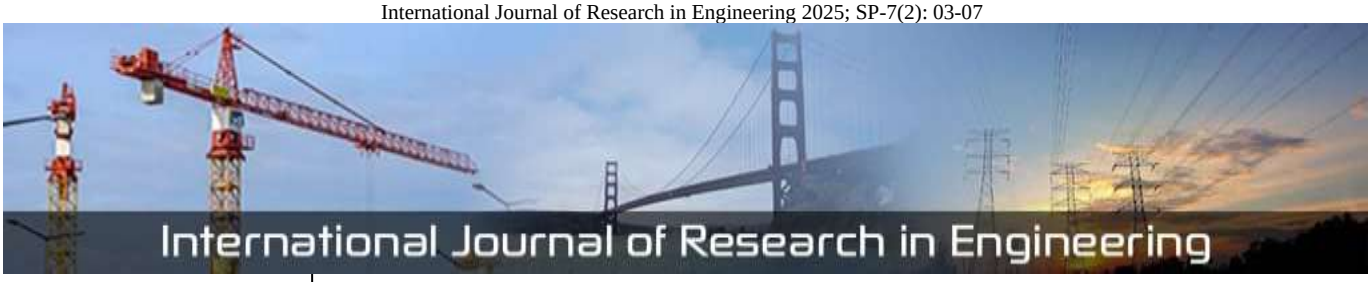

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Swati Rathore
Department of Chemistry, Suraj PG Degree College, Sector 75 Gurugram, Haryana, India

Sharvi Mishra
Department of Chemistry, Suraj PG Degree College, Sector 75 Gurugram, Haryana, India

Preeti Rewalliya
Department of Chemistry, Suraj PG Degree College, Sector 75 Gurugram, Haryana, India

Nirmla Devi
Department of Chemistry, Suraj PG Degree College, Sector 75 Gurugram, Haryana, India

Rakhi Devi
Department of Chemistry, Suraj PG Degree College, Sector 75 Gurugram, Haryana, India

Sushmita Gandash
Department of Chemistry, Suraj PG Degree College, Sector 75 Gurugram, Haryana, India

Correspondence
Swati Rathore
Department of Chemistry, Suraj PG Degree College, Sector 75 Gurugram, Haryana, India

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To study the microbiological quality of Gairatpur Bas pond water and stagnant water of Sector -28, Gurugram using chemically defined culture medium

Swati Rathore, Sharvi Mishra, Preeti Rewalliya, Nirmla Devi, Rakhi Devi and Sushmita Gandash

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Abstract
This study proposes an innovative technique for assessing fecal coliforms in water. The indiscriminate disposal of industrial and municipal waste contaminates groundwater. Water quality is significantly influenced by human and animal waste, household wastewater, industrial effluents, and agricultural pesticide runoff. Insufficient, poorly managed, or absent water and sanitation infrastructure renders drinking water susceptible to pollution. The contamination of water by pathogenic microorganisms poses a significant risk to human health. Diverse microorganisms act as major indicators for evaluating water quality. Our research identifies Escherichia coli (E. coli) as the most appropriate indicator organism for evaluating the potential presence of pathogens in drinking water. This study examines the presence and concentration of fecal coliforms in pond and stagnant water. Sample a (Gairatpur Bas) and B (Sector 28) were obtained for analysis. A test was conducted to identify potential pathogens utilizing a chemically defined culture medium, and the total coliform concentration in water was assessed using the Most Probable Number (MPN) method. The samples were incubated at 39^{O}C with Bromothymol blue as an indicator dye. The results demonstrated a distinct colour shift from teal blue to yellow, accompanied with gas production and increased turbidity in the fermented broth after 9 hours, so proving the presence of E. coli and, consequently, pathogens. The research revealed substantial discrepancies in fecal coliform concentrations, with pond water and surface water exhibiting elevated contamination levels. Sample a exhibited markedly elevated contamination levels compared to Sample B, as seen by the total coliform count. These findings underscore the necessity for enhanced water purification and effective management measures.

Keywords: Escherichia coli, bromothymol blue, bacteria, pathogenic microorganisms.

Introduction
Access to potable water is crucial, and enhancing the quality of drinking water from diverse sources can avert 10% of worldwide diseases [1]. Microbial water analysis is a critical process for assessing drinking water quality, quantifying bacterial presence, and facilitating the recovery of microorganisms for identification. The identification of bacterial indicators in potable water may indicate the presence of pathogenic organisms linked to waterborne illnesses. Indicator microorganisms have enhanced survival and longevity due to their reliable and stable characteristics, facilitating their identification by traditional laboratory methods [2]. The quality of water is significantly influenced by human and animal waste, home sewage, industrial effluents, and various contaminants. Insufficient, poorly managed, or absent water and sanitation systems render drinking water susceptible to pollution [3].
Water from taps, wells, and springs was gathered for the cross-sectional study. The identification and quantification of bacteria (Escherichia coli, Salmonella, Shigella, Proteus, Staphylococcus aureus, and total coliforms) in water samples were conducted utilizing several methodologies. Escherichia coli was enumerated using the membrane filtration technique; Salmonella, Shigella, and Proteus were examined by a two-step enrichment process; Staphylococcus aureus was evaluated by the surface spread method, and total coliforms were determined using the most likely number method. Mannitol salt agar was used to quantify Staphylococcus aureus, whereas violet red bile agar was applied to count

total coliforms and Escherichia coli [4]. About 80 residential well water samples were analysed employing the multiple fermentation tube method to ascertain the presumptive coliform count/most probable number of coliforms, with isolates identified by conventional procedures and subsequently assessed for susceptibility [5].

2. Materials and Method

I. Formulation and Selection of New Chemically Defined Culture Medium

A range of chemically defined culture media has been developed and evaluated to ascertain the bacteriological quality of water, ensuring stability, cost-efficiency, and prompt results. In accordance with their recommendations, BR medium has been chosen for implementation in the New Proposed approach.

Culture media is essential for microbiological assays: to get pure cultures, proliferate and measure microbial cells, and develop and select microorganisms. The deficiency of superior quality medium diminishes the likelihood of attaining accurate, consistent, and reproducible microbiological test results [6].

A microbiological culture medium is a substance that promotes the growth, sustenance, and viability of microorganisms. The culture medium comprises nutrients, growth-promoting substances, energy sources, buffering salts, minerals, metals, and gelling agents for solid media [7].

Selection of Indicator Organism: Escherichia coli is selected as the ideal indicator organism for our research.

Selection of suitable Dye to be used with Newly Formulated BR culture medium: For our study, Bromothymol blue dye was selected for analysis of microbiological quality of water.

II. Description of New Technique

The technique employs newly formulated BR culture medium

Composition of medium (BR broth)

Lactose .....15.0 g
L-Asparagine .....5.0 g
Urea .....1.0 g
Disodium hydrogen orthophosphate.....2.0 g
Sodium desoxycholate .....2.0 g
Sodium lauryl sulphate .....0.1 g
Bromothymol blue dye .....0.01 g
Distilled water .....1 L

Procedure: Dissolve about 2.5 g of the specified medium in a 10 ml water sample within a sterile inoculating tube (graduated at 10 ml and 110 ml) by applying gentle heat with a spirit lamp or burner. The contents are cooled, and the volume is made upto 110 ml using the inoculum (the water sample being analysed).It is thereafter covered with a loose lid and incubated at 39 ± 0.5°C in an incubator, with periodic stirring using a pointed glass rod provided inside the tube. A positive test is shown by a colour transition from teal blue to yellow, accompanied by gas evolution and the development of turbidity in the fermented broth. This signifies the existence of fecal coliforms (E. coli) and possible presence of pathogens.

III. MPN Method (Most Probable Number)

The MPN method is a statistical methodology employed in microbiology to estimate the quantity of viable microorganisms in a sample, particularly when direct enumeration is challenging. The method ascertains the presence or absence of microorganisms across many dilutions and subsequently use a statistical table (MPN table) to estimate the most probable number of germs in the original sample.

Procedure: Prepare three sets, each comprising three test tubes and inoculation tubes. The initial set comprises 10 ml of double-strength medium (BR medium with bromothymol blue dye), whereas the second and third sets each contain 10 ml of single-strength medium. Autoclave subsequent to the insertion of cotton plugs. Inoculate each tube with the water sample. Inoculate the first, second, and third sets with 10ml, 1ml, and 0.1ml of the contaminated water sample, respectively, and incubate at 37 degrees Celsius for 24- 48 hours for bacterial growth.

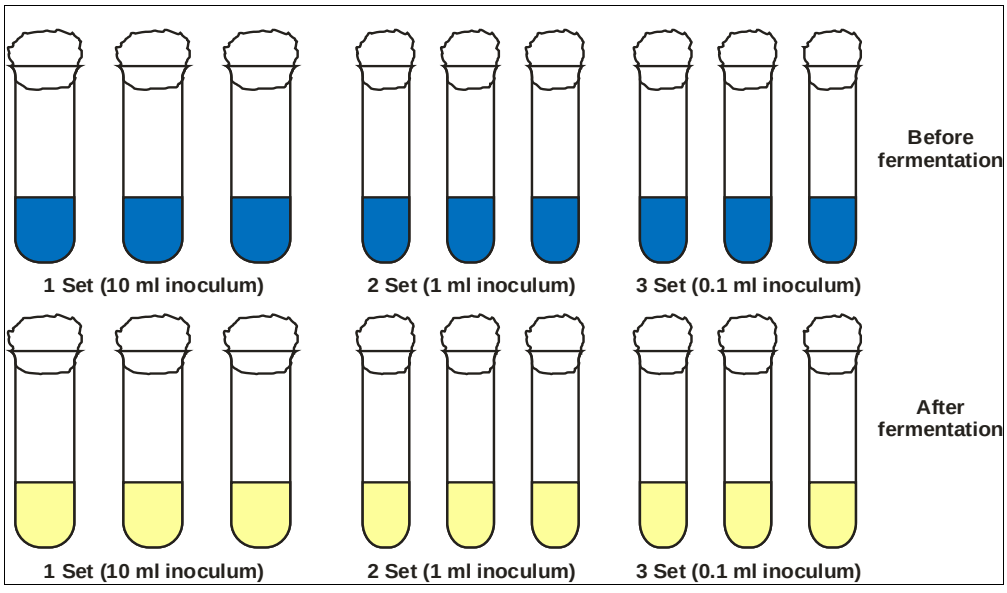

Fig 1: Conceptual Framework of Employee Engagement

3. Results and Discussion

A) Detection: Laboratory analyses were performed to ascertain the possible presence of pathogenic bacteria in Sample A (Gairatpur Bas pond) and Sample B (Sector 28). The outcomes were recorded as follows:

1) Sample A: A favourable result is signified by a color transition from teal blue to yellow, incubated at 39±0.5°C, together with concurrent gas production and turbidity in the fermented broth after 8-9 hours.

Sample B: A favourable result is indicated by a color transition from teal blue to yellow during incubation at 39±0.5°C, along with simultaneous gas production and turbidity in the fermented broth after 10 hours.

Sample A
(Gairatpur Bas Pond)

Before Fermentation **After Fermentation**

Sample B
(Stagnant water, Sector-28)

Before Fermentation **After Fermentation**

B) Calculation of MPN

The test tubes inoculated with 10ml, 1ml, and 0.1ml of the water sample were incubated for 24 to 48 hours at 37 °C, as outlined in the Materials and Methods. A positive test is indicated by gas evolution, turbidity, and a colour transition from teal blue to yellow.

The Most Probable Number (MPN) of coliforms might be ascertained utilizing the subsequent table, which correlates to the quantity of positive tubes.

Table 1: Most Probable Number of Coliforms per 100 ml Based on Positive Tube Combinations

Combination* of positive tubes	MPN of coliforms / 100 ml	Combination * of positive tubes	MPN** of coliforms / 100 ml
0-0-1	2	3-0-0	23
0-1-0	3	3-0-1	39
1-0-0	4	3-0-2	64

1-0-1	7	3-1-0	43
1-1-0	7	3-1-1	75
1-1-1	11	3-1-2	120
1-2-0	11	3-2-0	93
2-0-0	9	3-2-1	150
2-0-1	14	3-2-2	210
2-1-0	15	3-3-0	240
2-1-1	20	3-3-1	460
2-2-0	21	3-3-2	1100
2-2-1	28	3-3-3	>1100

3 tubes of 10 ml, 3 tubes of 1 ml and 3 tubes of 0.01 ml.

a) The MPN value for Sample A was recorded as 3-3-0, corresponding to an MPN value of 240 per 100 ml.
b) The MPN value for Sample B was recorded as 3-1-2, corresponding to an MPN value of 120 per 100 ml.

During a nine-month period from September to May, 116 drinking water samples were aseptically collected in sterile containers from diverse sources. The Most Probable Number (MPN) test was performed to detect coliforms in potable water samples [8].

C) Comparative Study of Sample A and Sample B

a) Looking at the results, it can be inferred that time taken by Sample B to indicate positive test is longer that as compared to Sample A
b) The total coliform count per 100ml of Sample A is more as compared to Sample B which indicates presence more pathogenic organisms in Sample A. It indicates that Microbial contamination in Sample A is greater as compared to Sample B.

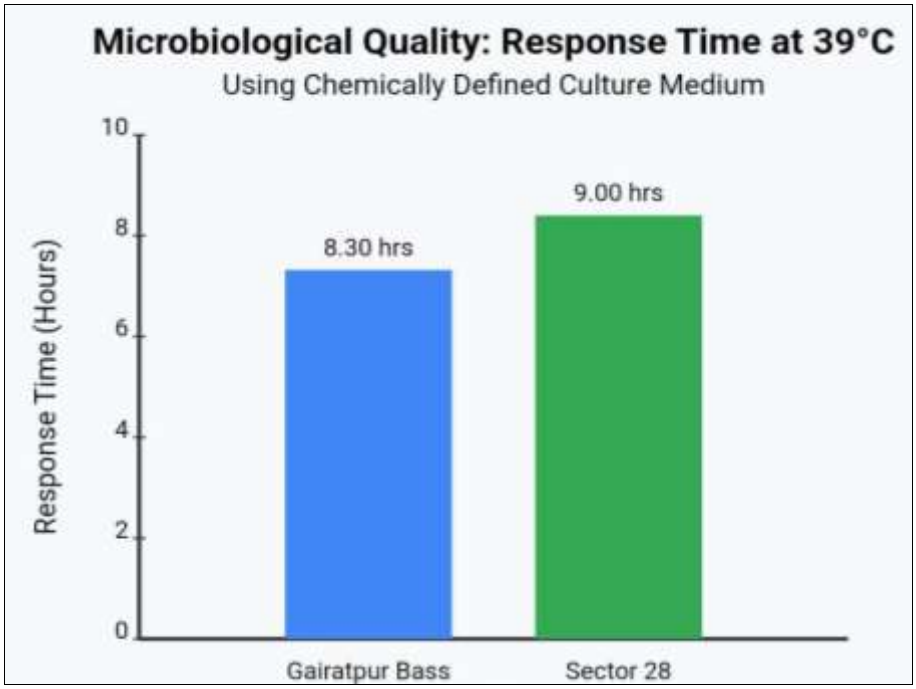

Fig 2: Microbiological Quality Assessment Based on Response Time at 39°C in Gairatpur Bass and Sector 28 Using Chemically Defined Culture Medium

One hundred samples (76 from tap water and 24 from bottled water) were analyzed for bacteriological cleanliness and pH levels. The methodologies adopted comprised the spread plate technique for total plate count (TPC) and the membrane filter technique for total coliform count (TCC), fecal coliform count (FCC), and fecal streptococcal count (FSC). Of the tap water samples, 55.3% tested positive for total coliforms, compared with 25% of the bottled water [9].

D) Evaluate the Stability/Shelf Life of BR Medium:

Small pouches were constructed using aluminum foil lined with polyethylene. The dehydrated BR medium was measured, packaged in bags, and sealed. In the single sample field, the contents of one bag may be employed. The stability or shelf life of BR medium was evaluated at regular intervals and found to be one year. A cross-sectional study was performed to determine the shelf life of liquid culture media, namely EC Broth [10].

4. Conclusion

This study conducted various tests, revealing that water sample A (from Gairatpur Bas pond) and sample B (Sector-28, Gurugram) were contaminated. The investigation detected pathogens in the collected water samples. Sample A exhibited a higher level of fecal contamination compared to Sample B. Microbial contamination of water samples presents considerable public health hazards, potentially resulting in numerous waterborne diseases.

Therefore, proper water treatment and monitoring are essential for alleviating these health risks. The devised test/technique is sensitive, intuitive, rapid, economical, and produces dependable results. The technology can be

included into existing water testing field kits.

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