et al., 2010).

Colonies

A viable cell is defined as a cell which is able to divide and form a population (or colony). A viable cell count is usually done by diluting the original sample, plating aliquots of the dilutions onto an appropriate culture medium, then incubating the plates under proper conditions so that colonies are formed. After incubation, the colonies are counted and, from a knowledge of the dilution used, the original number of viable cells can be calculated. For accurate determination of the total number of viable cells, it is critical that each colony comes from only one cell, so chains and clumps of cells must be broken apart. However, since one is never sure that all such groups have been broken apart, the total number of viable cells is usually reported as colony-forming units (CFUs) rather than cell numbers.

One fifty seven (157) colonies were isolated, with different aspects: circular viscosity, sharp pointed and brilliant, opaque, pumpkin, white and cream colored. All the colonies were confirmed as bacteria of the non-fermentative gram-negative, strictly aerobic, positive for indole and negative for oxidase.

Total Bacterial Count

The isolated bacteria was quantified by calculating Colony Forming Unit (C.F.U) i.e. Colony Forming Unit. The obtained C.F.U values are represented in the following table. The numbers of bacterial colonies were isolated by pour plate technique. The highest bacterial populations were present in nutrient agar plate In the present study total bacterial density was range from 3.28×10^6 (CFU/g).

Fig.1: Bacterial colony

Table.1: bacterial colony counting in polluted water

Sample	Number of colonies	Results (CFU/ml)
Water	157	3.28×10^6

Morphology characterization of bacteria

Five isolates were characterized on the basis of colony morphology and the staining characteristics. It was observed that two isolates were gram (-ve) rod and Bacilli and one isolate was gram (+ve) rod (Fig.2). Among the three isolates, two were motility rest of them are non motility.

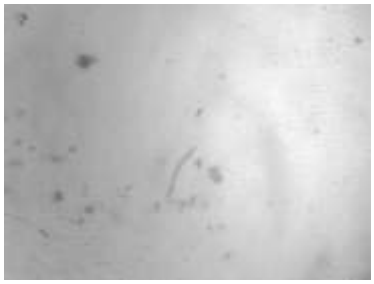
Table.2: Morphological characters of isolated bacterial species

Microorganisms	Morphology	Gram straining	Motility
Bacillus subtilis	Rod	+	NM
Escherichia coli	Rod	-	M
Salmonella typhi	Bacilli	-	M

(+) Positive (-) Negative M= Motile NM= Non motile

Escherichia coli

Bacillus subtilis



Salmonella typhi

Fig 2: Morphological characters of isolated bacterial species

Biochemical Characterization

Biochemical tests are the tests used for the identification of bacteria species based on the differences in the biochemical activities of different bacteria. The three isolates were characterized on the basis of biochemical tests (Table. 3). The tests performed to characterize the isolates were Indole, MR, VP and citrate test (Sherman, 2009).

Table 3 Biochemical characters of bacteria

Microorganisms	Indole test	Methyl Red test	Vogesproskauer test	Citrate test	Catalyst test
Bacillus subtilis	+	+	+	+	+
Escherichia coli	+	+	-	-	-
Salmonella typhi	-	+	-	+	-

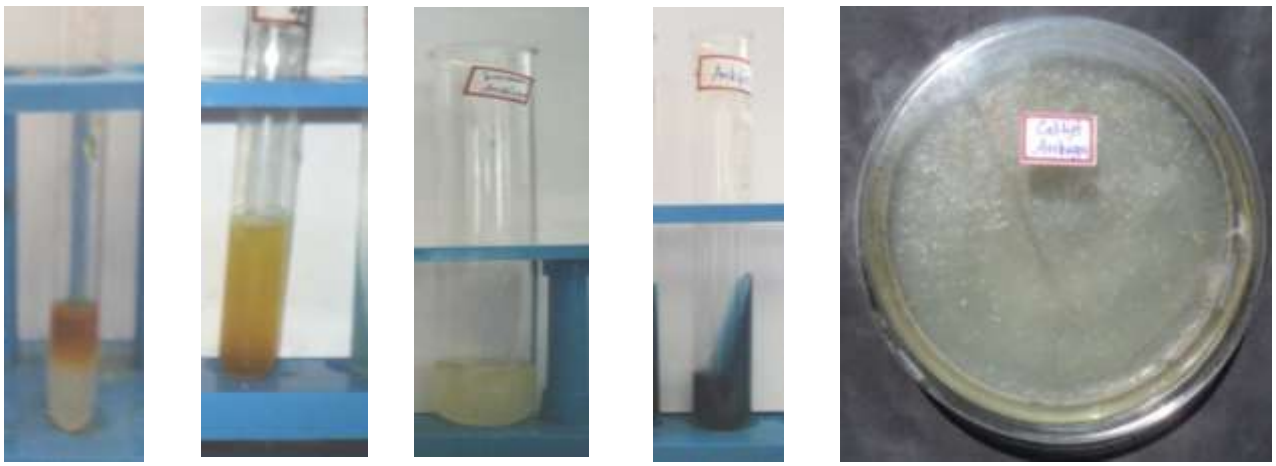
(+) Positive (-) Negative

Indole test looks for the presence or absence of tryptophanase enzyme production of the bacteria. If the enzyme is present, it will degrade the aminoacid tryptophan in the media and will produce Indole, ammonia and pyruvic acid. Indole will react with Kovac's reagent to produce a cherry red complex, which indicates a positive indole test. The absence of red color is indicative of tryptophan hydrolysis due to the lack of tryptophanse enzyme.

Methyl Red test detects the ability of microorganism to ferment glucose and to produce acidic end products. Enteric organism produces pyruvic acid from glucose metabolism. Some enteric will then use the mixed acid pathway to metabolize pyruvic acid to other acidic products such as lactic acid, acetic acid and formic acids. This will reduce the pH of the media. Methyl red is a pH indicator which is red at the acidic pH (below 4.4) and yellow at alkaline pH (above 7). The formation of red color after the addition of Methyl red reagent indicates the accumulation of acidic end products in the medium and is an indicative of positive test.

Vogesproskauer test determines the ability of microorganism to ferment glucose. The end products of glucose metabolism,pyruvic acid, is further metabolized by using Butylene glucol pathway to produce neutral end such as acetoin and 2,3 butanediol. When Barrit's reagent A (40% KOH) and Barrit's reagent B (5% solution of alpha naphthol) is added it will detect the presence of acetoin, the precursor in the 2,3- butanediol synthesis. Acetoin in the presence of Oxygen and Barrit's reagent is oxidized to diacetyl, where alpha naphthol act as a catalyst. Diacetyl then reacts with guanidine components of peptone to produce a cherry red colour.

Citrate test determines the ability of microorganism to utilize Citrate. Some bacteria have the capability to convert the salts of organic acids, for example, Sodium citrate to alkaline carbonates. Sodium citrate is one of the important metabolite of Kreb's cycle. Certain bacteria use citrate as the sole carbon source. Citrate utilization requires a specific membrane transporter and citrate lyase activity. Citrate is converted to Oxalo acetic acid by citrate lyase and oxaloacetate decarboxylase activity will convert oxaloacetate to pyruvate with the release of carbondioxide. The other products of the reaction are acetate, Lactic acid, formic acid etc. The carbondioxide reacts with sodium and water to form sodium carbonate. On the basis of morphology and biochemical characterization, the *Klebsiella pneumoniae*, *Escherichia coli*, *Vibrio chlorae.* were found to be in water sample.



Indole test **Methyl red test** **Vogesproskauer test** **Citrate test** **Catalase test**

Fig.3: Biochemical characters of bacteria

The results of the present study concluded that on the basis of morphology and biochemical characterization, the *Klebsiella pneumoniae*, *Escherichia coli* and *Vibrio chlorae* were found to be in Samuthuram lake water sample. These microorganisms possess potential pathogenic organism's causes fever and cholera. The following are recommended to avoid the microbial contaminations are to avoid bathing of animals, to avoid to accumulating wastes in lake and to use protective measures around lake.

IV. CONCLUSION

The results of the present study concluded that on the basis of morphology and biochemical characterization, the *Klebsiella pneumoniae*, *Escherichia coli* and *Vibrio chlorae* were found to be in Samuthuram lake water sample. These microorganisms possess potential pathogenic organism's causes fever and cholera. The following are recommended to avoid the microbial contaminations are to avoid bathing of animals, to avoid to accumulating wastes in lake and to use protective measures around lake.

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