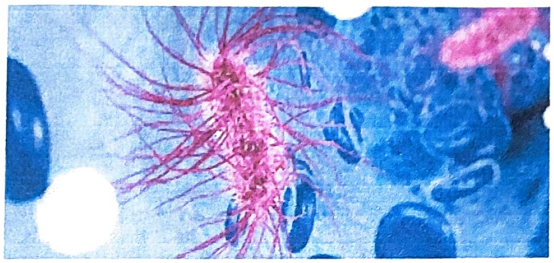
INVESTIGATION OF WATER SAMPLE FOR PRESENCE OF *E. coli*

A FIELD PROJECT REPORT

Submitted for partial fulfillment of undergraduate degree in Microbiology



SESSION – 2021 - 2022

SRI SATHYA SAI COLLEGE FOR WOMEN, BHOPAL

DATE: 20/12/2021 - 10/02/2022

SUBMITTED BY- ANJANI DUBEY

B.Sc. I year Microbiology

GUIDED BY

Dr. SHIKHA MANDLOI
Asst. Prof. Microbiology

Dr. NISHI YADAV
Asst. Prof. Microbiology

DECLARATION

I hereby declare that I have completed my project work (December 20th to February 10th) on the topic "Investigation of Water sample for presence of *E. coli*". This field project is my original work in which the published and unpublished work has been duly acknowledged and used. I also declare that no part of the project has been presented / submitted for any other degree or diploma in any other College or University.

Signature with date - Anjani

Name of student - Ku. Anjani Dubey

Class - B.Sc. I Year

APPROVAL LETTER

This is to certified that the present field report has been completed under my guidance. This has been presented in Department of Microbiology, Sri Sathya Sai College for Women, Bhopal after my approval.

Dr. SHIKHA MANDLOI
Asst. Prof. Microbiology

Date 16.4.22

Place Bhopal.

Dr. NISHI YADAV
Asst. Prof. Microbiology

Date: / / Page No.:

ACKNOWLEDGEMENT

The path of this research has become beautiful due to grace of almighty and good wishes of many people who have been with me along the way.
My heart felt gratitudes are due to of them. It is a matte of profound priviledge to pay my sincere and heartfelt thanks and gratitude to our principal Dr. Asha Agarwal for always motivating me.
We cherish an inexplicable sense of indebtness and my heartfelt gratitude to our Head, Department of Botany an Microbiology Dr. Renu Mishra mam for allowing me to do this study.
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We would like to thanks our lab technicians Seema Sahay and Sushma Upadhyay for giving me a conductin atmosphere to work.
At last we thank our lab attender Mrs. Thangum for always being there for us.
Last but not the least I thank my whole team memb Aastha Priya, Mahima Rajhansa, Shreshta Singh and Swastika Chaturvedi for being such a great research team. Our team work and hardwork has made this research work accomplished.

ॐ श्री साई राम OM SRI SAI RAM

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CERTIFICATE

This is to certify that *Ms. Anjani Dubey* student of B.Sc. I year Microbiology from Sri Sathya Sai College for Women, Bhopal has completed the field project on the topic entitled "Investigation of Water sample for presence of *E. coli* ". This work was carried out from 20 December 2021 to 10 February 2022 from this institute.

Ms. Anjani Dubey is hard working, dedicated and result oriented. She has done excellent work in institute during project period. We wish her for bright future.

Place- Bhopal

Date- 16.4.22

16.4.22

Institute Seal
SRI SATHYA SAI COLLEGE
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INTRODUCTION

Water covers over 71% of the earth's surface and it is a very important natural resources for people. Yet only 2.5% of the earth's water is fresh and thus suitable for consumption. Not only that but out of 2.5% more than 2/3rd is locked away in glaciers and not particularly able to ~~Keep~~ help meet the growing demands of society.

The increase in population coupled with unplanned urbanization and industrialization has resulted a large damage and deterioration in drinking water supply for human everyday needs.

The mismanagement of wastes and sewages deteriorates the water quality of area. Water pollution affects drinking waters, rivers, lakes and oceans all over the world, which consequently harms human health and the natural environment. Water pollution may not cause immediate effect on the health of individual but can prove fatal in the long run. Microbial pollutants like colliforms which include E. coli, Enterobacter proteus, etc., which often results in the infectious diseases like chlorella, typhoid and diarrhoea.

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LITERATURE REVIEW

To carry out the study on bore water problems following literature review was done.

In a study on bacteriological contamination of drinking water was rejected in Aligarh to investigate the possible contamination of ground water by the percolation of waste water. water samples from hand pumps near major lagoons and drains were analysed using standard methods. The results showed the presence of E.coli above the permissible limit by several times in drinking water. (Ayub et al ; 2011)

In an another study from the bores of Bangalore, a total coliform of 4231 bore water samples were analysed using standard methods for sewage contamination statistical analysis for the results indicated that 43.48% of bores were contaminated with coliform. The report concluded that the water from bores walls was not safe for human use. (Alias et al ; 2015)

In another study, MP techniques were used for the analysis of bacteriological contamination of ground water of Gehana by inspecting the water samples taken from 8 bore holes. It was indicated that 25% concentration of Escherichia coli was present in both wells and bore holes. Thus, the studies revealed some level of contamination in sampled water which raised question about its

sustainability (Kasalku et al; 2018)
In yet another study done by water quality where coliform count and physio-chemical parameter were studied from Sikkim. The methods used was membrane filtration method for microbial tuting. Microbial confirmatory testing indicated severe faecal contamination showing the presence of E. coli and Enterococcus with high counts. (Singh et al; 2019).

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MEDIA

* **MEDIA:** While carrying out our experiment we use various types of media for growth of the E.coli which were EMB and NAM.

1. **EMB:-** Eosin Methylene Blue

Composition per 1000 ml

- Pectic digest of animal tissue - 10.0 g
- Dipotassium phosphate - 2.0 g
- Lactose - 5.0 g
- Sucrose - 5.0 g
- Eosin - 0.4 g
- Methylene Blue - 0.065 g
- Agar-Agar - 13.5 g

We prepared 250 ml of EMB media on which 9 g of pre-prepared EMB powder was used.

* **PROCEDURE:-**

1. We weighed and suspended 9 g of dehydrated media in 250 ml distilled water.
2. Mixed until the suspension is uniform and heat to boiling to dissolve the medium completely.
3. Sterilised the prepared media by autoclaving at 15 lb/inch pressure and at 121°C temprature for 15 minutes.
4. Cooled to 40-45° C and with frequent gentle swirling, pour media into sterile petri dishes.

5. Labelled with initials of the name of medium, date of preparation and stored the plates upside down (lids below) in the refrigerator until used.

2. NAM:- Nutrient Agar Media

Composition per 1000 ml

- Peptone - 5g
- Distilled water - 1000 ml
- NaCl - 5g
- Beef extract - 3g
- Agar-Agar - 20g

Since we prepared 500 ml of NAM media as per our requirement for which following compositions were used

- Peptone - 2.5 g
- Distilled water - 500 ml
- NaCl - 2.5g
- Beef extract - 1.5 g
- Agar-Agar - 10g

* PROCEDURE:-

1. Chemical ingredients of NAM was accurately measured.
2. All the chemical except agar were transferred to a beaker containing 500 ml distilled water.
3. pH of this solution was adjusted to 7.2 by using either HCl or NaOH.
4. For making the medium, agar-agar was added to the solution of distilled water was added gradually to make volume 1 litre.

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5. The content were gently heated with slight agitation to dissolve the ingredients.
6. The medium was autoclaved at 121°C and at 15 lb/inch^2 pressure for about 15-20 minutes.
7. And after that media was ready for making NAM plates.

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BROTH

* BROTH:- In our experiment we used two broths namely Lactose Broth and Brilliant Green Broth.

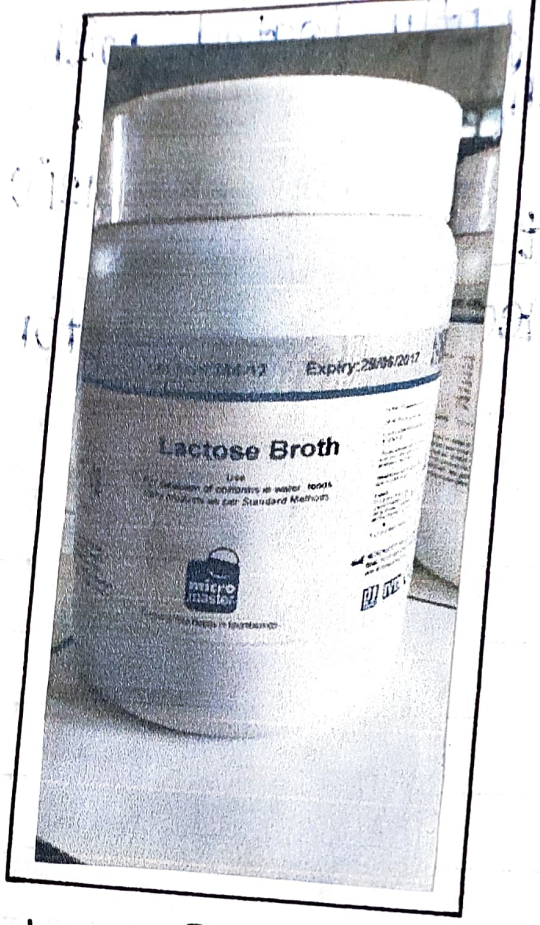
1. Lactose Broth :-

Composition

- Lactose - 5.0 g
- Beef extract - 3.0 g
- Peptide digest of animal tissue - 5.0 g
- Distilled water - 1000 ml

* PREPARATION:-

1. We used pre-prepared powder of lactose broth.
2. We prepared two concentrations of broth that is 1x and 2x in 300 ml and 150 ml of distilled water respectively.
3. In 1000 ml of distilled water, as instructed in description, 13 g of Lactose broth was to be used.
4. We prepared 300 ml solution (1x) in which 3.9 g of Lactose broth powder was used.
5. The weighed lactose broth of 3.9 was again added but this time for 2x concentration in 150 ml distilled water.
6. The required lactose broth was weighed on electronic balance and mixed in required amount of water and autoclaved.



Lactose Broth Powder

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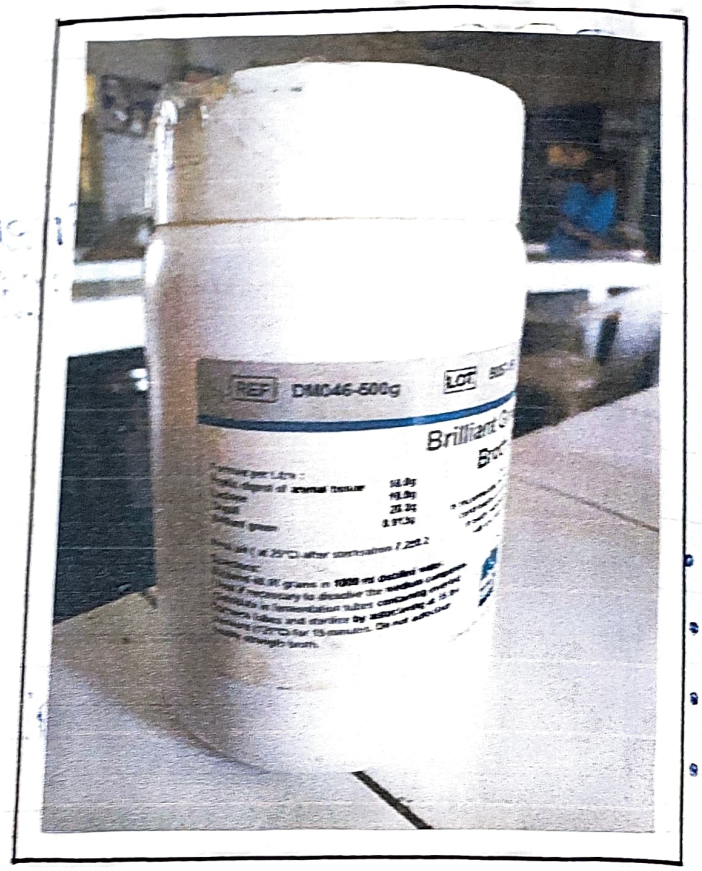
2. Brilliant Green Broth:-

Composition

- Peptic digest of animal tissue - 10.0 g
- Lactose - 10.0 g
- Oxgase - 20.0 g
- Brilliant green - 0.013 g
- Distilled water - 1000 ml

* PREPARATION:-

1. We use pre-prepared powder of Brilliant green broth.
2. We prepared two concentration i.e., 1x and 2x in 300 m and 150 ml of distilled water respectively for 1x concentration.
3. For 2x concentration, 12 g of Brilliant green ~~broth~~ powder was used.
4. The weighed Brilliant green broth powder was measure on electronic balance and mixed in required amount of water and autoclaved.



Brilliant Green Broth Powder

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INSTRUMENTS

* Instruments play very crucial role in our experiment to complete. They increase standardization used to explore the internal structure and to some extent more rapid processing of specimens and reporting of results.

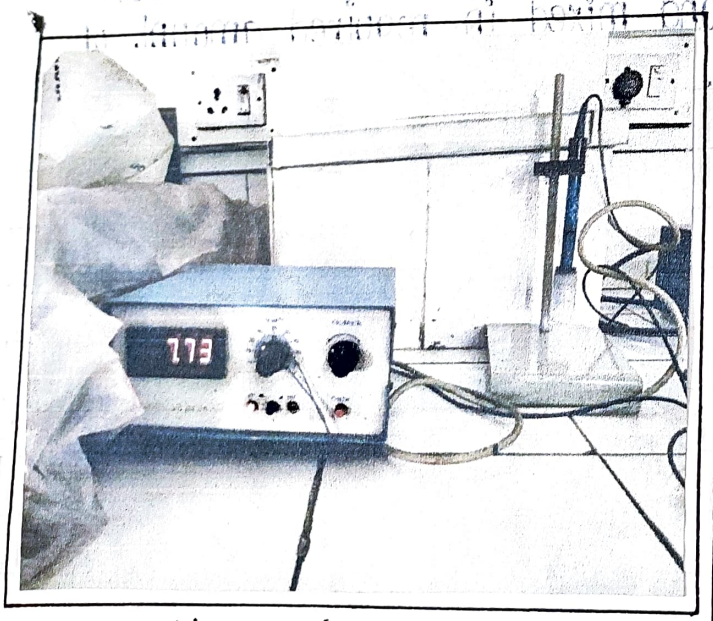
• Instruments that we used included the following :-

1. Electronic Balance :- Electronic balance is an instrument used in the accurate measurement of weight of material. It is a significant instrument for the laboratories for precise measurement of chemicals which are used in various experiments in laboratory. Electronic balance provides digital results of measurement.

2. pH meter :- A pH meter is a precise instrument that measures the H^+ ion movement in water based suspension, showing its acidity and alkanity expressed as pH. It is also called as "potentiometric" pH meter" because it measures variation in electrical potential between a pH electrode and a reference electrode.

3. Autoclave :- An autoclave is a pressure chamber used to carry out industrial process requiring elevated temprature and pressure different from ambient air pressure. Autoclave are used in medical application

Electronic Balance



pH meter

Autoclave

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METHODS

(1) Collection of Water Sample :-

Collection of water samples from three different taps of the college was done. These samples were well labelled and refrigerated.

- Water sample 1 was taken from Tap-1 on the first floor near the IT cell.
- Water sample 2 was taken from Tap-2 near scooter stand.
- Water sample 3 was taken from Tap-3 near the library.

(2) Determination of MPN count -

Step 1 : Presumptive Coliform Test :-

The presumptive coliform test is used to detect coliforms in water sample. In this test lactose fermentation tubes were inoculated with different water volumes and production of acid and gas from the fermentation of lactose in any of the tubes in a presumptive evidence of coliforms in the water sample. The lactose broth used in this test is selective for the isolation of coliform because of the addition of bile and sulphate or brilliant green. A pH indicator such as Bromocresol purple is also added to lactose broth for the detection of acid. The colour of the indicator changes to yellow with the protection of acid from lactose.

← Collection of water sample from Tap-1

Collection of water → sample from Tap-2

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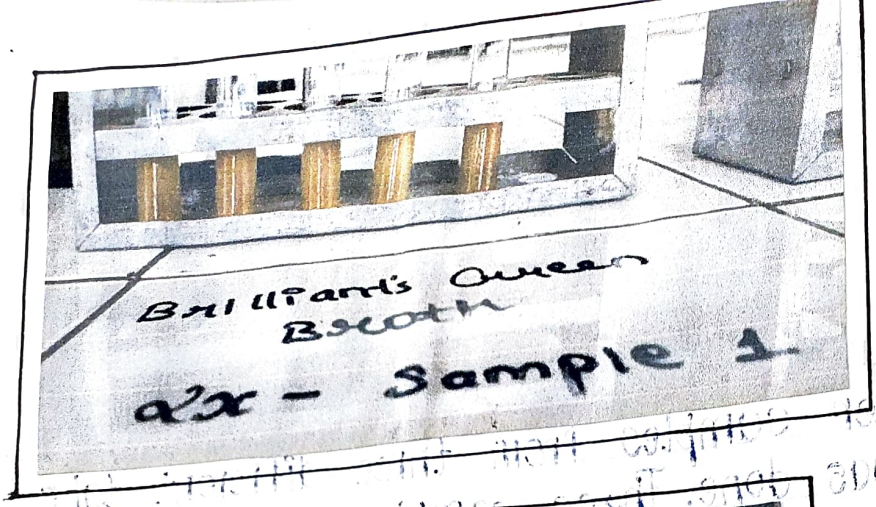
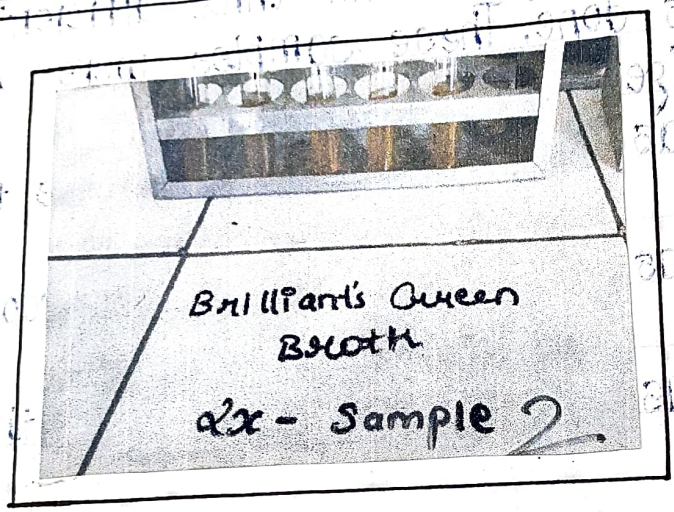
Step 2: Confirmed Coliform Test :-

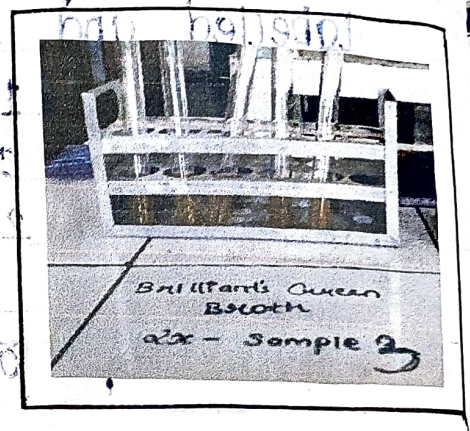
This test used to confirm the presence of coliforms and to determine the MPN value in water samples showing positive or doubtful presumptive. In the confirmed test, water samples from all the positive presumptive lactose broth tubes were innoculated into tubes of brilliant green lactose bile broth and incubated at 35°C for 48 hrs. Positive confirmed tubes were used to determine MPN. A statistical method is used to estimate the population of coliforms, which means that the result obtained is expressed as the most probable number (MPN) of coliforms. A count of number of lactose fermentation tubes / brilliant green lactose bile broth showing production of gas following the incubation period was taken and MPN was found by matching the results with those provided in the statistical table.

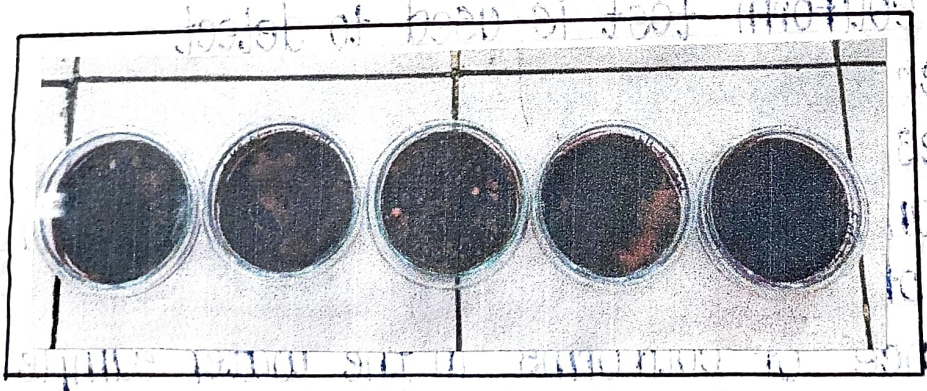
Step 3: Completed coliforms test :-

It is used to establish the presence of coliform bacteria and as a confirmatory test for the presence of E.coli in a water sample. In the completed test, the samples from the positive brilliant green lactose bile broth from the confirmed test were streaked onto a selective differential medium for coliforms and innoculated into eosin methylene blue plates as well as on nutrient agar plate to perform Gram-staining. The medium commonly used is EMB that is selective in nature because of the dye methylene blue which

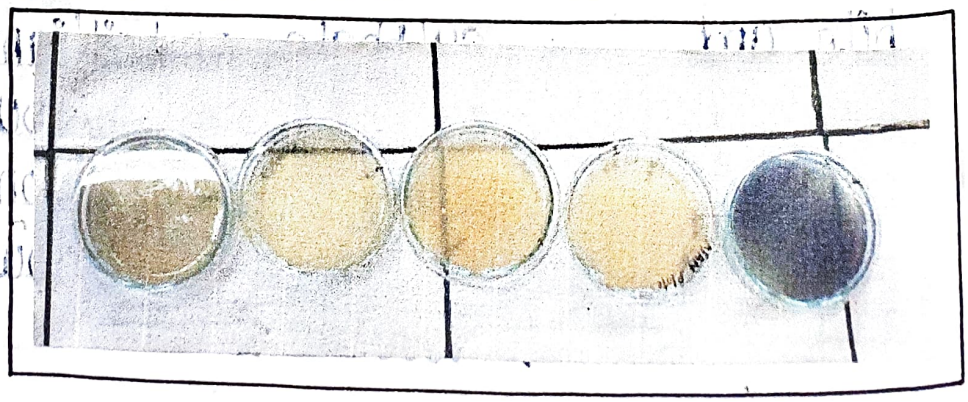








EMB plates



NAM plates

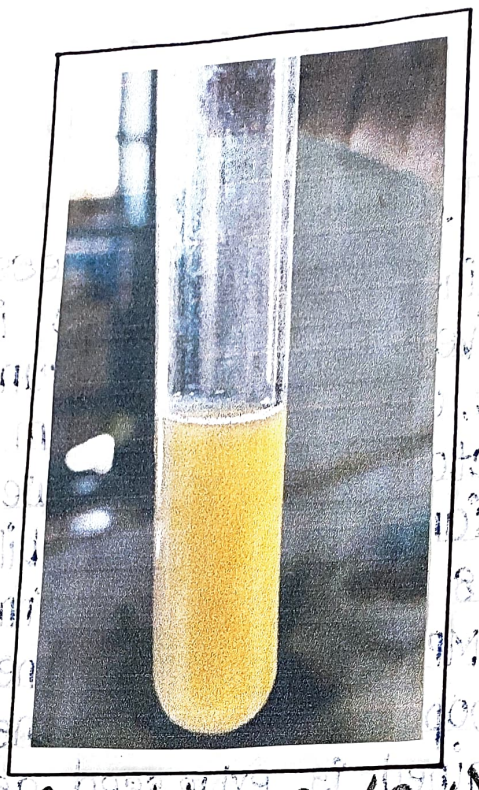
inhibits the growth of gram positive bacteria, allowing the growth of gram negative bacteria. EMB is differential in nature, in that lactose fermenting bacteria gave coloured colonies due to formation of a complex in EMB that precipetated out onto the coliforms colonies. Non-lactose fermentors produce colourless colonies on EMB agar plates, of acid and gas in the innoculated lactose broth showed gram negative reaction confirmed the presence of E.coli in the water sample and thus was considered a positive completed test.

(3) Isolation of E.coli on NAM and EMB:
The tubes of Brilliant green broth were taken. The NAM and EMB plates were inoculated from them. These plates were then incubated and observation were taken.

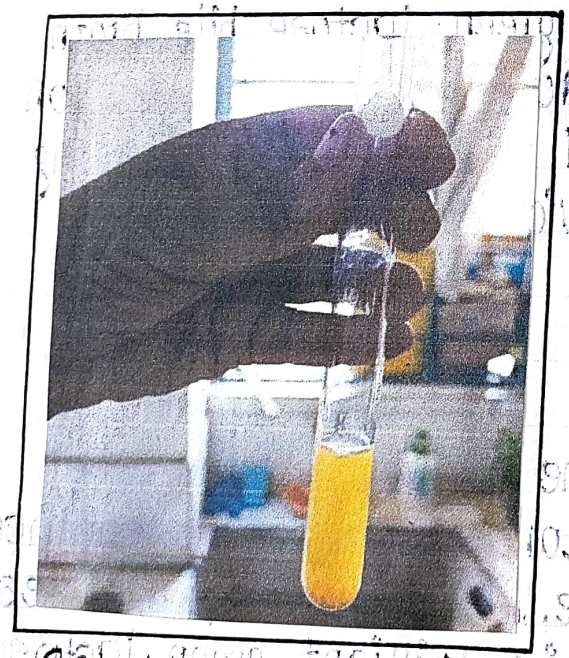
(4) Staining of colony and microscopic identification: Colonies obtained on NAM were stained by Gram's stain and then observed for the presence of Gram negative rods.

(5) Determination of physio-chemical parameters: Parameters like odour, smell, temprature, transparency, turbidity, pH, etc was ruled by standard methods.

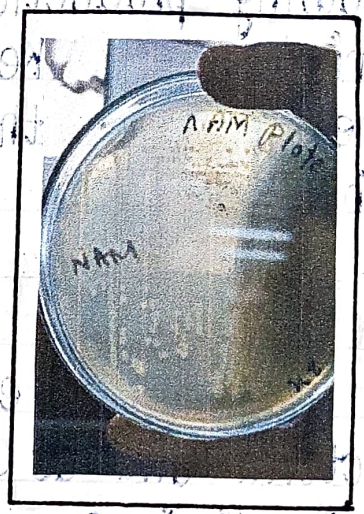
Sample-1 (2x)



Sample-2 (2x)



Sample-3 (2x)



Streaked NAM

PARAMETERS	SAMPLE-1	SAMPLE-2	SAMPLE-3
Colour	colourless	colourless	colourless
Opacity	nil	nil	nil
pH	~6.8	~6.7	~6.9
Odour	odourless	odourless	odourless
Temprature	~38°C	~36.5°C	~37°C

RESULT AND ANALYSIS

S.NO.	SAMPLES	2α	1α	0.1α
1.	Tap-1	2	2	1
2.	Tap-2	3	2	0
3.	Tap-3	3	2	2

Observation of test tube containing Brilliant green broth with air bubbles.

On analysing the above table it was concluded that the tap-1 water, tap-2 water and tap-3 water have MPN count per 100 ml of water sample as 7, 13 and 16 respectively.
All the three samples were inoculated on EMB and NAM plates using streak plate method. Out of which the EMB plate containing tap-3 water sample gave the metallic

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pH	~6.8	~6.7	~6.9
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Temprature	~38°C	~36.5°C	~37°C

RESULT AND ANALYSIS

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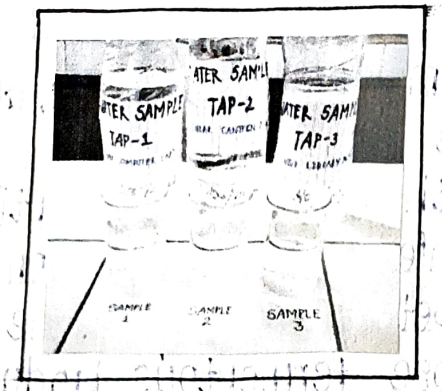

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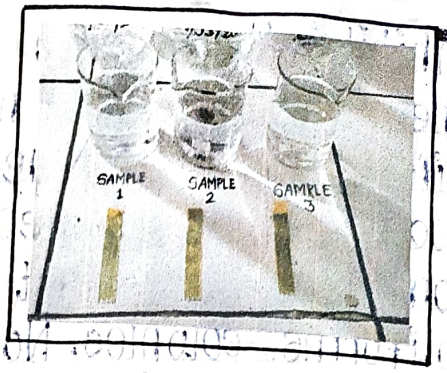
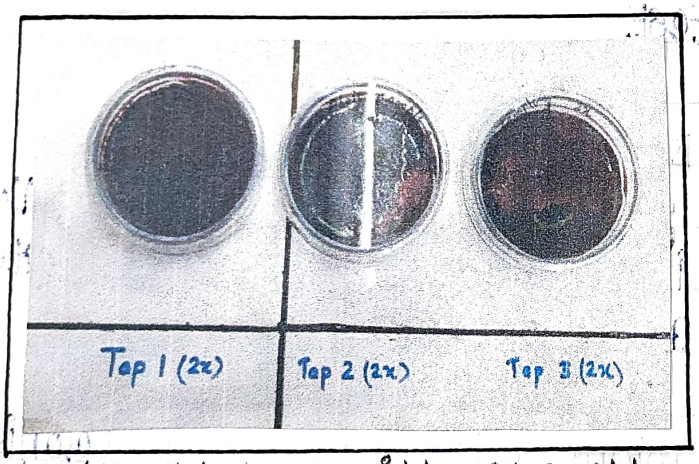
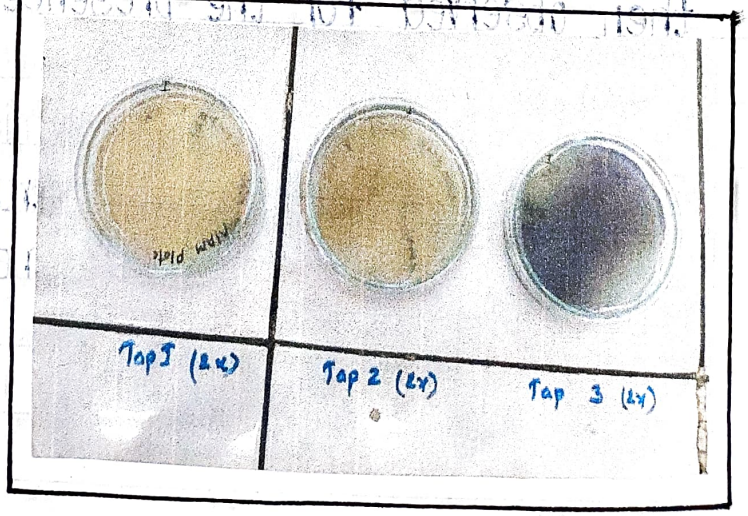


pH of 3 samples



EMB plate with growth



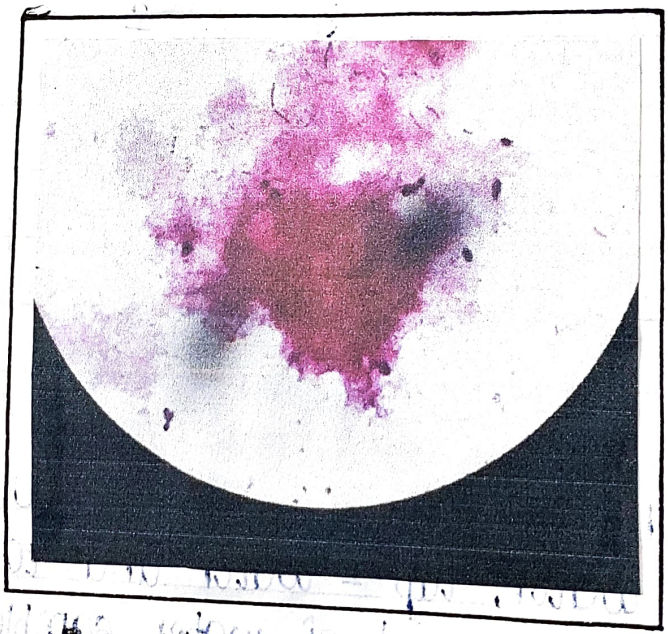
NAM plate without growth

green appearance. This colony showing of the metallic green was Gram stained under the microscope. It showed as pink, rod-shaped confirming it to be E. coli. Since, all the readings were under the permisible limit which is considered to be below the 50 MPN count, hence it is safe for drinking purpose and therefore water is potable.

However from the results of the study done by Kasalku et al 2018. It was concluded that on analysing water samples from boreholes, 25% concentration of the E. coli was found in the area of Gehana. In yet another study from Sikkhim by Singh et al in 2019. Water from four districts was revealed with traces of toxic heavy metals. Microbial confirmatory testing indicated severe faecal contamination of water courses with high counts of total coliforms, E. coli and Entemcoccus.

In another study on E. coli, E. coli as an indicator of contamination and risk in envison. Mental water was done by Price et al in 2017. A study by Giri et al; in year 2020 gave the result regarding bacteriological community revealed the presence of total coliforms in large numbers in all sampling stations which reflected environmental contamination and made water non-potable.

EMB plate with E.coli growth



E.coli under microscope

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CHALLENGES FACED DURING WORKING

(1) Electricity cut off at times disturbed the planning of work.

(2) To examine the potability of water MPN count is effective for examination of bacteria but does not provide reliable results for the enumeration of fungi.

(3) Water samples that were collected need to be stored at low temprature and should be tested as early possible.

(4) To complete the targeted work for one day the team needs to work in well co-ordinated and planned way. so that work could be finished in time. otherwise the work and practical both get delayed.

CONCLUSION

In this study water samples tested have been found to be safe for drinking. Since the E.coli count has been found to be below 50 value. However according to WHO guidelines that bacterial coliforms are not to be seen in any 100 ml of water for domestic consumption. This implies that though coliforms bacteria be in small quantity the water is not wholely safe for drinking purpose. Therefore it is recommended that their water quality should be checked routinely. Also any leak from sewage pipes should be properly repaired to prevent contamination of drinking water.

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RECOMMENDATION

On the basis of above conclusion it is recommended that this work should be repeated once more to get more authentic results and hence presence of E.coli could be detected.

The tap-1 that is connected with concern, its R.O. should also be listed for proper working.

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