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Original Research Paper

Assessment of Water Quality in Madiwala Lake, Bangalore in Relation to Faecal Contamination

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ABSTRACT

Tanks in and around urban areas of Bangalore receive considerable amount of sewage, which has caused a severe and persistent microbial pollution. The main objective of this study was to measure level of faecal contamination in Madiwala lake using different techniques of *E. coli* estimation. Results show that the golden green colonies of coliforms with metallic sheen showed a luxuriant growth at 35°C with M-Endo Agar (M1106) at incubation time of 24-48 hrs. The growth of both *E. coli* and *Enterobacter aerogens* using M-Endo Agar plate was also luxuriant at 35°C temperature. But *E. aerogens* colonies were pink and mucoid while that *of E. coli* varied from pink to rose-red with metallic sheen. The confirmatory test using Agar M-392 confirmed the presence of *Enterococcus fecalis* in yellow colour colonies at luxuriant growth of 35°C temperature. The presumptive test results of different volumes of the lake samples after 24 and 48 hours using lauryl tryptose broth indicated significant colour change, gas production, and turbidity. Confirmed results showed gas production in brilliant green using lactose bile broth at 37°C in 48 hrs. The completed result with EMB agar inoculated with positively confirmed test tube incubated at for 24 hrs at 35°C showed greenish metallic sheen colonies while the coliform colonies inoculated on Nutrient Agar slant and a broth tube incubated for 48 hrs at 35°C showed growth as observed on slant with gas production in the broth tube. Moreover, Gram staining results showed Gram negative with sporing rods in the tested samples. The MPN Index of the lake sample was found to be 38/100mL. Water is generally considered safe for drinking if it contains fewer than 4 coliforms/100 mL. It is also considered safe if it contains less than 2 *Enterococcus* bacteria colonies/100mL of a sample. These observations indicate that environmental status of Madiwala lake with respect to microbial pollution is continuing to deteriorate.

INTRODUCTION

Wetlands are important ecosystems and are diverse in terms of habitats, biota, distribution, functions, and uses (Madhyashta 2000). According to Kumar (2000), tanks in and around urban areas of Bangalore receive considerable amount of sewage inflow. This has caused a severe and persistent microbial pollution in the urban tanks of the city. But as the urban sprawling continues, the number of live tanks in Bangalore has also dwindled from 262 in the middle of last century to just about 81 tanks (Sridhar et al. 2000). In spite of the sewerage systems and STPs (Sewage Treatment Plants) in large cities, open drains still carry sewage and other mixed effluents discharging them in water bodies (Subir Paul 2007). This poses a serious threat to the ecological survival of these lakes as well as the health of urban dwellers living around these areas.

Rajakumar et al. (2007) imply that the limnological studies of the polluted water bodies are of considerable importance. A regular monitoring of water bodies with the required number of

environmental parameters including that of bacteriological characteristics can help prevent outbreak of diseases and occurrence of health hazards associated with aquatic pollution. Hence water quality survey and monitoring programs are essential to estimate the pollution level, rate at which additional pollutants are getting added and causes of pollution (Shivakumar 2007).

The main objective of this study was to measure the level of faecal contamination in Madiwala lake using several different techniques of *E. coli* estimation and confirmation. Faecal coliforms are a group of Gram negative, facultative, anaerobic, rod shaped bacteria that ferment lactose to produce acid and gas within 48 hrs at 35°C. *E. coli* bacteria is an excellent indicator of faecal contamination. This is based upon that the *E. coli* is abundant in human and animal faeces and not usually found in other niches. Moreover, faecal coliforms are a subset of total coliforms that grow and ferment lactose at elevated incubation temperature, hence also referred to as thermo-tolerant coliforms. This group consists mostly of *E. coli* but some other enterics such as *Klebsiella* can also ferment lactose at these temperatures and, therefore, can be considered as faecal coliform.

MATERIALS AND METHODS

Study area: Madiwala lake is located in the southern fringes of the Bangalore city. It is one of the biggest lakes in the city and is situated between 12°54'28" north and 77°37'0" east in Bangalore city. It is home to many migratory birds. The lake covers full extension of 114.16 ha and a water spread area of 7,80,802 m^2. The wetland area which acts as a buffer and dilution zone for the treated wastewater coming from the nearby waste water treatment plant is about 24.74 ha. The water holding capacity of the lake is 2,26,757 m^3.

Sample collection: Water samples from four fixed stations of Madiwala lake were collected in the month of September 2007 when the monsoon season was at its peak. The samples were collected in suitable bottles previously cleaned and rinsed with distilled water and sterilized by autoclaving (APHA 1998). In collecting the samples, extreme care was taken to avoid contaminating parts of bottle coming in contact with water. The sampling stations were equidistant to each other and parallel to the western shoreline of the lake. The bottles were tight-sealed without removal of protective cover. The bottles were filled to three quarters of their capacity. All the samples were stored in cool ice box and immediately brought to the laboratory within one hour for analysis (Kavitha & Siva Priya 2005).

Membrane Filter Technique (MFT): The water sample was filtered through the cellulose filters having the porosity of 0.45 µm and diameter of 47 mm. The membrane filter was then placed on M-Endo Agar plate recommended for enumeration of coliforms in water using a two-step membrane filter method (Aneja 2003). The plates were incubated at 37°C and 45°C for 24-48 hours. For the confirmatory test this method was also carried out to check the presence of *Enterococcus* in the lake sample.

Most Probable Number (MPN) Technique: The standard test to estimate the number of coliforms may be carried out by multiple tube dilution technique. There are three steps to estimate the coliform groups by MPN technique, presumptive test, confirmed test and completed test. For the presumptive test, single strength and double strength lauryl tryptose broth were prepared, sterilized in test tubes containing Durham's tubes to show the gas production. Three sets were used, with each set having 5 tubes. The first set of tubes was filled with 10 mL double strength medium, and the other two sets with 5mL single strength medium. 10 mL of water sample was inoculated in the first set and 1.0 mL and 0.1 mL in the second and the third set respectively. Finally, all the tubes were incubated at 37°C for a period of 24-48 hrs. The tubes were analysed after the incubation for gas production.

In the confirmed test, the water samples from all the presumptive tests with lauryl tryptose broth tubes were inoculated into tubes of brilliant green lactose bile broth (BGLB) with Durham's tubes and incubated at 37°C for 48 hrs. After incubation the formation of gas in any amount constituted the positive confirmed test. In the completed test, the samples from the positive BGLB broth were streaked onto selective differential medium (EMB) agar for coliforms and inoculated in lactose broth tube. In the *Enterococcus* confirmatory tests, M392 agar was used. The plate was incubated at 37°C and at 44°C for 24 to 48 hrs.

RESULTS AND DISCUSSION

Membrane Filter Technique: The results are presented in the Tables 1-3. It was observed that the golden green colonies of coliforms with metallic sheen showed a luxuriant growth at 35°C with M-Endo Agar (M1106) at incubation time of 24-48 hrs. The growth of both *E. coli* and *Enterobacter aerogens* using M-Endo Agar plate was also luxuriant at 35°C. But *E. aerogens* colonies were pink and mucoid while that *E. coli* varied from pink to rose-red with metallic sheen. The confirmatory test using Agar M-392 confirmed the presence of *Enterococcus fecalis* in yellow colour colonies at luxuriant growth of 35°C.

Most Probable Number Test: The results of this test are presented in Tables 4-6. The presumptive test results of different volumes of the lake samples after 24 and 48 hours using lauryl tryptose broth indicated significant color change, gas production and turbidity. Confirmed results showed gas production in brilliant green lactose bile broth at 37°C in 48 hrs. The completed result with EMB Agar inoculated with positively confirmed test tubes showed greenish metallic sheen colonies, while the coliform colonies inoculated on nutrient agar slant and a broth tube incubated for 48 hrs at 35°C showed growth as observed on slant with gas production in the broth tube. Moreover, Gram staining results showed Gram negative with sporing rods in the tested samples. The MPN Index of 100 mL of the lake sample was found to be 38. Water is generally considered safe for drinking if it contains fewer than 4 coliforms/100 mL. It is also considered safe if it contains less than 2 *Enterococcus* bacterial colonies/100mL of the sample.

CONCLUSION

It is concluded here that environmental status of Madiwala lake with respect to microbial pollution is continuing to be deteriorated. The presence of pathogens such as *E. coli* in the lake at dangerous and epidemic levels underpins the failure of the restoration efforts and especially the ineffectual operations of the nearby wastewater treatment plant. Moreover, lake encroachment continues unabated with raw sewage spilling openly across the western buffer zone of the lake into the water. It must be noted here that Madiwala lake is an important stop-over sanctuary for thousands of migratory bird species.

Table 1: Membrane filtration technique using M-Endo agar plate (MO29).

Organisms	Growth	Colonies	Temperature	
			35°C	45°C
Enterobacter aerogens	Luxuriant	Pink; Mucoid	++	+
Escherichia coli	Luxuriant	Pink to rose red with metallic sheen	++	+

Note: ++ = Higher Growth; + = Less Growth

Table 2: Membrane filtration technique using M-Endo agar (M1106).

Organisms	Growth	Colonies	Incubation Time	Temperature	
				35°C	45°C
Coliforms	Luxuriant	Golden green colonies with metallic sheen	24-48 hrs	++	+

Note: ++ = Higher Growth; + = Less Growth

Table 3. MFT confirmatory test using *Enterococcus* confirmatory agar (M392).

Organisms	Growth	Colonies	Incubation Time	Temperature	
				35°C	45°C
Enterococcus fecalis	Luxuriant	Yellow Color	24-48 hrs	++	+

Note: ++ = Higher Growth; + = Less Growth

Table 4: MPN Test: Presumptive test results after 24 and 48 hrs of incubation.

Lake Sample	Color Change		Gas Production		Turbidity	
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
Single Strength Medium for 0.1 mL	+	+	+	+	+	+
Single Strength Medium for 1mL	+	+	+	+	+	+
Double Strength Medium for 10 mL	+	+	+	+	+	+

++ = Higher Growth; + = Less Growth

Table 5: MPN Test: Confirmatory test results after 48 hrs of incubation.

Sample	Results
Lake Sample	Gas production in brilliant green lactose bile broth at 37°C at 48 hrs.

Table 6: MPN Test: Completed test results after 24 and 48 hrs of incubation.

Agar used	Incubation temperature	Results
EMB Agar inoculated with positively confirmed test tube	Incubation at 24 hrs at 35°C	Greenish metallic sheen colonies
Coliform colonies inoculated on Nutrient Agar slant and a broth tube	Incubation at 48 hrs at 35°C	Growth as observed on slant with gas production in the broth tube.

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