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Classification of Some Selected Lakes on the Basis of Water Quality Criteria: Most Probable Number

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Abstract:
Water is well known vehicle for transmission of water borne diseases. Water polluted with fecal matter or sewage is likely to contain many intestinal pathogens and may transmit diseases like giardiasis, amoeba is, hepatitis, typhoid, cholera, dysentery etc. to susceptible consumers. Natural water may also be contaminated with microbes, with soil, vegetation and other sources. Bacteriological quality of water evaluation is based on total microbial content coliform count (MPN) and presence of fecal streptococci. Detection of fecal E.coli and indicator of water fecal contamination is considered to be the best method of the judge the portability of drinking water. The presence of typical intestinal organism in water serve as an index of fecal contamination. According to the present study, the MPN count of three water bodies, Rewalsar, Kuntbhyog and Prashar lake was observed respectively. Rewalsar lake showed high MPN number of coliformsas compared to Kuntbhyog and Prashar lake. This is due to entry of sewage in Rewalsar lake. The Escherichia coli are entirely of human origin bit their exclusive estimation is difficult and hence the entire coliforms are used as indicator. In routine tests of potable waters, the actual number of coliforms is not reported but their Most Probable Number (Commonly called as MPN of coliforms) is enumerated. The water of these lakes are very important for religious basis, domestic basis, drinking and irrigation basis. So the collection of scientific data on these lakes are very important for conservation and sustainable development.

Keywords: *MPN, Coliform, Durham tubes*

1. Introduction

The waste from industries and domestic use might include unconsumed organic and inorganic media components, suspended particles, microbial cells and human sewage. The dispose of waste directly to convenient area of land or into nearby water coarse the disposal of the untreated sewage causes the contamination of the surface of the water bodies. Thus, it may cause the health risk. After its reuse for the domestic purposes drinking by men and animals as well as irrigation by the farmers. With increasing density of population and industrial expansion as well as awareness of the damage caused by pollution, the need for treatment and controlled disposal of waste has and will continue to grow (Farrington, 2005).

The discharge of untreated sewage and the ensuing bacterial contamination of surface water bodies pose a health risk in its reuse, be it for a variety of domestic purposes including safe drinking water, as well as exposing farmers who often use raw sewage or polluted streams to meet their irrigation needs. Not a single city in India has been able, in entirety, to deal with its sewage problem. The gap between sewage generated and the capacity to treat is on the rise, as urban planning and infrastructure is in a shambles. These lakesare worshipped by Hindus, Sikhs and Buddhists. The water of these lakes is being used for the purpose of drinking and irrigation and domestic purposes.

2. Materials and Methods

Samples of water were collected from the sites where sewage is discharged and they were diluted to an appropriate concentration with distilled water. 7.5 grams of Mac Conkey's broth were dissolved in 100 ml. of distilled water, and is heated at 80 ^{0}C, and is then cooled to room temperature. 10 ml of this solution were filled in 5 test tubes each and a durhams tubes were added in inverted position in each test tube, plugged with non-absorbent cotton plug. Then another 5 test tubes were with 10 ml of this solution and 1 ml of diluted sample water were added and a durhams tube were added in each test tube and pluged with cotton plug.

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2.1. Procedure

1. Select dilutions according to the expected bacterial count. In the waters known to be having low density (like potable waters) only 10ml undiluted water sample is inoculated, for the waters suspected of having high density several dilutions are used. Table. 1 provides the guidelines for selecting suitable dilutions.
2. Take the tubes for each sample as directed in Table 1. Put on Durham's vial inverted in each test tube and put the media required. Sterilize the tubes at 121^0C for 15 minutes.
3. Shake all the water sample vigorously immediately before removing sample aliquots to inoculate the series of test tubes.
4. Add sample using sterilized pipettes to the test tubes selected for the test and mix thoroughly. Use separate pipettes for different samples as well as for dilution. While withdrawing sample portion, the tip of the pipette should never be submerged more than one inch below the surface of the sample. This procedure minimizes the accumulative drainage from exterior of pipette into the media.
5. incubate within 30 min, all these in an incubator at 35-37^{0}C.
6. After 48 hrs. examine each tube carefully. Those showing gas in the Durham's vial are recorded as positive (+). Gas in any quantity even a tiny bubble is recorded as (+). The tubes showing positive test are subjected to confirmatory test, as gas production is not the only criterion for a positive test. Discard all the tubes showing negative test. It is however advisable to examine the tube first at the completion of 24 hrs. Subject the tubes showing positive test immediately to confirmatory test. Incubate negative tubes to further 24 hrs.

Type of water	Dilution and tubes
1. Potable waters (finished, after chlorination)	5tubes of 10ml double strength medium with 10ml sample
2. Relatively unpolluted water (Also raw waters for drinking and Finished water without chlorination).	5 tubes of 10 ml double strength medium with 10ml samples, 5 tubes with 1 ml sample and 5 tubes of 0.1 ml sample. (Additional dilution 0.01 ml may also be taken for precision)
3. Polluted waters, suspected to be Receiving domestic swage.	5 tubes with b10 ml single strength medium and 0.1 ml sample, 5 tubes with 10 ml single strength medium end 0.01 ml sample and 5 tubes with 10 ml single strength medium and 001 ml sample. (Additional dilution, 0.0001 ml may also be taken for precision).

Table 1

**Mc Conky's broth is suitable for this test.*

3. Calculation of Most Probable Numbers

The calculation of MPN of coliforms is done by combination of positive and negative results in the multiple tube test. The values can be calculated for any of the combination given it table 2 and 3. For estimation of MPN, if the tubes of only one sample portion (10 ml) have been used, as is usually done for potable waters, if three combinations, e.g. 10 ml, 1 ml and 0.1 ml have been used refer Table 3. The important things to remember is that the positive and negative combinations can be used of any one test e.g. if a test has been carried out only up to the presumptive test stage, than the positive and negative combinations of this test can be used to calculate the MPN, if all three tests has been carried out the MPN can be calculated on the basis of other presumptive or confirmatory or completed test.

For example, if 5 tubes each of 10 ml, 1ml and 0.1 ml sample portions have been used and the results are as follows

10 ml Portion -2 tubes positive, 3 tubes negative

1 ml Portion-2 tubes positive, 3 tubes negative

0.1 ml Portion – All tubes negative

The combination can be written as 2-2-0 and the MPN index according to the table 13 will be 9 cell/100ml. If combination other than those given in the table appear, then perhaps the test has been carried out according to the instructions.

The Table 3 is given for a starting dilution 20 ml and 1/10th and 1/100th part of it (10, 1 and 0.1 ml). If any other dilution pattern is used, e.g. 100 ml, 10 ml, and 1 ml), the same table can be used, but here 100, 10 and 1 will be supposed us 10, 1 and 0.1, this table value will be put in the following formula to get MPN/100ml.

$$\text{MPN/100ml} = \frac{\text{MPN table value -10}}{\text{String dilution}}$$

For example, if the result is same. as previously given 2.2-0, but the dilutions used are 100 ml and 1 ml than according to the formula MPN/ 100 ml will be:

$$= \frac{9 \times 10}{100} = 0.9$$

If more than three dilutions are used, than select three successive dilutions for the calculations of MPN, staring from the highest dilution [lowest sample fractional] which gives positive results in all five tubes and next two higher dilutions.

In case of unlikely combinations, use following formula to calculative MPN/100 ml.

$$\text{MPN/100ml} = \frac{\text{No. of positive tubes x 100}}{\text{Total sample [ml] in negative tubes}}$$

X total sample in test
These tables can be similarly used for fecal coliforms.

No of tubes giving positive results out of fives	MPN/10ml
0	2.2
1	2.2
2	5 1
3	9.2
4	16.0
5	16.0

Table 2: MPN /100ml values when the five tubes of only 100ml are used.

Combinations	MPN/100ml	Combinations	MPN/100ml
0-0-0	2	4-3-0	27
0-0-1	2	4-3-1	33
0-1-0	2	4-4-0	34
0-2-0	4	4-4-0	23
1-0-0	2	5-0-0	31
1-0-1	4	5-0-2	43
1-1-0	4	5-1-0	33
1-1-1	6	5-5-1	46
1-2-0	6	2-1-2	63
2-0-0	5	5-2-0	49
2-0-1	7	5-2-1	70
2-1-0	7	5-2-2	94
2-1-1	5	5-3-0	79
2-2-0	9	5-3-1	100
2-3-0	12	5-3-2	140
3-0-0	8	5-5-3	180
3-0-1	11	5-4-0	130
3-1-0	11	5-4-1	170
3-1-1	14	5-4-2	220
3-2-0	14	5-4-3	280
3-2-1	17	5-4-4	350
4-0-0	13	5-5-0	240
4-0-1	17	5-5-1	350
4-1-0	17	5-5-2	540
4-4-1	21	5-5-3	920
4-1-2	26	5-5-4	1600
4-2-0	22	5-5-5	2400
4-2-1	26		

Table 3: MPN/100 ml for various combinations of posting results when 5 tubes each of 10ml, 1 ml and 0.1ml sample fractions are used.

4. Result and Discussion

From all the results obtained by applying MPN test it is clear that the water of the Rewalsar is highly polluted due to disposal of sewage as compared to Kuntbhyog and Prashar lakes. Results of MPN tests are positive which confirms the presence of coliform bacteria in lake waters. Durham tubes float due to releasing of co_2 in water. In Rewalsar lake out of 10ml 5 tubes are positive, 1ml 5 tubes are positive and 0.1ml only 1 tubes are positive, 5-5-3. The value of MPN in Rewalsar lake is 920/100 ml of water. In kuntbhyog lake out of 10ml 5 tubes are positive, 1ml 3 tubes are positive and 0.1ml only 1 tubes are positive, 5-3-1. The value of MPN in Kuntbhyog lake is 100/100 ml of water. In Prashar lake out of 10ml, 1ml and 0.1ml no one tubes are positive 0-0-0 (fig.1- 3). The value of MPN in Prashar lake is 2/100 ml of water Caputo and Huffman, (2004).

S. No.	Parameter/ Water Quality	A	B	C	D	E
1.	Dissolved oxygen (mg L^{-1})	6.0	5.0	4.0	4.0	-
2.	Biochemical oxygen demand (BOD) (mg L^{-1})	2.0	3.0	3.0	-	-
3.	pH	6.5-8.5	6.5-8.5	6-9	6.5-8.5	6.5-8.5
4.	Free ammonia (mg L^{-1})	-	-	-	1.2	-
5.	Electric conductivity (µmhos cm^{-1})	-	-	-	-	2,250
6.	MPN 100 ml^{-1}	<50	<500	<5000	-	-

*Table 4: Water quality criteria for various uses (Central Pollution Control Board – 1978) *.*
**www.cpcb.nic.in/classi.htm *www.Lenntech.com*

On the basis of classification of waters for various uses given by Central Pollution Control Board, the water of the Rewalsar Lake can be categorized as 'C-D', whereas water of Kuthbhyog Lake as 'B-C' and water of Prashar Lake belongs to 'A-B'. Low values of D.O. (3.05-9.98 mg L^{-1}) at Rewalsar Lake indicated entry of organic matter in the lake, is an alarming condition and treatment is required before its use for various purposes Table
According to the water quality criteria of ISI (1991) and WHO (1993), dissolved oxygen, turbidity and carbon dioxide in all the three lakes have crossed the desirable limits. BOD values at Rewalsar and Kuntbhyog lakes have crossed the desirable limit, whereas in Prashar Lake is under desirable limit. Sulphate values have crossed the desirable limit only in the Rewalsar Lake. Prashar lake water is used for Drinking without conventional treatment but after disinfection, Kuntbhyog lake water is used for outdoor bathing. Rewalsar lake water is used for conventional treatment followed by disinfection and wild life fisheries.

Figure 1: Dhrum tube in different water sample

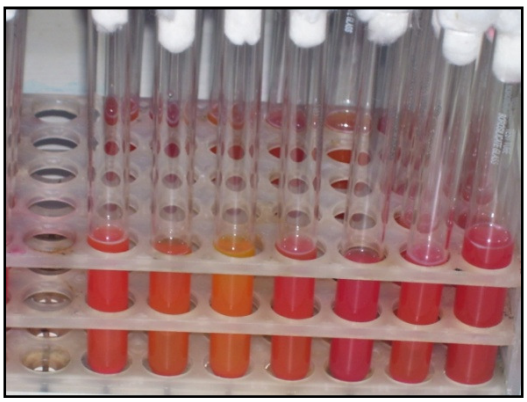

Figure 2: colour of different water sample

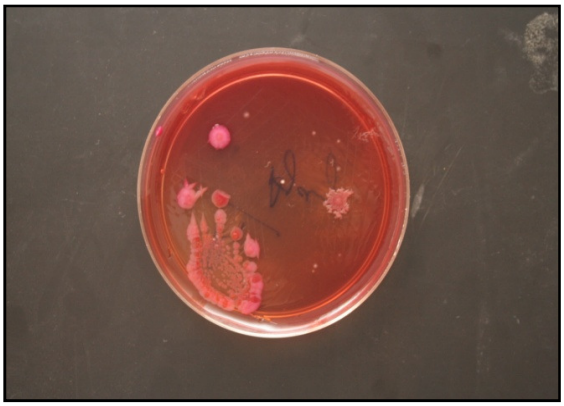

Figure 3: E. coli culture on agar palte

5. Conclusion

So we conclude that only water of Prashar lakes are used for drinking and domestic purposes. There is urgency to take conservation steps to prevent Rewalsar, Kuntbhyog and Prashar lakes respectively from further contamination. We strongly recommend to the concerned authorities of the city corporation to take restoration programs and minimize the anthropogenic activities in and around the lakes.

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