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PHYSICOCHEMICAL AND MICROBIOLOGICAL ANALYSIS OF POTABLE WATER FROM DIFFERENT REGIONS OF AURANGABAD

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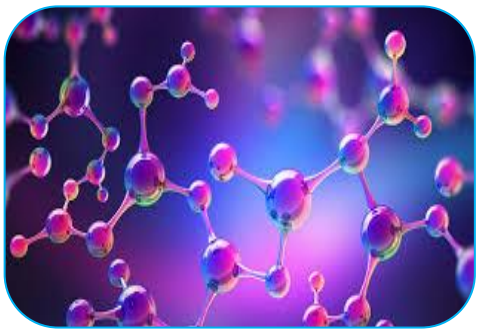
ABSTRACT



The Godavari River, commonly known as the Vriddh (Old) Ganga or the Dakshin (South) Ganga, is India's largest river among all peninsular rivers. Regrettably, pollution levels in this river have been rapidly increasing to unsafe levels. The residents of Aurangabad depend on the water from the Godavari River for household, industrial, and agricultural use. Access to clean and safe drinking water is crucial for survival, but many individuals in India lack it and suffer from waterborne bacterial infections, resulting in numerous fatalities. The most prevalent bacterial diseases transmitted through water include cholera, typhoid fever, and bacillary dysentery. Water samples from various areas of Aurangabad city were analyzed to evaluate the suitability of the water for drinking purposes. The analysis involved the examination of physical and chemical parameters such as TDS, turbidity, and pH, as well as microbiological assessments including MPN, SPC, and IMViC to detect the presence of Escherichia coli, an indicator of fecal contamination. The findings from the sampling sites suggest that the drinking water used within households is considerably more contaminated.

KEY WORDS: *Bore well, Physico-chemical analysis TDS, pH, MPN, SPC, IMViC.*

1. INTRODUCTION

Water pollution in India has now reached a point of crisis due to urbanization and rapid growth of industrialization. The entire array of life in water is affected due to pollution in water. The problem of water quality deterioration is mainly due to human activities such as disposal of dead bodies, discharge of industrial and sewage wastes and agricultural runoff which are major causes of ecological damage and pose serious health hazards to humans and other animals (Odigie J.O., 2014). Sufficient water sources are available in India. But many people in India do not have access to clean and safe drinking water and many die due to waterborne bacterial infections. Drinking water sources likely lakes and rivers are most important. Any change in water's physico-chemical characteristics alters its quality and disturbs its environment (Mohammad A.H., 2018).

Aurangabad city (19053'06.68' N and 750 19' 10.600E) and nearby villages receive drinking water from the Jayakwadi Dam constructed on the Godavari river. The Godavari River receives an enormous amount of domestic sewage and industrial waste with high physicochemical characteristics. The river is polluted due to the discharge of domestic sewage and industrial effluents from different parts of the Aurangabad district. This river water is purified in the water plants of the municipal corporation and then supplied to the public through water pipelines to their home for drinking

purposes (Patil S.S and Kaushik G.,2016). The U.S. Public Health Service's drinking water standards state that pollution is nothing but the presence of any foreign substance (organic, inorganic, radiological or biological) in water which tends to degrade its quality and influence hazardous effects on the ecosystem surrounding it. The quality of water is described by its physical, chemical and microbiological characteristics (Islam R.*et al*., 2017).

Water which is free from disease-producing microorganisms and chemical substances deleterious to health is called potable or drinking water. Contaminated water or. Waterborne disease outbreaks in affluent communities pose health risks associated with drinking water, either directly or indirectly, by human or animal excreta, (particularly faeces). If contamination is present also it includes carriers of communicable enteric disease (i.e. pathogenic microorganisms) that cause diseases in humans drinking or using such water in food preparation may result in new cases of infection (Lanrewaju A.A *et al*., 2022). The pathogenic agents involved include bacteria, viruses, and protozoa, which may cause diseases that vary from mild gastroenteritis to severe and sometimes fatal diarrhoea, dysentery, hepatitis, or typhoid fever. Many of these microorganisms are present throughout the world (Gebra C.P, 2015).

Microbiological analysis of Water is essential to determine its sanitary quality and its suitability for general drinking purposes. Indicator organisms (IOs), which reside in the gastrointestinal tracts of humans and animals, are used throughout the world to assess the microbiological safety of drinking water. Microorganisms can be used as indicator organisms for water analysis if it fulfils most of the following criteria. It should be present in abundant numbers. Isolation, identification and enumeration of indicator organisms should be easy. It should not grow or multiply but can survive longer in the water than other pathogens. Tests for the detection and enumeration of indicator organisms rather than pathogens are used. The coliform group of bacteria is used as an indicator organism for the suitability of water for domestic, industrial and other purposes (Some S.,2021). There are several such organisms belonging to the *Enterobacteriaceae* family. The finding of *Escherichia coli* or *Clostridium perfringens* and *Streptococcus faecalis* is sufficient evidence that the water sample is not safe for drinking purposes since enteric pathogens may be presumed present. The primary cause of groundwater contamination is the uncontrolled disposal of industrial and urban waste and the use of chemical substances (fertilizers, pesticides, herbicides) (Matte. G. *et al.,*2014).

Methods of differentiation of the coliform group included a presumptive test, confirmed test and completed test. Such differentiation of the coliform is generally considered of limited value in assessing drinking water quality because the presence of the coliform bacteria indicates that the water is unsafe for drinking purposes. In the presumptive test enumeration of coliforms by using multiple tube fermentation procedure as a most probable number (MPN) index was carried out to find out whether water contains lactose fermenting, gas-producing bacteria or not. This test is followed by a confirmed test. It is meant for differentiating coliforms of non-coliforms as well as Gram-negative bacteria & Gram-positive bacteria. In this test, EMB agar contains methylene blue which inhibits Gram-positive bacteria and confirms the presence of Gram-negative lactose fermenting bacteria (Hrudey S.E and Hrudey E. J., 2014).

Confirmation test further confirmed Gram-negative lactose fermenting non-spore-forming bacteria. IMViC test is carried out to investigate the presence of *Escherichia coli* or *Enterobacter aerogenes* (Patil S.S and Kaushik G.,2016). U.S. Environmental Protection Agency recommends the use of *Escherichia coli*, a member of the faecal coliform group, as an indicator organism for recreational waters in freshwater bodies .The standard plate count method is used for the enumeration of the bacterial colonies (Dietrich A.M and Burlingame G.A., 2015)

Total Dissolved Solids (often abbreviated TDS) is a measure of the combined content of all organic and inorganic substances, ionized or micro-granular (colloidal solution) suspended form. Total dissolved solids are normally discussed only for freshwater systems, as salinity comprises some of the ions constituting the definition of TDS (Kulinkinaa A.B *et al*., 2017). The principal application of TDS is in the study of water quality for streams, rivers and lakes, although TDS is not generally considered a

primary pollutant (e.g. it is not deemed to be associated with health effects) it is used as an indication of the presence of a broad array of chemical contaminants (Jingxi M *et al*.,2020)

Primary sources for TDS in receiving waters are agricultural and residential runoff, leaching of soil contamination and point source water pollution discharge from industrial or sewage treatment plants. TDS constituents are calcium, phosphates, nitrates, sodium, potassium and chloride[16]. The chemicals may be cations, anions, or molecules. More exotic and harmful elements of TDS are pesticides arising from the surface. Certain naturally occurring total dissolved solids arise from the weathering and dissolution of rocks and soils. The United States has established a secondary water quality standard of 500 mg/l to provide for the palatability of drinking water (Mohit S.A *et.al*., 2016)

Most people think of TDS as being an aesthetic factor. In a study by the World Health Organization, a panel of tasters came to the following conclusions about the preferred level of TDS in water:

TABLE 1 Taste of Water with Different TDS Concentrations:

Level of TDS (milligrams per litre)	Rating
Less than 300	Excellent
300 - 600	Good
600 - 900	Fair
900 - 1,200	Poor
Above 1,200	Unacceptable

www.who.int/water_sanitation_health/dwq/chemicals/tds.pdf

The objective of the present study is to identify microbiological quality of drinking water in Aurangabad.

2. MATERIAL AND METHODS

Sample collection

Ten Water samples of municipal water supply were selected from various regions of Aurangabad city. Samples were collected into 55ml sample bottles (Pre-sterilized at 121^{0}C for 15 minutes).

Microbiological analysis

Most probable number

This test is divided into three parts: Presumptive, Confirmed and Completed tests. In the presumptive test, 9 tubes of MacConkey's broth were prepared according to the size of the water sample i.e. 3 of 0.1 ml, 3 of 1 ml, 3 of 10 ml respectively. For a 10ml sample double strength, MacConkey's broth was prepared. Water samples were added 0.1ml in each tube in 1, 2, 3 and 1ml in each 4, 5, 6 and 10ml in each 7, 8, 9. Tubes were then incubated at 35^{0}C for 24 hours for gas production. MPN Index was analysed by standard methods. (Dubey R.C and Maheshwar D.K., 2002).

In the Confirmed test, positive results of the confirmatory test were spread plated on the EMB agar and incubated at 37^{0}C for 24 hours for colony appearance. (Dubey R.C and Maheshwar D.K., 2002). In the completed test colonies on the EMB agar were selected and inoculated into Lactose broth streaked on nutrient agar slants and incubated at 37^{0}C for 24 hours for final examination of the gas production. Gram's reaction has been performed (Dubey R.C and Maheshwar D.K., 2002)

IMViC (Indole, Methyl Red, Vogus Proskauer, Citrate utilization)

Indole Production

Isolates were inoculated in 5mL of tryptone water and Incubated at 37°C for 24 hrs. After incubation, a few drops of the Kovacs reagent were added to Observe for dark red-colored ring formation. Based on red-coloured ring formation bacteria are differentiated as indole positive or

negative. Pure cultures of E. coli (indole-positive control) and (indole-negative control) (Parija S C., 2005).

Methyl Red (MR) Test

Isolates were inoculated in Five mL of prepared MR-VP broth and incubated at 37°C for 24 hrs. After incubation, a few drops of methyl red indicator solution were added to the tubes. Based on the colour change from colourless to cherry red of MR-VP broth, bacteria are differentiated as Methyl Red (MR) positive or negative. Pure cultures of MR-positive bacteria *E. coli* (Positive Control) and MR-negative bacteria *Enterobacter aerogenes* (Negative Control) were taken (Parija S C., 2005)

Vogus- Proskauer (VP) test

Isolates were inoculated in Five mL of prepared MR-VP broth and incubated at 37°C for 24 hrs. After incubation, 0.5 ml (4-5 drops) of the α-naphthol (Baritt A reagent Himedia) solution and 0.5 ml of the 40% KOH solution containing 0.3% creatine (Baritt B reagent- Himedia) was added to the tubes. Based on the colour change to the red of MR-VP broth, bacteria are differentiated as VP positive or negative. Pure cultures of VP-positive bacteria *Enterobacter aerogenes* (Positive Control) and VP-negative bacteria *E. coli* (Negative Control) were taken (Parija S C., 2005)

Citrate utilization test

Isolates were streaked on Simmon's Citrate agar slants and incubated at 37°C for 48 hours. Growth on the medium accompanied by a rise in pH and change the medium from its initial green colour to deep blue. Results were compared with the Pure culture of citrate-positive bacteria *Enterobacter aerogenes* (Positive Control) and Citrate -negative bacteria *E. coli* (Negative Control) (Parija S C., 2005)

TABLE 2. Correlation of IMViC Results with Probable Identification (Dimri A.G *et al.*,2020)

Indole	Methyl red	Voges- Proskauer	Citrate	Probable identification
+	+	-	-	*Escherichia coli*
-	-	+	+	*Enterobacter or Klebsiella*
-/(+)	-	-	+	*Citrobacter*

Standard plate count

For SPC three tubes liquefied containing Plate count agar cooled at 45^0 C. 1 ml Water sample and medium was transferred to each three sterile Petri dish. Samples were mixed and incubated at 37^{0}C for 24 hours. Colony-forming units (CFU) were observed and a number of colonies were counted (Dubey R.C and Maheshwar D.K., 2002).

Physico-chemical characteristics:- Physico-chemical parameters such as TDS and pH were measured at the spot.

P^H :- Dissolved gases in potable water and industrial wastes such as heavy metals or other compounds affect the pH value of water and this finally changes the test of drinking water. P^H of the samples were determined with the help of a pH meter . The tolerance pH limit is 6.5 to 7.5 and other such as odor and colour were measured (Joline E.L *et.al.*, 2015)

Total Dissolved Solids:- The presence of different types of water-soluble minerals and organic matter denote total dissolved solids. The concentration of dissolved solids in water is an important parameter that determines the quality of drinking water. The analytical data for all the water samples in our investigation ranged from 218 mg/L to 241 mg/L, which is lower than the WHO and National Drinking Water Quality Standard (NDWQS) value (1000 mg/L). TDS Meter is used for measurement. Other physicochemical characteristics such as odour and Colour also tested (Jingxi M *et al.*,2020).

3. RESULTS AND DISCUSSIONS

The results obtained from analysis of water samples are summarized in table 3.

Microbiological analysis

The presumptive test showed all positive results (Photo1). Gas production in all samples. MPN index varies of each. Water Samples 2A (BANSILAL NAGAR), 7A (GARKHEDA) and 8A (N-8, CIDCO) contained highest 2400MPN/100ml coliform population and water sample 5A (N-13, HUDCO) contained least 28MPN/100ml coliform population. 6A (OSMANPURA) and 9A (SHIVAJI NAGAR) contained 1100MPN/100ml coliform population. 4A (HARSUL) and 10A (JYOTI NAGAR) water samples contained 210MPN/100ml coliform population. 1A (NIRALA BAZAR) and 3A (N-2, CIDCO) contained 43MPN/100ml coliform population. Confirmed test showed positive results. All samples showed small, dark, metallic sheen colonies on EMB agar. This sheen is due to the precipitation of methylene blue in the medium (Photo2). Completed test of all samples showed gas production in the lactose broth and their by confirmed presence of Gram negative coliform bacteria. According to Indian Standard Institute (ISI) specification desirable limit of coliform population is 1-10/100ml for drinking water (Dubey R.C and Maheshwar D.K., 2002).

IMViC (Indole, Methyl Red, Vogus Proskauer, Citrate utilization)

IMViC test carried out to differentiate between Gram negative Coliform. Either it is *E. coli*, *Enterobacter, Klebsiella or Citrobacter*. After all results were compared with table 1 it was evident that bacteria showed positive result is Escherichia coli. According to Indian Standard Institute (ISI) specification, No sample should contain *E. coli* in 100ml otherwise gastrointestinal infection will be caused.

PHOTO 1. Gas production in Presumptive test

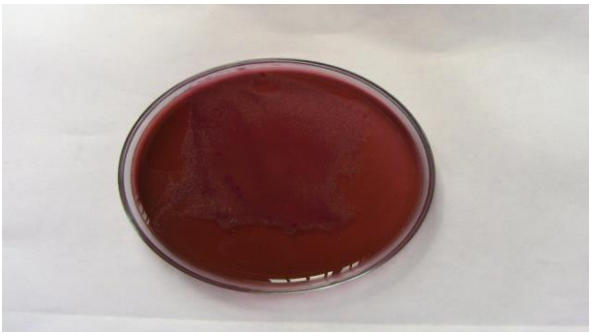


PHOTO 2. Confirmed Test. Metallic sheen colony appeared on EMB Agar

Standard plate count

A standard plate count is recommended for water analysis. Bacterial colonies were counted by colony counter and the average number of colonies was recorded in the range of 113cfu/ml to 277cfu/ml and others were too numerous to count.

Physico-chemical characteristics

PH is considered the most important in determining the corrosive nature of water. pH of all municipal water samples in the range of 6.93-7.56. Total dissolved solids calculated by the TDS meter were in the range of 218-241mg/l.

TABLE 3 Examination of Microbiological and Physiological analysis of water samples

SAMPLES → TESTS ↓	**1A**	**2A**	**3A**	**4A**	**5A**	**6A**	**7A**	**8A**	**9A**	**10A**
MPN Presumptive test-gas production	+	+	+	+	+	+	+	+	+	+
MPN INDEX PER 100ml	43	2400	43	210	28	1100	2400	2400	1100	210
Confirmed test-Metallic sheen colonies for *E. coli*	+	+	+	+	+	+	+	+	+	+
Complited Test-Gas production	+	+	+	+	+	+	+	+	+	+
Gram's nature	Gram - ve	Gram - ve	Gram - ve	Gram - ve	Gram - ve	Gram - ve	Gram - ve	Gram - ve	Gram - ve	Gram – ve
SPC Plate 1	124cfu/ml	TNTC	134cfu/ml	267cfu/ml	110cfu/ml	TNTC	260cfu/ml	270cfu/ml	TNTC	245cfu/ml
Plate2	165cfu/ml	TNTC	157cfu/ml	279cfu/ml	122cfu/ml	TNTC	TNTC	TNTC	TNTC	257cfu/ml
Plate3	143cfu/ml	TNTC	146cfu/ml	285cfu/ml	107cfu/ml	TNTC	TNTC	TNTC	290cfu/ml	283cfu/ml
Average	144cfu/ml	TNTC	145cfu/ml	277cfu/ml	113cfu/ml	TNTC	TNTC	TNTC	TNTC	262cfu/ml
IMViC										
Indole	+	+	+	+	+	+	+	+	+	+
MR	+	+	+	+	+	+	+	+	+	+
VP	-	-	-	-	-	-	-	-	-	-
Citrate	-	-	-	-	-	-	-	-	-	-
TDS	241mg/l	218mg/l	220mg/l	223mg/l	219mg/l	226mg/l	219mg/l	236mg/l	230mg/l	221mg/l
PH	7.13	7.11	7.50	6.93	7.19	7.56	7.05	7.00	7.22	7.30
Odor	Agree-able	Agree-able	Agree-able	Agree-able	Agree-able	Agree-able	Agree-able	pungent	Agree-able	Agree-able
Color	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless

Note: TNTC- Too numerous to count, cfu – colony forming units

4. CONCLUSION

Present study results show that Physiological parameters such as TDS and pH values are in the permissible limits. Bacteriological parameters of drinking water were found above the allowable limits of ISI. *E. coli* indicates that water is highly contaminated with fecal coliform. Water contamination may be due to leakage in supply pipes, bad sanitary conditions in the water purification plant, wastewater contamination, old water purification plant, short distance between the water supply network and sewage supply lines is some of the major problems associated with the presence of the indicator organism in the Aurangabad municipal corporation drinking water.

TABLE 3 Samples and their locations

SAMPLE	LOCATION
1-A	NIRALA BAZAR
2-A	BANSILAL NAGAR
3-A	N-2, CIDCO
4-A	HARSUL
5-A	N-13, HUDCO
6-A	OSMANPURA
7-A	GARKHEDA
8-A	N-8, CIDCO
9-A	SHIVAJI NAGAR
10-A	JYOTI NAGAR
A- Aurangabad Municipal Corporation	

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