Journal of Materials Science & Surface Engineering, 6(6): 905-908
ISSN (Online): 2348-8956; 10.jmsse/2348-8956/6-6.6

Biological analysis of Yamuna River

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ABSTRACT

Water pollution is a very common cause of major health problems across the globe. The most common and widespread health risk associated with drinking water is contamination. The pathogenic agents involved include bacteria, viruses, and protozoa, which may cause diseases that vary in severity from mild gastroenteritis to severe and sometimes fatal diarrhea, dysentery, hepatitis, or typhoid fever, most of them are widely distributed throughout the world. Biological testing methods are progressively often used for determining the surface water quality. In the biological analysis of the water samples using methods like, most probable number (MPN) method, glutamate starch phenol red agar and hektoen enteric agar, we observed various organisms like Coliform bacteria, *Aeromonas*, *Pseudomonas*, *Salmonella*, and *Shigella*, which are harmful for consumption of population to be present in the river water. The biological methods are used for analyzing water quality involves collection, counting and identification of micro organisms, measurement of metabolic activity rates, and processing and interpretation of biological data. In this paper, we have done a comparative analysis of microbes present in samples collected from different places and their impact on water quality.

ARTICLE HISTORY
Received 30-03-2019
Revised 01-09-2019
Accepted 06-09-2019
Published 01-12-2019

KEYWORDS
Biological testing
Contamination
Harmful organism
Water pollution

Introduction

The aim of this study was to do a seasonal assessment of physical and chemical parameters to assess Yamuna river water quality. Samples were collected from three stretches of Delhi and two stretches of Mathura in different seasons (from winters to summers) and were assessed for their suitability for human consumption. Supply of potable water is important for a country's development. Clean water sustains a healthy population and it contributes to the quality of life of households by providing the basic needs of water and sanitation [1]. It is reported that 80% of all diseases in developing countries is related to water and sanitation. Water of poor quality can cause social and economic damages through water-related epidemics such as cholera which in turn causes a rise in medical treatment costs. Supply of pollution free drinking water is the basic need for all human beings on Earth. In most of the developing countries bacteria and microorganisms have regularly infected drinking water supplies, sometimes causing severe epidemic in a town. Hence, water quality is amongst the very big issue today [2].

The work involving MPN analysis will contribute in:

1. Identification of coliform bacteria, *Pseudomonas*, *Aeromonas*, *Salmonella*, *Shigella*, indicating type of contamination;
2. Identification the nature, extent and biological effects of pollution;
3. Seasonal wise variation analysis.

According to water quality parameters, the maximum acceptable concentration of microorganisms present in drinking water should be as follows:

1. No total coloiforms or *E.coli* should be detected [3]
2. Less than 3-4 CFU per ml of *Pseudomonas* should be detected [3]
3. No *Aeromonas* species should be detected [3]
4. No *Salmonella* species should be detected [3]
5. No *Shigella* species should be detected [3]

Experimental

Sampling locations

A consistent analysis of Delhi stretch of Yamuna river water was done, as this stretch starts from Palla village (upstream), to Kashmiri gate (mixing point), till Okhla barrage (downstream). These three sites were selected as these are amongst highly developed sites that shows maximum number of polluted parameters that has to be taken under consideration while water analysis. The main sources of pollution in the Yamuna River:

- Household disposal
- Municipal disposal sites
- Untreated industrial discharge
- Malfunctioning of sewage treatment plants

Selection of the sites was done to compare the level of pollution of the state from the entry point to the exit of the flow of river by sampling upstream, mid-point and downstream sites:

Sites selected for Yamuna River in Delhi:

- Upstream flow – Palla Village (Figure 1)
- Midpoint – Kashmiri Gate (Figure 2)
- Downstream flow – Okhla Barrage Bridge (Figure 3)

Sites selected for Yamuna River in Mathura:

- Upstream flow – Gokul Barrage Bridge (Figure 4)
- Downstream flow – Lalit Bagh (Figure 5)

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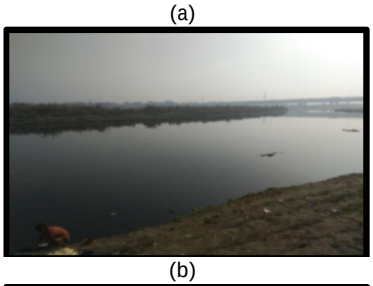

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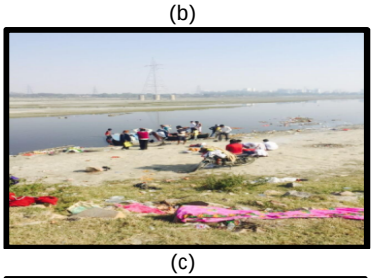

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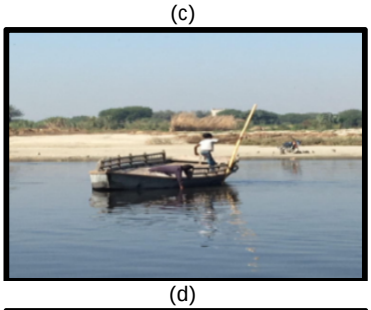

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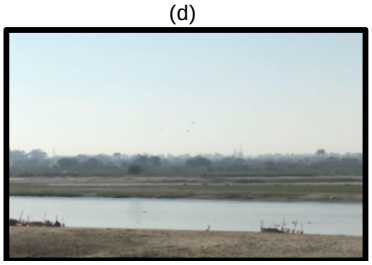


(e)

Figure 1: (a) Palla Village (Delhi), (b) Kashmiri Gate (Delhi), (c) Okhla (Delhi), (d) Gokul Barrage Bridge (Mathura), (e) Lalit Bagh (Mathura)

Materials and methods

Methods involves collection, counting and identification of micro organisms (MPN method), measurement of metabolic activity rates, and processing and interpretation of biological data.

Identification of Coliform

The Most Probable Number (MPN) test was performed by inoculating 5 test tubes (with inverted Durham tubes) each of 10 ml containing diluted sample to 10ml of double strength MacConkey Broth, 1ml diluted sample to 10ml of single strength MacConkey Broth and 0.1ml diluted sample to 10ml single strength MacConkey Broth. Incubation of the test tubes was done at 37ºC for 24-48 hours. Then, the test tubes were observed for turbidity, acid and gas production. 0.1ml of sample from MacConkey Broth tubes was taken which showed positive results for gas and acid production and then the tubes were inoculated in 10ml of BGBB (Brilliant Green Bile Broth) and turbidity, acid and gas production in the tubes were observed. In this test streaking of samples from the tubes showing positive results for gas and acid production on EMB (Eosin Methylene Blue agar) was done. The petri plates were incubated and observed for growth of faecal coliform.

Identification of Pseudomonas and Aeromonas species

1 ml of sample water was inoculated in 9 ml of peptone water with 1% NaCl (w/v) at pH 8.6 adjusted with sodium hydroxide. After incubation at 37ºC for 24-48 hr, the streaking of the cultures were done on Glutamate Starch Phenol Red agar (GSP) and were further incubated at 37ºC for 24-28 hr.

Identification of Salmonella and Shigella species

1 ml of sample water is inoculated in 9 ml of water with 1% NaCl (w/v). Further serial dilution was done till 5 test tubes. Then the prepared dilution was streaked on Hektoen Enteric Agar and left for incubated at 37ºC for 24- 48 h.

Identification of E.coli

Sterilized test tubes were taken containing 4 ml of tryptophan broth. Inoculation of the tubes was done by taking growth culture from 18 to 24 hrs. Then, tubes were incubated at 37°C for 24-28 hours. 0.5 ml of Kovac's reagent was added to the broth culture and observed for presence or absence of ring.

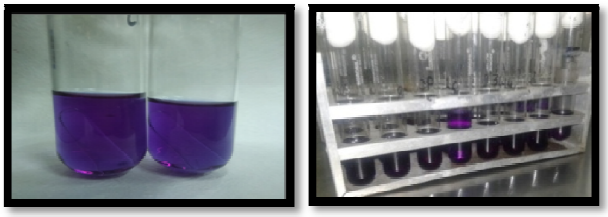
Results and Discussion

Coliform

The sample collection was done during two different seasons i.e. summer season (September- October) and winter season (December- January).

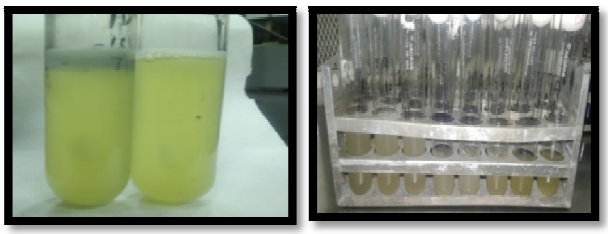
Pre-confirmatory test for the presence of coliform

The MPN test was performed; the MacConkey Broth tubes (Figure 2) were incubated with the sample for 24-48 hours.



(a) (b)

Figure 2: (a) Test tubes with MacConkey Broth; (b) Set of test tubes with MacConkey Broth and sample in it before incubation.



(a) (b)

Figure 3: (a) Test tubes with gas production and turbidity after incubation; (b) Test tubes with gas production and turbidity after incubation

Occurrence of bubble formation and change in the colour of broth from violet to yellow due to the acid production indicated the presence of coliform (Figure 3). Therefore, samples were further analyzed for confirmation.

According to the results, MPN values were analyzed from the standard MPN index table for each sample; Higher the MPN value more will be the contamination of the sample and the values are shown in Table 1.

Table 1: Comparison between the MPN values of different samples collected during two different seasons as mentioned above.

S.NO.	SAMPLE	SUMMER SEASON (MPN index value per 100ml)	WINTER SEASON (MPN index value per 100ml)
1.	DELHI UPSTREAM	9	4
2.	DELHI MIDPOINT	15	14
3.	DELHI DOWNSTREAM	17	17
4.	MATHURA UPSTREAM	17	15
5.	MATHURA DOWNSTREAM	9	14

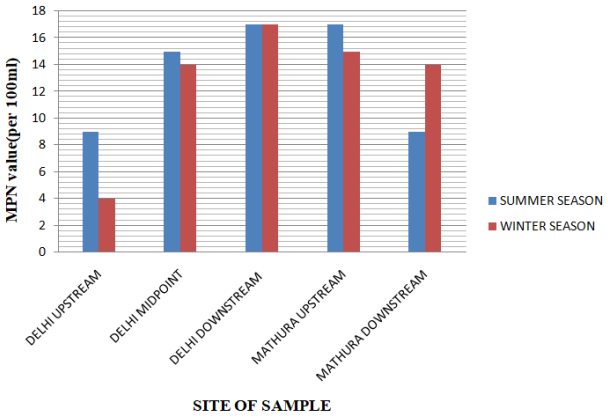
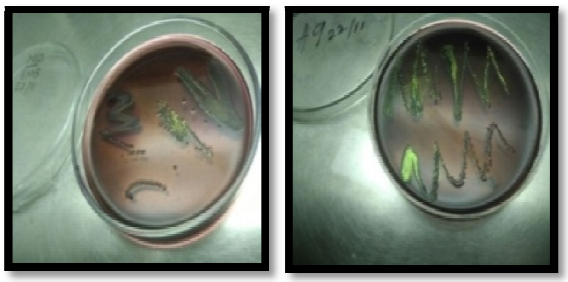


Figure 4: Comparison between the MPN values of different samples collected during two different seasons.

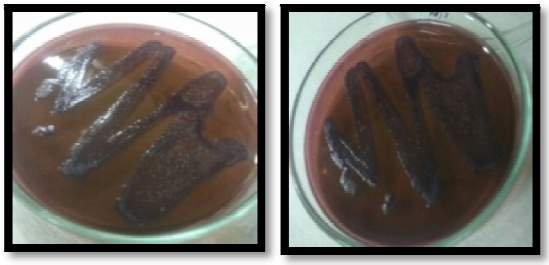
EMB Agar: Confirmatory Test for the presence of coliform

Samples with positive result in the pre-confirmatory test were selected for confirmation for the presence of feacal coliform. Growth of Green Metallic Sheen on EMB agar will indicate the presence of feacal coliform. Interpretation of the Plates according to results obtained due to difference in the seasons is given below (Figure 5-7):



(a) (b)

Figure 5: (a) E.coli growth on EMB medium during the summer season; (b) E.coli growth on EMB medium during the summer season



(a) (b)

Figure 6: (a) Represents the no E.coli growth on EMB medium during the winter season; (b) No E.coli growth on EMB medium during the winter season

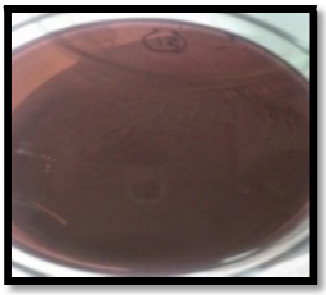
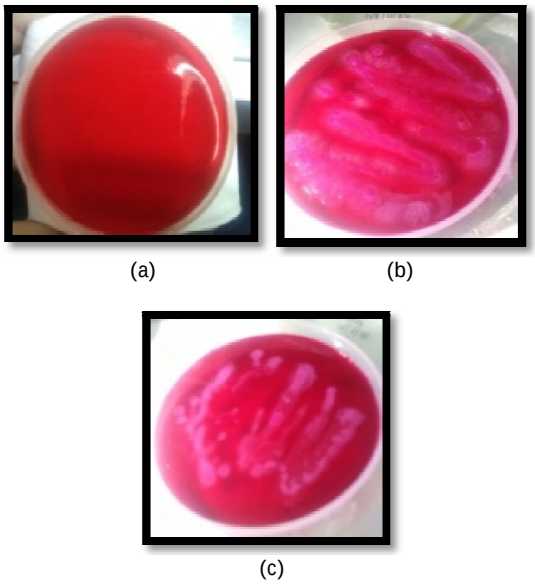


Figure 7: Control on the EMB medium

Presence of Fecal Coliform were observed in water samples of Mathura Upstream, Mathura Downstream, Delhi Upstream, Delhi Midpoint and Delhi Downstream during summer season, whereas the absence of Fecal Coliform in water samples of Mathura Upstream, Mathura Downstream, Okhla, Kashmere gate and Palla Village were observed during winter season.

Aeromonas and Pseudomonas species

Samples were checked on GSP agar for Aeromonas and Pseudomonas species and the observations are as follows (Figure 8, Table 2):



(a) (b)

(c)

Figure 8: (a) Control on the GSP Agar; (b) The presence of pseudomonas species on GSP Agar; (c) The presence of pseudomonas species on GSP Agar

Table 2: Observations of the *Aeromonas sp.* and P*seudomonas sp.* in the different samples

S.NO.	SAMPLE	*Aeromonas sp.*	*Pseudomonas sp.*
1.	DELHI UPSTREAM	Absent	Present
2.	DELHI MIDPOINT	Absent	Present
3.	DELHI DOWNSTREAM	Absent	Present
4.	MATHURA UPSTREAM	Absent	Present
5.	MATHURA DOWNSTREAM	Absent	Present

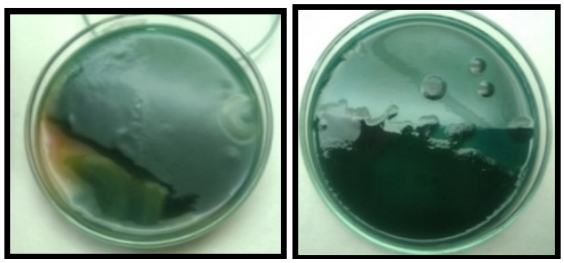
Growth of light pink colonies on GSP agar which exhibits the presence of *Pseudomonas sp.* in water samples of Mathura Upstream, Mathura Downstream, Delhi Upstream, Delhi Midpoint and Delhi Downstream was observed, whereas the no presence of yellow colored colonies on GSP agar which exhibits the absence of *Aeromonas sp.* in water samples of Mathura Upstream, Mathura Downstream, Delhi Upstream, Delhi Midpoint and Delhi Downstream was observed.

Salmonella and Shigella species

Samples were checked on Hektoen Enteric agar for Salmonella and Shigella species and the observations are as follows (Table 3, Figure 9-10):

Table 3: Observations of the Salmonella and Shigella in the different samples

S.NO.	SAMPLE	Salmonella sp.	Shigella sp.
1.	DELHI UPSTREAM	Present	Absent
2.	DELHI MIDPOINT	Present	Absent
3.	DELHI DOWNSTREAM	Present	Present
4.	MATHURA UPSTREAM	Present	Absent
5.	MATHURA DOWNSTREAM	Present	Absent



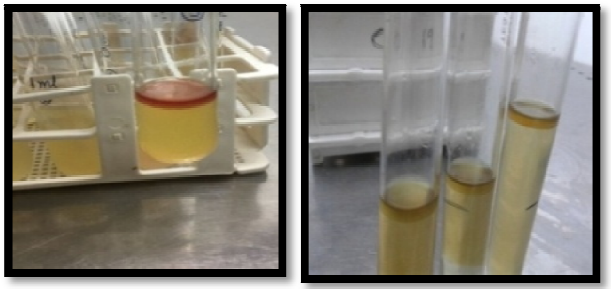
(a) (b)

Figure 9: a) The presence of Salmonella and Shigella on Hektoen Enteric Agar; b) The presence of Salmonella on Hektoen Enteric Agar

Growth of dark green colonies on Hektoen Enteric Agar which exhibits the presence of Salmonella sp. in water samples of Mathura Upstream, Mathura Downstream, Delhi Upstream, Delhi Midpoint and Delhi Downstream were observed, whereas the presence of orangish-yellow colored colonies on GSP agar which exhibits the presence of Shigella sp. in water samples of Okhla and Haridwar were observed.

Escheria coli

Formation of a pink to red color ring in the reagent layer on top of the medium within seconds of adding the reagent in samples has shown positive Indole test which confirms the presence of Escherichia coli in the samples of Mathura Upstream, Mathura Downstream, Delhi Upstream, Delhi Midpoint and Delhi Downstream.



(a) (b)

Figure 10: a) Positive result of Indole test; b) Negative result of Indole test

Conclusions

Depending on the seasonal variation, a major difference in the growth of the *E. coli* coliform was observed. The presence of *E.coli* in the summer season shows that this season provided ambient conditions for the growth of the organism. But in winter season, there was no growth of the suspected organism. With various reasons, like untreated industrial discharge as it is a major hub of factories, domestic waste runoff as it is the downstream of Yamuna river through Delhi, increase in pollutants in the Delhi atmosphere due to industrial, automobile effluents, it was found out that sample of site "Okhla" to be the most polluted due to presence of several microorganisms and higher MPN value among other samples in our study. This study is creating awareness of current scenario of high pollution levels in Delhi and Mathura. Yamuna River is becoming one of the most polluted rivers; therefore future research on its treatment will be of great importance.

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