Enumeration of coliform bacteria; A tool for evaluating water quality

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Abstract
Microbial indicators are used to determine whether or not water is safe for use. Recently, concerns have been raised about the appropriate use of microbial indicators to regulate recreational use of water bodies. The present study is conducted to assess the water quality of fresh water reservoir-Adharwadi Lake, Kalyan, Dist. Thane, Maharashtra, India by using, the coliform group of bacteria as indicators of microbiological contamination. On the basis of result, it can be concluded that bacterial fauna showed seasonal variation. Wherein, the highest proportion of indicator coliforms was observed during monsoon season and lowest proportion was observed during winter season. Further it can be concluded that the water from the reservoir is not fit for human consumption as per CPCB standard limits and corrective measures are necessary.

Keywords: Microbial indicators, fresh water reservoir, microbiological contamination

Introduction
Aquatic environment act as a natural habitat of diverse range of microorganisms, with both beneficial and pathogenic characteristics. Microorganisms are widely distributed in nature. Their diversity and density may be used as an indicator for the suitability of water (Okpokwasili and Akujobi, 1996) [11]. Use of bacteria as water quality indicators can be viewed in two ways. First, the presence of such bacteria can be taken as an indication of faecal contamination of water and second it can be taken as an indication of the potential health risk that faecal contamination poses. The coliform bacteria are persistently present in human intestine in enormous numbers. These entities live longer in water than intestinal pathogens and hence easily identified, compared to existent pathogens. If these indicator bacteria are present in large numbers, the contamination is considered to be recent and/or severe, which increases the risk of water borne diseases. Hence, monitoring the quality of water using microbial indicators is of pivotal importance in combating the problems associated with public health. Resume of literature reveals that voluminous work has been carried out all over the world. Some of the noteworthy and recent publications are (Singh A.K., 1985; Sharma and Mall., 1988; Patralek L. N.,1992; Khatavkar and Trivedy., 1994; Parihar et. al., 2003; Mohan et al., 2007; Abbu AA and Lyimo TJ, 2007; Siddhi Sharma et al., 2008; Aw Sadat et al., 2011; Abbot B et al., 2011; Jaini E et al., 2013; Shafi Sana et al., 2013; Olapade O., 2013; Birander N. V. et al., 2014; Shirude M et al., 2014; Kokali A. and Sulce S., 2016) [21, 22, 14, 8, 13, 10, 2, 18, 5, 1, 7, 16, 12, 6, 20, 3]. However practically no investigation has been carried out on the water bodies located in and around KDMC area on this aspect. Hence, present investigation was carried out to enumerate the occurrence of coliform bacteria as indicators of contamination level in one of the fresh water bodies- Adharwadi lake of Kalyan city, Maharashtra, India.

Brief history of water bodies in and around KDMC area
Kalyan and Dombivali cities, the parts of the Mumbai metropolitan region and are located in Thane district between 19°4’ and 19°14’ North and longitude 72°29’ and 73°17’ East. Kalyan is one of the oldest municipal council in Thane district, with the history of about 150 years Dombivali is well-known as cultural and educational hub near Mumbai. KDMC has in total 29 Lakes in its area. Despite the fact that a good clean, fresh water body bestows inculpable, economic, ecological and aesthetic benefits to a people, very little efforts have been paid for the conservation of these Lakes. KDMC has undertaken the beautification
programme to restore these Lakes. Out of 29 Lakes situated in KDMC area 9 Lakes listed below are on priority list.

**List of Lakes under the project of recreation**

1. Kala Talav [Prabhodankar Thakare Sarover (Lake)]
2. Umbarde Lake
3. Gauripada Lake
4. Rahatale Lake
5. Sapadale Lake
6. Adharwadi Lake
7. Bhatale Lake
8. Chole Lake
9. Titwala Lake

Hence, detail investigation was carried out on three Lakes namely Kala Talav, Gauripada Lake and Adharwadi Lake during the study period 2009-2011. Incidentally these lakes are also selected for beautification programme.

**Description of the lake**

Adharwadi Lake is located between latitude 19°15’ 25” North and longitude 73° 7’ 34” East. It spreads over 10,165 m² and is 3.0 to 4.0 m deep. It is located near Bhiwandi-Murbad Kalyan by-pass road. It is very close to the famous Adharwadi Jail, Kalyan. The Lake do not have developed surroundings. On its eastern side, is located a Hindu temple. It has a ghat for immersion of idols during religious festivals on the same side (Sampling station-II). The domestic waste and nirmalya (religious offerings) are found dumped at this side of the Lake. The western side of the Lake is used by the local people for various purposes (Sampling station-I). There is a prolific growth of the Eichhornia due to eutrophic condition of the Lake. Earlier aquaculture was practiced in this water. But due to present pathetic condition fishermen do not find it commercially viable.
Methodology
Sample collection and analysis
In the present study water samples from station I and station II of the selected water body were collected between 7.00 a.m. to 8.00 a.m. in sterilized glass bottles and transferred to the laboratory for microbiological analysis. The bacteriological examination was conducted within 24hrs after collection for most probable number (MPN) index for coliforms in water sample using standard multiple tube fermentation technique (MTFT) (APHA, AWWA, WPCI 2004). The study was carried out for the period of two years 2009-2011. However, observations reported during the year 2009-2010 are discussed in the paper.

Microbiological studies
Microbiological studies comprises of following analysis:
- Presumptive coliform test was performed by using Mac Conkey’s broth
- Confirmatory test was performed by using Brilliant Green Lactose Bile Broth (BGLB).

Total coliform and faecal coliform count
Presumptive Test
Coliform count was obtained using the five tube assay of the most probable number (MPN) technique. Presumptive coliform test was performed using Mac Conkey’s broth. All the three sets of five tubes had 10ml Mac Conkey’s broth containing inverted Durham’s tubes before sterilization. The first set of five tubes received 0.1 ml of water sample using sterile pipette. Similarly 0.01 ml and 0.001 ml of water sample was inoculated aseptically in the remaining second and third set of five tubes. All the three sets of tubes were incubated at 37 °C for 24-48 hrs. After 48 hrs the tubes were examined carefully for acid-gas production. Acid production was determined by colour change of the both from reddish to yellow and gas production was checked by entrapment of gas in the Durham’s tube and recorded as positive (+). The tubes showing positive test were subjected to confirmatory test, as gas production is not the only criterion for a positive test.

Confirmatory Test
Confirmatory test was performed using fermentation tube with 10ml BGLB (Brilliant Green Lactose Bile Broth) medium and inverted Durham’s tube for Total coliform and 10ml EC (E. Coli) medium and inverted Durham’s tube for faecal coliform in sterile condition. A loopful of medium was transfer from a positive tube from the presumptive test in both the tubes containing BGLB and EC medium. The tubes were incubated at 37 °C for 24-48 hrs for total coliform and 44.5 °C for 24-48 hrs for faecal coliform and observed for gas production.

Result
Table 1 shows the range and mean values of coliform and faecal coliform counts of the water samples collected seasonally. The water quality standards prescribed by Central Pollution Control Board (CPCB) are depicted in Table 2.

During the period of investigation the total coliform count ranged from 0.04 x 10^4 to 2.2 x 10^4 MPN/100 ml at station I and 0.05 x 10^4 to 2.2 x 10^4 MPN/100 ml at station II. The faecal coliform count varied from 0.04 x 10^4 to 1.1 x 10^4 MPN/100ml and 0.02 x 10^4 to 0.79 x 10^4 MPN/100 ml at station I and II respectively. The mean value recorded for total coliform and faecal coliform during the study period is depicted in Table 1. The total coliform showed maximum MPN index of 1.8 x 10^4 MPN/100ml and 1.4 x 10^4 MPN/100 ml during monsoon season at station I and II. The minimum MPN index of 0.10 x 10^4 MPN/100 ml at station I and 0.79 x 10^4 MPN/100 ml at station II was noted during post monsoon season. Similar trend of observations were recorded for faecal coliforms with highest MPN index of 0.79 x 10^4 and 0.74 x 10^4 MPN/100ml at station I and II during monsoon season. The lowest MPN index obtained was 0.04 x 10^4 MPN/100 ml and 0.25 x 10^4 MPN/100ml at station I and II during post monsoon season respectively.

Table 1: Seasonal variation of microbial characteristics of Adharwadi Lake

<table>
<thead>
<tr>
<th>Season</th>
<th>Months</th>
<th>Total Coliform (MPN/100ml)</th>
<th>Faecal Coliform (MPN/100ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Station I</td>
<td>Mean</td>
</tr>
<tr>
<td>Monsoon</td>
<td>July – 09</td>
<td>1.4 x 10^4</td>
<td>1.8 x 10^4</td>
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<tr>
<td></td>
<td>Sept – 09</td>
<td>2.2 x 10^4</td>
<td>1.7 x 10^4</td>
</tr>
<tr>
<td>Post Monsoon</td>
<td>Nov – 09</td>
<td>0.04 x 10^4</td>
<td>0.10 x 10^4</td>
</tr>
<tr>
<td></td>
<td>Jan – 09</td>
<td>0.17 x 10^4</td>
<td>0.79 x 10^4</td>
</tr>
<tr>
<td>Pre Monsoon</td>
<td>Mar – 10</td>
<td>0.79 x 10^4</td>
<td>1.49 x 10^4</td>
</tr>
<tr>
<td></td>
<td>May – 10</td>
<td>2.2 x 10^4</td>
<td>2.2 x 10^4</td>
</tr>
</tbody>
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Table 2: Water quality standards by Central Pollution Control Board (CPCB)

<table>
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<tr>
<th>Designated Best Use</th>
<th>Class</th>
<th>Criteria</th>
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| Drinking Water Source without conventional treatment but after disinfection | A     | 1. Total Coliforms Organism MPN/100ml shall be 50 or less  
2. pH between 6.5 and 8.5  
3. Dissolved Oxygen 6mg/l or more  
4. Biochemical Oxygen Demand 5 days 20 °C, 2mg/l or less |
| Outdoor bathing (Organised) | B     | 1. Total Coliforms Organism MPN/100ml shall be 500 or less  
2. pH between 6.5 and 8.5  
3. Dissolved Oxygen 5mg/l or more  
4. Biochemical Oxygen Demand 5 days 20 °C, 3mg/l or less |
| Drinking water source after conventional treatment and disinfection | C     | 1. Total Coliforms Organism MPN/100ml shall be 5000 or less  
2. pH between 6 and 9  
3. Dissolved Oxygen 4mg/l or more  
4. Biochemical Oxygen Demand 5 days 20 °C, 3mg/l or less |

Source: [hppt://cpcb.nic.in/water_Quality_Criteria.php](http://cpcb.nic.in/water_Quality_Criteria.php)
Discussion

The water of Adharwadi Lake showed significant deterioration in quality in view of global standards. The lake water is contaminated with coliform bacteria resulting mainly from anthropogenic activities, especially discharging of domestic and agricultural wastes directly into the lake. Some studies revealed that coliform count has positive relation with anthropogenic activities (Sharma et al., 2010; Sadat et al., 2011) [17, 18]. Microbiological studies conducted by Sharma Riddhi et al., (2008) [18] reported total coliform colonies ranged between 27.5 x 10^3 to 84.17 x 10^3 MPN/100 ml and faecal coliform ranged between 109 to 2400/100 ml during their comparative studies on four Lakes namely Pichhola Lake, Fatehsagar Lake, Swaroopsagar Lake and Udaisagar Lake from Udaipur (Rajasthan, India). They further concluded that the higher values of microbial parameters give clear indication of very poor water quality. Anilda Kokali and Sulejman Sulce (2016) [3] reported similar range of total coliform and faecal coliform in the fresh waters of Drini river and Lezha lagoons. They reported total coliform ranged from 15 CFU/100 ml to 2.4 x 10^4 CFU/100 ml and faecal coliform values ranging from 3 CFU/100 ml to 18.9 x 10^3 CFU/100 ml. result of their study showed that there is an origin mixed of faecal contamination in the Drini river rural and urban discharges, livestock and industrial flow into the river.

Biradar N.V. et al. (2014) [6] carried out a study on Kotur lake to assess microbiological parameters and reported monthly variation in the results. They recorded 150 MPN/100 to 2400 MPN/ 100 ml at station-I and 93 MPN/ 100 ml to 460 MPN/100 ml at station-II. They recorded higher MPN values were recorded during summer months i.e. in January, February and March. It may be due to higher temperatures recorded during these months. The lower MPN values were attributed in October, November and December due to low temperature. Further they concluded that the Kotur Lake exceeds the limits and hence water is not fit for pot ability.

Mohammad Armin et al. (2015) [9] reported total coliform and E. coli values which exceeded the recommended limits of 100 MPN/100 ml and 200 MPN/100 ml respectively. According to them these results are indicators of faecal contamination of the lake and shows the presence of pathogenic microorganisms in the water.

In the present investigation maximum bacterial load was recorded during monsoon season whereas minimum count was recorded during post-monsoon season. Higher bacterial population during monsoon months may be due to transport of organic matter from various sources through surface run off from the catchment areas. Similar trend have been reported by Sharma Riddhi et al., (2008) [18] during their study on Udaipur Lake. Findings of the present investigation are also supported by Anilda Kokali and Sulejman Sulce (2016) [3] who reported similar seasonal variation in the fresh waters of Drini river and Lezha lagoons. Lower bacterial load during the post monsoon months may be due to lower temperature which suppresses the growth of coliform bacteria. This is in agreement with findings of Singh (1985) [21], Sharma and Mall (1998), Patralek (1992) [14] and Biradar et al. (2014) [6] opined that temperature also governs the bacterial population.

Conclusion

- The microbial parameters were found to be in a very high range. This range was much higher than the standards laid down for fresh water bodies by Central Pollution Control Board (CPCB).
- High concentration of coliform bacteria in the lake indicate that its microbiological quality has been impaired.
- Further, it can be concluded that anthropogenic activities have resulted in elevated levels of total coliforms. This might have resulted in decline in survival rate of fishes, thus affecting aquaculture.
- Thus, the water of the lake under study is not good for direct domestic use and the occurrence of pathogens is likely to pose a threat to public health.

Recommendations (Scope for Further Work)

- As the presence of indicator bacteria do not clearly reflect the occurrence of pathogenic microorganisms in water, further tests are needed to identify the actual risk to public health.
- There is need to increase awareness of the community towards the dangers associated with the use of contaminated water.

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References

7. Jaiani E, Kokashvili T, Mitashvili N, Elbakidze T, Janelidze N, Lashkhi N et al. Microbial water quality of...


