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Molecular characterization of fluorine degrading bacteria from various sampling station water samples for its industrial exploitation

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ABSTRACT

The object of the present study is to carry out the Physico–Chemical analysis of well and bore well water samples from ten sampling stations .They are Hyderabad, Vijayawada, Guntur, Tadepalli, Chirala, Bapatla, Srisailam, Mahanandi, Yaganti and Guntakal (Rural area) for a period of 3 months from December 2015 to April 2016. The analysis of different parameters namely-temperature, pH, color, chlorine and estimation of bacteria in different places water samples were carried out as per standard methods. The results were compared with the values stipulated by WHO and ICMR standards. The results indicate that the chloride in some sampling stations was found above the permissible limits probably due to contamination with sea water.

Keywords: Temperature, pH, color, chlorine and bacterial content.

INTRODUCTION

Water is a transparent fluid and is the major constituent of the fluid of organisms. It is a natural resource vital for the survival of humanity and all species on earth. Chlorine was first used in drinking water to reduce waterborne infectious diseases. It occurs in combined form because of it's highly reactiveness. The chlorine gas is greenish-yellow in color and combines readily with nearly all other elements. The Chlorine gas is two and one half times as heavy as air, has an intensely disagreeable suffocating odor, and is exceedingly poisonous. In its liquid and solid form it is a powerful oxidizing, bleaching, and disinfecting agent. Chlorine is widely used in manufacture of many products directly and indirectly i.e., in paper product production, antiseptic, food, insecticides, paints, petroleum products, medicines, textiles, solvents etc. Chlorine causes environmental harm at low levels .It is especially harmful to organisms living in water and in soil. Breathing small amounts of chlorine for short periods of time adversely affects the human respiratory system. Along with chlorine the Bacteria beyond the levels present in the water sample is also harm i.e., for drinking it is not safe to us. The aim of the present study is to determine the amount of chlorine content and Bacteria in the water samples which are collected from ten different sampling stations and also the optimum values of the parameters like Temperature, pH, colour and chlorine.

MATERIALS AND METHODS

Estimation of chlorine content in water from different sampling stations:
Materials:
Water samples, potassium dichromate indicator, silver nitrate (0.0282N), sodium chloride, conical flask, pipette, burette.

Reagents:
Potassium dichromate indicator, 0.0282N $AgNo_3$, sodium chloride

PREPARATION OF REAGENTS
STANDARDIZED CHLORINE SOLUTION
- Weigh 1.648gms of sodium chloride.
- Transfer the content to the beaker containing 100ml distilled water and mix the content well.

PREPARATION OF THE SILVER NITRATE ($AgNo_3$)
- Initially take the beaker and clean it.
- Weigh 4.791gms of silver nitrate and add it to the beaker.
- Fill the beaker with 100ml distilled water and mix the content well.
- Standardized it against 0.0282Nacl solution.

PREPARATION OF POTASSIUM DICHROMATE INDICATOR:
- Weigh 25gms of potassium dichromate.
- Transfer it to the beaker contains distilled water.
- Add few drops of $AgNo_3$ solution, until slight red precipitate is formed.
- Allow it to stand for 12hrs.
- After 12hrs filter the solution, using the filter paper and dilute the filtrate to 1000ml using distilled water.

METHODS
BLANK TITRATION
- Take 20ml of distilled water in a clean 250ml conical flask.
- Add 1ml of potassium dichromate indicator to get light yellow color.
- Titrate the distilled water with indicator against silver nitrate solution until color changes from yellow color to brick red color.
- Note the volume of silver nitrate added for distilled water.

TESTING OF WATER SAMPLE
- Before starting the titration, rinse the burette with $AgNo_3$ solution.
- Fill the burette with $AgNo_3$ solution of 0.0282N.
- Adjust to zero and fix the burette in stand.
- Take 20ml of sample in a clean 250ml conical flask.
- Add 1ml of potassium dichromate indicator to get light yellow color.
- Titrate the sample against silver nitrate solution until color changes from yellow to brick red color.
- Note the volume of silver nitrate added for distilled water.

Estimation of Bacterial content on water from different sampling stations
For the estimation of the bacterial content the following methods are performed.
- Qualitative analysis of water from different sampling stations
- Isolation of microorganisms from water
- Streak plate method
- Gram staining
- Culturing of Bacteria in LB broth

Qualitative analysis of water from different sampling stations
Materials
Test tubes, Measuring cylinder, Durham tube, Lactose Fermentation Broth, water samples.
Preparation of the Lactose Fermentation Broth:
- Initially weigh 1.3gms of lactose and add 100ml of distilled water to the conical flask.
- Mix the content well.
- Later keep the lactose fermentation broth in autoclave at 121^0 c for 30minutes for sterilization.

Method
- Initially 10ml of lactose fermentation broth is taken into a sterilized test tube .
- Add 1ml of water sample to it and mix the content well.
- Place the Durham tube into test tube in an inverted manner, without any air bubbles.
- Later cover the test tube with cotton plug or foil and incubate it.
- After 24 hours a bubble is formed in the durhan tube, then it indicates presence of E coli bacteria.
- If bubble is not formed, it indicates the absence of coliform bacteria.

Isolation of microorganisms from water
Serial dilution technique is used here for the isolation of pure culture from water.

Materials
Test tubes, pipette, Distilled water, nutrient broth and water samples.

Method
- Take 6 test tubes and sterilize them in autoclave at 121^0c for 30min.
- Fill each with 10ml of nutrient broth.
- Make each test tube in decreasing power (10,.).
- Fill the first tube with 1ml of sample and mix well.
- Now, from the 1st test tube, extract 1ml of sample and add to the 2nd test tube.
- Similarly, add 1ml of sample from 2nd tube to the 3rd repeat till the last tube.
- Now add 1ml of the water sample to the 6th test tube and incubate it for 24 hours.

Streak plate method
Materials
24 - 48 hrs. Incubated nutrient both (mixed culture), nutrient agar medium, spirit lamp, inoculating loop, 95% ethanol, petri plates.
Preparation of nutrient agar media:
- 1st nutrient agar is weighed and it is dissolved in 100 ml of distilled water and autoclaved for 30min at 121^0c.
- Later nutrient agar is poured into the sterilized petri plates and allows it to solidify for about 10mins.
- After that streaking is done on the solidified agar plate.

Method
- Initially sterilize the inoculation loop with the help of spirit lamp.
- Later collect the sample with the help of inoculation loop.
- Now placing the inoculation loop in the angle of 45^0C and streak the water sample on the agar plate in zigzag manner.
- Now place the agar plates in incubator.
- After 24 hours we will find the colonies of microorganisms on the incubated agar plates.

Gram staining
It is done to difference between two principle groups of Bacteria (gram positive and gram negative).

Materials
24 hrs. Inoculated culture, glass slide, inoculation loop, spirit lamp, crystal violet, iodine, 95% ethanol, saffron and also microscope.

Method
- Take a slide, wash it and mark an area.
- Sterilize the inoculation loop under spirit lamp.
- Collect the culture with the help of inoculation loop then, spread culture on slide by smear preparation technique.
- Follow with heat fixation.
- Stain it with crystal violet as primer strain.
- After a minute add iodine, mordant.
- After 30 seconds add decolorizing agent, alcohol.
- Later add the counter strain-saffron, and wait.
- Now wash the slide and observe under microscope.
- If the bacteria appears blue in color, it is gram positive, or if it is present in pink color, it is gram negative.

Culturing of Bacteria in LB broth
Materials
Conical flasks, inoculation loop, spirit lamp, culture, LB media and organic compounds such as Aniline, Benzene, Toluene, Acetone.

Method
- Take 5 conical flasks of 250ml and clean them.

- Later take 10 ml of LB media in each conical flask. Among them keep one conical flack with LB media as control.
- To those add 1ml of organic compound in each conical flask.
- After that sterile the inoculation loop with the help of spirit lamp.
- Now take a loop full colony of water sample from the nutrient agar plate and add it to LB media in the conical flask.
- Similarly repeat the above 2 steps for remaining conical flasks except the fifth conical flask and consider it as a control.
- Sterile the inoculation loop with the help of spirit lamp.
- Now take a loop full of sample (colonies) from the nutrient agar plate and add in the lb media in the each of the conical flask.
- Add 1ml of the organic compound in each conical flask in 4 different samples.
- Now place the cotton plug for the samples and incubate then in orbital shaker at 120rpm at 37^0c for 1 week.

RESULTS AND DISCUSSION

TEMPERATURE

A rise in temperature of water leads to the speeding up of chemical reactions in water, reduces the solubility of gases and amplifies the tastes and odours. The average temperature of the present study ranged from 27.85 - 31.94^0 C.

It is known that p^H of water (7.5 to 9.9) does not has no direct effect on health. But lower value below 5.0 produce sore taste and has higher value above 8.9 are of alkaline taste. The p^H values of the present investigation were within the ICMR standards (7.0 – 8.9). Conductivity varies with the season as well as ions present in water.

CHLORIDE

Chloride happens in a wide range of common waters. The high grouping of chloride is thought to be an indication of contamination by sewage misuse of creature starting point. Businesses are likewise critical wellsprings of chloride in water. Chloride values got in the study are observed to be higher (212.4 mg/lit) in S1 inspecting station than different stations.

Table1: Physico–chemical properties of the water samples collected from different sampling stations

S.No	Sampling Station	Temperature (^{0}C)	Colour	pH	Chlorine(mg/lit)
1	Hyderabad	29	Colorless	6.99	18.35
2	Vijayawada	28	Colourless	7.16	168.51
3	Guntur	30	Colourless	6.81	161.84
4	Tadepalli	28	Colourless	7.0	133.48
5	Chirala	32	Colourless	6.9	0
6	Bapatla	30	Colourless	7.06	0
7	Srisailam	34	Colourless	7.14	118.46
8	Mahanandi	27	Colourless	6.95	130.14
9	Yaganti	28	Colourless	6.05	28.03
10	Guntakal	28	Colourless	6.98	76.751

From the above table1, we observed that the water sample available from Vijayawada has more chlorine i.e. 168.51 mg/ml and the water sample from chirala and bapatla has least chlorine i.e.0mg/ml along with this parameters, we observed the color and we found that all the water which have been considered in the experiment are colorless and also the water sample available from srisailam has more temperature i.e.34^0c and the water sample from Mahanadi has least temperature i.e.27^0c.

Estimating the Bacterial content for various water samples
Qualitative analysis of water in various water samples

Figure 1: Bubble formed in the Durham tube

Figure 1 indicates that bubble is formed in the Durham tube so the bacteria present in the various places water samples and the type of bacteria determined with the help of streak plate method.

Streak plate method:

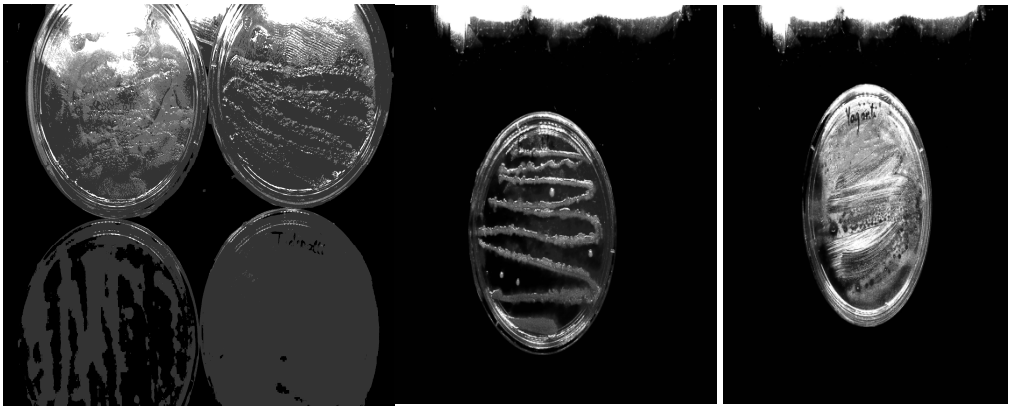

Figure 2: Bacterial colonies in a various places water samples

Figure 2 showed that mixed cultures in the form of bacterial colonies from various places water samples

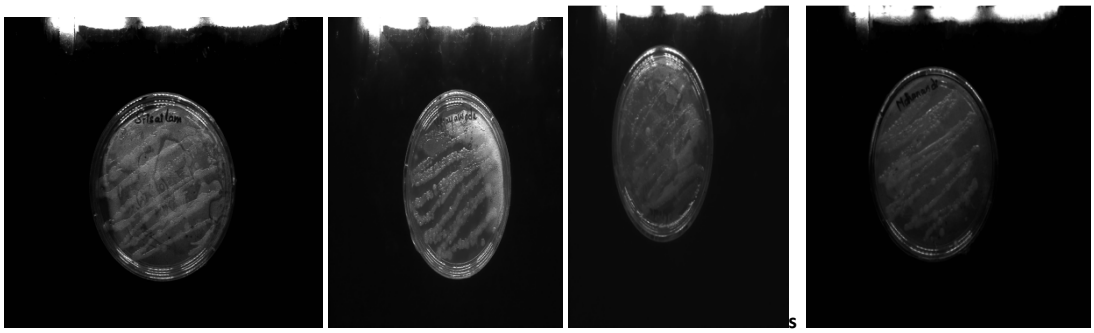

Fig3: Represents the Bacterial colonies are formed.

From the above fig. colonies of bacteria are formed i.e., from the water samples of different sampling stations micro organisms are isolated.

Gram Staining:
By performing Gram staining the resultant Bacteria obtained is Gram negative (-ve) i.e., in pink color and its morphology is observed under the microscope and identified it as Bacilli (rod shaped).

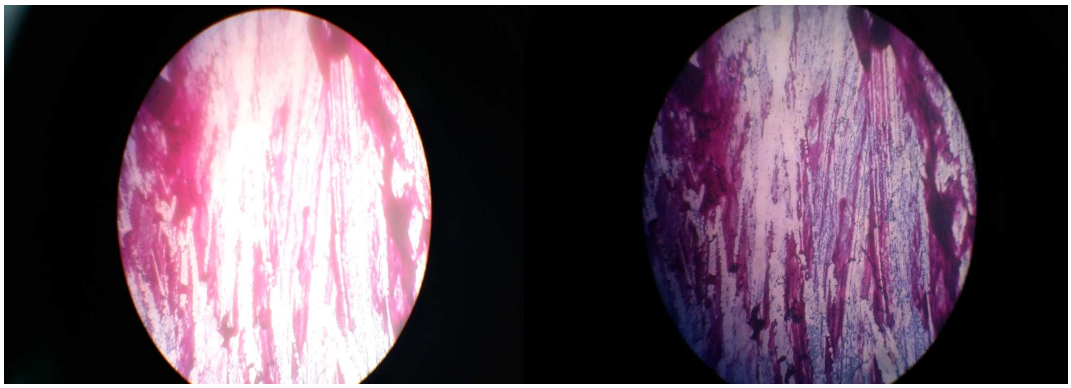

Figure 4: Represents the rod shaped bacteria bacilli which are in pink color

The obtained result from the above fig. represents that the nature of bacteria is gram negative, pinkish in color, Bacilli i.e., is rod shape.

Colony morphology:

Table 2: Colony morphology and various biochemical testing methods of different places water samples

S.No	Sampling Stations	Gram nature	Color	Bacteria shape
1	Hyderabad	Gram negative	Pink	Bacilli(rod shape)
2	Vijayawada	Gram negative	Pink	Bacilli(rod shape)
3	Guntur	Gram negative	Pink	Bacilli(rod shape)
4	Chirala	Gram negative	Pink	Bacilli(rod shape)
5	Bapatla	Gram negative	Pink	Bacilli(rod shape)
6	Tadepalli	Gram negative	Pink	Bacilli(rod shape)
7	Srisailam	Gram negative	Pink	Bacilli(rod shape)
8	Mahanandi	Gram negative	Pink	Bacilli(rod shape)
9	Yaganti	Gram negative	Pink	Bacilli(rod shape)
10	Guntakal	Gram negative	Pink	Bacilli(rod shape)

From the above table2, we observed that the gram nature of the bacterial colonies present in the water samples is gram negative because it is pinkish in colour and also the shape of bacteria i.e. bacilli (rod shaped) are obtained through gram staining from the water samples of ten sampling stations.

Culturing of Bacteria in LB broth:

Figure 5: Represents culturing of Bacteria for various sampling station water samples in LB broth with organic compounds

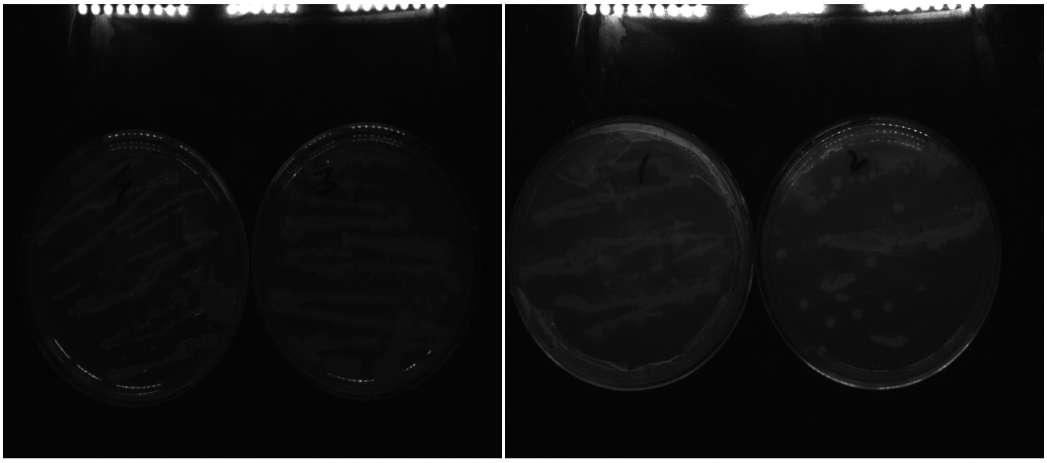

Figure 6: Showed that the colonies of bacteria formed

Figure 6 shows that the bacteria present in the various places water samples.

CONCLUSION

From the above experiments we conclude that the water sample available from Vijayawada has more chlorine i.e. 168.51 mg/ml and the water sample from chirala and bapatla has least chlorine i.e.0mg/ml along with chlorine level in water we found out a presence of colonies of bacteria in water that is Bacilli (rod shaped) which is pinkish in color with gram negative nature.

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